INNOVATIONS IV
In Cancer Prevention and Research Conference

PROGRAM & ABSTRACTS

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Cancer Prevention & Research
Institute of Texas

November 9-10
2015
Renaissance Hotel
Austin, Texas

The 2015 CPRIT INNOVATIONS IN CANCER PREVENTION AND RESEARCH PROGRAM AND ABSTRACTS BOOK is published by the Cancer Prevention and Research Institute of Texas (CPRIT).

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INNOVATIONS IV
In Cancer Prevention And Research Conference

Program Guide

Abstracts

Academic Research

Product Development Research

Prevention

Greetings:

Texas has always been home to innovative people determined to accomplish big things. Our can-do spirit resulted in the invention of everything from liquid paper to integrated circuits, artificial hearts and the first space sui

Today, Texas is the front line in the battle against cancer, a scourge as old as mankind and one that affects thousands of our citizens every year. All of you—scientists, physicians, clinical researchers, product development entrepreneurs, public health professionals, patients, advocates and health care providers—are essential to this undertaking. It brings us great pleasure, therefore, to welcome you to "Innovations in Cancer Prevention and Research IV." This conference is designed to facilitate an exchange of ideas and showcase recent advances in the fight against cancer, many made by you right here in Texas thanks to funding provided through the Cancer Prevention and Research Institute of Texas (CPRIT).

Since the last Innovations conference, CPRIT grantees have been quite active: 417 new awards have been announced, 39 stellar researchers have come to our state and 14 companies have been added to the CPRIT early translational research company portfolio. You might also be interested to know that in all, CPRIT has funded the delivery of 2.5 million cancer prevention services to Texans in all 254 counties.

Welcome to Austin, and on behalf of the citizens of Texas and everyone in the world, thank you for participating in this historic effort and investing your time, energy and talents in this important cause.

Respectfully,

Greg Abbott	Dan Patrick	Joe Straus
Governor	Lieutenant Governor	Speaker of the House

Cancer Prevention & Research Institute of Texas

ABOUT CPRIT

Texas voters overwhelmingly approved a constitutional amendment in 2007 establishing the Cancer Prevention and Research Institute of Texas and authorizing the state to issue $3 billion in bonds to fund groundbreaking cancer research and prevention programs and services in Texas. CPRIT's goal is to expedite innovation in cancer research and product development, and to enhance access to evidence-based prevention programs throughout the state.

CPRIT's objective is to position Texas as a world-class leader in research and prevention through collaboration with a variety of entities, including public and private institutions of higher education, governmental and nongovernmental organizations, and private companies and others involved in the figh against cancer. CPRIT supports innovation in the selection of research projects emphasizing immediate or long-term medical breakthroughs; product development translational opportunities for research, and prevention services and health education for citizens with culturally appropriate information about ways in which their risks of developing and dying from cancer can be reduced. CPRIT accepts applications and awards grants for a wide variety of cancer-related research and for the delivery of cancer prevention programs and services by public and private entities located in Texas.

Since 2009, CPRIT has awarded more than $1.35 billion in grants to Texas researchers, institutions and organizations. CPRIT provides funding through its academic research, prevention and product development research programs. Programs made possible with CPRIT funding have reached all 254 counties of the state, brought nearly 100 distinguished researchers to Texas, advanced scientific and clinical knowledge, and provided more than 2.5 million life-saving education, training, prevention and early detection services to Texans.

More information about CPRIT is available at www.cprit.texas.gov. Follow CPRIT on Twitter @CPRITTexas and Facebook.

SCHEDULE AT A GLANCE

MONDAY, NOVEMBER 9

8:30am-8:45am — Plenary
Welcome — Ballroom A
Wayne Roberts, CPRIT;
Pete Geren, CPRIT

8:45am-10:30am — Plenary
The Promise and Perils of Immunotherapy — Ballroom A
Jim Allison, PhD, The University of Texas MD Anderson Cancer Center;
Malcolm Brenner, MD, PhD, Baylor College of Medicine;
Cassian Yee, MD, The University of Texas MD Anderson Cancer Center;
Margaret Kripke, PhD, CPRIT

10:30am-10:45am — *Coffee Break* — TBA

10:45am-11:30am — Plenary
Modern Epidemiology: Dark Wood, Glimmer of Hope — Ballroom A
David Katz, MD, MPH, Yale-Griffin Prevention Research Cente

11:30am-12:30pm — *Lunch Provided - No Program* — Ballroom B & Arbor

12:30pm-1:15pm — Academic Research & Prevention
Environmental Chemicals and Breast Cancer: What Do We Know? — Ballroom A
Julia Brody, PhD, Silent Spring Institute

Product Development Research
Elements of Successful Product Development Research Applications — Glass Oaks
Jack Geltosky, PhD, CPRIT;
Kristen Doyle, CPRIT;
Margaret Sampson, PhD, Baker Botts LLP;
Rob Sarisky, PhD, CPRIT;
Craig Rosenfeld, MD, CPRIT;
David Shoemaker, PhD, CPRIT

1:30pm-2:15pm — Prevention
Adapting and Disseminating Evidence-Based Prevention Programs — Wedgwood
Ross Brownson, PhD, CPRIT

Academic Research & Product Development Research
Moving From Research to Reality — Ballroom A
Barry Maurer, MD, PhD, Texas Tech University Health Sciences Center;
George Georgiou, PhD, The University of Texas at Austin

2:30pm-3:15pm — Academic Research
Research Advances: Updates from CPRIT Grantees — Ballroom A
Melanie Cobb, PhD, The University of Texas Southwestern Medical Center;
Philip Lupo, PhD, MPH, Baylor College of Medicine

Prevention
Prevention in Practice: Academic-Community Collaborations — Wedgwood
Linda Ross, DNP, Angelo State University;
Navkiran Shokar, MD, MPH, Texas Tech University Health Sciences Center El Paso;
Andrea Caracostis, MD, MPH, HOPE Clinic;
Stephen Wyatt, DMD, MPH, CPRIT

Product Development Research
Resources Part I – Texas Incubators — Glass Oaks
Emmanuelle Schuler, PhD, Johnson & Johnson Innovation;
Terry Zrubek, Office of the Governo , State of Texas;
Emma Wollschlager Schwartz, MPH, Medical Center of the Americas Foundation;
Tom Kowalski, Texas Healthcare & Bioscience Institute

3:15pm-4:45pm — Poster Session Presentations & Coffee Break
Poster Group A — Rio Grande

TUESDAY, NOVEMBER 10

8:00am-8:45am	Plenary ***The Evolution of Precision Oncology – Biological Complexity, Big Data & Big Price*** George Poste, PhD, Arizona State University	Ballroom A
8:45am-9:30am	Plenary ***Accelerating HPV Vaccine Uptake: The President's Cancer Panel Report*** Abby Sandler, PhD, National Cancer Institute	Ballroom A
9:30am-9:45am	*Coffee Break*	TBA
9:45am-10:30am	Academic Research & Prevention ***Liver Cancer in Texas: Causes and Cures*** Hashem El-Serag, MD, MPH, Baylor College of Medicine; Barbara Turner, MD, The University of Texas Health Science Center at San Antonio	Ballroom A
	Product Development Research ***University & Biotech Company Alliances*** Ferran Prat, PhD, The University of Texas MD Anderson Cancer Center; Patricia Hurn, PhD, The University of Texas System; Clifford Stocks, Theraclone Sciences, Inc.; Andrew Strong, Pillsbury Winthrop Shaw Pittman LLP; Harpreet Singh, PhD, CEO, Immatics US, Inc.	Trinity
10:30am-12:00pm	Poster Session Presentations ***Poster Group B***	Rio Grande
12:00pm-1:00pm	*Lunch Provided - No Program*	Ballroom B & Arbor
1:00pm-1:45pm	Academic Research ***Research Advances: Updates from CPRIT Scholars*** Raghu Kalluri, MD, PhD, The University of Texas MD Anderson Cancer Center; Yun Huang, PhD, Texas A&M University Health Science Center	Glass Oaks
	Product Development Research ***Resources Part II – Investors*** Chau Q. Khuong, MPH, Orbimed Advisors, LLC; Asish Xavier, PhD, Johnson & Johnson Innovation – JJDC, Inc.; Evan Melrose, MD, Spindletop Capital	Trinity
	Prevention ***Elements of Successful Prevention Applications*** Nancy Lee, MD, CPRIT; Rebecca Garcia, PhD, CPRIT	Wedgwood

TUESDAY, NOVEMBER 10

2:00pm-2:45pm	Academic Research ***Research Advances: Updates from CPRIT Grantees*** George Georgiou, PhD – Abstract #295, The University of Texas System; Jessica Zavadil – Abstract #296, The University of Texas Health Science Center at San Antonio; Vincent Bernard – Abstract #1, The University of Texas MD Anderson Cancer Center	Glass Oaks
	Product Development Research ***CPRIT Companies in Action: Early Stage Successes*** David G. Lowe, PhD, Aeglea BioTherapeutics; Paul Lammers, MD, Mirna Therapeutics, Inc.; Annemarie Moseley, MD, PhD, Bellicum Pharmaceuticals; Matt McManus, MD, PhD, Asuragen	Trinity
	Prevention ***Prevention in Practice: Implementing Programs in Rural Communities*** Simon Craddock Lee, PhD, MPH, The University of Texas Southwestern Medical Center; Carol Rice, PhD, RN, Texas A&M AgriLife Extension Service; Rakhshanda Layeequr Rahman, MD, Texas Tech University Health Sciences Center; Nancy Lee, MD, CPRIT	Wedgwood
2:45pm-3:30pm	Prevention – Prevention Networking Interest Groups	Wedgwood
	Academic Research ***Research Advances: Updates from CPRIT Grantees*** Vijetha Kumar, MS - Abstract #175, Baylor College of Medicine; Caroline Ahrens, PhD - Abstract #176, Texas Tech University Institute of Biosciences and Technology; Peter Davies, MD, PhD - Abstract #297, Texas A&M University System Health Science Center	Glass Oaks
	Product Development Research ***CPRIT Companies in Action: Early Stage Successes*** *(Continued)* David G. Lowe, PhD, Aeglea BioTherapeutics; Paul Lammers, MD, Mirna Therapeutics, Inc.; Annemarie Moseley, MD, PhD, Bellicum Pharmaceuticals; Matt McManus, MD, PhD, Asuragen	Trinity
3:45pm-4:15pm	Plenary ***CPRIT: A Look Forward*** Wayne Roberts, CEO, CPRIT	Glass Oaks

DETAILED SESSION DESCRIPTIONS AND SPEAKER INFORMATION

MONDAY, NOVEMBER 9

8:30 – 8:45 AM - PLENARY

Ballroom A

Welcome

Speakers

Wayne Roberts
Chief Executive Office
CPRIT

Mr. Roberts was named CEO of the Cancer Prevention and Research Institute of Texas in November 2013 after serving since December 2012 as the interim executive director. Prior to four years with The University of Texas Health Science Center at Houston as associate vice president for public policy, his career was weighted towards public finance and budget especially with respect to higher education. He held numerous senior positions under Governor Rick Perry in which, among other things, he authored the white paper and draft legislation creating the Texas Emerging Technology Fund to catalyze economic development, including transferring research conducted at universities to the Texas marketplace. He was Lieutenant Governor Bob Bullock's special assistant for budget and human services following 18 years with the Legislative Budget Board. Mr. Roberts received a bachelor's degree with honors and special honors in government from The University of Texas at Austin and a master's from the Lyndon B. Johnson School of Public Affairs at UT.

Pete Geren
Presiding Office
CPRIT Oversight Committee

Mr. Geren is the president of the Sid W. Richardson Foundation, which provides grants to educational, health, human service and cultural nonprofit organ zations in Texas. From 2001 to 2009, he served in the U.S. Department of Defense as special assistant to the secretary of defense, acting secretary of the Air Force, under-secretary of the Army and secretary of the Army. He also served four terms in the U.S. House of Representatives and was formerly assistant to U.S. Sen. Lloyd Bentsen. Geren received his bachelor's from The University of Texas in 1974 and a law degree from The University of Texas Law School in 1978.

8:45 – 10:30 AM - PLENARY

Ballroom A

The Promise and Perils of Immunotherapy

Harnessing the immune system to combat cancer has been an elusive goal in the history of cancer therapy. Recent advances in our understanding of how the immune system is regulated provide new optimism that immunotherapy can be an effective addition to other forms of cancer treatment. Three such approaches to immunotherapy will be presented by CPRIT grantees. A brief panel discussion on the promise and pitfalls of immunotherapy will follow the presentations.

Moderator

Margaret Kripke, PhD
Chief Scientific Offic
CPRIT

Dr. Kripke has served as CPRIT's chief scientific officer since January 2013. Previous , she was the executive vice president and chief academic officer at The University of Texas MD Anderson Cancer Center. During her tenure there, Dr. Kripke founded the Department of Immunology and was also a professor of immunology. She served for almost a decade on the three-person President's Cancer Panel, an honor reserved for the most distinguished oncology scientists in the nation. She holds a doctorate in immunology from the University of California at Berkeley and is a world-renowned expert in the immunology of skin cancers. Due to her esteemed position within the scientifi community, particularly as a champion of diversity in the workplace, the Margaret L. Kripke Legend Award was established by MD Anderson in 2008 to honor individuals who have enhanced the careers of women in cancer medicine and cancer science.

Speakers

Jim Allison, PhD
Chair, Immunology Department
The University of Texas MD Anderson Cancer Center

Dr. Allison serves as the chair of The University of Texas MD Anderson Cancer Center Immunology Department and is the executive director of the Moon Shots Program Immunotherapy Platform. Dr. Allison's pioneering research has led to treatment that unleashes the body's immune system against cancer, and to the the approval of Yervoy, which became the first FD approved checkpoint inhibitor when it was authorized for the treatment of advanced melanoma in 2011. This year, Dr. Allison was named winner of the Lasker-DeBakey Clinical Medical Research Award, one of the world's most prestigious scientific awards. He also received the Science of ncology Award by ASCO and the Pezcoller Foundation-AACR International Award for Cancer Research. Dr. Allison received the Giants of Cancer Care award for scientific advances in 2014. A CPRIT grant helped bring him back to his native Texas in 2012.

Malcolm Brenner, MD, PhD
Director, Center for Cell and Gene Therapy
Baylor College of Medicine

Dr. Brenner is the founding director of the Center for Cell and Gene Therapy at Baylor College of Medicine (BCM), Texas Children's Hospital and The Methodist Hospital. Currently, he serves as a professor in the Departments of Pediatrics and of Medicine at BCM. Dr. Brenner's clinical research interests span many aspects of stem cell transplantation, using genetic manipulation of cultured cells to obtain therapeutic effects. Efforts in his laboratory to analyze the cell of origin when relapse occurs in patients with acute myelogenous leukemia led Brenner's team to be the first to label autologou bone marrow cells genetically after purging, prior to being reintroduced to the patient. He is studying the effects of gene transfer into autologous neuroblastoma cells and the use of gene-modifie EBV-specific cytotoxic T lymphocytes for prevention and treatment of lymphoproliferative disorders, Hodgkin's disease, lung cancer and neuroblastoma. His group recently pioneered the first clinica use of a new safety switch for cellular therapy. Dr. Brenner is editor-in-chief of Molecular Therapy and a former president of the American Society for Gene and Cell Therapy (ASGCT) and the International Society for Cell Therapy. He has won many awards for his work, including the ASGCT Outstanding Achievement Award and the American Society of Hematology Mentor Award. Dr. Brenner received his PhD and medical degree from Cambridge University, England.

Cassian Yee, MD
Professor, Department of Melanoma Medical Oncology and Department of Immunology;
Director, Solid Tumor Cell Therapy Program
The University of Texas MD Anderson Cancer Center

Dr. Yee is a Burroughs Wellcome Scientist in Translational Research, an elected member of the American Society for Clinical Investigation, and co-leader of the Stand Up to Cancer Immunology Dream Team. His research was among the first to show that adoptive T-cell therapy holds great promise for treating melanoma. Additionally, he demonstrated for the first time that human T-cells can become long-lasting memory cells after infusion and, when combined with a checkpoint inhibitor, halt tumor growth in patients with metastatic melanoma. CPRIT funding helped bring him to Texas from the Fred Hutchinson Cancer Research Center at the University of Washington.

10:30 – 10:45 AM - COFFEE BREAK

10:45 – 11:30 AM - PLENARY

Ballroom A

Modern Epidemiology: Dark Wood, Glimmer of Hope

This session explores the importance of the "big picture" in patient care and health promotion at the population level. The case will be made for consensus on priority behavioral risk reduction strategies that have the greatest impact on population health. The value of a holistic approach to clinical care will be discussed, as will the potential of a short list of lifestyle factors to eliminate up to 80 percent of all chronic disease.

Speaker

David Katz, MD, MPH
Founding Director, Yale-Griffin Prevention Research Cente
Yale University

Dr. Katz is the founding director of Yale University's Prevention Research Center, president of the American College of Lifestyle Medicine, editor-in-chief of the journal Childhood Obesity, chief science officer for Nu al LLC, and director of the Integrative Medicine Center at Griffin Hospital. A clinician, researcher, author, inventor, and journalist, Dr. Katz has authored nearly 200 scientific papers an chapters, 15 books, and hundreds of online columns and blogs, with a resulting social media following of more than 400,000. A two-time diplomat of the American Board of Internal Medicine and a board-certified specialist in reventive medicine and public health, he is recognized globally for expertise in nutrition, weight management, and the prevention of chronic disease. Dr. Katz earned his undergraduate degree from Dartmouth College, his medical degree from the Albert Einstein College of Medicine and his master's in public health from the Yale University School of Public Health. The recipient of numerous awards and recognitions, Dr. Katz has been a widely supported nominee for the position of U.S. surgeon general, and has been recognized by Greatist.com as one of the 100 most influential people in health and fitness in the worl

11:30 AM – 12:30 PM - LUNCH

Ballroom B & Arbor

12:30 – 1:15 PM - ACADEMIC RESEARCH & PREVENTION

Ballroom A

Environmental Chemicals and Breast Cancer: What Do We Know?

Dr. Brody reviews the chemical pathways to breast cancer, evaluates the concordance between animal and human evidence, and considers what types of evidence should guide risk reduction. She'll discuss new rapid-screening technologies that can be adapted from drug discovery for use in testing consumer product chemicals for safety. Participants will learn about compelling new research opportunities and discover how to communicate with patients and public health leaders about what is known and yet to be discovered about environmental links to breast cancer.

Speaker

Julia Brody, PhD
Executive Director
Silent Spring Institute

Dr. Brody is the executive director of Silent Spring Institute and a leader in research on breast cancer and the environment, as well as community-based research and public engagement in science. Her research focuses on methods for reporting to people on their own exposures to hormone disruptors and other emerging contaminants when health effects are uncertain. Dr. Brody recently led a project connecting breast cancer advocacy and environmental justice in a study of household exposures to endocrine disruptors and air pollutants. Since 1996 Dr. Brody has been the principal investigator of the Cape Cod Breast Cancer and Environmental Study, a case-control study of 2,100 women that includes testing for 89 endrocrine disruptors in homes, along with historical mapping. She led a two-year study of scientific review of evidenc on animal mammary gland carcinogens and epidemiologic studies of breast cancer and environmental pollutants, diet, body size, and physical activity.

12:30 – 1:15 PM - PRODUCT DEVELOPMENT RESEARCH

Glass Oaks

Elements of Successful Product Development Research Applications

An in-depth review of the elements that go into a successful CPRIT Product Development Research application from members of the Product Development Review Council and the Oversight Committee, along with intellectual property counsel and CPRIT's deputy executive officer and general counsel. This session is relevant to anyone interested in applying for a CPRIT Product Development Research award.

Moderator

Craig Rosenfeld, MD
Oversight Committee; Product Development Research Subcommittee Chair
CPRIT

Dr. Rosenfeld is well-known in the field of biotechnology and onc logy after a distinguished career as a physician, advocate and entrepreneur. At nearly every turn, he has led essential cancer-fighting initiatives ranging from direct patient care an teaching medical students to directing bone marrow transplant facilities and founding biotechnology startups. He currently serves as CEO at Collaborative Medical Development, a firm purs ing therapies for neurogenerative and psychiatric diseases including Alzheimer's, Parkinson's and ALS. Dr. Rosenfeld is a member of numerous medical organizations including the American Society of Clinical Oncology and the New York Academy of Science. He was also highly involved in the Texas Israel Chamber of Commerce, having previously served on its board of directors.

Speakers

Jack Geltosky, PhD
Chair, Product Development Research Review Council
CPRIT

Dr. Geltosky, the managing director of JEG and Associates, LLC, has over 30 years of experience in the pharmaceutical industry, evenly split between R&D and licensing. He is a senior adviser to Arizona Technology Enterprises (AzTE), Arizona State University's intellectual property management and technology transfer organization. Dr. Geltosky has worked at DuPont, Johnson & Johnson, SmithKline Beecham, and was vice president of external science, technology and licensing at Bristol Myers Squibb. He holds a Bachelor of Science in chemistry from Memphis State University and a PhD in biochemistry from the California Institute of Technology.

Kristen Doyle
Deputy Executive Officer and General Counse
CPRIT

Ms. Doyle is responsible for overseeing the legal issues that arise as part of CPRIT's operations, including grant award contract negotiations, intellectual property and revenue sharing agreements, conflicts of interest and confidentiali , and regulatory/compliance issues. Prior to joining CPRIT, Ms. Doyle was a partner at an Austin-based law firm and served a Vice President of the Board of Directors for the Central Texas Chapter for the Leukemia and Lymphoma Society. Ms. Doyle has spent the majority of her legal career practicing administrative law, with an emphasis in the field of energy and regulatory la . She received her undergraduate degree in Public Policy, *magna cum laude*, from Indiana University and her doctorate of Jurisprudence from The University of Texas at Austin School of Law. In 2012, Ms. Doyle received her masters of science in technology commercialization from the McCombs School of Business at The University of Texas at Austin. She has been recognized four times as a Super Lawyers Texas Rising Star by Texas Monthly and named to the 2010 edition of The Best Lawyers in America.

Margaret Sampson, PhD
Partner
Baker Botts LLP

Dr. Sampson has a global, strategic intellectual property transaction and patent counseling practice focused in the areas of life sciences, pharmaceuticals, research tools, and medical devices. Dr. Sampson has extensive experience advising clients in evaluating patent portfolio positions; analyzing freedom-to-operate issues; identifying and evaluating targets for potential investment, mergers, or acquisitions; and assisting with joint development, inbound, and outbound licensing agreements. Dr. Sampson received a PhD in molecular and human genetics from the Baylor College of Medicine and her doctorate of jurisprudence from The University of Texas School of Law, where she was editor-in-chief of the Texas Intellectual Property Law Journal.

Rob Sarisky, PhD
Product Development Research Peer Review Panel
CPRIT

Dr. Sarisky is an experienced scientific and business professiona from the pharmaceutical industry, most recently serving as chief business officer of FORM Therapeutics, and vice president of Oncology Business Development and Licensing within Janssen Pharmaceuticals, a Johnson & Johnson company. Prior to that role, he held management positions at Centocor and GlaxoSmithKline Pharmaceuticals. Dr. Sarisky received a bachelor of science degree in biology from the University of Scranton, a PhD in genetics from the Pennsylvania State University College of Medicine, completed his postdoctoral training at the Johns Hopkins School of Medicine and holds an MBA in marketing from Lehigh University. He has authored more than 120 publications and patents and served on the editorial boards of two scientifi journals and the University of Pennsylvania Executive Advisory Committee for the HHMI Graduate Training in Medical Sciences.

David Shoemaker, PhD
Vice Chair, Product Development Review Council
CPRIT

Dr. Shoemaker is senior vice president, research and development, at Rho, a contract research organization. At Rho, he ensures company compliance with applicable regulations and guidance documents through consultations with other departments, training and review of procedural documentation. Dr. Shoemaker also represents Rho in client and auditor meetings regarding regulatory affairs. He graduated from Trinity College in Connecticut with a BS in chemistry and subsequently received his PhD in physiology and pharmacology from Duke University. Dr. Shoemaker has extensive experience in many therapeutic areas ranging from oncology, hematology, circulatory, respiratory, nervous system, transplants and many others.

1:30 – 2:15 PM - PREVENTION

Wedgwood

Adapting and Disseminating Evidence-Based Prevention Programs

This session explores recent advancements in disseminating and implementing science relevant to cancer prevention. It focuses on issues of program development and program adaptation, with a discussion on funding opportunities in community settings.

Speaker

Ross Brownson, PhD
Prevention Review Council
CPRIT

Dr. Brownson is the Bernard Becker professor at Washington University in St. Louis, with appointments in the Brown School and the Alvin J. Siteman Cancer Center. He is involved in numerous community-level studies designed to understand and reduce modifiable risk factor such as physical inactivity, obesity, and tobacco use. In particular, he is interested in the impacts of environmental and policy interventions on health behaviors and he conducts research on dissemination of evidence-based interventions with a focus on policy settings and health departments. Dr. Brownson is the author of seven books and over 400 peer-reviewed articles. He is active in numerous professional associations such as the American Public Health Association and the Missouri Public Health Association. He is the immediate past-president of the American College of Epidemiology.

Ballroom A

Moving From Research to Reality

Two CPRIT grantees offer insights and perspectives on how their projects moved across the continuum from research to product development.

Speakers

Barry Maurer, MD, PhD
Associate Professor
Texas Tech University Health Sciences Center

Dr. Maurer is a board-certified pediatric oncologist and academic developmental cancer researcher conducting basic laboratory investigations and early phase clinical trials. His major interests are the cellular mechanisms, translational development, and clinical testing of the cytotoxis retinoid, fenretinide, as a dihydroceramide-increasing agent, both as a single agent and in combination with other modulators of ceramide pathways in adult and pediatric tumor systems. He currently holds two investigator-initiated, FDA investigational new drug applications related to this research.

George Georgiou, PhD
Cockrell Chair in Engineering
The University of Texas at Austin

Dr. Georgiou holds the Cockrell Chair in Engineering at The University of Texas at Austin, where he serves on the faculties of chemical engineering, molecular biosciences and the Institute for Cell and Molecular Biology. He received his BSc degree from the University of Manchester, U.K. and his master's and PhD from Cornell University. His group is working in molecular biotechnology, antibody engineering and human B cell immunology. Dr. Georgiou was selected in 2013 by Nature Biotechnology as one of the top 20 translational researchers in the world. He has authored more than 220 research publications and is the co-inventor of over 78 issued and pending U.S. patents, of which more than 70 percent have been licensed to 21 biotech and pharma companies. Dr. Georgiou was elected to the National Academy of Engineering (NAE) and to the U.S. Institute of Medicine (IOM) of the National Academy of Sciences. In 2008 he was named as one of the top "100 Chemical Engineers of the Modern Era" by the American Institute of Chemical Engineers. He founded GGMJD LLC (acquired by NASDAQ-traded Maxygen) in 2000, Austin-based Aeglea Biotherapeutics, Inc., and Kyn Therapeutics LLC.

Ballroom A

Research Advances: Updates from CPRIT Grantees

Come along on a journey from ideas to discoveries. Two CPRIT grantees take you behind the scenes in their labs to talk about their research and the advances they've made in the fight against cance .

Speakers

Melanie Cobb, PhD
Professor, Department of Pharmacology
The University of Texas Southwestern Medical Center

Dr. Melanie Cobb received her undergraduate degree in biochemistry from the University of Chicago and her PhD in biological chemistry from Washington University in St. Louis in the laborabory of Garland Marshall. Following postdoctoral work with Ora Rosen at the Albert Einstein College of Medicine in New York, she joined the Department of Pharmacology at The University of Texas Southwestern Medical Center, where she is currently a professor and holds the Jane and Bill Browning, Jr. chair in medical science. Her interests are in cellular regulatory mechanisms.

Philip Lupo, PhD, MPH
Assistant Professor of Pediatrics
Baylor College of Medicine

Dr. Lupo is a molecular epidemiologist and assistant professor of pediatrics at Baylor College of Medicine. As a member of the epiCENTER in the Texas Children's Cancer Center, his particular interests are in the genetic and environmental determinants of childhood cancer. He is currently collaborating with other researchers and clinicians, both nationally and internationally, to examine the role of genes and prenatal exposures on the development of childhood cancer, as well as the identification of novel risk factors for late ffects among childhood cancer survivors. Dr. Lupo is an active collaborator with the Centers for Disease Control and National Birth Defects Prevention Study (NBDPS). Additionally, he is part of a multi-disciplinary team of experts assessing the intersection of childhood cancer and birth defects. As part of that effort, he is currently the principal investigator of the GOBACK (Genetic Overlap between Anomalies and Cancer in Kids) study. The ultimate goal of Dr. Lupo's research is to discover factors that can be used in childhood cancer prevention efforts and targeted interventions to limit the adverse consequences of childhood cancer treatment.

Wedgwood

Prevention in Practice: Academic-Community Collaborations

This interactive session explores the value, challenges and opportunities associated with community organization-academic partnerships on CPRIT Prevention proposals. Panelists include academics and a community organization leader, who will respond to questions about collaboration opportunities across the life span of a CPRIT project, including planning, implementation and evaluation.

Moderator

Stephen Wyatt, DMD, MPH
Chair
CPRIT Prevention Review Council

Dr. Wyatt is chair of the CPRIT Prevention Review Council and former dean of the College of Public Health at the University of Kentucky. He began serving as dean in November 2004. Prior to that, he focused for six years on research and teaching at UK while serving as the associate director for cancer control at the Markey Cancer Center. During his tenure at UK, Dr. Wyatt was the principal investigator for several large cancer control grants, including the NCI-funded Appalachia Cancer Network and Cancer Information Service and the CDC-funded Prevention Research Center and Comprehensive Cancer Control. Dr. Wyatt is currently the senior associate director for UK's Center for Clinical and Translational Science and the vice president for research for Norton Healthcare in Louisville, Kentucky.

Speakers

Linda Ross, DNP
Executive Director, Center for Wellness Engagement and Development;
Director, Laura W. Bush Institute for Women's Health
Angelo State University

Dr. Ross has more than 30 years of experience in nursing and healthcare/nursing administration. She currently serves as the executive director of the Center for Wellness Engagement and Development and director of The Laura W. Bush Institute for Women's Health at Angelo State University. She co-authored and served as project director for a $1.27 million grant to implement an accelerated/online LVN-RN program. Prior to joining the nursing faculty at Angelo State University in 2005, she served as administrator of a multi-speciality physician clinic. She has worked in various areas of hospital and nursing administration. Dr. Ross is a Certified Professional in Healthcare Quality (CPHQ) a d Healthcare Quality Management, and a fellow of the American Institute of Healthcare Quality. She has a DNP from Chatham University, a master of science degree from Texas Woman's University and a bachelor of science degree in nursing from Baylor University.

Navkiran Shokar, MD, MPH
Director, Cancer Prevention and Control
Texas Tech University Health Sciences Center, El Paso

Dr. Shokar is associate professor of Family and Community Medicine and Biomedical Sciences, vice chair for research, Department of Family and Community Medicine, and director of Cancer Prevention and Control in the Center of Excellence for Cancer at Texas Tech University Health Sciences Center Paul L. Foster School of Medicine, El Paso. Her research interests include interventions in cancer prevention and control, medical decision making, health services research, and cancer health disparities. She is the principal investigator on CPRIT-funded prevention grants for colorectal, breast, and cervical cancer screening programs in El Paso and Hudspeth Counties. They consist of culturally tailored and community-based education, clinical service delivery for screening and diagnosis, and navigation.

Andrea Caracostis, MD, MPH
Chief Executive Office
HOPE Clinic

Dr. Caracostis has been the CEO of the Asian American Health Coalition - HOPE Clinic since 2007. She has been recognized for her work in health care access and is well known for her spirit of collaboration and partnership building. Under the leadership of Dr. Caracostis, HOPE Clinic has grown from a staff of seven and $700,000 operation in 2008 to a staff of 80 and budget of $8 million. It provides care to patients 60 hours a week in 14 different languages. Prior to joining the HOPE Clinic, Dr. Caracostis worked as a provider in the Migrant Health Center (MHC), a consultant to Migrant Clinicians Network, and provided technical assistance to Community Health Centers around the country. She has been active in the Bureau of Primary Health Care's Health Disparities Collaboratives, as a board member of the Texas Community Health Centers, and as a member of many national health care advisory committees.

2:30 – 3:15 PM - PRODUCT DEVELOPMENT RESEARCH

Glass Oaks

Resources, Part I - Texas Incubators

The two resources panels will explore how incubators, accelerators, and investors enable talented researchers in Texas to take innovation to the next level, furthering a goal of helping entrepreneurs advance science through transformational solutions for cancer patients.

Moderator

Tom Kowalski
President
Texas Healthcare & Bioscience Institute

Mr. Kowalski is president and CEO of the Texas Healthcare and Bioscience Institute (THBI) in Austin, Texas. The Institute is a public policy research organization whose purpose is to promote medical research, development and manufacturing in Texas. THBI consists of leading biotechnology, medical device, agriculture and pharmaceutical companies, universities, private research institutions, chambers of commerce and economic development corporations. Mr. Kowalski was appointed in 2002 to Governor Rick Perry's state council on science and biotechnology development. With over thirty years of political and policy experience, Mr. Kowalski was appointed in 1989 by Governor Bill Clements to the board of regents of the Texas State University System. He served as chairman of the board of regents in 1995. In addition, Mr. Kowalski was chairman of the Texas State University Development Foundation and is the current chairman of the McCoy College of Business Administration advisory council. He is the former chairman of Bio's Council of State Biotechnology Associations.

Speakers

Emmanuelle Schuler, PhD
Head of JLABS @TMC
Johnson & Johnson Innovation

Dr. Schuler is the site leader for JLABS @TMC, Johnson & Johnson's latest life science incubator. She focuses on bringing together executives, investors, entrepreneurs, thought leaders, clinicians and inventors to create innovative solutions that impact healthcare delivery and patients' lives. She collaborates with J&J Innovation and J&J's corporate venture capital group to attract companies in alignment with their strategic efforts in biopharma, diagnostics, medical devices and consumer healthcare. She brings over 10 years of experience in intellectual capital management, strategic alliances, and joint ventures. Prior to joining J&J, she led IP-based transactions at The University of Texas MD Anderson Cancer Center. Before

that, she was responsible for industry-based strategic collaborations at the University of Houston. She consulted with the European Commission on innovation policy issues. She also worked on science policy projects under the mentorship of Nobel Laureate Dr. Richard E. Smalley at Rice University. Dr. Schuler earned a bachelor of science degree in chemistry from Université du Québec à Montréal, a PhD in chemistry from McGill University, and a master's in business administration from Rice University.

Terry Zrubek
Director of Economic Development
Office of the Governo , State of Texas

Mr. Zrubek has valuable and productive experience in both the private and governmental sectors. After receiving a degree in economics from Texas State University, he worked for Newell Manufacturing Company, a Fortune 500 Company that later merged to become Newell Rubbermaid. During his time there, Zrubek focused on numerous aspects of finance marketing and sales dealing with consumer interests and preferences. Since 2003, he has been employed with the State of Texas, where he has developed an expert level of understanding of state government and its budget, legislative, and policy processes. Zrubek holds an MBA degree from St. Edwards University, with a concentration in corporate finance During his years as a state governmental employee he served in various capacities for the Texas Department of Public Safety, state Senator Steve Ogden, the Texas Commission on Environmental Quality, the Lower Colorado River Authority, and Governors Rick Perry and Greg Abbott.

Emma Wollschlager Schwartz, MPH
President
Medical Center of the Americas Foundation

Ms. Schwartz is the president of the Medical Center of the Americas (MCA) Foundation. She leads, advocates for, and coordinates the development of the MCA campus. She also manages the foundation's day-to-day operations and coordinates the activities of the foundation's board of directors. Ms. Schwartz is the founder and president of Wollschlager Consulting, LLC, d/b/a W Consulting, LLC, a healthcare management and regulatory compliance consulting company. Prior to launching her own consulting firm, Ms. Schwartz wa the director of compliance consulting for Sinaiko Healthcare Consulting, Inc. in Los Angeles, California. Before that, she was the assistant director of legal compliance for Sierra Providence Health Network, a multi-hospital Tenet Healthcare System in El Paso, Texas. At Tenet, she directed both legal and contract compliance efforts. Ms. Schwartz received her bachelor's degree from Stanford University in human biology with a concentration in comparative health policy, and she received her master of public health from the UCLA School of Public Health in health services management.

3:15 – 4:45 PM - POSTER SESSION PRESENTATIONS

Rio Grande

Poster Group A

The poster sessions provide a great opportunity to network and exchange ideas. Presenters are assigned to either Group A or Group B. Group A is scheduled on Monday and Group B on Tuesday to discuss their work and answer questions. Abstracts not designated as Group A or B are published in the program book but not presented.

DETAILED SESSION DESCRIPTIONS AND SPEAKER INFORMATION

TUESDAY, NOVEMBER 10

8:00 – 8:45 AM - PLENARY

Ballroom A

The Evolution of Precision Oncology - Biological Complexity, Big Data & Big Price

Despite the enormous promise of precision oncology, technical progress alone is insufficient. D . Poste discusses how large scale adoption of molecular profiling in cancer will enerate unprecedented amounts of data and the need for new data analytics.

Speaker

George Poste, PhD
Chief Scientist, The Complex Adaptive Systems Initiative and the Del E. Webb Professor of Health Innovation
Arizona State University

Dr. Poste is chief scientist, Complex Adaptive Systems Initiative, Regents' professor and Del E. Webb Chair in Health Innovation at Arizona State University (ASU). He was the founder and director of the Biodesign Institute at ASU. Dr. Poste serves on the board of directors of Monsanto, Exelixis, Caris Life Sciences, and the scientific adv sory board of Synthetic Genomics. Prior to working at ASU, Dr. Poste served as chief science and technology office and president, R&D, of SmithKline Beecham, where he shepherded the successful registration of 31 drug, vaccine and diagnostic products. He has won numerous awards, published over 350 research papers and edited 14 books on pharmaceutical technologies and oncology. A fellow of the Royal Society, Dr. Poste is currently a member of the U.S. Institute of Medicine Board on Global Health and has served on advisory committees for multiple U.S. government agencies in the areas of defense, national security and healthcare.

8:45 – 9:30 AM - PLENARY

Ballroom A

Accelerating HPV Vaccine Uptake: The President's Cancer Panel Report

During 2012-2013, the President's Cancer Panel held workshops to explore underuse of HPV vaccines and ways to accelerate vaccine uptake. In its February 2014 report, the Panel provided specific, targeted, and actionabl recommendations to address barriers to uptake of this cancer prevention vaccine. The Panel's report, and actions taken by various stakeholders to implement the report's recommendations, will be discussed.

Speaker

Abby Sandler, PhD
Special Assistant to the Director
National Cancer Institute

Dr. Sandler has worked at the National Cancer Institute (NCI) since 1999 and has served as executive secretary of the President's Cancer Panel since January 2005. Since 2013, she also has served as special assistant to the director, NCI Center for Cancer Research, on the Rare Tumors Initiative. Prior to working at NCI, Dr. Sandler was a program director for the Department of Veterans Affairs Medical Research Service in Washington, D.C., and was also a scientist for Pro-Virus, Inc. (now called Wellstat Biologics) in Gaithersburg, Maryland. Her research background focuses on molecular tumor virology and gene therapy. She received her bachelor's in biology from Rensselaer Polytechnic Institute and her PhD in biology from The Johns Hopkins University. Dr. Sandler carried out her postdoctoral research at NCI.

9:30 – 9:45 AM - COFFEE BREAK

9:45 – 10:30 AM - ACADEMIC RESEARCH & PREVENTION

Ballroom A

Liver Cancer in Texas: Causes and Cures

The session includes a discussion of the epidemiology and prevalence of Hepatocellular Carcinoma focusing on Texas and primary risk factors including Hepatitis C virus (HCV) and non-alcoholic fatty liver disease. It also provides an overview of two CPRIT-funded projects approved last May. Drs.Turner and El-Serag review HCV prevention opportunities through baby boomer screening and access to new direct-acting drugs to cure HCV. Attendees will hear about findings from a previously completed rogram of inpatient baby boomer screening and plans for engaging diverse practices in South-Central Texas and Dallas.

Speakers

Hashem El-Serag, MD, MPH
Chief, Gastroenterology and Hepatology Section
Baylor College of Medicine

Dr. El-Serag obtained his medical degree from Al-Arab Medical University in Libya. He completed his internship and residency in internal medicine at Greenwich Hospital, Yale University, and completed a fellowship in clinical gastroenterology at the University of New Mexico, where he also earned a master's degree in public health. In 1999 Dr. El-Serag joined the Michael E. DeBakey VA Medical Center and Baylor College of Medicine in Houston, where he became chief of the Section of Gastroenterology and Hepatology and leader of the Cancer Prevention and Population Sciences at the Dan L. Duncan Cancer Center. His research focuses on the clinical epidemiology and outcomes of several digestive disorders, including hepatocellular carcinoma. His seminal work on hepatocellular carcinoma, "Rising Incidence of Hepatocellular Carcinoma in the United States," was published in the New England Journal of Medicine and has been cited more than 2,000 times.

Barbara Turner, MD
Director, ReACH Center
The University of Texas Health Science Center at San Antonio

Dr. Turner trained in medicine at the University of Pennsylvania and was a tenured professor of medicine at the University of Pennsylvania School of Medicine when she and her husband were recruited to The University of Texas Health Science Center in San Antonio in 2010. Dr. Turner now directs the ReACH (Research to Advance Community Health) Center, a collaboration of the Health Science Center and The University of Texas School of Public Health. She has more than 140 peer-reviewed publications and has led several national physician organizations, serving as regent of the American College of Physicians, a national organization or more than 125,000 internists, and as president of the Society of General Internal Medicine (SGIM), a national organization of all the leading researchers and educators in general medicine.

9:45 – 10:30 AM - PRODUCT DEVELOPMENT RESEARCH

Trinity

University & Biotech Company Alliances

Texas prides itself on the preeminence of its academic medical centers such as MD Anderson, UT Southwestern, Baylor College of Medicine, UTMB and others. Forming strategic alliances with these institutions can be a significant achievement for biotech companies that are advancin novel cancer vaccines, therapeutics or diagnostics. The institutions are creative in the ways in which they support the growth and success of these companies and, in some cases, invest in the companies with which they are partnering. The panel of experts will review the types of alliances that have been formed in the past and what we can expect in the future.

Moderator

Andrew Strong
Partner
Pillsbury Winthrop Shaw Pittman LLP

Mr. Strong is a partner in the Pillsbury law firm s Houston and Austin offices. His practice i focused on the biotech side of life sciences where he assists companies on general corporate, capital market, partnering, IP, employment and regulatory matters. Mr. Strong is the former president and CEO of Kalon Biotherapeutics, LLC, a biologics and manufacturing company formed by The Texas A&M University System in 2011. In the span of three years, Kalon grew to a staff of over 100, was a CPRIT awardee and secured over $90 million in contracts from GlaxoSmithKline, MD Anderson, BARDA and others. In 2014 he oversaw a process that led to the successful sale of Kalon in December to a subsidiary of FUJIFILM Corporation and

Mitsubishi Corporation. Prior to joining Kalon, Mr. Strong served as the general counsel and compliance officer f r The Texas A&M University System where he was responsible for all legal matters of its 11 universities, seven state agencies and health science center. He received a bachelor of science degree from Texas A&M University in civil engineering and is a registered professional engineer in Texas. He also received a doctorate of jurisprudence from South Texas College of Law.

Speakers

Ferran Prat, PhD
Vice President, Strategic Industry Ventures
The University of Texas MD Anderson Cancer Center

Dr. Prat is vice president of Strategic Industry Ventures, helping the faculty and researchers at The University of Texas MD Anderson Cancer Center develop collaborative opportunities with pharmaceutical, biotech, diagnostics, imaging, laboratory medicine and other industry partners. Previously, Dr. Prat held a number of industry and academic positions, including vice president, oncology and women's health, at Alere Inc., VP for licensing at Biosite Inc., management consultant at McKinsey & Co., engineer at Chromogenia-Units, and researcher at the University of California at Los Angeles (UCLA). Dr. Prat has a PhD in organic chemistry from UCLA and a law degree from the University of San Diego School of Law.

Patricia Hurn, PhD
Vice Chancellor for Research and Innovation
The University of Texas System

Dr. Hurn is vice chancellor for research and innovation at The University of Texas System. She serves as the chief health research officer to the U System and its six academic health center campuses. She is also an active neuroscientist and is internationally known for her work in understanding the cellular and molecular basis of gender differences in response to experimental brain injury. Dr. Hurn serves as a research professor in neurobiology in The University of Texas at Austin's College of Natural Sciences. She directs a translational laboratory that studies the role of hormone in post-stroke immunology. She holds bachelor's and master's degrees of science in nursing, and earned a doctorate in physiology from The Johns Hopkins University, where she went on to become a full professor. At Oregon Health and Science University (OHSU), Dr. Hurn served as professor and vice chair for research in the Department of Anesthesiology and Perioperative Medicine, and as professor of neurology and of physiology and pharmacology.

Clifford Stocks
Chief Executive Office
Theraclone Sciences, Inc.

Mr. Stocks is CEO of Theraclone Sciences, Inc., a therapeutic antibody company focused on immuno-oncology and the treatment of infectious disease. Previously he helped form and served as chief business officer of Calistoga Pharmaceuticals, nc. Mr. Stocks brings more than two decades of experience in the biotech industry. His career includes 15 years at ICOS Corporation, where he served as an executive officer and vice p esident of business development. While at ICOS, he led acquisitions and joint venture activities as well as alliance formation, strategy, licensing and deal making. He played an instrumental role on the leadership team that developed and launched Cialis, and was a key architect of the Lilly ICOS joint venture partnership leading to their $2.3 billion merger in 2007. Previously in his career, Mr. Stocks was a management consultant in the health services practice of Booz, Allen & Hamilton, and his early career includes academic research on staff in the department of immunology at the University of Utah and on staff in the department of molecular genetics and cell biology at the University of Chicago, where he also received an MBA degree from the University of Chicago Booth Graduate School of Business.

Harpreet Singh, PhD
Chief Executive Office
Immatics US, Inc.

Dr. Singh co-founded Immatics Biotechnologies GmbH, the parent company of Immatics US, Inc., during his PhD studies in 2000. Since then he has served as managing director and chief scientific officer of Immatics GmbH, which is dedicated t the translation of science into highly innovative cancer immunotherapeutics. At Immatics GmbH, Dr. Singh leads a team dedicated to Target and TCR discovery, immunology, CMC and translational development. He co-founded Immatics US, Inc. this year with help from a CPRIT grant.

Dr. Singh completed his PhD in immunology at the University of Tuebingen with Hans-Georg Rammensee, a pioneering immunologist who discovered basic principles of all T-cell based immunotherapies – the presentation of peptides by HLA receptors – in the 1990s. Dr. Singh holds numerous patents and is the co-author of 30 publications in peer-reviewed journals, including Nature Medicine, Nature Biotechnology, Journal of Experimental Medicine, and Brain and Blood.

10:30 AM – 12:00 PM – POSTER SESSION PRESENTATIONS

Rio Grande

Poster Group B

The poster sessions provide a great opportunity to network and exchange ideas. Presenters are assigned to either Group A or Group B. Group A is scheduled on Monday and Group B on Tuesday to discuss their work and answer questions. Abstracts not designated as Group A or B are published in the program book but not presented.

12:00 – 1:00 PM - LUNCH

Ballroom B & Arbor

Glass Oaks

Research Advances: Updates from CPRIT Scholars

Since its inception, CPRIT funding has helped recruit almost 100 world class cancer researchers to Texas institutions. In this session, two of them will discuss their work to advance our knowledge of cancer.

Speakers

Raghu Kalluri, MD, PhD
Chairman, Department of Cancer Biology
The University of Texas MD Anderson Cancer Center

Dr. Kalluri is chairman and professor of the Department of Cancer Biology and the director of the Metastasis Research Center at The University of Texas MD Anderson Cancer Center, where he also holds the Rebecca and Joseph Brown endowed chair. This year Dr. Kalluri's lab at MD Anderson received widespread attention for research which could lead to the discovery of a blood test that detects pancreatic cancer early on. Dr. Kalluri received his PhD in biochemistry and molecular biology from the University of Kansas Medical Center and his medical degree from Brown University Medical School. He was a postdoctoral fellow and a research associate at the University of Pennsylvania Medical School and served as professor of medicine at Harvard Medical School (HMS). Dr. Kalluri held appointments in the Department of Biological Chemistry and Molecular Pharmacology at HMS, Harvard MIT division of health sciences and technology, Harvard Stem Cell Institute. He was a research fellow of the HMS Peabody Society. Dr. Kalluri was honored this year with the prestigious Jacob-Henle Medal for his discoveries related to autoimmune and genetic kidney diseases, organ fibros s and cancer biology. CPRIT funding helped bring Dr. Kalluri to Texas from HMS in 2012.

Yun Huang, PhD
Assistant Professor, Center for Epigenetics and Disease Prevention
Texas A&M University Health Science Center

Dr. Huang joined the newly created Center for Epigenetics and Disease Prevention at the Texas A&M Health Science Center Institute of Biosciences & Technology in Houston last year. Her research focuses on epigenetics, or the "software" that runs the human genome, which is one of the most promising targets for disease prevention. Dr. Huang received her doctorate in biochemistry from Georgia State University. In 2009 she joined Dr. Anjana Rao's laboratory at the Immune Disease Institute at Harvard Medical School as a GSK-Immune Disease Institute Alliance fellow and Leukemia and Lymphoma Society fellow. During her postdoctoral training, Huang significantly contributed to the groundbreaking discovery of TET enzymes, a new type of epigenetic code writer, and was among the first to characterize their biological func ions in myeloid cancers and embryonic stem cells. Her discovery resulted in more than 15 peer-reviewed publications in top scientific journals and 1,500 citations in four short years A CPRIT recruitment grant helped bring her to Texas from the La Jolla Institute for Allergy and Immunology in California.

Trinity

Resources, Part II - Investors

The two resources panels will explore how incubators, accelerators, and investors enable talented researchers in Texas to take innovation to the next level, furthering a goal of helping entrepreneurs advance science through transformational solutions for cancer patients.

Moderator

Evan Melrose, MD
Founding Managing Director
Spindletop Capital

Dr. Melrose has 25 years of experience in a variety of health care investments, including research, clinical practice, education, and health policy. Prior to founding Spindletop Capital, he was the founding managing director of PTV Sciences, a Texas-based venture capital firm and a director with Burrill & Company, a San Francisco-based life science private equity/ venture capital firm. He has been a board member and advisor to many organizations including BayBIO-BayBioNest, BioHouston and MD Anderson Technology Review Committee. Dr. Melrose received his bachelor's degree from the University of Pennsylvania, his MD from Indiana University School of Medicine and an MBA from The Wharton School. In 2013 the National Association of Corporate Directors (NACD) elected Dr. Melrose as a NACD Board Leadership Fellow, the highest level of credentialing for corporate directors and corporate governance professionals.

Speakers

Chau Q. Khuong, MPH
Private Equity Partner
Orbimed Advisors, LLC

Mr. Khuong joined OrbiMed in 2003 as a private equity partner. His experience includes start-up operations and business development at Veritas Medicine, Inc. and in basic science research at the Yale School of Medicine and at Massachusetts General Hospital. He was a summer associate in the new ventures technology transfer group at Columbia University. Mr. Khuong is a Yale Univesity graduate with a bachelor of science degree in molecular, cellular and developmental biology and an MPH with a concentration in infectious disease.

Asish Xavier, PhD
Vice President, Venture Investments
Johnson & Johnson Innovation - JJDC, Inc.

Dr. Xavier previously worked in business development at BioRexis Pharmaceutical, Inc., which was acquired by Pfizer in 2007. While at BioRexis, he ass sted the company in raising a $30 million second round of financing. D . Xavier has worked in business development at Structural GenomiX, Inc., acquired by Eli Lilly in 2008, and was a project leader at Message Pharmaceuticals, Inc. Dr. Xavier received a PhD from the University of Houston and a master of business administration from the Wharton School of the University of Pennsylvania, where he graduated with honors. He received a bachelor of technology in chemical engineering from the Indian Institute of Technology, Kanpur, India.

1:00 – 1:45 PM - PREVENTION

Wedgwood

Elements of Successful Prevention Applications

CPRIT Prevention Review Council member Dr. Nancy Lee and Chief Prevention Program Officer D . Rebecca Garcia, review the elements of a successful CPRIT Prevention program application. This is relevant to anyone interested in applying for a CPRIT Prevention award.

Speakers

Nancy Lee, MD
Prevention Review Council
CPRIT

Dr. Lee is the deputy assistant secretary of women's health and director of the Office o Women's Health (OWH) at the U.S. Department of Health and Human Services. For most of her career, she was employed at the Centers for Disease Control (CDC), where she focused on cancer screening and epidemiology, safety of contraceptive methods, and HIV infection among women. She has extensive experience in women's health, surveillance systems, and cancer prevention and control. Dr. Lee has published more than 100 articles in scientifi journals. From 1999-2004, she served as director of the CDC's division of cancer prevention and control. She joined the OWH in 2011, where her work has concentrated on the Affordable Care Act and women's preventive services, women's health across the life span, and violence against women.

Rebecca Garcia, PhD
Chief Prevention and Communications Office
CPRIT

Dr. Garcia heads up CPRIT's prevention and communications efforts. Her responsibilities include directing the prevention program and fostering collaboration among the cancer and disease prevention community to maximize CPRIT's impact. In addition, she is responsible for overseeing CPRIT's strategic communications efforts. Prior to joining CPRIT in 2009, she served as vice president, continuing professional development, for Physicians' Education Resource (PER), a medical education and communications company. Previously, Dr. Garcia was vice president of health sciences for the Susan G. Komen for the Cure where she managed Komen's research grants and education programs. Dr. Garcia received a bachelor's degree in medical technology from the University of Texas Health Science Center at Dallas. She obtained a master's degree in instructional design from the Department of Biomedical Communications at the University of Texas Health Science Center at Dallas and a PhD in adult education from the Department of Higher Education at the University of North Texas.

2:00 – 2:45 PM - ACADEMIC RESEARCH

Glass Oaks

Research Advances: Updates from CPRIT Grantees

Three CPRIT grantees discuss their work and what it means in the fight aga nst cancer.

Speakers

George Georgiou, PhD
Abstract #295
The University of Texas System

Jessica Zavadil
Abstract #296
The University of Texas
Health Science Center at San Antonio

Vincent Bernard
Abstract #1
The University of Texas
MD Anderson Cancer Center

Wedgwood

Prevention in Practice: Implementing Programs in Rural Communities

A discussion of how to innovatively collaborate to achieve effective partnerships that result in advancing delivery of evidence-based preventive services in rural communities. Panelists will explain how unique partners can help bridge the geographic distances, develop new resources, provide flexibility and identify promising new approaches

Moderator

Nancy Lee, MD
Prevention Review Council
CPRIT

Dr. Lee is the deputy assistant secretary of women's health and director of the Office o Women's Health (OWH) at the U.S. Department of Health and Human Services. For most of her career, she was employed at the Centers for Disease Control (CDC), where she focused on cancer screening and epidemiology, safety of contraceptive methods, and HIV infection among women. She has extensive experience in women's health, surveillance systems, and cancer prevention and control. Dr. Lee has published more than 100 articles in scientifi journals. From 1999-2004, she served as director of the CDC's division of cancer prevention and control. She joined the OWH in 2011, where her work has concentrated on the Affordable Care Act and women's preventive services, women's health across the life span, and violence against women.

Speakers

Simon Craddock Lee, PhD, MPH
Assistant Professor of Clinical Sciences
The University of Texas Southwestern Medical Center

Dr. Lee is an assistant professor of clinical sciences and a member of the Population Sciences and Cancer Control program at The University of Texas Southwestern Harold C. Simmons Comprehensive Cancer Center. A medical anthropologist, Dr. Lee was recruited to Dallas from the National Cancer Institute, where he completed postdoctoral training as a cancer prevention fellow. His research examines the culture and organization of cancer prevention and care delivery in safety-net settings, both urban and rural. As a CPRIT grantee, he led the design and implementation of a de-centralized regional delivery model for breast cancer screening and patient navigation across 17 rural and underserved counties serving 18,000 unique women, now supported by a CPRIT competitive renewal grant.

Carol Rice, PhD, RN
Professor and Health Specialist; Assistant Director
Texas A&M AgriLife Extension Service

Dr. Rice serves as project director for a CPRIT prevention services project, Increasing Breast and Cervical Cancer Screening and Diagnostic Rates in Rural, Frontier, and Border Counties. The project provides education, navigation, transportation and access to clinical services in over 50 counties for uninsured women. She currently is a professor and extension health specialist at Texas A&M AgriLife Extension Service. She specializes in community health and wellness programs delivered through the extension system. Dr. Rice's past experience includes a variety of clinical positions with a community focus and 21 years teaching undergraduate and graduate nursing at several schools within The University of Texas System. Since coming to the Texas A&M AgriLife Extension Service in 1995, she has worked to support and extend health education conducted by county extension agents. She serves as project director for early detection and cancer awareness for rural Texans and reducing tobacco use by youth in rural communties. Dr. Rice received her bachelor's degree from the University of Wisconsin, a master's from

the University of California at San Francisco and a PhD from The University of Texas at Austin. All of her degrees are in nursing.

Rakhshanda Layeequr Rahman, MD
Professor of Surgery; Director, Breast Center of Excellence
Texas Tech University Health Sciences Center

Dr. Rahman leads a CPRIT-funded prevention project, Access to Breast and Cervical Care for West Texas, which provides screenings and education for residents in 26 Texas Panhandle counties. She joined the Texas Tech University Health Sciences Center (TTUHSC) in 2009 after serving as the founder and director of the interdisciplinary breast fellowship program at the University of Massachusetts. At TTUHSC, Dr. Rahman spearheaded formation of the firs nationally accredited Breast Center of Excellence in the Texas Panhandle. Currently, she is the director of the Amarillo Breast Center of Excellence and professor of surgery for the TTUHSC School of Medicine. Dr. Rahman serves on numerous local and national boards and committees including the American Society of Breast Surgeons and the Amarillo Area Breast Health Coalition. She graduated from the Aga Khan University Medical College in Karachi, Pakistan, where she completed her internship and residency. She then spent several years furthering her studies, completing fellowships in breast and general surgery in 2001 at Aga Khan, followed by another fellowship at the University of Arkansas for the medical sciences division of surgical oncology.

2:00 – 2:45 PM & 2:45 – 3:30 PM - PRODUCT DEVELOPMENT RESEARCH

Trinity

CPRIT Companies in Action: Early Stage Successes

To date, CPRIT has awarded over $250 million in Product Development Research grants. Panelists will discuss the projects their companies are working on and detail early stage successes. In addition, their experiences with CPRIT and the significance of CPRI support to their companies will be covered.

Moderator

Matt McManus, MD, PhD
Chief Executive Office
Asuragen

Dr. McManus joined Asuragen in August 2014 with more than 20 years of clinical diagnostic leadership experience, and was previously the CEO and president of PrimeraDx, Inc., a molecular diagnostics company marketing a novel, multiplexed, multi-modal, molecular diagnostics instrument for oncology, infectious disease, and genetic testing. Dr. McManus also served as head of Cleveland Clinic Laboratories and chief operating officer of the Patholog and Laboratory Medicine Institute at the Cleveland Clinic. He received an MD and PhD from the University of Pennsylvania School of Medicine, MBA from Boston College and his bachelor's from the College of the Holy Cross.

Speakers

David G. Lowe, PhD
President and Chief Executive Office
Aeglea BioTherapeutics

Dr. Lowe has been a research scientist leading and managing drug discovery efforts, a venture capitalist investing in emerging and mid-stage life science companies, and most recently a biotechnology entrepreneur. He started his career as a molecular biologist, joining Genentech in 1985 as a postdoctoral fellow and ultimately became a research director. Dr. Lowe was responsible for drug discovery activities in biologics and small molecule therapeutics, efforts that resulted in eight drugs that progressed to clinical development, including two eventual products. In 2002 he joined Skyline Ventures as a Kauffman fellow. As a partner and later managing director, Dr. Lowe led financings in start-ups to mid-stag therapeutics, diagnostics and platform technology companies. In 2013 he became co-founder and CEO of Aeglea BioTherapeutics, an Austin, Texas-based biotechnology company developing engineered human enzymes to treat inborn errors of metabolism and cancer. He also sits on the board of directors and investment committee of MaRS Innovation, a seed stage accelerator.

Paul Lammers, MD
President and Chief Executive Office
Mirna Therapeutics, Inc.

Dr. Lammers joined Mirna Therapeutics in November 2009 as its president, CEO, and board director. Previously, he was president of Repros Therapeutics. Dr. Lammers served for six years as the chief medical officer for EMD Serono Inc., a divis on of Merck KgA, leading a clinical development, medical and regulatory team of 150 people. He began his career with Organon, spending eight years in the commercial and clinical operations in Europe and the U.S. He also served four years as the senior VP of clinical and regulatory affairs at Zonagen in The Woodlands, Texas. Dr. Lammers obtained his medical and master's degrees from Radboud University in Nijmegen, The Netherlands, and moved to the U.S. in 1992.

Annemarie Moseley, MD, PhD
Chief Operating Officer and Executive ice President of Clinical Development
Bellicum Pharmaceuticals

Dr. Moseley joined Bellicum in 2011. She has over 20 years of industry experience in translational medicine and clinical development of stem cell therapies, immunotherapies, biological devices, and combination products, including overseeing the first late-stage Graf versus Host Disease (GvHD) study in patients who underwent hematopoietic stem cell transplant. Dr. Moseley has held CEO positions at Osiris and Cognate Therapeutics, and prior management positions at Guidant, Novartis and Rhone-Polenc Rorer. She completed her medical degree, internal medicine residency and genetics fellowship at Baylor College of Medicine, Houston. She received her PhD from Utah State University in Physiology/ Biochemistry.

2:45 – 3:30 PM - PREVENTION

Wedgwood

Prevention Networking Interest Groups

Prevention grantees will have the opportunity to network, share ideas and best practices, and discuss challenges and solutions to implementing their CPRIT-funded projects.This session is for CPRIT Prevention program grant recipients and potential grant recipients.

2:45 – 3:30 PM - ACADEMIC RESEARCH

Glass Oaks

Research Advances: Updates from CPRIT Grantees

Three CPRIT grantees discuss their work and the advances they've made in their fields

Speakers

Vijetha Kumar, MS
Abstract #175
Baylor College of Medicine

Caroline Ahrens, PhD
Abstract #176
Texas Tech University Institute of Biosciences and Technology

Peter Davies, MD, PhD
Abstract #297
Texas A&M University System Health Science Center

3:45 – 4:15 PM - PLENARY

Glass Oaks

CPRIT: A Look Forward

As CPRIT nears the halfway point of its authorization in funding, CEO Wayne Roberts leads a discussion about the challenges and operational planning for the remaining five year and beyond.

Speakers

Wayne Roberts
Chief Executive Office
CPRIT

Mr. Roberts was named CEO of the Cancer Prevention and Research Institute of Texas in November 2013 after serving since December 2012 as the interim executive director. Prior to four years with The University of Texas Health Science Center at Houston as associate vice president for public policy, his career was weighted towards public finance and budget especially with respect to higher education. He held numerous senior positions under Governor Rick Perry in which, among other things, he authored the white paper and draft legislation creating the Texas Emerging Technology Fund to catalyze economic development, including transferring research conducted at universities to the Texas marketplace. He was Lieutenant Governor Bob Bullock's special assistant for budget and human services following 18 years with the Legislative Budget Board. Mr. Roberts received a bachelor's degree with honors and special honors in government from The University of Texas at Austin and a master's from the Lyndon B. Johnson School of Public Affairs at UT.

CPRIT OVERSIGHT COMMITTEE

CPRIT is governed by nine dedicated Texans who together comprise the Oversight Committee. Oversight Committee members are appointed by the Governor, the Lieutenant Governor, and the Speaker of the House to serve staggered terms. The Oversight Committee meets at least once every quarter.

Pete Geren, Fort Worth, *Presiding Office*

Will Montgomery, Dallas, *Assistant Presiding Office*

Amy Mitchell, Austin, *Secretary*

Angelos Angelou, Austin

Ned Holmes, Houston

Donald “Dee” Margo, El Paso

Cynthia Mulrow, MD, San Antonio

William Rice, MD, Austin

Craig Rosenfeld, MD, Dallas

CPRIT EXECUTIVE TEAM

CPRIT’s efforts are guided by an executive team that is committed to fulfilling CPRIT’s mission to find and fund the best cancer prevention, academi research, and product development research projects in Texas.

Wayne Roberts, *Chief Executive Office*

Kristen Doyle, *Deputy Executive Officer and General Counse*

Vince Burgess, *Chief Compliance Office*

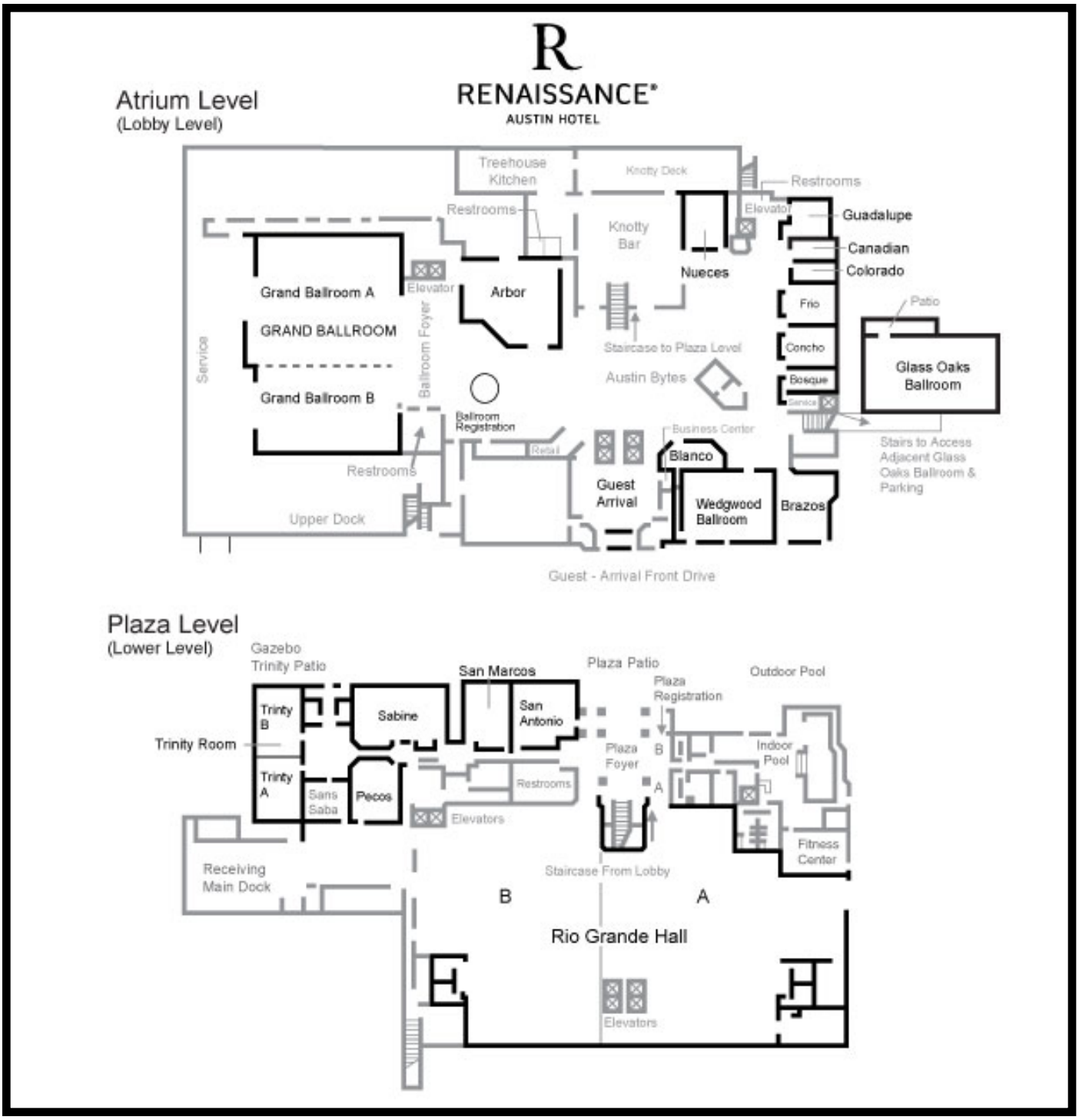
Rebecca Garcia, PhD, *Chief Prevention and Communications Office*

Margaret Kripke, PhD, *Chief Scientific Offic*

Michael Lang, *Chief Product Development Office*

Heidi McConnell, *Chief Operating Office*

RENAISSANCE HOTEL FLOOR PLANS



Renaissance Austin Hotel

9721 Arboretum Boulevard
Austin, Texas 78759 USA
Phone: 512-343-2626
Toll-free: 1-800-468-3571

Airport

Austin-Bergstrom International Airport - AUS
Airport Phone: 512-530-2242
Hotel direction: 18 mile(s) NW

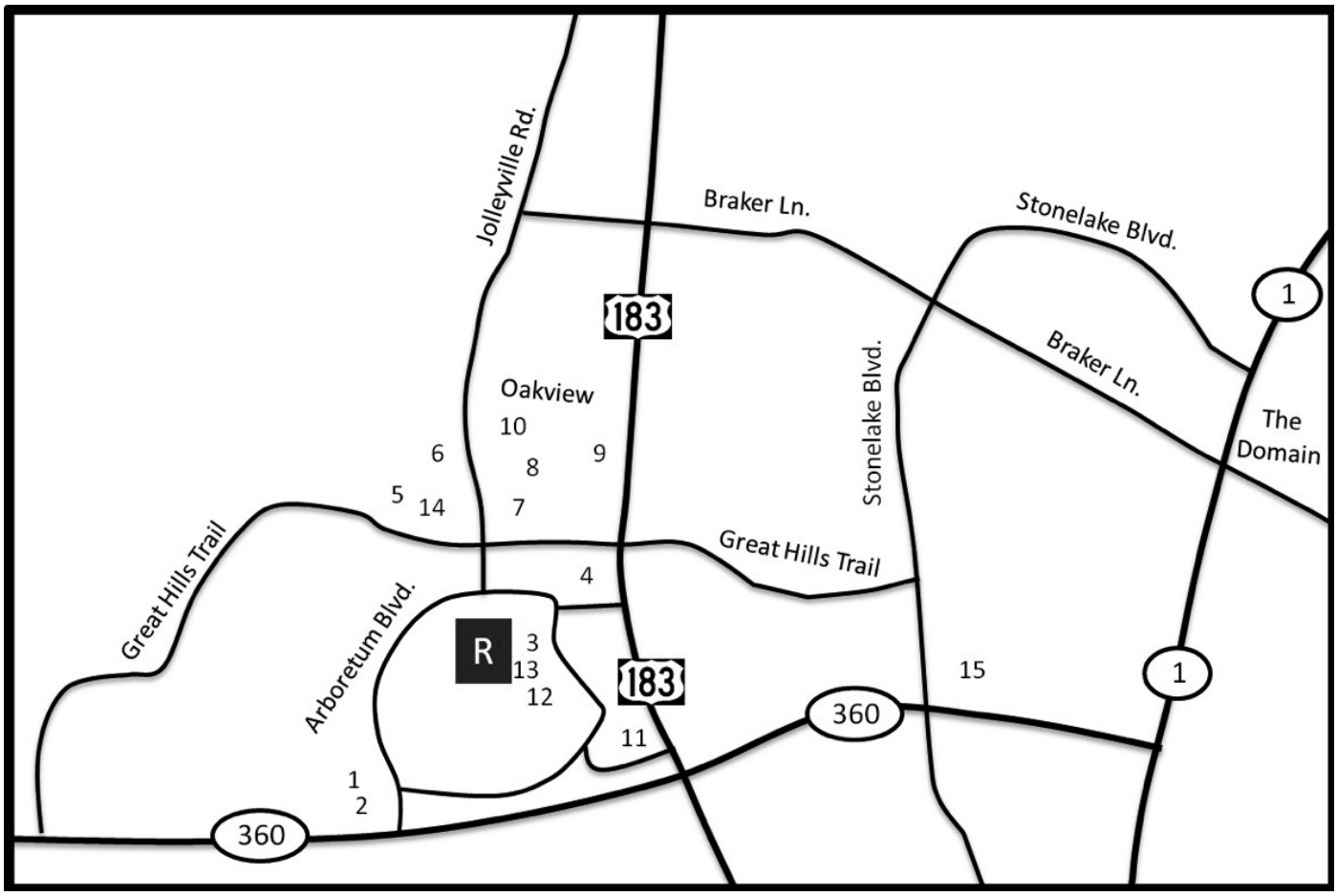
Parking

Complimentary self-parking
Valet $22/overnight, $16/daily

Check-in and Check-out

Check-in: 4:00 PM
Check-out: 11:00 AM

AUSTIN ARBORETUM MAP



Nearby Restaurants:

1.	Eddie V's	Seafood/ Steakhouse
2.	Z Tejas	Southwestern
3.	Cheesecake Factory	American
4.	Blue Baker	Bakery/Café
5.	Newk's Express Kitchen	Café
6.	P.F. Chang's	Asian
7.	Elevation Burger	Burgers
8.	Fire Bowl Café	Asian
9.	La Madeleine	French Bistro Café
10.	Manuel's	Mexican
11.	Five Guys	Burgers
12.	Amy's Ice Cream	Dessert
13.	Zoe's Kitchen	Mediterranean
14.	Corner Bakery	Bakery/Café
15.	North by Northwest	American/Brewery

Arboretum Shopping:

Saks Fifth Avenue
Barnes & Noble
Sephora
Nine West
Pottery Barn
Gap
Z Gallerie
and more

The Domain Shopping Mall:

Neiman Marcus
Dillard's
Macy's
Apple
and more

Movie Theaters:

Regal Arbor Cinema
iPic Theaters (Domain)

ABSTRACTS

1 **Oral Presentation**
CPRIT Grantee
Poster Session A

Molecular Characterization of Circulating Tumor Cells in Pancreatic Cancer *V. Bernard, The University of Texas M.D. Anderson Cancer Center; F. San Lucas, The University of Texas M.D. Anderson Cancer Center; K. Allenson, The University of Texas M.D. Anderson Cancer Center; P. Gascoyne, Advanced Electronic Systems, LLC; C. Davis, Avera Institute for Human Genetics; G. Davies, Avera Institute for Human Genetics; E. Ehli, Avera Institute for Human Genetics; G. Varadhachary, The University of Texas M.D. Anderson Cancer Center; R. Wolff, The University of Texas M.D. Anderson Cancer Center; H. Alvarez, The University of Texas M.D. Anderson Cancer Center; A. Maitra, The University of Texas M.D. Anderson Cancer Center*

Introduction: Effective management of pancreatic ductal adenocarcinoma (PDAC) has remained elusive. Even with large scale sequencing efforts of cancer genomes providing potential for guidance in informing tumor characterization and therapeutic strategies, the inaccessibility of primary and metastatic tissue precludes the opportunity for further workup following initial tumor sampling. To circumvent this issue, we utilize circulating tumor cells (CTCs), or those cells shed by primary and metastatic tissue, to noninvasively monitor tumor evolution and changing patterns of drug susceptibility. We hypothesize that CTCs isolated from patients with metastatic PDAC harbor the molecular signatures that dictate emerging chemoresistance patterns and potential therapeutic vulnerability nodes. **Methods:** A total of 8mL of peripherally drawn blood was collected from PDAC patients at various points throughout disease progression for antigen-independent CTC enrichment. Whole genome amplification (WGA) and whole transcriptome amplification (WTA) of single cell and clustered CTCs was performed using multiple displacement amplification strategies. Amplification efficiency was confirmed using PCR based methods. Those that passed quality control underwent subsequent whole exome sequencing (WES) and RNA-seq. Identified aberrant events were compared across CTCs and primary tumor tissue to assess heterogeneity and define actionable mutations. **Results:** CTCs were reliably isolated from 8mL of peripheral blood from three metastatic PDAC patients throughout disease progression. DNA and RNA was effectively isolated and amplified to sufficient quality for WES and RNA-seq. RNA-seq confirmed the identity of CTCs of pancreatic tissue origin. Genome sequencing of CTCs revealed the presence of characteristic driver mutations of PDAC tumors including *KRAS, TP53*, and *SMAD4*. Serial sampling revealed new mutations shared across CTC clones that were not present on initial primary and metastatic tissue sampling such as *NOTCH2*. This suggests the possibility of potential therapeutic targets or new emerging driver mutations. **Conclusion:** This study provides a proof of concept of the potential of CTC genomics in PDAC. Noninvasive monitoring of tumor evolution through CTCs may have a use in detecting changing patterns of drug susceptibility for therapeutic stratification in PDAC patients.

2 **CPRIT Grantee**
Poster Session B

Nanomanipulation Bioworkstation: Single Cell Analysis at the Cancer Forefront *G. Verbeck, University of North Texas; M. Phelps, University of North Texas; J. Hamilton, University of North Texas*

Introduction: Typical cellular chemical analysis employs the application of lysing groups of cells to observe biomolecules of interest in their roles of metabolism. Individual cells have unique physiologies and produce different metabolites even within cells of the same tissue. As such, a method for investigating a single cell without destroying it or surrounding cells is desired to reduce enzymatic responses. Micromanipulators have been introduced to study single cells, but their mechanical perturbation of living cells often induces a distress response, leading to cell necrosis. Therefore, a more minimally invasive extraction technique is necessary to analyze cell-to-cell heterogeneity and provide a better understanding of the true metabolic processes. **Methods:** The application of directing femtosecond laser pulses at cell membranes to create sub-micron dissections has been accomplished in mammalian cells without inducing the distress response. In combination with nanomanipulation and Raman microscopy, a novel bioworkstation has been developed capable of providing accurate and localized single cell chemical analysis. Here we analyze single cells for metabolic chemistries through Raman microscopies, while cell cycles were visually identified bright-field. Localized cellular chemistries were then extracted through nanosurgery, using the 800nm Ti:Saph laser, followed by extraction with the nanomanipulator. The sample of interest was then analyzed by nanospray ionization mass spectrometry (NSI-MS) to produce a metabolic profile. **Results:** Here we demonstrated the capabilities via nanoextraction of adipocytes from tumorous and healthy breast tissue. A distinct difference between healthy and tumorous adipocytes was identified with the m/z 878 and 902 peak ratios. The extraction of triglycerides from individual cells within these tissues provided evidence of lipid heterogeneity amongst healthy and tumor adipocytes. Preliminary data suggests that the triglyceride make-up appears to be influenced by the cellular location within tissue, evident by the ratios of the triglyceride peaks. The most significant of these peaks is the m/z 902 peak which has been identified as an oleic acid containing triglyceride via collision induced dissociation. The intensity of the m/z 902 peak decreases relative to the most intense m/z 878 peak as the location of cell nears the center of the tumor. **Conclusion:** With incorporation of the femtosecond laser and Raman microscopy into the bioworkstation, we were able to analyze cancer cells in vivo to access accurate localized biochemistry and provide further insights into the mechanisms in which cancer proliferates. The lipid heterogeneity identified here supports the notion that oleic acid may play a role in tumor cell proliferation.

3 **CPRIT Grantee Poster Session A**

NCOA1 Promotes Angiogenesis in Breast Tumors by Simultaneously Enhancing both HIF1α- and AP-1-mediated VEGFa Transcription *L. Qin, Baylor College of Medicine; Y. Xu, Baylor College of Medicine; L. Liao, Baylor College of Medicine; J. Xu, Baylor College of Medicine*

Introduction: Nuclear receptor coactivator 1 (NCOA1) is overexpressed in a subset of breast cancer and its increased expression positively correlates with disease recurrence and metastasis. Although NCOA1 is known to promote breast cancer metastasis through working with multiple transcription factors to upregulate the expression of Twist1, ITGA5, CSF-1, SDF1 and CXCR4, the role of NCOA1 in breast tumor angiogenesis has not been investigated. In this study, we defined the promoting role of NCOA1 in angiogenesis in breast tumors. **Methods:** Immunohistochemistry and immunofluorescence staining were performed to examine microvascular density (MVD) and VEGFa expression in mammary tumors from Tg(Ncoa1$^{-/-}$)×Tg(MMTV-PyMT) mice, Tg(MMTV-NCOA1)×Tg(MMTV-TVA/RCAS-PyMT) mice with overexpresion of NCOA1 in epithelial cells and corresponding control mice. In vivo Matrigel angiogenesis assay was carried out with two Ncoa1 knockout mammary tumor cell lines (K1 and K2) and two wildtype mammary tumor cell lines (W1 and W2) in SCID mice. CHIP assay was performed to assess the association of NCOA1, HIF1α and c-Fos with VEGFa promoter in MDA-MB-231 cells and VEGFa promoter luciferas assay was assayed to determine the transcription activation by NCOA1, HIF1α and c-Fos upon VEGFa transcription. Finally, the correlation of NCOA1 expression and MVD was evaluated in a cohort of 140 human breast cancer specimens. **Results:** We found that the microvascular density (MVD) was significantly decreased and increased in NCOA1-knockout and NCOA1-overexpressing mammary tumors, respectively, in several breast cancer mouse models. Knockout or knockdown of NCOA1 in breast cancer cell lines also markedly compromised their capability to induce angiogenesis in Matrigel plugs embedded subcutaneously in mice, while this compromised capability could be rescued by VEGFa treatment. At the molecular level, NCOA1 upregulates VEGFa expression in both mouse mammary tumors and cultured breast cancer cells, and it does so by associating with both c-Fos, which is recruited to the AP-1 site at bp -938 of the VEGFa promoter, and HIF1α, which is recruited to the HIF1α-binding element at bp -979 of the VEGFa promoter, to enhance VEGFa transcription. In 140 human breast tumors, high NCOA1 protein correlates with high MVD and patients with both high NCOA1 and high MVD showed significantly shorter survival time. **Conclusion:** This study revealed a novel mechanism that NCOA1 potentiates breast cancer angiogenesis through upregulating HIF1α and AP-1-mediated VEGFa expression, which reinforces the rational of targeting NCOA1 in controlling breast cancer progression and metastasis.

4 **CPRIT Grantee Poster Session B**

Daam2 Functions as a Novel Convergence Point of Signaling Pathways in Glioblastoma *W. Zhu, Baylor College of Medicine; H .Lee, Baylor College of Medicine; C. Mohila, Texas Children's Hospital; B. Deneen, Baylor College of Medicine*

Introduction: Glioblastoma multiforme (GBM) is one of the most aggressively malignant gliomas in humans. Genes involved in glia development have been linked to GBM tumorigenesis. Considering tumorigenesis is viewed as a convergence of genetic mutation and developmental context, we want to understand the mechanisms which govern glia development contribute to GBM formation. Our lab had identified Daam2 inhibits oligodendrocyte differentiation through Wnt pathway. The Wnt signaling pathway also has well defined functions in several cancers except GBM, which accentuates the importance of our study. Moreover Daam2 was reported also by our lab to function through a direct interaction with PIP5K during development, which raises the possibility that Daam2 interacts with genes unrelated to Wnt pathway in GBM. **Methods:** I am using a novel mouse model that uses PiggyBac(PB) and In Utero Electroporation (IUE) to target astro-glial lineages with oncogenes, which can efficiently generate GBM in three weeks. **Results:** We first characterized that Daam2 is highly expressed in both human GBM xenograft and tissue array samples. To further analyze the function of Daam2 in GBM, we took advantages of both human GBM xenograft cell line model and mouse GBM brain tissue generated from IUE model. We found that Daam2 potentiates tumor proliferation and migration. In detail, in human GBM model, the result from the growth curve and agar assay indicated that Daam2 over-expression increases proliferation and migration. Coherent results were observed from mouse IUE model coinjected with oncogene and Daam2. In these tissues, we observed more proliferation as indicated by staining of pH3, a mitotic marker. In parallel, brain tissues over-expressed with Daam2 were dissociated and showed more migration through transwell assay. These results were further supported by the complementary study performing IUE on Daam2 knockout mice. Next, we delineate how Daam2 is participated in GBM formation/progression. We first verified that Daam2 functions through Wnt pathway. TOP reporter signal in GBM cell lines was dramatically increased with the over-expression of Daam2. To fully explore the mechanism, MS and RPPA were applied on mouse brain tissues. It shows that Daam2 binds to PI3K and also negative correlates with VHL expression, both of which have also been verified in mouse model. **Conclusion:** In sum, we demonstrated that Daam2 plays critical roles in GBM tumorigensis, which potentiates proliferation and migration. Its roles were found not only in Wnt signaling pathway, but also involved in PI3K/AKT and VHL, which made Daam2 as a convergence point of multiple pathways in GBM.

5 **CPRIT Grantee Poster Session A**

ESR1 Mutations Escape Hormone Therapy through Activation of IGF1R in Breast Cancer *S. Fuqua, Baylor College of Medicine*

Introduction: There has been a recent rediscovery of estrogen receptor (ESR1) mutations in metastatic breast cancer, however we do not know how to best treat these patients. There have also been widely contradictory reports on the frequency of ESR1 mutation in primary breast cancer which need to be addressed. **Methods:** We have modeled the three most frequent hormone binding ESR1 (HBD-ESR1) mutations using stable lentiviral vector transduction in human breast cancer cell lines. Effects on growth was examined in response to hormonal and targeted agents, and mutation-specific changes were studied using microarray and alterations using proximity ligation assay and western blot analysis. **Results:** We determined that the HGD-ESR1 mutations confer relative resistance to tamoxifen (Tam) in a cell-type specific manner due to distinct epigenetic changes. Resistance was only observed with concomitant engagement and activation of the insulin growth factor signaling pathway (IGF1R). The ESR1 mutants also exhibited enhanced binding with insulin growth factor receptor beta (IGF1Rβ). The selective estrogen degrader, fulvestrant, significantly reduced the anchorage-independent growth of ESR1 mutant-expressing cells, while combination treatment with the mTOR inhibitor, everolimus, restored Tam sensitivity. In vivo tumor growth experiments are underway and the metastatic potential of cells expressing the Y537S ESR1 mutant will be presented. **Conclusion:** Since we detected relatively high frequencies of these three mutations in primary breast tumors, our results suggest that clinical targeted sequencing of both primary and metastatic tumors may be justified, and that combination therapies with IGF-1 inhibitors should be considered earlier in the course of treatment to block metastatic progression.

6 **CPRIT Grantee Poster Session B**

Telomerase Positive Human Cancer Cells May Engage Alternative Lengthening of Telomeres During The Telomerase Inhibition Therapy *J. Min, The University of Texas Southwestern Medical Center at Dallas; W. Wright, The University of Texas Southwestern Medical Center at Dallas; J. Shay, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Telomeres are composed of TTAGGG repeated sequences located at the end of chromosomes. In human somatic cells, cell divisions are accompanied with progressive telomere length shortening due to lack of or insufficient telomerase activity. Cancer cells need to acquire a telomere maintenance mechanism in the early stage of tumorigenesis to proliferate indefinitel . It is widely known that ~90% of human cancer cells maintain their telomere length *via* telomerase reactivation. Therefore anti-telomerase cancer therapy has been considered an almost universal target and one that should not affect somatic cells that are telomerase silent. However one concern of anti-telomerase therapy is potential acquired resistance and engagement of the DNA recombination ALT mechanism. Here we will provide evidence about activation of ALT in telomerase-positive human cancer cells by knocking out telomerase using genome editing techniques. **Methods:** We generated telomerase RNA component (*hTR/hTERC*) knockout (KO) in human telomerase-positive cancer cell lines by using the CRISPR/Cas9 genome editing system. *hTR* KO cells should proliferate for a while and enter crisis which is accompanied by massive cell death. We predicted only a small fraction of cells would escape from the crisis by acquiring a telomerase-independent telomere maintenance mechanism. We performed various telomere analyses to verify how survival clones engaged a telomere maintenance mechanism. **Results:** We generated *hTR* KO in SW39 cell line (IMR90_SV40 origin immortalized cell line, telomerase positive). From the fluctuation analysis using SW39 *hTR* KO cells, we obtained three survival clones out of 12 million cells in crisis resulting in a frequency of immortalization = $2.5x10^{-7}$. The survival clones maintained their telomere length by acquiring the ALT mechanism which that is characterized by heterogeneity in telomere length, abundant C-circle levels, and increases in telomere sister chromatid exchanges. At present we are determining the molecular factors involved in ALT mechanism from the *Cas9_sgRNA* and *shRNA* library screening which can be potential anti-ALT cancer targets. **Conclusion:** These results demonstrate that combination therapy of telomerase/ALT inhibitor may be a significant anti-cancer approach. Various molecular approaches will be initiated to identify novel ALT cancer specific targets by using our isogenic telomerase positive and ALT background cells.

7 **CPRIT Grantee Poster Session A**

Down-regulation of Bcl2-related Ovarian Killer (BOK) By MiR-296-5p Protects Breast Cancer Cells From Paclitaxel-induced Apoptosis *B. Onyeagucha, The University of Texas Health Science Center at San Antonio; S. Rajamanickam, The University of Texas Health Science Center at San Antonio; P. Subbarayalu, The University of Texas Health Science Center at San Antonio; S. Bansal, The University of Texas Health Science Center at San Antonio; H. Bansal, The University of Texas Health Science Center at San Antonio; S . Timilsina, The University of Texas Health Science Center at San Antonio; V. Eedunuri, The University of Texas Health Science Center at San Antonio; R. Guzman, St Mary's University; N. Abdelfattah, The University of Texas Health Science Center at San Antonio; T. Fraker, The University of Texas Health Science Center at San Antonio; M. Rao, The University of Texas Health Science Center at San Antonio*

Introduction: BCL-2 family proteins are essential for normal development and are often dysregulated in cancers. The molecular mechanisms that cause their altered expression are largely unknown. In this study, we investigate the mechanism by which BCL2-related Ovarian Killer (BOK) protein, which has a crucial role in the regulation of apoptosis, is frequently deregulated in several human cancers, including breast cancer. **Methods:** A bioinformatics approach identified putative microRNA binding sites within the 3'-UTR of the BOK. We confirmed the regulation of BOK expression at the post-transcriptional level by miR-296-5p in human breast cancer cells using qRT-PCR, Western blot, and luciferase reporter assay. Also, we examined the effects of paclitaxel and ectopic BOK expression on human breast cancer cells using immunofluorescence, flow cytometry, cell viability count. **Results:** Our results demonstrated that ectopic miR-296-5p down-regulated BOK expression in human breast cancer cells. Transfection of miR-296-5p significantly suppressed luciferase reporters containing wild-type BOK-3'-UTR constructs. In contrast, mutant BOK-3'-UTR constructs were unaffected by ectopic miR-296-5p. Additionally, over-expression miR-296-5p significantly attenuated BOK expression level in the presence of paclitaxel treatment compared to the control cells. Over-expression of BOK or paclitaxel treatment significantly altered human breast cells' morphology. **Conclusion:** Our data provide new insights on the regulation of BOK expression by miR-296-5p, its effects on paclitaxel induced apoptosis and suggests that therapeutic strategies against miR-296-5p and BOK may be warranted.

8 **CPRIT Grantee**
Poster Session B

Dynamic analysis of Alternative PolyAdenylation from RNA-Seq (DaPars) Reveals landscape of 3' UTR usage across 7 tumor types *Z. Xia, Baylor College of Medicine; W. Li, Baylor College of Medicine*

Introduction: The dynamic usage of mRNA 3′ untranslated region (3′UTR) resulting from alternative polyadenylation (APA) is emerging as a pervasive mechanism to regulate approximately 70% of human genes. The importance of APA in human diseases such as cancer is only beginning to be appreciated. Current APA profiling protocols use the partitioning and fragmentation of mRNA to enrich for polyA sites followed by high throughput sequencing (polyA-seq). These polyA-seq protocols, although powerful, have not been widely adopted. Therefore, global studies of APA in cancer are very limited. In contrast, whole transcriptome RNA-seq has been broadly employed in almost every large-scale genomics project, including The Cancer Genome Atlas (TCGA). **Methods:** We therefore developed a novel bioinformatics algorithm, termed Dynamic analysis of Alternative PolyAdenylation from RNA-Seq (DaPars), to directly infer dynamic APA events through standard RNA-seq. DaPars used a linear regression model to identify the exact location of the de novo APA site, and quantify the lengthening or shortening of 3′UTRs between different conditions. **Results:** When applied to 358 TCGA tumor/normal pairs across 7 tumor types, DaPars reveals 1,346 genes with recurrent and tumor-specific APAs. Most APA genes (91%) have shorter 3' UTRs in tumors that can avoid miRNA-mediated repression, including glutaminase (GLS), a key metabolic enzyme for tumor proliferation. Interestingly, selected APA events add strong prognostic power beyond common clinical and molecular variables, suggesting their potential as novel prognostic biomarkers. Finally, our results implicate CstF64, an essential polyadenylation factor, as a master regulator of 3' UTR shortening across multiple tumor types. **Conclusion:** Our work demonstrates the feasibility of dynamic analysis of APA from RNA-seq using DaPars, reveals the importance of dynamic APA in cancer and expands our knowledge of the mechanisms and consequences of APA regulation during tumorigenesis.

9 **CPRIT Grantee**
Poster Session A

Genome-wide Annotation of MicroRNA Primary Transcript Structures Reveals Novel Regulatory Mechanisms *T. Chang, The University of Texas Southwestern Medical Center at Dallas; M. Pertea, Johns Hopkins University School of Medicine; S. Lee, The University of Texas Southwestern Medical Center at Dallas; S. Salzberg, Johns Hopkins University School of Medicine; J. Mendell, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Precise regulation of microRNA (miRNA) expression is critical for diverse physiologic and pathophysiologic processes. Nevertheless, elucidation of the mechanisms through which miRNA expression is regulated has been greatly hindered by the incomplete annotation of primary miRNA (pri-miRNA) transcripts. While a subset of miRNAs are hosted in protein-coding genes, the majority of pri-miRNAs are transcribed as poorly-characterized noncoding RNAs that are 10's-100's of kilobases in length and low in abundance due to efficien processing by the endoribonuclease DROSHA, which initiates miRNA biogenesis. Accordingly, these transcripts are poorly represented in existing RNA-seq datasets and exhibit limited and inaccurate annotation in current transcriptome assemblies. To overcome these limitations, we developed a highly effective experimental and computational approach that allows genome-wide detection and mapping of miRNA primary transcript structures. **Methods:** We performed deep RNA-seq in cells expressing a dominant negative DROSHA mutant protein, which resulted in dramatic enrichment of intact pri-miRNAs and much greater coverage of these transcripts compared to standard RNA-seq. Moreover, a computational pipeline was developed that produces highly accurate pri-miRNA assemblies, as confirmed by extensive validation. **Results:** Our experimental and computational strategy for pri-miRNA assembly was applied to a panel of human and mouse cell lines, providing pri-miRNA transcript structures for 1291/1871 human and 888/1181 mouse miRNAs, including 594 human and 425 mouse miRNAs that fall outside protein-coding genes. The new assemblies uncovered unanticipated features and new potential regulatory mechanisms, including links between pri-miRNAs and distant protein coding genes, alternative pri-miRNA splicing, and transcripts carrying subsets of miRNAs encoded by polycistronic clusters. **Conclusion:** We established an experimental and computational strategy for pri-miRNA reconstruction, and generated a genome-wide map of human and mouse pri-miRNA structures. This map provides a valuable resource of miRNA gene structures that can be easily accessed by investigators in the field, thereby facilitating the study of the mechanisms that control miRNA expression in normal physiology and disease.

10 **CPRIT Grantee**
Poster Session B

Preclinical Intravital Microscopy of Prostate Cancer Lesions in Bone *E. Dondossola, The University of Texas M.D. Anderson Cancer Center; S. Alexander, The University of Texas M.D. Anderson Cancer Center; S. Alexander, The University of Texas M.D. Anderson Cancer Center; B. Holzapfel, Queensland University; C. Logothetis, The University of Texas M.D. Anderson Cancer Center; D. Hutmacher, Queensland University; P. Friedl, The University of Texas M.D. Anderson Cancer Center*

Introduction: Bone metastases are the initial site of progression and account for many of the complications experienced by men with metastatic prostate cancer (PCa). Although current therapies have been proven to prolong survival, most men will advance to metastatic disease due to the emergence of therapy resistance. Besides cell intrinsic mechanisms, signals received from the bone microenvironment critically contribute to support PCa cell survival and growth. Recent improvements in intravital microscopy enable to investigate these complex cell-cell and cell-matrix interactions and allow preclinical examination of tumor responses to both conventional and targeted therapy. **Methods:** We developed a mouse model amenable to intravital multiphoton microscopy (iMPM) to longitudinally study PCa-stromal cell interactions and therapy response in a partially humanized neobone, established in the dermis of the mouse. **Results:** We generated tissue engineered bone constructs, TEBCs, by functionalizing polymeric polycaprolactone scaffolds with human mesenchymal stem cells (hMSCs) differentiated to osteoblasts. We then analyzed hMSCs distribution and calcium deposition after cell seeding, showing that hMSCs create a 3D niche-like environment, including calcified matrix. Functionalized scaffolds differentiated for 30 days in osteogenic conditions were then implanted under the skin of the mouse. Maturation of bone was complete within 30 days, as monitored by microCT and iMPM analysis. At day 30 post-implantation, human fluorescent PCa cells (PC3) were co-implanted and followed by multi-parameter iMPM through a body window for: collagen and bone matrix (SHG), bone remodeling (fluorescent bisphosphonates), osteoclasts (cathepsin K), bone surface (THG), blood vessels and stromal phagocytes (fluorescen dextran), and PC3 cells (nuclear H2BeGFP, cytoplasmatic DsRed2). Multi-parameter 3D reconstruction of tumor growth, invasion and reactive remodeling of the stroma was monitored longitudinally, as baseline for evaluating preclinical disease and future studies on therapy response.

Conclusion: This imaging model combines innovative tissue engineering with optical windows, state-of-the-art fluorescence reporter technology and intravital MPM, as an interdisciplinary effort for developing better tools for the analysis of PCa bone lesions. Because of these strengths, we expect our model will provide mechanistic insight and efficacy predictions for innovative therapeutics not delivered by existing approaches used to monitor treatment response in bone.

11 **CPRIT Grantee Poster Session A**

Wild Type N-Ras, Overexpressed In Basal-Like Breast Cancer, Promotes Tumor Formation By Inducing IL8 Secretion Via JAK2 Activation *Z. Zheng, Baylor College of Medicine; L. Tian, Baylor College of Medicine; W. Bu, Baylor College of Medicine; C. Fan, University of North Carolina; X. Gao, Baylor College of Medicine; X. Zhang, Baylor College of Medicine; C. Yu, Baylor College of Medicine; H. Wang, Baylor College of Medicine; Y. Liao, National Taiwan University Hospital; Y .Li, Baylor College of Medicine; M. Lewis, Baylor College of Medicine; D .Edwards, Baylor College of Medicine; T. Zwaka, Mount Sinai School of Medicine; S. Hilsenbeck, Baylor College of Medicine; D. Medina, Baylor College of Medicine; C. Perou, University of North Carolina; C. Creighton, Baylor College of Medicine; D. Liu, Baylor College of Medicine; Z. Songyang, Baylor College of Medicine; X. Zhang, Baylor College of Medicine; E. Chang, Baylor College of Medicine*

Introduction: "Basal-like" breast cancer (BLBC) is a very aggressive subtype of breast cancer. BLBC has very poor prognosis — median time to distant recurrence is just 2.6 years vs. 5 years overall, and survival time from diagnosis of distant metastatic disease is 9 months vs. 22 months. BLBC tumors usually do not express ER, Her2, or progesterone receptor. As such, they cannot be treated by the current targeted therapies, which target these molecules. What drive the formation and progression of BLBCs is largely unclear. **Methods:** We examined differential gene expression patterns by microarrays, and the results were validated by examing protein levels in cell lines and PDXs. Gene functions were validated by gene silencing and overexpression. Tumor formation was examined using either human breast cancer xenograft or genetically enginnered mouse models. Ras and effector binding was analyzed by BiFC. **Results:** Ras GTPases are best known for mediating growth factor signaling. Oncogenic mutations in the *RAS* genes, *K-RAS* in particular, are found in more than 30% of human tumors. Surprisingly, oncogenic RAS mutations are rare in breast cancer. However, we found that wild-type *N-RAS* is overexpressed in BLBCs, possibly partly via promoter demethylation, but not in other breast cancer subtypes. Repressing *N-RAS* inhibits transformation and tumor growth, while overexpressing it enhances these processes even in preinvasive BLBC cells. In contrast, in breast cancer cells of other subtypes, repressing N-RAS expression does not affect growth and transforming activities. We identified N-Ras-responsive genes, most of which encode chemokines and cytokines, e.g., IL8. High expression levels of these N-Ras-responsive genes as well as of *N-RAS* itself in tumors correlate with poor patient outcome. N-Ras, but not K-Ras, induces IL8 by binding and activating the cytoplasmic pool of JAK2; IL8 then acts on both the cancer cells and stromal fibroblasts **Conclusion:** N-Ras drives BLBC by promoting transformation in epithelial cells, which may in turn remodel the tumor microenvironment to create a proinvasive state. Although oncogenic mutations affecting RAS are common in many other human cancers, tumorigenesis in an important subset of breast cancers is driven instead by increasing activity of wild-type N-Ras. Thus, to fully assess the impact of Ras on tumorigenesis, the role of wild-type as well as mutant Ras proteins must be carefully examined.

12 **CPRIT Grantee Poster Session B**

Metabolic Diversity in Non-small Cell Lung Cancer Provides Insight Into Selection of New and Existing Therapies *P. Chen, The University of Texas Southwestern Medical Center at Dallas; L. Cai, The University of Texas Southwestern Medical Center at Dallas; J. Kim, The University of Texas Southwestern Medical Center at Dallas; E. McMillan, The University of Texas Southwestern Medical Center at Dallas; K .Huffman, The University of Texas Southwestern Medical Center at Dallas; L. Girard, The University of Texas Southwestern Medical Center at Dallas; M. Peyton, The University of Texas Southwestern Medical Center at Dallas; M. White, The University of Texas Southwestern Medical Center at Dallas; J. Minna, The University of Texas Southwestern Medical Center at Dallas; R. DeBerardinis, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Cancer cells display oncogene-driven rewiring of metabolism to produce energy and macromolecules for growth. Inhibition of growth-promoting metabolic pathways may prove to be a useful therapeutic strategy in cancer. However, neither the full breadth of cancer cell metabolic diversity, nor the complement of mechanisms by which tumor mutations elicit metabolic reprogramming, are known. We set out to characterize cell-autonomous metabolic heterogeneity in non-small cell lung cancer (NSCLC) and to use orthogonal high-content data sets to understand the mechanisms by which metabolic phenotypes are established in lung cancer. A major goal is to understand whether these metabolic phenotypes predict therapeutic liabilities to novel metabolic inhibitors, targeted therapies, or conventional chemotherapeutic agents. **Methods:** We used a highly annotated panel of more than 80 NSCLC cell lines and non-transformed immortalized bronchial epithelial cells to develop the most comprehensive database of cancer cell metabolism to date. The cancer cell lines were analyzed for a set of ~100 metabolic parameters derived from nutrient utilization, nutrient addiction, and isotope labeling patterns following culture with ^{13}C-glucose or ^{13}C-glutamine. Orthogonal data sets included analysis of the genome, epigenome, transcriptome and proteome, as well as sensitivity to over 40 chemotherapeutic agents. Several cell lines were also subjected to high-throughput chemical compound and genome-wide siRNA screens. **Results:** NSCLC cell lines display a surprising degree of cell-autonomous metabolic heterogeneity in culture. Many canonical hallmarks of cancer cell metabolism, including the Warburg effect, were observed to span at least a 10-fold range among

cell lines grown under identical conditions. Affinity propagation clustering using metabolic features alone produced families that were largely distinct from clusters based solely on gene expression. Nevertheless, databases of metabolic features and orthogonal data sets could be cross-queried to identify robust, novel relationships connecting metabolic preferences to oncogenotypes, transcriptomic phenotypes and therapeutic responses. Two specific relationships were assessed in greater detail: sensitivity to folate antagonists in cell lines displaying high flux through the de novo serine biosynthetic pathway, and addiction of LKB1/KRAS co-mutant cell lines to the urea cycle enzyme carbamoyl-phosphate synthase-1. **Conclusion:** NSCLC cell metabolism is highly heterogeneous in every parameter so far assessed. This diversity produces an opportunity to derive novel functional families that cannot be recapitulated solely through analysis of the genome or transcriptome. Functional metabolic families are significant because they predict sensitivity to existing therapies and nominate new therapeutic targets from a set of reprogrammed metabolic networks.

13 **CPRIT Grantee Poster Session A**

RB and E2F1 Direct Histone H3K56 Acetylation and Promotes DNA-End Resection and Homologous Recombination at Double Strand Breaks *R. Velez-Cruz, The University of Texas M.D. Anderson Cancer Center; S. Manickavinayaham, The University of Texas M.D. Anderson Cancer Center; A. Biswas, Colombia University; R. Clary, Trinity Valley Community College; D. Johnson, The University of Texas M.D. Anderson Cancer Center*

Introduction: The retinoblastoma (RB) tumor suppressor is widely recognized as a master regulator of the transcriptional program that controls entry into the S phase of the cell cycle. RB represses the expression of genes important for cell cycle progression by binding to members of the E2F family of transcription factors and recruiting chromatin-modifying proteins, such as histone deacetylases, to the promoters of E2F target genes. **Methods:** We used fluorescenc confocal microscopy to study the dynamics of recruitment of RB and other repair factors to DSBs, and to measure DNA-end resection after IR. We also employed the homing endonuclease I-PpoI in U2OS cells to induce site-specific DNA double-strand breaks (DSB) and chromatin immunoprecipitation to monitor the recruitment of different factors and the induction of chromatin modifications to DSBs. We used flow cytometry to measure DNA-end resection, homologous recombination, and cell cycle progression. **Results:** Our work shows that RB is recruited to DSBs after IR and its recruitment depends on E2F1 and ATM kinase activity. RB-deficient cells are impaired in the clearance of gamma-H2AX foci, DNA-end resection, homologous recombination, and display increased frequency of chromosomal abnormalities upon IR. In response to DNA damage, RB stabilizes a phospho-dependent interaction between E2F1 and TopBP1, forming a TopBP1-E2F1-RB complex. This complex is responsible for the recruitment of the histone acetyltransferases p300 and CBP, and acetylation of histone H3K56 at DSBs. A knock-in mutation of the ATM phosphorylation site on E2F1 prevents recruitment of E2F1, RB, p300, and CBP to DSBs, abolishes H3K56 acetylation at damaged sites, impairs DNA repair, and renders knock-in mice hypersensitive to IR. **Conclusion:** This study reveals a novel, non-transcriptional function for RB in modifying chromatin structure at DSBs, an activity that facilitates DNA repair and may contribute to chromosomal instability associated with RB loss in human cancers.

15 **CPRIT Grantee Poster Session A**

The Genomic Landscape of Allelic Imbalance in the Normal-Appearing Airway Field of Cancerization *P. Scheet, The University of Texas M.D. Anderson Cancer Center; Y. Jakubek, The University of Texas M.D. Anderson Cancer Center; W. Lang, The University of Texas M.D. Anderson Cancer Center; S. Vattathil, The University of Texas M.D. Anderson Cancer Center; M. Garcia, The University of Texas M.D. Anderson Cancer Center; L. Huang, The University of Texas M.D. Anderson Cancer Center; Z. Weber, Avera Institute for Human Genetics; G. Davies, Avera Institute for Human Genetics; C. Behrens, The University of Texas M.D. Anderson Cancer Center; N. Kalhor, The University of Texas M.D. Anderson Cancer Center; C. Moran, The University of Texas M.D. Anderson Cancer Center; J . Fujimoto, The University of Texas M.D. Anderson Cancer Center; R. Mehran, The University of Texas M.D. Anderson Cancer Center; J. Fowler, The University of Texas M.D. Anderson Cancer Center; E. Ehli, Avera Institute for Human Genetics; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; H. Kadara, The University of Texas M.D. Anderson Cancer Center*

Introduction: The phenomenon of field cancerization has been observed in various cancers, including those of the lung. We have recently demonstrated that “normal” airway cells carry expression profiles that are often characteristic of the adjacent tumor. A better understanding of mechanisms driving these field changes may provide important biological insights into lung tumorigenesis. Loss-of-heterozygosity (LOH) and other forms of acquired chromosomal alterations (allelic imbalance; AI) have an established role in oncogenesis. However, the relationship between AI and field cancerization has not been studied comprehensively across the genome. Here we address this void by interrogating a rich collection of normal airways from non-small cell lung cancer (NSCLC) patients. **Methods:** We applied Illumina 1M SNP arrays to characterize whole genome copy number alterations in 435 samples from 45 early-stage NSCLC patients [31 adenocarcinomas (ADCs), 14 squamous cell carcinomas (SCCs)]. Each patient set comprised samples from the primary tumor and adjacent airways paired with blood cells and/or uninvolved normal lung tissue. A subset of these included brushings from large mainstem bronchi and from the nasal cavities as well as multi-region tumor biopsies for intra-tumoral analysis. To characterize the field in airways at a genome-wide scale, we applied a haplotype-based computational program, hapLOH, to profile AI events (loss, gain, copy neutral LOH) in a paired mode contrasting signals in the blood or normal lung. **Results:** We detected 247 AI events in airways of 21 of 45 patients. Of the 21 patients, 19 had events in the adjacent airway, 3 had events in the large airway, and no events were observed in nasal brushings, indicating a pronounced AI field gradient. We detected AI in the airways of ~30% of ADCs regardless of smoking status (2 of 7 non-smokers, 8 of 24 smokers), and 79% (11 of 14) of SCCs, clearly indicating squamous histology as a greater predictor of genomic field effects ($P < 0.01$). The most frequently observed airway alterations were in 9p and 9q, affecting 13 smoker patients. Finally, we note that AI events were present in the airways of 4/5 (80%) of patients with recurrence and only in 17/40 (43%) patients without recurrence, signaling a prognostic value in studying the field in NSCLC. **Conclusion:** Although preliminary, our findings suggest that chromosomal aberrations are common in the airway field of cancerization and can provide insights into the biology of lung cancer pathogenesis.

16 **CPRIT Grantee**
Poster Session B

The ribosomal biogenesis profiling identified Rio kinase 1 and RRP9 as are highly sensitive factors to ATP depletion *D. Sarbassov, The University of Texas M.D. Anderson Cancer Center; V. Kiyan, The University of Texas M.D. Anderson Cancer Center*

Introduction: Ribosomal biogenesis is a fundamental and the most energy consuming process in cells responsible for a rate of protein synthesis and cell growth. It takes place in a specific organelle defined as nucleolus in all eukaryotes. Deregulation of ribosomal biogenesis drives an accelerated growth of cancer cells that remains poorly characterized. Because of a major gap in our knowledge of ribosomal biogenesis and particular its nutrient-dependent regulation, we propose to carry a characterization of this process based on the functional profiling and biochemical isolation of the critical nucleolar complexes sensitive to metabolic stress caused by the nutrient deprivation. **Methods:** We carried out biochemical sub-cellular fractionations of the nutrient-deprived human cancer cells to isolate and examine ribosomal biogenesis factors within different subcellular fractions (the cytoplasmic, soluble nuclear, and nuclear insoluble fractions) that represent the different functional localization sites. Following the nutrient deprivation of cancer cells for different time points and sub-cellular fractionation, we have examined 20 different ribosomal biogenesis factors by detection these proteins and also purification of their native complexes. In the present time, we are in a process of extending our study to the proteomics based nutrient-sensitive profiling of nucleolar fractions. **Results:** Our initial characterization of the ribosomal biogenesis factors indicated that the nuclear insoluble fraction represents the nucleolar enriched fraction. The nutrient deprivation studies determined that a glucose dependent ATP depletion but not serum or amino acid starvation caused a substantial effect on the posttranslational modifications of Rio kinase 1 and also ribosomal RNA processing 9 factor as detected by their altered protein mobility only in the nuclear but not in the cytoplasmic fraction. We also found that Rio kinase 1 is co-purified with the methylosome 50 and arginine N-methyltransferase proteins suggesting its role in regulation of a protein methylation complex required for ribosomal assembly. **Conclusion:** Our pilot study indicates that a glucose-dependent ATP depletion is a highly informative approach to reveal the critical regulators of ribosomal biogenesis sensitive to metabolic stress. We believe that our profiling and functional studies of ribosomal biogenesis will lead to novel valuable targets controlling accelerated growth of cancer cells.

17 **CPRIT Grantee**
Poster Session A

Estrogen Receptor β2 Induces Hypoxia Signature of Gene Expression by Stabilizing HIF-1α in Prostate Cancer *M. Faria, University of Houston; P. Dey, The University of Texas M.D. Anderson Cancer Center; L. Velazquez-Villegas, University of Houston; A. Turner, University of Houston; P. Jonsson, University of Houston; P. Webb, The Methodist Hospital Research Institute; C. Williams, University of Houston; J. Gustafsson, University of Houston; A. Strom, University of Houston*

Introduction: The estrogen receptor (ER) β variants ERβ2 and ERβ5 are expressed in aggressive castration-resistant prostate cancer and have been shown to correlate with decreased overall survival. The variants are known to counteract ERα activity by forming a heterodimer that increase degradation and inhibit function of ERα, however, since prostate cancer expresses very low levels of ERα another mechanism of oncogenic activity independent of ERα is more likely. **Methods:** Genome-wide expression analysis of ERβ2 expressing in prostate cancer cells. Transient transfection of ERβ2 responsive promoters linked to luciferase. Chromatin immunoprecipitation assay (ChIP). Endogenous biotin labeling of ERβ2 and ERβ5 by transfection of biotin ligase and biotinylation consensus linked to the receptors. Pulldown using streptavidin magnetic beads followed by detection of interacting proteins. **Results:** Genome-wide expression analysis revealed that hypoxia was an overrepresented theme. We show that ERβ2 interacts with and stabilizes HIF-1α protein under normoxic conditions, thereby inducing a hypoxic gene expression signature in prostate cancer cells. HIF-1α is known to stimulate metastasis by increasing expression of Twist1 and increasing vascularization by directly activating VEGF expression. We found that ERβ2 interacts with HIF-1α and piggybacks to the HIF-1α response element present on the proximal Twist1 and VEGF promoters. **Conclusion:** Since expression of HIF-1α in advanced prostate cancer have been shown to correlate to castration resistance these findings suggest that expression of ERβ2 could enhance castration resistance in prostate cancer. In addition, the findings suggest that at least part of the oncogenic effects of ERβ2 is mediated by HIF-1α and that targeting of this ERβ2 – HIF-1α interaction may be a strategy to treat prostate cancer.

18 **CPRIT Grantee**
Poster Session B

Increase in Matrix Modulus Leads to Tumor Progression In Vivo *S. Allen, The University of Texas at Austin; N. Ebelt, The University of Texas at Austin; R. Stowers, Stanford University; C. Van Den Berg, The University of Texas at Austin; L. Suggs, The University of Texas at Austin*

Introduction: Soft tissue tumors become stiffer through disease progression. This increase in stiffness has been shown to correlate to malignant phenotype in vitro. Our group has previously created a system which allows for dynamic stiffening of an alginate-matrigel composite hydrogel to model the native dynamic process. Work with this model has shown that dynamic increases in stiffness in vitro lead to previously non-proliferative MCF10A adopting a more invasive and proliferative phenotype. Here we present an extension of this work in an in vivo model, allowing the ability to investigate the role of tissue stiffness on later stages of a tumor. **Methods:** Primary tumor cells were isolated from transgenic mice using the mouse mammary tumor virus to drive oncogene expression. These cells were pooled and mixed with alginate, matrigel, liposomes, calcium carbonate, and glucono-delta-lactone and injected into the mammary fat pad of FVB/N mice to form soft (150 Pa) gels with the ability to be stiffened upon irradiation in those mice injected with $CaCl_2$-loaded liposomes. Gels underwent transdermal irradiation to externally increase the modulus to an intermediate stiffness 5 days post injection; 10 days after injection a sub-group was irradiated again to a fina stiffness of 1200 Pa. Control groups consisted of mice injected with gels which initially formed soft and stiff gels, containing NaCl-loaded liposomes such that transdermal irradiation did not result in a change in gel modulus. Following transdermal irradiation, a subset of mice in each group were given an intraperitoneal injection of NSC23766, a Rac1 inhibitor, at 2.5 mg/kg/day. **Results:** Mice injected with control stiff gels exhibited larger tumors and shorter survival time relative to control soft gels. Additionally, mice with gels which were irradiated twice (Day 5&10) exhibited larger tumors and shorter survival time relative to those which were irradiated only once (Day 5). Interestingly, treatment with NSC23766 led to smaller tumors and extended survival time in experimental groups ($CaCl_2$-loaded liposomes). **Conclusion:** The increased size and decreased survival time in the control stiff group relative to the soft group indicates a role for matrix stiffness in tumor progression and development. The Rac1 mechanotransduction pathway is indicated as important in this as mice given NSC23766, a Rac1 inhibitor, exhibited smaller tumors and longer survival time relative to those given a vehicle control. Additionally, dynamic stiffening is demonstrated as important to tumor progression as those mice with gels which were stiffened twice exhibited larger tumors than those which were stiffened only once.

19 **CPRIT Grantee Poster Session A**

PPARD Promotes Wnt/Beta-Catenin–Driven Colorectal Tumorigenesis *I. Shureiqi, The University of Texas M.D. Anderson Cancer Center; X. Zuo, The University of Texas M.D. Anderson Cancer Center*

Introduction: Aberrant Wnt/b-catenin signaling activation due to mutations in the adenomatous poliposis coli gene, APC, is a critical event in colorectal cancer (CRC). However, to drive CRC tumorigenesis, aberrant Wnt/b-catenin activation requires additional enhancing regulatory mechanisms. The peroxisome proliferator-activated receptor delta gene, PPARD, which is upregulated in CRC, is a downstream target of aberrant Wnt/B-catenin activation in human colon cancer cells. However, PPARD has also been reported to inhibit intestinal tumorigenesis in mice with germline APC^{min} mutations. Thus, the mechanistic interaction between PPARD and Wnt/B-catenin remains poorly understood. PPARD is a druggable protein for which agonists and antagonist are being developed. Determining PPARD effects on Wnt/B-catenin activation would defin the direction of its therapeutic targeting in cancer and other diseases. **Methods:** We developed a novel mouse model that simulates PPARD overexpression in CRC by inducing PPARD overexpression in intestinal epithelial cells via a villin promoter (villin-PPARD mice). We bred villin-PPARD mice with mice that have APC^{580} mutations in intestinal epithelial cells induced by CDX2–Cre recombinase expression (Apc580mu mice) to generate Apc580mu-PPARD-Gut mice. Apc580mu-PPARD-Gut and control mice were monitored for CRC tumorigenesis. We also assessed the effects of PPARD expression modulation on Wnt/b-catenin signaling in human colon cancer cell lines. **Results:** PPARD overexpression in colonic epithelial cells increased CRC tumorigenesis in mice with APC580 mutations. At 10 weeks of age, 100% of Apc580mu-PPARD-Gut mice but only 60% of Apc580mu mice had tumors of any size, and the mean number of tumors per Apc580mu-PPARD-Gut mouse (1.6 ± 2.5) was significantly higher than that per Apc580mu mouse (0.6 ± 0.25; p=0.02). Tumors >3.5 mm were present in all Apc580mu-PPARD-Gut mice but only 20% of Apc580mu mice, and the mean number of tumors >3.5 mm per Apc580mu-PPARD-Gut mouse (1.4 ± 2.5) was significantl higher than that per Apc580mu mouse (0.2 ± 0.2; p = 0.005). In mice, colonic epithelial PPARD overexpression increased activated B-catenin protein levels and Wnt/B-catenin target gene mRNA levels. In human colon cancer cells, PPARD increased the levels of activated B-catenin, its nuclear localization and transcriptional activity. **Conclusion:** Our finding indicate that PPARD augments Wnt/B-catenin signaling to promote CRC tumorigenesis and thus support the targeted inhibition of PPARD to suppress CRC tumorigenesis.

20 **CPRIT Grantee Poster Session B**

Transcriptome Profiling Reveals Enrichment of Epithelial Cells with both Luminal and Basal Markers in Aged Murine Mammary Gland *X. Gu, The University of Texas Health Science Center at San Antonio; H. Gao, The University of Texas Health Science Center at San Antonio; A. Wu, The University of Texas Health Science Center at San Antonio; A. Bandyopadhyay, The University of Texas Health Science Center at San Antonio; Q. Dong, The University of Texas Health Science Center at San Antonio; L. Sun, The University of Texas Health Science Center at San Antonio*

Introduction: Aging is the number one risk factor for breast cancer development. Increasing evidence suggests the potential of mammary stem cells (MaSCs) and their progenitors to generate certain types of breast cancers through neoplastic transformation. Our previous study has shown increased percentage of MaSC-enriched basal cell population ($Lin^-CD49f^{high}CD24^{med}$) and increased MaSC frequency during aging in murine models. On the other hand, a recent study from another group showed an increased frequency of $CD49f^{high}$ cells in the human luminal population ($CD227^+$) during aging, indicating possible aberrant expression of CD49f in the aged luminal cells. However, how these age-related luminal cells with basal markers are generated and how they contribute to potential breast cancer development remains unknown. **Methods:** We apply bioinformatics analysis on Next Generation Whole Transcriptome Sequencing data of MaSC-enriched basal cell population ($Lin^-CD49f^{high}CD24^{med}$) and luminal progenitor-enriched cell population ($Lin^-CD49f^{low}CD24^{high}$) of both young (4 to 6 months) and old (26 to 31 months) mouse mammary gland to test the hypothesis that age-associated increase of basal cell population and MaSCs may be due to the gain of basal cell markers and features by luminal cells. **Results:** By Gene Set Enrichment Analysis (GSEA) we found a significant loss of basal cell and basal mammosphere signatures and a significant enrichment of luminal cell and luminal mammosphere signature in the old basal cell population and mammospheres in comparison with the young basal cell population and mammospheres. The core enrichment luminal genes from GSEA are able to cluster the old MaSC-enriched basal cell population as well as mammospheres closer to the cluster of luminal population than to the young basal population. **Conclusion:** These analyses indicate that aging may be associated with an expansion of aberrant MaSCs with both basal and luminal markers in mice, which may be the precursors of certain types of breast cancer. We are now studying the potential function of the basal-like luminal cells in the aged basal population.

21 **CPRIT Grantee Poster Session A**

COMMD1 restrains tumor promoting activities of myeloid cells in colon cancer *N. Miyata, The University of Texas Southwestern Medical Center at Dallas; E. Burstein, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The transcription factor NF-kB plays essential roles in regulating immune and inflammatory responses. It also has an important role in tumorigenesis, especially in inflammation-driven cancer. COMMD1 is the founding member of the COMMD protein family, a group of highly conserved factors that remain relatively understudied. Previously we found that COMMD1 is a negative regulator of NF-kB that modulates tumor invasion in xenograft models. Moreover, we have identified common variants in the human *COMMD1* gene that modulate its expression, and these variants affect the risk for intestinal inflammation (ulcerative colitis). Our purpose in this study is to clarify the role of COMMD1 in colon cancer development and progression using murine genetic models. **Methods:** We utilized the Cre-LoxP recombination system to cause *Commd1* gene inactivation in specific tissues. In particular, we used the LysM-Cre knock-in mice to delete a LoxP-flanked *Commd1* allele in the myeloid lineage (*Commd1-Mye K/O*). These animals were bred with *ApcMin/+* mice, an animal model of Familial Adenomatous Polyposis (FAP), which develop intestinal adenomas. **Results:** In this model, we found that loss of *Commd1* in immune cells of myeloid origin specifically promotes colon tumor development. *Commd1-Mye K/O* had a doubling in the number of colonic tumors observed (4.7 tumors per animal in control mice, compared to 9.7 in *Commd1-Mye K/O*, $p<0.05$). This was associated with more severe anemia (Hct in control mice 28.9±3.6%, vs. 20.8±2.4% in *Commd1-Mye K/O*, $p<0.05$). Interestingly, colonic adenomas in *Commd1-Mye K/O* displayed similar rates of proliferation (Ki-67) and apoptosis (TUNEL staining). However, colonic adenomas in the *Commd1-Mye K/O* mice were noted to have up-regulation of *Ptgs2* (COX-2), a gene known for its role in promoting adenoma and colon cancer development. **Conclusion:** Altogether, our studies indicate that *Commd1* expression in cells of myeloid origin restrain their tumor promoting potential in the colon. The mechanism for this role of *Commd1* in the immune system is still unclear, but COX2 upregulation resulting from *Commd1* deficiency is an attractive target that may be driving the increased number of tumors noted. Furthermore, given that the effects of *Commd1* deficiency are specific for colonic tumors, a potential role for the microbiota can be posited. Ongoing experiments are examining these hypotheses. In addition, a possible role for Commd1 in the epithelial compartment is being evaluated using a complementary approach for enterocyte-specific deletion (Villin-Cre). Finally, these studies suggest that genomic loci that affect *COMMD1* expression in humans may be linked to a risk of adenoma development.

22 **CPRIT Grantee Poster Session B**

Regulation of Polycomb Subunit Composition Mediates Non-Canonical Functions During Blood Development and Cancers *J. Xu, The University of Texas Southwestern Medical Center at Dallas; Z. Shao, Boston Children's Hospital; H. Cao, The University of Texas Southwestern Medical Center at Dallas; X. Liu, The University of Texas Southwestern Medical Center at Dallas; D. Li, Harvard College; Y. Zhang, The University of Texas Southwestern Medical Center at Dallas; G. Yuan, Dana-Farber Cancer Institute; S. Orkin, Boston Children's Hospital*

Introduction: The epigenetic machinery plays crucial roles in cellular identity, and its deregulation drives the pathogenesis of human cancers. Polycomb Repressive Complex 2 (PRC2) is a major epigenetic repressor that catalyzes histone H3K27 di/tri-methylation (or H3K27me2/3). The canonical PRC2 complex consists of EED, SUZ12, and the methyltransferase EZH2. While overexpression of PRC2 proteins is common in many cancers, inactivating mutation in PRC2 is frequently found in hematopoietic malignancies, indicating that PRC2 can be oncogenic or tumor suppressive in different cellular contexts. In light of recent efforts to therapeutically target EZH2 activities or canonical EZH2-PRC2 functions in various hematopoietic malignancies, it will be critical to fully assess the context-specific activity of this epigenetic complex in normal and malignant developmental processes. **Methods:** We first measured the expression of EZH1 and EZH2 during normal and neoplastic hematopoiesis. We next examined the in vivo stoichiometry of the PRC2 complexes by quantitative proteomics. We performed genome scale chromatin occupancy (by ChIP-seq) and transcriptional profiling (by RNA-seq) analyses to define the similarities and differences between the canonical and non-canonical PRC2 complexes. Furthermore, we started characterizing the role of canonical and non-canonical PRC2 in leukemia initiation and maintenance using PRC2 individual and combination knockout mouse models. **Results:** We revealed that the PRC2 enzymatic subunits EZH1 and EZH2 undergo an expression switch during hematopoiesis. EZH2 is highly expressed in hematopoietic stem/progenitor cells and progressively downregulated during lineage specification, whereas EZH1 is significantly upregulated during differentiation. We next revealed the existence of an EZH1-SUZ12 sub-complex lacking EED subunit. EZH1 together with SUZ12 form a non-canonical PRC2 complex, occupy active chromatin domains, and positively regulate gene expression. Furthermore, loss of EZH2 leads to global repositioning of EZH1 to EZH2 targets, and EZH1 complements EZH2 loss within canonical PRC2 target genes. We identified and characterized an erythroid-selective enhancer element indispensable for the activation of EZH1. Moreover, a switch from GATA2 to GATA1 expression controls the developmental EZH1/2 switch by differential association with EZH1 enhancers during erythroid differentiation. **Conclusion:** Our findings demonstrate that the developmental regulation of PRC2 subunit composition leads to a switch from canonical silencing to non-canonical PRC2 functions. Hence, the combinatorial assembly of multi-protein chromatin modifying complexes plays a critical role to specify the context-dependent activities in development and cancers. Ongoing work is focused on elucidating the regulation of EZH1 and EZH2 during normal and malignant hematopoiesis, and to develop therapeutic approaches to target oncogenic EZH2 for cancer intervention.

23 **CPRIT Grantee Poster Session A**

Tenascin-C as an Effector of Prostate Cancer Bone Metastasis

R. San Martin, Baylor College of Medicine; K. Pienta, The Johns Hopkins University; D. Rowley, Baylor College of Medicine

Introduction: The reactive stroma response in prostate cancer initiates early and is predictive of biochemical recurrence. Reactive stroma myofibroblasts are smooth muscle alpha actin (SMA) and vimentin positive, and are a critical component in wound repair biology. Myofibroblasts remodel the stromal compartment, in part, via deposition of tenascin C. Tenascin C is an extracellular matrix protein expressed during development and is critical for neuronal patterning and osteogenesis; in contrast, expression of tenascin C in adult tissues is restricted to regions of wound repair, tissue remodeling and pathological conditions. This microenvironment shift may foster the progression of prostate cancer via differential adhesion patterns and transient EMT induction. **Methods:** To characterize the microenvironment changes present in prostate cancer derived bone metastasis in the context of a reactive tissue phenotype, we used dual immunohistochemistry protocols and spectral deconvolution microscopy on human bone metastasis tissue microarrays. In order to evaluate the mechanisms involved in tenascin C induced biology, we developed an in vitro 3D osteogenic organoid model with human mesenchymal stem cells induced to osteoblastic differentiation and co-culture with prostate cancer metastatic cell lines. The interaction of prostate derived metastatic cell lines and tenascin C was also assessed in vitro, in a 2D - osteomimetic cell culture surface and in an 3D bone matrix scaffold. **Results:** Immunohistochemical studies identified a tenascin-C expression pattern at trabeculae-associated metastatic foci, suggesting the evolution of a reactive endosteum. The osteogenic organoid system, successfully recapitulates a reactive endosteum phenotype and its co-culture with the metastatic prostate cancer cell line VCaP showed that these cells preferentially bind at sites high in tenascin C deposition. Also, metastatic cells were capable of adherence to tenascin C in vitro, forming 3D colonies. These studies identified alpha9 beta1 integrin as the key mediator of cancer cell adhesion to tenascin C–rich, osteomimetic surfaces. Preliminary data has also identified signaling through the EGFR-WNK1 pathway as additional candidate mechanism that mediates tenascin-C induced biology in prostate cancer cells that are metastatic to bone surfaces. **Conclusion:** These studies characterize a reactive endosteum phenotype at sites of metastatic prostate cancer foci and suggest that elevated tenascin-C at these sites mediates cancer cell adhesion, and colony formation. This study may provide data from which to develop novel therapeutic approaches to target the bone metastatic niche.

24 **CPRIT Grantee Poster Session B**

Bispecific Antibodies Targeting Heregulin Induced HER3 Signaling in Breast Cancer Cells

J. Kang, Texas A&M University System Health Science Center; J. Poovassery, The University of Texas Southwestern Medical Center at Dallas; P. Bansal, The University of Texas Southwestern Medical Center at Dallas; S. You, Texas A&M University System Health Science Center; I. Manjarres, The University of Texas Southwestern Medical Center at Dallas; R. Ober, Texas A&M University; E. Ward, Texas A&M University System Health Science Center

Introduction: Epidermal growth factor receptor 2 (HER2) overexpressing breast cancer is aggressive and has an unfavorable prognosis. Single agents, such as lapatinib (a tyrosine kinase inhibitor that targets HER1 and HER2) and trastuzumab (a HER2-specific antibody), have limited efficacy in the treatment of HER2 overexpressing breast cancer. One of the reasons for the limited efficacy can be attributed to the HER3 receptor and its ligand heregulin. Heregulin can be expressed in either a paracrine or autocrine manner; ligand bound HER3 can dimerize with HER2 and potently activate the PI3K/Akt pathway, leading to tumor escape. Our study focuses on the creation of a bispecific antibody that is designed to overcome this escape. **Methods:** Bispecific anti-HER2 and anti-HER3 antibodies were generated by fusing an anti-HER3 single chain variable fragment (scFv) to the CH3 domain of an anti-HER2 antibody, trastuzumab. HER2 overexpressing cell lines were treated with combinations of lapatinib, heregulin, anti-HER2 and anti-HER3 antibodies. Cell proliferation was analyzed using an MTS assay. Cell lysates of treated cells were also analyzed by immunoblotting. **Results:** SK-BR-3 and BT-474 cells treated with lapatinib show significantly decreased proliferation, decreased Akt signaling, and upregulation of the HER2 and HER3 receptors on the cell surface. Addition of heregulin to these cancer cells reverses the anti-proliferative effects of lapatinib. The engineered bispecific antibody that binds to both HER2 and HER3, in combination with lapatinib, reverses the proliferative effects of heregulin. Consistent with the anti-proliferative effects of the combination treatment, heregulin induced PI3K/Akt signaling is inhibited. **Conclusion:** Antibodies targeting HER2 and HER3, such as the HER2-specific trastuzumab, have limited efficacy in inhibiting proliferation and signaling in HER2 overexpressing breast cancer cells. The effects of small molecule inhibitors, such as lapatinib, can be overcome by HER2/HER3 upregulation and signaling. In both cases, heregulin plays a critical role in reversing the inhibition of tumor cell proliferation and signaling by antibody or lapatinib alone. In our study, we have shown that a bispecific antibody is capable of binding to both HER2 and HER3, blocking the interactions of both these receptors with other tyrosine kinase receptors. The addition of lapatinib to the HER2/HER3 complexes formed by the bispecific antibody prevents HER2 from trans-phosphorylating HER3, creating a locked HER2-HER3 heterodimer that cannot signal. This suggests the bispecific antibody utilized in combination with small molecule inhibitors has potential for treatment of cancer.

25 **CPRIT Grantee Poster Session A**

Stromal Effects of Adrenergic Signaling in Ovarian Carcinoma

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Introduction: Catecholamine-mediated effects driven by elevated adrenergic signaling are known to increase tumor growth and tumor metastasis by direct effects on tumor cells. However, knowledge of effects of adrenergic signaling on other cells within the tumor microenvironment is extremely limited. Cancer-associated fibroblasts (CAFs), a constituent of tumor stroma implicated in increased metastasis and angiogenesis in ovarian cancer. We hypothesize that of adrenergic signaling and norepinephrine can play can accelerate this process. Here, we address the functional and biological consequences of adrenergic-induced recruitment and transformation of fibroblasts in promoting ovarian cancer. **Methods:** Tumor samples from mice exposed to daily restraint-stress or non-stressed controls were assessed for a-smooth muscle actin (a-SMA) expression. Normal fibroblasts (NoF 151 and NoF 182) were treated with conditioned media from non-treated and norepinephrine (NE) treated Skov3 and HeyA8 cells and analyzed for induction of CAF-phenotype by a-SMA expression. Cancer-associated fibroblast (CAF 148) was treated with NE as these were shown to express adrenergic receptors. Gene expression of cytokines and changes in migratory potential of fibroblasts were assessed. **Results:** Adrenergic signaling was associated with significantly increased levels of α -SMA, a marker for CAFs by both intensity and distribution in both Skov3 and HeyA8 tumors. These increases in α-SMA were abrogated when mice were treated with broad beta-blocker propranolol during restraint stress. Treatment of normal fibroblasts with conditioned media from cancer cells treated with NE accelerated the expression of α-SMA, indicating a role for NE in driving CAF transformation (3-fold change in protein levels). Normal fibroblasts treated with conditioned media from NE-treated cells showed a 3-fold increase in migratory potential compared to media from untreated tumor cells. These fibroblasts were also more responsive to NE directly and showed elevated levels of cytokines at gene level. CAF148 that was directly treated with NE also showed elevated expression of several cytokines. Tumors obtained from GFP mice with RFP-labeled bone marrow cells were stained for RFP, GFP and α-SMA. Consistent with literature, α-SMA co-localized with resident fibroblasts (GFP), but not RFP. **Conclusion:** Sustained adrenergic stimulation results in significant increases in the CAFs in-vivo and accelerates coversion of normal fibroblasts to CAFs in vitro. Adrenergic-mediated changes in CAF phenotype can increase production of pro-inflammatory cytokines and can play a role in driving inflammation. Further studies will focus on the role of CAFs in mediating immune-cell trafficking in tumors. This project provides a better understanding of the neuroendocrine influences on the cells within the tumor microenvironment.

26 **CPRIT Grantee Poster Session B**

PTP-PEST and β8 Integrin Cooperatively Regulate Tumor Cell Invasion in Glioblastoma

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Introduction: Grade IV astrocytoma, or glioblastoma (GBM), is a primary brain tumor that is resistant to post-operative radiation and chemotherapy. This resistance is due in large part to invasive cancer cells that escape surgical resection and invariably contribute to tumor recurrence. The mechanisms that promote GBM cell invasion during tumor progression following therapies remain enigmatic. In this project we will study an adhesion and signaling pathway, comprised of β8 integrin and its intracellular binding partner PTP-PEST that drives GBM cell invasion in the brain microenvironment. **Methods:** Mutagenesis strategies and biochemical approaches were used to identify domains in β8 integrin and PTP-PEST that mediate protein-protein interactions. Regulation of downstream signaling pathways, especially involving RhoGDI1 and the Rho GTPases Rac1/Cdc42, were analyzed by imaging fluorescen biosensors. Xenograft mouse models of GBM were developed to study functions for the β8 integrin-PTP-PEST protein complex in tumor growth and invasion. Lastly, immunohistochemistry was used to quantify levels of of β8 integrin and PTP-PEST protein expression in human GBM tissue microarrays. **Results:** β8 integrin (ITGB8) and PTP-PEST (PTPN12) gene expression are elevated in GBM as determined by Oncomine analysis. Integrin and PTP-PEST proteins are robustly expressed in human tumor lysates and primary GBM stem cells. Patient samples stained with β8 integrin and PTP-PEST antibodies, reveal protein expression primarily in GBM cells, although PTP-PEST expression was also detected in angiogenic blood vessels. β8 integrin and PTP-PEST proteins form complexes in tumor cells, as determined by co-immunoprecipitation and GST pull-down experiments. PTP-PEST and β8 integrin also form a complex with the Rho signaling effector, RhoGDI1. Silencing β8 integrin or PTP-PEST gene expression leads to hyperactive Rac1 and Cdc42 signaling and impaired tumor cell invasion. Use of Crispr-Cas9 genetic strategies in human GBM stem cells reveals a requirement for β8 integrin and PTP-PEST signaling in invasive cell growth in mouse xenograft models. **Conclusion:** These experiments reveal novel β8 integrin-regulated signaling events in GBM cell invasion, and may eventually lead to new strategies for inhibiting cell invasion during tumor progression and following anti-VEGF therapies. For example, small molecules that prevent formation of this signaling complex to block β8 integrin adhesion and/or PTP-PEST enzymatic activities could be used to inhibit pro-invasive signaling pathways in GBM cells.

27 **CPRIT Grantee Poster Session A**

PRDX2 and PRDX4 are the negative regulators of hypoxia-inducible factors under prolonged hypoxia *W. Luo, The University of Texas Southwestern Medical Center at Dallas; I. Chen, Johns Hopkins University; Y. Wang, The University of Texas Southwestern Medical Center at Dallas; G. Semenza, Johns Hopkins University School of Medicine*

Introduction: The hypoxia-inducible factors (HIFs) are master regulators of transcriptional responses to reduced oxygen availability. They control transcription of many genes that are crucial for pathogenesis of many human diseases, including cancer. The transcriptional activity of HIFs is rapidly increased upon acute hypoxia, but decreases during prolonged hypoxia. However, the underlying mechanism for feedback inhibition is not completely understood. **Methods:** Human cervical carcinoma HeLa cells were exposed to 20% or 1% O_2. The interaction of HIF-alpha with peroxiredoxins (PRDXs) was tested by mass spectrometry, co-immunoprecipitation, and GST pull-down assays. The effects of PRDXs on HIF transcriptional activity were measured by luciferase reporter assays and real-time RT-PCR assays. PRDX2 expression levels were examined by real-time RT-PCR assays and immunoblotting assays. Chromatin immunoprecipitation assays were performed to determine HIF binding to *PRDX2* gene in HeLa cells. The hypoxia response element on the *PRDX2* gene was tested by luciferase reporter assays. **Results:** We found that PRDX2 and PRDX4 interact with HIF-1alpha and HIF-2alpha, and inhibit transcription of a subset of HIF target genes in cancer cells exposed to prolonged hypoxia. The enzymatic activity of PRDX2 or PRDX4 is not required for inhibition of HIF-1 and HIF-2. We demonstrate that *PRDX2* is a HIF target gene and PRDX2 expression is induced by prolonged hypoxia. **Conclusion:** PRDX2 and PRDX4 are negative regulators of HIFs under prolonged hypoxia. These findings uncover a novel feedback mechanism for inhibition of HIF transcriptional activity by prolonged hypoxia.

28 **CPRIT Grantee Poster Session B**

Fate Determination in Mammary Stem Cells *A. Sreekumar, Baylor College of Medicine; K. Roarty, Baylor College of Medicine; J. Rosen, Baylor College of Medicine*

Introduction: Stem cells orchestrate homeostasis by balancing cell division, differentiation and apoptosis. This balance is disrupted as a consequence of dysregulated signalling during tumourigenesis. Stem cell-driven, pubertal mammary epithelial structures termed terminal end buds (TEBs) serve as a model to study the complex processes underlying homeostasis. We hypothesize that targeted disruption of the stemness-maintaining Wnt pathway components results in an abrogation of homeostasis that leads to tumourigenesis. These studies are pertinent to our understanding of breast cancers as multiple models of the aggressive basal-like subtype of breast cancers show aberrant Wnt pathway activity. **Methods:** To study homeostasis in the TEB, we employ the s-SHIP:eGFP mouse model that marks cap cells in the pubertal mammary gland (putative stem cells). Additionally, we use in vitro and in vivo imaging to observe cellular dynamics in real time. Finally, we perturb Wnt signalling locally in the TEB by administering Wnt pathway ligands and antagonists in the vicinity of TEBs or globally, utilizing the MMTV-Wnt1 overexpression model. The latter serves as a model of tumourigenesis. **Results:** We demonstrate that cap cells invaginate from their outer location in the TEB and intermix with body cells where a large percentage undergoes apoptosis. Apoptosis potentially acts as a mechanism of lumen formation and balances excessive cap cell divisions. We confirm previous finding that cap cells show both parallel and perpendicular planes of cell division and show that these are accompanied by differential localization of markers of asymmetric cell division; wherein a stem cell gives rise to daughter cells with divergent cell fates. Our laboratory and others have previously described that these cap cells in the TEB reside in a Wnt responsive niche. Preliminary data suggest that global hyperactivation of Wnt signalling in the MMTV-Wnt1 model results in defective TEB lumen formation, decreased apoptosis and an increase in parallel cap cell divisions. Future experiments will focus on studying the mechanisms by which the Wnt signalling pathway co-ordinates homeostasis. **Conclusion:** Our results demonstrate that the Wnt pathway finely tunes homeostatic processes in the normal mammary gland. Current studies are focused on studying the contribution of the disrupted Wnt pathway at different stages of tumourigenesis and inhibiting Wnt pathway components locally to study their short-term effects on homeostasis. Our studies may shed light on the complex regulation of stem cell homeostasis by the Wnt pathway and provide a rationale for treatment of a subset of breast cancer patients with Wnt inhibitors.

29 **CPRIT Grantee Poster Session A**

TRIM24 links Epigenetics and Metabolism in Breast Cancer *K. Thakkar, The University of Texas M.D. Anderson Cancer Center; S. Stratton, The University of Texas M.D. Anderson Cancer Center; S . Jiang, The University of Texas M.D. Anderson Cancer Center; M .Barton, The University of Texas M.D. Anderson Cancer Center*

Introduction: TRIM24, also known as Transcription Intermediary Factor 1-alpha (TIF1a), negatively regulates p53 as an E3-ubiquitin ligase, as discovered by our laboratory using breast cancer cell lines and embryonic stem cells. TRIM24 acts as a transcriptional co-regulator by "reading" a specific signature of histone post-translational modifications by means of a tandem plant homeodomain (PHD) and Bromodomain within the C-terminus of TRIM24. TRIM24 interacts with chromatin marked by H3K4me0-H3K23ac, including specific estrogen regulatory elements (EREs), recruits Estrogen receptor-a via TRIM24's LXXLL motif, and activates estrogen-dependent genes. However, the potential roles of TRIM24 expression in breast tumorigenesis remain largely unknown. **Methods:** I used immortalized human mammary epithelial cells (iHMECs), derived from reduction mammoplasty tissue, as a model system to study the effect of TRIM24-overexpression on cellular transformation. **Results:** I found that ectopic expression of TRIM24 in immortalized HMECs (TRIM24 iHMECs) greatly increased cellular proliferation and induced malignant transformation. Subcutaneous injection of TRIM24 iHMECs in nude mice led to growth of intermediate to high-grade tumors in 60–70% of mice. Molecular analysis of TRIM24 iHMECs revealed a glycolytic and tricarboxylic acid cycle gene signature, alongside increased glucose uptake and activated aerobic glycolysis. Consistent with in vitro findings, the glucose transport pathway was among the top 10 pathways positively correlated with TRIM24 expression in human breast tumors (n = 1008) from the TCGA database. Thus, TRIM24 is co-expressed with genes that regulate glucose metabolism in breast tumors, supporting the clinical relevance of our findings. In addition, I observed increased c-Myc expression and a decrease in p53 protein levels in TRIM24 over-expressing iHMECs. Since both c-Myc and p53 play key roles in regulating cellular metabolism, we proposed that these could be the possible mediators of TRIM24-induced metabolic reprogramming. C**onclusion:** In summary, our studies suggest a unique role for TRIM24 in early steps of mammary carcinogenesis that involves reprogramming of glucose metabolism. These results provide the groundwork to test

chemical therapeutics that disrupt TRIM24 functions, e.g. Bromodomain inhibitors. Currently, I am utilizing iHMECs as tools to define the functional domains responsible for TRIM24's oncogenic functions.

30 **CPRIT Grantee Poster Session B**

Image Analysis of Cancer Metastasis in the Zebrafish Model *S. Upadhyay, University of Houston; M. Bondesson, University of Houston; J. Gustafsson, University of Houston; I. Kakadiaris, University of Houston*

Introduction: In recent years, the zebrafish has gained an increasing popularity as a model for different human diseases, including cancers, largely because of its small size, transparency and the high number of embryos that can be obtained. In this project, we are investigating whether the zebrafish (ZF) vertebrate organism resembles mammalian models for tissue specific metastasis, which if so suggests that it can be used as a cost-efficient and rapid alternative to mammalian models in cancer metastasis research. We aim to: (i) use ZF to build a 4D (3D + time) atlas of different tissues; and (ii) using xenografts of human cancer cells investigate tissue specific metastasis. Our goal is to build a 2D + time atlas for zebrafish embryo development. **Methods:** Previously we built a 2D atlas of three day-old ZF embryos with five tissues (Le et al., ISBI 2014 750-753). We have now expanded the ZF atlas to include 2D + time. Through confocal time-lapse imaging techniques, we have imaged different developing tissues in transgenic fish. These fish expressed fluorescence in certain tissues or cells (e.g., the vascular endothelium, skeleton, liver or muscle cells). The fish were crossed so that each imaged embryo expresses several transgenic tissue markers. Next, we created a new framework for building a representative atlas of multiple tissue types during development. This allowed a direct visualization of tissue in ways impossible using mammalian models. **Results:** One of the prime challenges in observing embryo development over time is to fin a suitable mounting medium that restricts fish movement while allowing free growth. We solved this problem by using two layers to agarose with optimally chosen concentrations. We then used registration techniques introduced in Le et al. to develop an approximation to continuous time evolution of the 2D atlas. We have previously constructed fluorescentl labeled highly metastatic breast and prostate cancer cells. In the next phase of the project, these cancer cells will be xenografted onto the fis and the growth and migration of cancer cells will be followed. **Conclusion:** The expected outcome of this research is to enhance the knowledge of tissue specific cancer metastasis, and eventually the model can be used to investigate mechanisms related to tissue specificity for metastatic cells.

31 **CPRIT Grantee Poster Session A**

ASCL1 and NEUROD1 Identify Distinct Pulmonary Neuroendocrine Tumors and Bind Distinct Sites Within the Tumor Genomes *J. Johnson, The University of Texas Southwestern Medical Center at Dallas; M. Borromeo, The University of Texas Southwestern Medical Center at Dallas; T. Savage, The University of Texas Southwestern Medical Center at Dallas; R. Kollipara, The University of Texas Southwestern Medical Center at Dallas; M. He, The University of Texas Southwestern Medical Center at Dallas; A. Augustyn, The University of Texas Southwestern Medical Center at Dallas; L. Girard, The University of Texas Southwestern Medical Center at Dallas; J. Minna, The University of Texas Southwestern Medical Center at Dallas; A. Gazdar, The University of Texas Southwestern Medical Center at Dallas; M. Cobb, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC) are high-grade pulmonary neuroendocrine tumors. Patients with SCLC have a poor prognosis, largely due a lack of advances in treatment. As targetable mutations have not been identified that guide therapeutic decisions, identifying genes required for cell survival of the tumor cells is needed. The neural basic helix-loop-helix (bHLH) transcription factors ASCL1 and NEUROD1 have been shown to play crucial roles in promoting the malignant behavior and survival in distinct human SCLC cell lines. However, the in vivo requirement of these transcription factors for these tumors, and the identity of their downstream transcriptional targets is unknown. It also is unclear if these related bHLH factors bind and regulate similar genes to exhibit a common function in SCLC. We address these questions in this study. **Methods:** This study uses genomic/transcriptomic techniques applied to a panel of human SCLC tumor cell lines, and genetically engineered mouse models (GEMM) of lung cancer, to investigate the function of ASCL1 and NEUROD1 in pulmonary neuroendocrine cancers. **Results:** We find ASCL1(High) and NEUROD1(High) expression in human SCLC cell lines to be mutually exclusive and identify distinct neuroendocrine tumors, they bind distinct genomic loci, and they regulate mostly distinct genes. ASCL1 and NEUROD1 are often bound in super-enhancers that are associated with highly expressed genes in their respective SCLC cell lines defining different subtypes of SCLC. ASCL1 directly targets oncogenic genes such as MYCL1, RET, and NFIB, while NEUROD1 directly targets the oncogenic gene MYC. Although ASCL1 and NEUROD1 regulate different genes, many of these gene targets commonly contribute to neuroendocrine and cell migration function. ASCL1 in particular also regulates genes in the NOTCH pathway and genes important in cell-cycle dynamics. Furthermore, in GEMMs that delete Tp53, Rb1, and Rbl2 in lung epithelia to model SCLC and LCNEC, we also selectively deleted Ascl1 or Neurod1 to test their requirement for tumorigenesis and survival in vivo. We find that Ascl1 but not Neurod1 is required for SCLC and LCNEC tumor formation in current in vivo genetic mouse models of pulmonary neuroendocrine tumors. **Conclusion:** Taken together, our human tumor and GEMM data strongly suggest that tumor expression of ASCL1 and NEUROD1 define SCLC subtypes that may arise from different cell lineages, are driven by distinct oncogenic pathways, and need to be considered in developing new rational, targeted therapy for SCLC and related pulmonary neuroendocrine tumors.

32 **CPRIT Grantee Poster Session B**

The Effect of the Protein Kinase With No Lysine 1 (WNK1) on Autophagy *S. Gallolu Kankanamalage, The University of Texas Southwestern Medical Center at Dallas; M. Cobb, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The With No Lysine (WNK) is an atypical family of kinases in which a conserved lysine residue in the catalytic domain has shifted from the conventional position, conferring them unique structural and functional properties. WNKs are activated by osmotic stress and once activated; they phosphorylate and activate downstream kinases oxidative-stress responsive 1 (OSR1) and Ste20-related proline-alanine-rich kinase (SPAK). These in turn phosphorylate the SLC12 family of cation-chloride cotransporters, either activating or inhibiting them. Thus, the WNKs mediate the cellular response to changing osmotic conditions. They govern physiological functions such as blood pressure, secretion and reabsorption of ions in the kidney, inhibitory neurotransmission and muscle contraction. In addition, the WNKs regulate other cellular processes such as cell proliferation, migration, endocytosis and angiogenesis. They are mutated/differentially expressed in cancer, hypertension and neurological diseases. Out of four mammalian WNKs, only WNK1 is ubiquitously expressed. Unpublished work from our lab shows that WNK1 regulates autophagy. Autophagy is an intracellular degradation pathway that supplies cells with nutrients and protects them under stress. It starts with the formation of phagophores which engulf the cellular materials and extend to generate organelles called autophagosomes. Autophagosomes fuse with lysosomes to form autolysosomes where the degradation of cellular materials occurs. This pathway is regulated by ULK1 and Vps34 protein complexes. Autophagy is altered in different types of cancers and other human diseases. **Methods:** In HeLa cells, we depleted WNK1 in cells using siRNA and analyzed the changes in autophagy and key autophagy-related signaling pathways, using immunoblotting and immunocytochemistry. We also explored the interactions between WNK1 and autophagy proteins using in-vitro binding assays. In addition, we assessed the effects of OSR1 and SPAK on autophagy by knocking them down in cells using siRNA. **Results:** The depletion of WNK1 increased autophagy in cells under both fed and starved conditions. The loss of WNK1 increased the level of autophagy activator ULK1 and the pro-autophagic phosphorylation of ULK1 at S555. It also increased the activation of the pro-autophagic Vps34 complex. The C-terminus of WNK1 bound to several autophagy proteins in-vitro. OSR1 depletion had little effect, whereas SPAK depletion partially increased autophagy. **Conclusion:** WNK1 is an inhibitor of autophagy under both fed and starved conditions. SPAK also slightly inhibits autophagy. WNK1 inhibits the expression and pro-autophagic phosphorylation of ULK1, and the activation of Vps34. WNK1 binds to several autophagy proteins, possibly via its C-terminal region.

33 **CPRIT Grantee Poster Session A**

Coupling the Decision-making of EMT and Stemness: A Flexible 'Stemness Window' Model *D. Jia, Rice University; M. Jolly, Rice University; M. Boareto, Rice University; S. Mani, The University of Texas M.D. Anderson Cancer Center; K. Pienta, The Johns Hopkins University; E. Ben-Jacob, Rice University; H. Levine, Rice University*

Introduction: Metastasis and tumor relapse are two clinically insuperable aspects of cancer, and their interconnections remains elusive. Metastasis involves multiple cycles of epithelial-to-mesenchymal transition (EMT) and its reverse process – mesenchymal-to-epithelial transition (MET). Tumor relapse is caused by so-called Cancer Stem Cells (CSCs) that are drug resistant and can initiate a tumor. Cells undergoing EMT have been shown to be more likely to behave as CSCs; but this correlation has been blurred by recent studies elucidating that (a) EMT and MET are not 'all-or-none' processes; rather cells can attain an intermediate or a hybrid epithelial/mesenchymal phenotype that allows them to migrate collectively, and (b) CSCs and non-CSCs can interconvert among themselves. **Methods:** To decipher the EMT-stemness interplay, we devise a mechanism-based mathematical model that incorporates the bidirectional coupling between the decision-making modules of EMT (miR-200/ZEB) and stemness (LIN28/let-7) - miR-200 inhibiting LIN28 (feed-forward coupling) and let-7 inhibiting ZEB (feed-backward coupling); and investigate the likelihood of different EMT phenotypes to gain stemness at different relative strengths of coupling between these two circuits. We further incorporate the effect of OVOL, a transcription factor known to maintain the hybrid E/M phenotype. **Results:** We find that depending on the relative strengths of the two coupling links between the modules, all three phenotypes - complete EMT (M), partial EMT (E/M) and epithelial (E) - can acquire stemness; thereby proposing a flexible 'stemness window' on the 'EMT axis'. Further, we analyze the effect of transcription factor OVOL on these coupled circuits, and find that it can also promote cells in hybrid E/M or epithelial phenotype to gain stemness, i.e. it prevents the 'stemness window' from completely sliding to the M end of the EMT axis. **Conclusion:** Our results show that the 'stemness window' can be flexible on the 'EMT axis' and unify many apparently contradictory studies claiming that stemness associates with E, E/M, or M phenotypes. Specificall , we present OVOL as a modulating factor that can fine tune the EMT-stemness interplay and hypothesize that the 'phenotypic stability factors' that can maintain the hybrid E/M phenotype may also increase the association between hybrid E/M phenotype and stemness, or, in other words, maintain the 'stemness window' around the midpoint of the 'EMT axis'.

34 **CPRIT Grantee**
Poster Session B

Development of Epithelial to Mesenchymal Transition (EMT) Pathway Sensors for Targeting Therapy Resistance in Breast Cancer *M. Toneff, Baylor College of Medicine; J. Taube, The University of Texas M.D. Anderson Cancer Center; S. Habib, Baylor College of Medicine; L. Xin, Baylor College of Medicine; S. Mani, The University of Texas M.D. Anderson Cancer Center; J. Rosen, Baylor College of Medicine*

Introduction: Breast cancer cells undergoing epithelial to mesenchymal transition (EMT) acquire cancer stem cell (CSC) properties. Therapies frequently target the bulk tumor population but are ineffective in targeting EMT/CSCs, which leads to relapse. Moreover, EMT endows cells with metastatic properties. Inhibition/reversal of EMT in breast cancer may improve the response to conventional therapies, decrease metastasis and improve disease free survival (Knezevic et al. Oncogene 2015). EMT is associated with both loss and gain of expression of several genes. Sensors that accurately report the expression of these bona fide molecular determinants of EMT (and CSCs) will provide important biomarkers and enhance our understanding of EMT/CSCs. Moreover, these sensors could be utilized in high-throughput screening assays to identify compounds that can reverse/inhibit EMT and sensitize CSCs to standard therapies. **Methods:** We validated several lentivirus-based fluorescent sensors of EMT including: GFP-based Zeb1 3'UTR sensor, mCherry-based miR-203 3'UTR sensor, and RFP driven by the E-cadherin proximal promoter. Using these, we isolated and observed cells displaying an EMT/MET via fluorescence-activated cell sorting (FACS) and live cell imaging. We isolated mesenchymal-like cells from heterogeneous cellular populations and assessed their behavior using tumorsphere assays and live cell imaging. We employed the miR-203 sensor in a pilot screen of 320 FDA-approved agents to identify candidate compounds that can reverse EMT. **Results:** We reversed EMT and confirmed sensor function by overexpressing miR-200c in mesenchymal-like claudin-low breast cancers. EMT induction in epithelial-like breast cancer generated an EMT fluorescence signature through combinatorial use of Zeb1 and E-cadherin sensors. This signature is present in a heterogeneous breast cancer population largely comprising epithelial-like cells. Cells displaying this signature were mesenchymal compared to the bulk tumor cell population. Moreover, this mesenchymal subpopulation displays greatly enhanced CSC activity versus their epithelial counterparts. Decitabine, a DNA-methyltransferase inhibitor, reduced mCherry expression from our miR-203 sensor. A screen using this sensor identified potential inhibitors of EMT, including a c-Met inhibitor. **Conclusion:** The epithelial or mesenchymal state of individual breast cancer cells can be determined using molecular EMT sensors. These sensors can be used to isolate and assess the behavior of a subpopulation of cells displaying EMT among a heterogeneous population consisting primarily of epithelial-like cells. They can also identify subpopulations of cancer cells with EMT/CSC properties that are responsible for therapeutic resistance and metastasis and can be employed in high throughput screens to identify compounds and/or genes that affect EMT/CSC function in breast cancer to more effectively treat this disease.

35 **CPRIT Grantee**
Poster Session A

Macrophage-Mediated Trogocytosis Leads to Death of Antibody-Opsonized Tumor Cells *R. Velmurugan, Texas A&M University System Health Science Center; D. Challa, Texas A&M University System Health Science Center; S. Ram, The University of Texas Southwestern Medical Center at Dallas; R. Ober, Texas A&M University; E. Ward, Texas A&M University System Health Science Center*

Introduction: Understanding the complex behavior of effector cells such as monocytes or macrophages in regulating cancerous growth is of central importance for cancer immunotherapy. Trogocytosis is one such interaction between immune cells and antibody-opsonized tumor cells wherein small parts of cancer cell material are engulfed by the immune cells. Earlier studies using CD20-specific antibodies have demonstrated that the Fcγ receptor-mediated transfer of the targeted receptors from tumor cells to these effector cells through trogocytosis can enable escape from antibody therapy, leading to the viewpoint that this process is pro-tumorigenic. **Methods:** We use unique properties of two macrophage cell lines to study the effect of phagocytosis and trogocytosis separately. We use advanced microscopy methods such as multifocal-plane microscopy (MUM) and flow cytometry to quantify and characterize the trogocytosis process. Long-term coculture assays were used to study the possibility of macrophage-mediated trogocytosis to kill breast cancer cells in an antibody-dependent fashion. Antibody engineering was used to identify if increasing antibody-Fcγ receptor interactions can affect this process. **Results:** We demonstrate that persistent trogocytic attack by macrophages results in the killing of HER2-overexpressing breast cancer cells in an antibody-dependent fashion. Using MUM, we also find that trogocytosis involves the extrusion of tubular extensions from cancer cells that are engulfed by macrophages. Further, we observe that antibodies engineered to have enhanced affinity for Fcγ receptors have enhanced tumoricidal activity under physiological conditions. **Conclusion:** Our study establishes trogocytosis as a process that can cause cancer cell death in an antibody-dependent fashion, adding one more facet to the complex repertoire of activities of macrophages in a tumor environment. The results have implications for the development of effective antibody-based therapies.

36 **CPRIT Grantee**
Poster Session B

Development and Application of Proteomic Technologies to Profile Kinome and Transcription Factor Activities in Breast Cancer *D. Chan, Baylor College of Medicine; N. Le, Baylor College of Medicine; M. Ellis, Baylor College of Medicine*

Introduction: Aberrant protein phosphorylation has been linked to many human diseases, including breast cancer. In order for precision medicine to be a reality for the treatment of cancer, we must have the ability to identify the kinase that is aberrantly regulated in patients. To assess the kinome of a tumor, we make use of kinase-inhibitors coupled to a bead support to enrich for protein kinases. Due to the promiscuous nature of these inhibitors, multiple kinases will bind to any given drug that can be identified by mass spectrometry. Previous studies have used multiple drug-beads in combination, named "multiplexed kinase inhibitor beads" (MIB), and has been demonstrated to be a powerful technique to identify and quantify up to 400 protein kinases, corresponding to roughly 80% of the human kinome. Importantly, the MIB approach allows us to detect changes in the kinome in response to environmental stimuli (such as in response to drug treatment) and to quantify the differences between tumor samples (for example to identify the kinases that maybe over activated in a particular patient tumor). Many of the kinase signaling pathways ultimately lead to regulation of transcription factors, thus a technology platform that enables us to measure activities of transcription factors would complement our kinome profiling efforts. DNA-binding transcription factors are typically very low in abundance and nearly impossible to detect by mass spectrometry without an enrichment technique. To enrich for transcription factors, we use a 2.8kb biotinylated DNA that contains tandem copies of 100 binding sites for transcription factors. Using this TFRE approach, we can readily detect and quantify more than 300 DNA-binding transcription factors from a tumor. **Methods:** We have developed novel proteomic methods that enable us to profile and quantify the kinome and transcription factor activities changes between two or more different states. **Results:** We can routinely profile and quantify more than 250 protein kinases and more than 300 DNA binding transcription factors from a tumor or cell line sample. **Conclusion:** The kinome and transcription factor profiling platforms are powerful approaches that will not only provide a deeper understanding of how signaling programs contribute to breast cancer pathophysiology, but potentially offer new insights for oncologists to treat patients.

37 **CPRIT Grantee Poster Session A**

EpCAM and LEF-1 Co-regulated Transcription Alters Nanomechanical Phenotypes of Endometrial Cancer Cells for Promoting Invasion *Y. Hsu, The University of Texas Health Science Center at San Antonio; P. Osmulski, The University of Texas Health Science Center at San Antonio; Y. Wang, The University of Texas Health Science Center at San Antonio; Y. Huang, Medical College of Wisconsin; J. Ruan, The University of Texas at San Antonio; V. Jin, The University of Texas Health Science Center at San Antonio; N. Kirma, The University of Texas Health Science Center at San Antonio; M. Gaczynska, The University of Texas Health Science Center at San Antonio; T. Huang, The University of Texas Health Science Center at San Antonio*

Introduction: Endometrial carcinoma is one of the most common female malignancies and the progression of this disease often leads to poor 5-year survival outcome. As local invasions play critical steps initiating tumor aggressiveness programing, we aimed to study the mechanisms which involve in gain of invasion as well as characterize morphological changes of cancer cells in such progression. Our study revealed the epidermal growth factor (EGF), which is the ligand for EGFR that constantly activated in tumors, is able to stimulate regulated intramembrane proteolysis of EpCAM and translocation of its C-terminal fragment EpICD into nucleus. EpICD then forms a complex with Lef-1, FHL2 and β-catenin to bind to target genes. **Methods:** We performed ChIP-Seq and pathway enrichment analysis revealed that their binding targets are highly associated in tumor-related pathways, in particular enriched in mobility related pathways. Further studies showed that EpICD is an essential component for this EpICD/Lef-1 complex to function as co-regulator, as in absence of EpICD reduced the expression activation and also reduced Lef-1 occupancies by EGF stimulation. We also investigated the cell morphologies and behaviors in nano-mechanical aspects by Atomic Force Microscopy (AFM). **Results:** Our results suggested that when endometrial cancer cells are stimulated with EGF, they became more elastic and less adhesive compared to non-treated control cells, suggesting they were under the progress of becoming more aggressive in response to EGF. In comparison, cells that lost EpICD showed no or weak changes in elasticity and adhesion in response to EGF, suggesting EpICD indeed plays crucial roles in mediating EGF-stimulated aggression in endometrial cancer cells. Further, by using AFM tip conjugated with anti-EpCAM antibody, we were able to detect the interaction between EpCAM-decorated tip and EpCAM molecules on the cell surface. **Conclusion:** In this study we proposed for the first time how EpCAM molecules redistributed on the surface and how the topographical changes by EpICD internalization and regulates its downstream gene expression to promote tumor progression.

38 **CPRIT Grantee Poster Session B**

Signaling at the primary cilia during brain development and disease: Studying the ciliary GPCR, Gpr161 in cerebellar development and disease *B. Somatilaka, The University of Texas Southwestern Medical Center at Dallas; I. Shimada, The University of Texas Southwestern Medical Center at Dallas; S. Hwang, The University of Texas Southwestern Medical Center at Dallas; S. Mukhopadhyay, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Signaling mediated by the primary cilium is involved in cell cycle control, cellular differentiation, and cellular polarity. Medulloblastoma (MB) is the most common malignant pediatric brain tumor, arising either from the cerebellum or brainstem; however, genetic alterations driving MB initiation and progression remain poorly understood. Shh drives proliferation of cerebellar granule neuron progenitors (CGNPs) to organize cerebellar patterning and to dictate its size in a cilia-dependent manner; however, the role of cilia-generated signaling in cerebellar proliferation is unclear. The orphan, constitutively active G-protein-coupled receptor (GPCR), Gpr161 localizes to the primary cilium, and negatively regulates the Shh pathway via cAMP signaling. As Gpr161 regulates Shh signaling in a cilia-dependent manner, we intend to study the role of this receptor in later embryonic development, including cerebellar development, and in the pathogenesis of MB. In addition, studying Gpr161 helps us dissect the role of signaling in intact cilia during development and disease. **Methods:** To study the role of Gpr161 during cerebellar development, we have generated a conditional allele, which is in a separate exon from the previously characterized null allele. Generating a complete knockout (ko::lacZ) by crossing with CAAG-Cre mice recapitulates the mid-gestation embryonic lethality and neural tube phenotypes of the previously characterized allele, suggesting that the new allele is functionally null. **Results:** Using this conditional allele, we are studying the following: **(a) Examining Gpr161 expression and subcellular localization in the cerebellum** using the ko::lacZ heterozygous allele, and immunostaining with an in-house anti-Gpr161 antibody, respectively. **(b) Studying the role of Gpr161 in granule neuronal proliferation/differentiation during cerebellar development** by crossing the conditional allele with (i) the CAAG::Cre-ERT2 and tamoxifen induction at different time points during embryonic development, and (ii) GFAP-Cre. **(c) Testing the role of Gpr161 in CGNP proliferation** *in vitro***.** We have standardized CGNP cultures in the lab, and are currently testing the wild type, *Gpr161*, and *Ift88* knockout CGNP cultures for proliferation with/without Shh pathway activation. We are performing these experiments by crossing the conditional allele with CAAG::Cre-ERT2 and tamoxifen induction during later quarter of embryonic development. **(d) Testing the role of Gpr161 in Shh-driven MB.** Initially, we are testing the *Gpr161* conditional knockout mice for development of MB. Next, we will be crossing them with the *Ptch1*$^{+/-}$ knockout mice to test for increased incidence of MB. **Conclusion:** Studying the role of Gpr161 during cerebellar development and disease promises to provide important insights into the role of cilia-generated signaling in MB progression.

39 **CPRIT Grantee Poster Session A**

Genome Wide Chromatin State Landscapes across Breast Cancer Cells *Y. Xi, Baylor College of Medicine; H. Franco, The University of Texas Southwestern Medical Center at Dallas; A. Nagari, The University of Texas Southwestern Medical Center at Dallas; W. Li, The University of Texas M.D. Anderson Cancer Center; J. Li, University of Houston; K. Tanaka, The University of Texas M.D. Anderson Cancer Center; D .Richardson, The University of Texas M.D. Anderson Cancer Center; K. Keyomarsi, The University of Texas M.D. Anderson Cancer Center; C . Chiang, The University of Texas Southwestern Medical Center at Dallas; O. Conneely, Baylor College of Medicine; M. Bedford, The University of Texas M.D. Anderson Cancer Center; M. Barton, The University of Texas M.D. Anderson Cancer Center; X. Shi, The University of Texas M.D. Anderson Cancer Center; W. Kraus, The University of Texas Southwestern Medical Center at Dallas; S. Dent, The University of Texas M.D. Anderson Cancer Center; W. Li, Baylor College of Medicine*

Introduction: Chromatin architecture is essential to transcriptional regulation. Cancer cells undergo critical chromatin remodeling processes that lead to activation or silencing of oncogenes or tumor suppressors. Histone modifications are key epigenetic marks that define different chromatin states across the genome that in turn dictate changes in transcriptional outcomes. Systematic profiling of combinatorial patterns of multiple histone modifications may identify chromatin state alterations in cancer cells that contribute to disease associated transcriptional outcomes. **Methods:** Using Chromatin Immunoprecipitation coupled to massive parallel sequencing (ChIP-seq), we systematically mapped 12 different histone modifications across 14 breast cancer cell lines representative of the five distinct molecular breast cancer subtypes. We adopted a computational pipeline to integrate multiple histone modificatio patterns and define genome wide chromatin state landscapes. These data were integrated with gene expression data measured by RNA-seq and GRO-seq to determine the transcriptional outcomes associated with the different chromatin states. **Results:** We observed distinct histone modification patterns across different breast cancer subtypes and were able to define 13 chromatin states based on 5 key histone modifications including H3K4me3, H3K4me1, H3K27me3, H3K36me3 and H3K9me3. We identified subtype specific chromatin state patterns in breast cancer and their associated regulatory elements, as well as transcription activity levels. We discovered putative regulators based on DNA binding motifs in active promoters/enhancers. Integration with RNA-seq and GRO-seq revealed specific gene expression signatures and key pathways relevant to each breast cancer subtype. **Conclusion:** Our study highlights a novel bioinformatics framework to decode chromatin architecture alterations in breast cancer. Our data also provide a comprehensive resource for histone modification profiling across breast cancer cell lines and subtypes. Ultimately, these data may provide clues to new the etiology of the different breast cancer subtypes and new avenues for therapy development.

40 **CPRIT Grantee Poster Session B**

Mining of potential human cancer genes in human exome databases and cancer databases using functional information from Drosophila screens *W. Charng, Baylor College of Medicine; J .Lupski, Baylor College of Medicine*

Introduction: Previously, we showed that phenotypic information from Drosophila forward genetic screens can largely facilitate the identificatio of several neurodevelopmental disease causing variants in exome database. Fly is also a good model for studying cancer biology and there are several fly screens carried out in search of novel tumor suppressor or proto-oncogenes. It has been reported that continuously proliferating cells in the imaginal discs can interfere with metamorphosis and lead to a defective pupation phenotype. On the other hand, the cellular mechanisms that cause many underdeveloped phenotypes are defects in cell proliferation, cell growth, cell differentiation, cell migration, and cell death, which are very similar to the cellular mechanisms involved in cancer development. For example, several chromatin regulators have been reported to be involved in both neural development and cancer progression. **Methods:** We will search the human homologs of the fl genes showing neurological features and defective pupal formation in Baylor-Hopkins Center for Mendelian Genomics (BHCMG), Exome Aggregation Consortium (ExAC), Oncomine, and TCGA database in order to identify potential cancer genes. We will further study the functions of these genes in fly and cell culture. **Results:** From the X chromosome screen published from the Bellen lab, we re-explored this whole screened fly gene list (165 genes) in BHCMG variant database which now has data from an additional 3,000 more exomes. Among these complementation groups, there are 16 with defective pupation phenotypes and 14 of them are mapped to single genes, corresponding to 24 to 29 human homologs (depends on the prediction programs). On the other hand, we use the 7 novel potential tumor suppressor genes from the defective pupation screen reported by Menut et al. as our positive controls in such mining process. The mining and analysis is still ongoing. **Conclusion:** We expect to identify genes involved in both neurodevelopmental processes and tumorigenesis. Comparison of the mutations and roles in both processes will provide us additional understanding in cancer progression. Consistent with this idea, among the 16 complementation groups from X screen with defective pupation phenotypes, 6 of them contain different alleles exhibiting neural developmental defects in fl .

41 **CPRIT Grantee Poster Session A**

Engineering and Functional Annotation of Fusion Genes in Cancer *H. Lu, Baylor College of Medicine; A. Pantazi, Brigham and Women's Hospital; T. Dogruluk, Baylor College of Medicine; C. Wu, The University of Texas M.D. Anderson Cancer Center; L. Yang, Harvard Medical School; A. Dogruluk, Baylor College of Medicine; N. Neill, Baylor College of Medicine; P. Park, Harvard Medical School; G. Mills, The University of Texas M.D. Anderson Cancer Center; R. Kucherlapati, Brigham and Women's Hospital; K. Scott, Baylor College of Medicine*

Introduction: Next generation sequencing (NGS) technologies are rapidly being incorporated into the clinic to facilitate decisions on cancer patient care. Recognizing this, large-scale efforts by The Cancer Genome Atlas (TCGA) and others are generating a compendium of genomic aberrations found across major cancer types with the goal of identifying new therapeutic targets. The challenge now is to find ways to identify functional "driver" aberrations, as targeting driver events or their activated pathways offers the greatest hope of improving patient outcomes. Oncogenic transcript fusions resulting from chromosomal rearrangements represent an important class of such events. The successful targeting of fusion oncoproteins such as BCR-ABL1 and EML4-ALK with imatinib and crizotinib, respectively, provide strong rationale for comprehensive testing of cancer fusion genes. **Methods:** The functional interrogation of fusion genes is complicated by the large quantity identified, inability to accurately predict those with driver activity, and significant technical roadblocks preventing fusion gene construction for biological assays. To circumvent these bottlenecks, we developed novel technologies permitting (1) rapid fusion gene construction using a novel multi-fragment DNA recombineering strategy with our platform of >35,000 human open reading frame gene clones, and (2) rapid lentiviral delivery of fusion genes to generalized and context-specific cell models to identify those with *in vitro* and *in vivo* driver activity and responsiveness to available therapeutics. **Results:** As proof-of-concept, we used this approach to engineer known fusion oncogenes (*BCR-ABL1*, *EML4-ALK*, and *ETV6-NTRK3*) and validated their transforming ability using our *in vitro* and *in vivo* driver screening systems, demonstrating our ability to rapidly deliver fusion genes with functional activity. In a pilot study of 15 fusion gene candidates identified from TCGA datasets with unknown driver activity, we identified multiple uncharacterized *BRAF*, *RAF1*, and *ALK* fusion events that exhibited potent transforming activity *in vitro* and *in*

vivo and conferred marked sensitivity to currently available therapeutics, indicating potential use of these agents for patients whose tumors harbor these events. **Conclusion:** We are now scaling these efforts for the comprehensive analysis of uncharacterized gene fusions, ultimately allowing functionalization of thousands of fusion events across diverse cancer types. These systems will reveal the highest priority fusion gene targets to enroll in deep mechanistic biology studies, drug discovery and development programs ultimately leading to personalized treatment strategies.

42 **CPRIT Grantee Poster Session B**

Personalized Functional Screens for Pancreatic Cancer Gene Drivers *N. Villafane, Baylor College of Medicine; Y. Tsang, Baylor College of Medicine; K. Scott, Baylor College of Medicine*

Introduction: Identifying early cancer biomarkers, as well other genes directly responsible for promoting cancer progression ("drivers") is of extreme importance in cancer research. The need for doing so is of particular importance in pancreatic ductal adenocarcinoma (PDAC), an aggressive disease that often presents up front with advanced metastases. Identifying and understanding the mechanism of action of PDAC drivers could lead to new diagnostic modalities and treatments. Recognizing this, The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) are cataloging genomic aberrations in PDAC with the goal of identifying new therapeutic targets and detection biomarkers. **Methods:** To functionally prioritize PDAC somatic aberrations identified by the TCGA, we are using several technologies developed in our laboratory: (1) a platform of ~32,000 sequence-verified human genes integrated into infrastructure enabling high content genetic screens, (2) a robotics driven, high-throughput modeling of gene mutations (missense, nonsense and small insertions/deletions) that permits construction of every mutation found within a given candidate cancer gene, and (3) a molecular barcoding approach that facilitates cost-effective detection of driver events following in vitro and in vivo pooled genetic screens. To maximize discovery potential, we are assembling barcoded wild-type and mutant genes into several screening libraries that include (1) the top mutations filtered by computational algorithms like CHASM and MutationAssessor and (2) unbiased tumor-specific libraries representing all somatic aberrations identified in given patients (i.e., personalized functional genomics). **Results: Conclusion:** Our screening approach, driver validation and mechanistic data support the notion that discovery of low frequency, functional aberrations may intersect or otherwise lead to important pathways representing known or novel therapeutic liabilities. When applied more broadly to aberration gene sets informed by biological importance and computational analyses, our functional screening technologies are revealing high priority PDAC targets to enroll in deep mechanistic biology studies and drug development programs with the ultimate goal of developing personalized treatment strategies critically needed for PDAC patients.

43 **CPRIT Grantee Poster Session A**

Zeb1 Induces LOXL2-Mediated Collagen Stabilization and Deposition in the Extracellular Matrix to Drive Lung Cancer Invasion and Metastasis *D. Peng, The University of Texas M.D. Anderson Cancer Center; P. Tong, The University of Texas M.D. Anderson Cancer Center; L. Byers, The University of Texas M.D. Anderson Cancer Center; J. Wang, The University of Texas M.D. Anderson Cancer Center; C. Creighton, Baylor College of Medicine; D. Gibbons, The University of Texas M.D. Anderson Cancer Center*

Introduction: Lung cancer is the leading cause of cancer-related death, primarily due to distant metastatic disease. Metastatic cancer cells undergo an epithelial-to-mesenchymal transition (EMT) regulated by a double-negative feedback loop between the microRNA-200 (miR-200) family and Zeb1, but the precise mechanisms of Zeb1-dependent EMT in promoting malignancy remain largely undefined. While the cell-intrinsic effects of EMT are important for tumor progression, the reciprocal dynamic crosstalk between mesenchymal cancer cells and the extracellular matrix (ECM) is equally critical in regulating invasion and metastasis. This study investigates the collaborative effect of EMT and ECM in the metastatic process. **Methods:** Bioinformatic analysis of TCGA dataset was done to correlate ECM-associated gene expression with EMT gene signature scores. Western blotting and qPCR analysis of epithelial and mesenchymal lung cancer cell lines were performed to determine expression levels of collagen, LOX, and LOXL2. Lung tumor tissues from non-metastatic KrasG12D and metastatic KrasG12D;p53R172H mutant mice were analyzed by immunohistochemistry, Masson's trichrome staining, and second harmonics generation for collagen, LOX, and LOXL2 expression as well as collagen fiber organization. Syngeneic primary tumors generated by subcutaneous injection of murine lung cancer cell lines were analyzed in a similar fashion. Promoter and 3'-UTR luciferase reporter assays were performed to determine direct regulation LOXL2 and LOX by Zeb1 and miR-200, respectively. **Results:** Our results reveal increased collagen deposition in metastatic tumor tissues as a direct consequence of amplified collagen gene expression in Zeb1-activated mesenchymal lung cancer cells. Additionally, collagen fibers in metastatic lung tumors exhibit greater linearity and organization as a result of collagen crosslinking by the lysyl oxidase (LOX) family of enzymes. Expression of the LOX and LOXL2 isoforms is directly regulated by miR-200 and Zeb1, respectively, and their upregulation in metastatic tumors and mesenchymal cell lines is coordinated to that of collagen. Functionally, LOXL2, as opposed to LOX, is the principle isoform driving lung cancer metastasis by crosslinking and stabilizing insoluble collagen deposition in primary tumor tissues. **Conclusion:** Our study demonstrates that mesenchymal lung cancer cells metastasize by modulating the compositional and structural properties of the ECM through LOXL-mediated collagen crosslinking and deposition. We are the first to validate direct regulation of LOX and LOXL2 by the miR-200/Zeb1 axis, delineate collagen as a prognostic marker for lung cancer, and identify LOXL2 as a potential therapeutic target against tumor progression.

44 **CPRIT Grantee**
Poster Session B

The role of cholesterol exporter in metastatic breast cancer: The other side of the coin *S. Prijic, The University of Texas Health Science Center at Houston; W. Zhao, The University of Texas Health Science Center at Houston; J. Chang, The University of Texas Health Science Center at Houston*

Introduction: Metastatic breast cancer currently lacks effective treatment, mainly because of the poorly understood mechanism of metastasis[1]. Metastasis, however, may require a change of cell phenotype known as the epithelial-mesenchymal transition (EMT)[2], which has been associated with reduced cholesterol content in the plasma membrane[3]. Level and distribution of plasma membrane cholesterol are maintained by ATP-binding cassette transporter A1 (ABCA1). We have recently discovered that high expression of ABCA1 in tumors of breast cancer patients reduces time to metastasis by 9 years[4], therefore promoting cancer aggressiveness. While ABCA1 has previously been related to decreased tumor proliferation rate[5], therefore contributing to anticancer activity, we find that it reprograms cancer cells to undergo an EMT. **Methods:** An innovative interdisciplinary approach, *i.e.* computational analyses combined with standard molecular biology techniques, provides us a platform for unraveling the role of cholesterol exporter channel ABCA1 in cancer progression, which holds the potential to expose novel targetable pathways for treatment and/or prevention of metastatic breast cancer. **Results:** We found support of ABCA1 being involved in an EMT, as it was considerably upregulated in various metastatic breast cancer cell lines as opposed to the non-metastatic ones as analyzed by qPCR and confirmed by Western blot. We revealed upregulation of *ABCA1* in the EMT after computational analysis, which we confirmed by qPCR *in vitro*. We ascertained that ABCA1 becomes expressed at higher level during the EMT upon activation of its proximal promoter. Deploying site-directed mutagenesis on the *ABCA1* promoter we found involvement of various transcriptional factors in the EMT program. Using mass spectrometry, we discovered a transcriptional repressor and a splicing factor as ABCA1 binding partners that we currently consider as potential targetable candidates for metastatic breast cancer. **Conclusion:** Taken together, we conclude that ABCA1 does play an important, perhaps dual role in breast cancer progression as its expression and activity dramatically change during the EMT program. Moreover, it could serve as a marker for early detection and prevention of the metastatic breast cancer progression.

45 **CPRIT Grantee**
Poster Session A

ARHI Induces Autophagy in Part by Binding to the Coiled-Coiled Domain of Beclin-1, Monomerizing Beclin-1 Dimers, and Displacing Bcl-2 *G. Huang, The University of Texas M.D. Anderson Cancer Center; L. Zhen, The University of Texas M.D. Anderson Cancer Center; M. Sutton, The University of Texas M.D. Anderson Cancer Center; A. Reger, Baylor College of Medicine; A. Hurwitz, Baylor College of Medicine; T. Palzkill, Baylor College of Medicine; C. Kim, Baylor College of Medicine; R. Bast, The University of Texas M.D. Anderson Cancer Center*

Introduction: ARHI (DIRAS3) is a maternally imprinted tumor suppressor gene downregulated in >60% of primary ovarian cancers. Downregulation of ARHI is associated with decreased progression-free survival. Reexpression at physiologic levels of ARHI in xenografts and in cultured cells induces autophagy, and knockdown of ARHI with siRNA blocks autophagy, suggesting that ARHI plays a critical role in this process. ARHI expression is increased upon nutrient deprivation and under these conditions ARHI colocalizes with Beclin-1, a known component of the Autophagosome-Initiation Complex (AIC). Additionally, ARHI binds directly to Beclin-1 in vitro and acts as a platform modifier of Beclin-1 dimers, causing their monomerization and facilitating the displacement of Bcl-2, an inhibitor of Beclin-1 dependent autophagy. ARHI stimulates the recruitment of Atg14L and PIK3C3, positive regulators of autophagy, to the AIC. Punctate expression of ARHI and LC3-II was found in 80% of positive second-look procedures in ovarian cancer patients who had completed cytoreductive surgery and conventional chemotherapy compared to 30% of primary ovarian cancers from the same patients. Forced expression of ARHI in xenograft models induced autophagy and tumor dormancy in human ovarian cancer cells. Outgrowth of cancer cells and reduction of ARHI was slowed by treatment with chloroquine. **Methods:** We hypothesized that, under low-nutrient conditions, ARHI promotes the survival of dormant tumor cells through induction of the autophagy pathway. We therefore sought to understand the molecular mechanism of autophagy induction resulting from the interaction of ARHI and Beclin-1. To map the specific sites of protein-protein interaction between ARHI and Beclin-1, we performed peptide array screening. **Results:** The regions identified from reciprocal peptide array analysis include the Switch 2 helix on the small-GTPase fold of ARHI, a region N-terminal to the BH3 domain of Beclin-1, and the coiled-coiled domain (CCD) of Beclin-1. **Conclusion:** Taken together, our findings lead us to propose that ARHI monomerizes Beclin-1 in part through the disruption of its CCD. We suggest that disrupting the interaction between ARHI and Beclin-1 may represent a possible strategy for preventing or delaying the outgrowth of dormant ovarian cancer cells.

46 **CPRIT Grantee**
Poster Session B

Acyl-CoA synthetase long-chain family member 3 dependent lipid homeostasis is required for mutant KRAS driven lung cancer *M. Padanad, The University of Texas Southwestern Medical Center at Dallas; G. Konstantinidou, The University of Texas Southwestern Medical Center at Dallas; N. Venkateswarn, The University of Texas Southwestern Medical Center at Dallas; M. Melegari, The University of Texas Southwestern Medical Center at Dallas; C. Yang, The University of Texas Southwestern Medical Center at Dallas; K. Batten, The University of Texas Southwestern Medical Center at Dallas; K. Huffman, The University of Texas Southwestern Medical Center at Dallas; J . Canales, The University of Texas M.D. Anderson Cancer Center; J . Shay, The University of Texas Southwestern Medical Center at Dallas; J. Minna, The University of Texas Southwestern Medical Center at Dallas; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; R .DeBerardinis, The University of Texas Southwestern Medical Center at Dallas; P. Scaglioni, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Lung cancer is the leading cause of cancer related deaths in the USA and worldwide. Lung tumorigenesis is a multistep process that involves several genetic aberrations. Activating mutations of the proto-oncogene KRAS (mutant KRAS) occur in ~30% of the cases of human non-small cell lung cancer (NSCLC), which is associated with aggressive, therapy-resistant disease. Despite the recent discovery of low affinit inhibitors, mutant KRAS is a challenging therapeutic target and there is a dearth of therapeutic options for these tumors. Mutant KRAS not only promotes tumorigenesis but also the survival of established lung cancer, both in mouse models and in certain human NSCLC lines. Therefore, in the absence of clinically-relevant effective inhibitors of mutant KRAS, there has been an intense clinical interest in the development of inhibitors of its downstream effectors. Importantly, mutant KRAS cancer cells undergo oncogene-directed metabolic reprogramming in order to meet the energetic and biosynthetic challenges of cell survival, growth and proliferation. Activation of certain pathways of fatty acid synthesis has been observed in many cancer types including lung cancer. Till date, fatty acid synthase (FASN) has been the candidate for drug development. Unfortunately, the inhibitors against FASN have poor pharmacokinetics and target related toxicity concerns. There is an urgent need for discovery of additional targets that inhibit lipid metabolism specifically in cancer cells

that could be exploited for therapeutic gain. The goal of our study was to identify oncogenic networks required for the maintenance of established tumors. **Methods:** We analyzed the transcriptome of mouse lung tumors undergoing mutant Kras extinction by database for Annotation, Visualization and Integrated Discovery-based functional enrichment analysis and Gene Set Enrichment Analysis between mutant KRAS extinguished versus non-extinguished tumors. **Results:** We found that Acyl-CoA synthetase long-chain family member 3 (ACSL3), which converts fatty acids into fatty Acyl-CoA esters, the substrate for lipid synthesis and β-oxidation, is required for the survival of mutant KRAS lung cancer cells. Furthermore, mutant KRAS promotes the cellular uptake, retention and β-oxidation of fatty acids in lung cancer cells in an ACSL3 dependent manner. Accordingly, ACSL3 suppression is associated with depletion of cellular ATP. Finally, ACSL3 is essential for mutant KRAS lung cancer tumorigenesis in vivo and is highly expressed in human lung cancer. **Conclusion:** Our data demonstrate that mutant KRAS reprograms lipid homeostasis in lung cancer, establishing a cancer specific metabolic vulnerability. Thus, ACSL3 could be a viable therapeutic target for NSCLC driven by mutant KRAS.

47 **CPRIT Grantee**
Poster Session A

Elevated TATA-Binding Protein expression drives VEGFA expression in colon cancer *S. Johnson, Baylor College of Medicine; J. Lin, Baylor College of Medicine; C. Coarfa, Baylor College of Medicine; D. Johnson, Baylor College of Medicine*

Introduction: The TATA-binding protein (TBP) plays a central role in transcription. TBP expression is up-regulated by oncogenic signaling pathways and this increase induces changes in cellular growth properties towards a transformed phenotype. However, the specific gene targets driving this response have not been elucidated. Here we demonstrate that TBP controls expression of the master regulator of angiogenesis, VEGFA, to drive cell migration, tumor growth and vascularization. **Methods:** To assess human tumor and normal colon tissues for TBP and VEGFA expression, published data sets were examined and mRNAs from 24 matched were used to assess TBP expression by RT-qPCR. Tumorigenecity assays with rat1A cells and athymic mice were used to measure tumor growth and vascularization. TBP expression was manipulated in both rat1A and HT-29 cells to examine VEGFA expression. Elisa assays measured VEGFA amounts in conditioned media from HT-29 cells. This media was further used in HUVEC transmembrane migration assays. Available ENCODE ChIP-seq data and ChIP assays were used to analyze TBP or RNA Pol II occupancy on the distal and proximal VEGFA promoters. VEGFA promoter-luciferase reporter constructs were used to determine sequences within the VEGFA promoter that drive TBP-mediated changes in its expression. Statistic analyses were measured using T-tests, one-way ANOVA followed by Tukey's multiple comparisons test, or a two-way ANOVA followed by Sidak's multiple comparisons test. **Results:** An increase in cellular TBP induces VEGFA expression and secretion and enhances tumor vascularization in tumorigenicity assays. TBP-mediated changes in VEGFA transcription require both an intact TATA sequence at the well-studied hypoxia-sensitive distal promoter transcription start site (TSS) as well as sequences within the region containing the hypoxia-insensitive proximal TSS. In silico ChIP-Seq analysis of ENCODE datasets revealed that TBP occupies both TSSs. However, only TBP occupancy at the proximal TSS is sensitive to changes in TBP expression. To determine the biological relevance of these findings, we analyzed TBP expression in human colon. The results reveal that TBP expression is significantly increased in both colorectal tumors as well as adenomas relative to normal colon tissue. Furthermore, TBP expression is positively correlated with VEGFA expression in colon tissue. **Conclusion:** Collectively, these studies identify VEGFA as a key target of TBP that is induced through oncogenic signaling early in colorectal cancer development. We further reveal a novel mechanism by which VEGF transcription is regulated under non-stress conditions using a non-conventional start site. These TBP-driven increases in VEGFA importantly contribute to TBP-mediated tumor development.

48 **CPRIT Grantee**
Poster Session B

Gene Expression Profiling Reveals Potential Predictors of Response to Immune Checkpoint Blockade *W. Roh, The University of Texas M.D. Anderson Cancer Center; P. Chen, The University of Texas M.D. Anderson Cancer Center; A. Reuben, The University of Texas M.D. Anderson Cancer Center; C. Spencer, The University of Texas M.D. Anderson Cancer Center; R. Bassett, The University of Texas M.D. Anderson Cancer Center; H. Jiang, The University of Texas M.D. Anderson Cancer Center; A. Lazar, The University of Texas M.D. Anderson Cancer Center; M. Davies, The University of Texas M.D. Anderson Cancer Center; J. Miller, The University of Texas M.D. Anderson Cancer Center; K. Wani, The University of Texas M.D. Anderson Cancer Center; P. Hwu, The University of Texas M.D. Anderson Cancer Center; S. Patel, The University of Texas M.D. Anderson Cancer Center; S . Woodman, The University of Texas M.D. Anderson Cancer Center; I. Glitza, The University of Texas M.D. Anderson Cancer Center; J. Allison, The University of Texas M.D. Anderson Cancer Center; P . Sharma, The University of Texas M.D. Anderson Cancer Center; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; J. Blando, The University of Texas M.D. Anderson Cancer Center; V. Prieto, The University of Texas M.D. Anderson Cancer Center; M . Tetzlaff, The University of Texas M.D. Anderson Cancer Center; R . Amaria, The University of Texas M.D. Anderson Cancer Center; J . Hu, The University of Texas M.D. Anderson Cancer Center; L. Chin, The University of Texas M.D. Anderson Cancer Center; A. Futreal, The University of Texas M.D. Anderson Cancer Center; J. Wargo, The University of Texas M.D. Anderson Cancer Center*

Introduction: Significant advances have been made through the use of immune checkpoint blockade in melanoma and other cancers, however responses are not universal and are not always durable. Genomic and immune predictors of response have recently been reported, however their predictive value is not robust and additional biomarkers of response are critically needed. **Methods:** To address this area of critical need, we assembled a cohort of 54 patients with metastatic melanoma who were initially treated with anti-CTLA-4 blockade and were treated with anti-PD-1 blockade at time of progression. Longitudinal tumor biopsies were collected at pre-treatment, on-treatment, and post-treatment for anti-CTLA-4 and anti-PD-1 respectively when feasible. We first performed immune profiling via a 12-marker immunohistochemistry panel and

demonstrated that the presence of an immune infiltrate in early on-treatment samples is highly predictive of response to immune checkpoint blockade (for oral presentation at the Society of Melanoma Research Meeting 2015). To validate these findings and to identify additional putative biomarkers of response, we performed gene expression profilin via a custom 788 gene Nanaostring panel in samples from each of these time points. We used t-test and fold change to identify differentially expressed genes between responders and non-responders and linear mixed effects model to identify genes with significant interaction between time and response status. **Results:** We compared gene expression profiles in responders versus non-responders (R vs. NR) at each time point (pre-treatment, on-treatment) on therapy (aCTLA-4, aPD-1). We also compared these across time points (pre-treatment versus on-treatment). When comparing gene expression profiles at individual time points (R vs NR), we found the most significant differences at the early on-treatment time point of patients treated with PD-1 therapy (consistent with our IHC data). Significant differences were also observed when comparing pre-treatment to on-treatment values for patients on CTLA-4 and PD-1 (R vs. NR). We then compared differences across each form of therapy, and demonstrated modest overlap in up-regulated and down-regulated genes (with 117 genes commonly up-regulated between CTLA-4 and PD-1 blockade) and a large number of up-regulated genes unique to patients on PD-1 blockade (in R vs. NR). **Conclusion:** Further studies (including whole-exome sequencing, TCR-seq, and other analyses) are currently underway to further characterize responses in this unique cohort. However, these early findings suggest that immune profiling in early-on treatment tumor biopsies (via IHC and NanoString technology) may best predict outcome to immune checkpoint blockade in the short-term, and may help guide therapy in melanoma (and other cancers).

49 **CPRIT Grantee**
Poster Session A

Histone H2A Variants and Genome Stability Maintenance *J. Leung, The University of Texas at Austin; K. Miller, The University of Texas at Austin*

Introduction: Genomic instability promotes cancer progression. DNA repair machinery plays an important role in maintaining genome stability by repairing DNA lesions. In the nucleus, all chromosomal DNA is stored as chromatin. Physiologically, chromatin is the bona fide substrate of the DNA damage response (DDR) pathway. Therefore, understanding the molecular regulation how chromatin responds to DNA damage is crucial for identifying therapeutic targets in cancer treatment strategy development **Methods:** Chromatin is composed of nucleosomes, which consist of heterogeneous histone octamer (H2A, H2B, H3 and H4). In particular, histone H2A has been implicated in the DDR pathway. However, as the most divergent histone, the exact role of the H2A variants is not well defined. Here, we employed genetic and proteomic approaches to elucidate the functions of the non-canonical H2A variants. We used CRISPR/Cas9 technology to create H2A variants knockout in human cancer cells for genetics studies. In addition, we profiled the H2A variants intereactome landscape using mass spectrometry. We also used in vivo and in vitro assays to identify new effector proteins in the DDR pathway **Results:** We found that macroH2A is important in DDR pathway. In addition, we identified a novel macroH2A interacting protein, ZMYM3, which participates in DDR pathway. ZMYM3 is recruited to DNA double strand breaks (DSBs) that are macroH2A dependent. ZMYM3 knockdown cells displayed hypersensitivity to DNA damaging agents and unfaithful DNA repair **Conclusion:** In current study, we discovered a novel DDR pathway mediated by histone variant macroH2A. We also identified a new macroH2A interacting protein, ZMYM3, which required for DNA repair and maintenance of genome stability.

50 **CPRIT Grantee**
Poster Session B

Whole Exome Sequencing of Primary Human Osteosarcomas *S. Madan, Baylor College of Medicine; Y. Bae, Baylor College of Medicine; S. Chen, Baylor College of Medicine; P. Katsonis, Baylor College of Medicine; O. Lichtarge, Baylor College of Medicine; P .Campeau, Baylor College of Medicine; R. Gibbs, Baylor College of Medicine; H. Liang, The University of Texas M.D. Anderson Cancer Center; B. Lee, Baylor College of Medicine*

Introduction: Osteosarcoma (OS) is the most common primary bone cancer. OS incidence has a bimodal distribution with patients diagnosed as children or adolescents or as adults over 70 years of age. Despite intervention by surgery followed by combination chemotherapy, the 5-year survival rate is 68% while the overall survival rate is a mere 10-15% due to recurrence and metastasis. Developing targeted therapy to improve patient survival would require better understanding of the genetic drivers of OS. So far, it is known that germ-line mutations in tumor suppressor genes such as RB1, p53 and RECQL4, associate with the risk of OS development. However, beyond these three genes not much is known about other genetic drivers of tumorigenesis **Methods:** In this study, we performed whole exome sequencing (WES) on paired tumor and blood samples from 6 osteosarcoma patients to identify novel somatic mutations. Mutations identified from the 6 paired samples were compared to mutations identified from our previous WES of independent cohort of 10 tumor samples, followed by Sanger sequencing to eliminate false positives.We then assigned scores to the variants, which were tabulated by an algorithm based on the magnitude of amino acid change and the functional importance of the position where the mutation was occurred. Functional studies of these candidates are now underway to determine their roles in tumor initiation and progression. **Results:** We found 68 novel variants (SNPs and INDELs) on comparing the 6 paired samples to our previous WES of independent cohort of 10 tumor samples. Of the 68 variants, we successfully validated 14 variants as de novo mutations by Sanger sequencing.These included mutations in four big families of genes including transcription factors, ion channels, cell adhesion proteins and receptors. The 14 variants included mutations in the following genes: ATXN1, AKAP6, ATRX, ANKRD11, HECW1, OPCML, ESR1, KCNH8, KCNN3, CACNA1F, MED13, UNC13C and ALG13. Of these, we found that mutations in KCNH8, MED13, ATRX, ESR1 and UNC13C may have tumor suppressive functions based on the functional relevance algorithm while mutations in HECW1 and OPCML were predicted to be oncogenic by this algorithm. **Conclusion:** This study highlights the power of whole exome sequencing for identifying novel, somatic mutations that could be primary drivers of disease.

51 **CPRIT Grantee Poster Session A**

Inhibition of ATM Kinase Activity by Phosphorylation *Y. Zhou, The University of Texas at Austin; J. Lee, The University of Texas at Austin; T. Paull, The University of Texas at Austin*

Introduction: The Ataxia-Telangiectasia mutated (ATM) protein is a key regulator of checkpoint activation in response toDNA double strand breaks (DSBs). The Mre11-Rad50-Nbs1 (MRN) complex acts as a DSB sensor for ATM and is essential for ATM recruitment to broken DNA ends and ATM activation. However, the precise mechanism for ATM activation upon DNA damage and ATM inactivation after DNA repair has remained poorly understood. Phosphorylation of ATM has been suggested to play important roles in this process. Autophosphorylation of ATM at four sites (S1981, S367, S1893, and S2996) has been shown to be essential for ATM activation and function in response to DNA damage in human cells. However, mutations at these four sites do not affect ATM kinase activity in vitro or in mouse models, suggesting that there are other uncharacterized mechanisms for regulation of ATM catalytic activity in cells. Previous studies suggest that there might exists inhibitory phosphorylation sites in ATM. It was suggested that under normal situation, serine/threonine phosphorylation at sites other than ATM autophosrylation sites represses ATM activity, and that removal of these inhibitory phosphates by certain phosphotase is essential for ATM activation upon DNA damage. **Methods:** In this study we sought to identify inhibitory phosphorylation sites in ATM using site-directed mutagenesis. **Results:** We characterized three sets of sites: S85/T86, T372/T373, S1985/T1987/T1988. The phosphomimetic mutations at these sites repress ATM activation both in vitro and in vivo. Overexpression of phosphomimetic mutants (S85D/T86E, S1985DT1987ET1988E) in ATM-deficient cells fails to restore cell survival upon ionizing radiation (IR) or camptothecin (CPT)treatment.We propose that DNA-dependent protein kinase (DNA-PK) is likely to mediate phosphorylation of some of these sites, supported by our observations that ATM is hyperactive when DNA-PK activity is blocked by DNA-PK specific inhibitor or when the DNA-PK gene is deleted. In addition, we have observed that overexpression of phospho-blocking ATM mutants fails to respond to DNA-PK inhibition in comparison to wild-type ATM. Lastly, we present in vitro evidence showing that pre-incubation of ATM protein with DNA-PK significantly decreases ATM kinase activity and that the phospho-blocking ATM mutants are resistant to this inhibition by DNA-PK. **Conclusion:** Taken together, our data suggests that the non-homologous end joining factor DNA-PK suppresses the catalytic activity of ATM through phosphorylation. Since ATM is known to promote homologous recombination pathways, this may provide a novel mechanism for DNA repair pathway choice upon DNA damage as well as ATM inactivation after DNA repair.

52 **CPRIT Grantee Poster Session B**

MiR-195 Has Both Tumor Suppressive Function and Oncogenic Potential in Non-small Cell Lung Cancer *X. Yu, The University of Texas Health Science Center at San Antonio; Z. Zhao, The University of Texas Health Science Center at San Antonio; X. Ma, The University of Texas Health Science Center at San Antonio; L. Du, The University of Texas Health Science Center at San Antonio; A. Pertsemlidis, The University of Texas Health Science Center at San Antonio*

Introduction: MicroRNAs (miRNAs) play important roles in nearly all cellular physiological and pathological pathways. Dysregulated miRNAs have been shown to contribute to cell proliferation and tumorigenesis of many cancers, including lung cancer, which is the leading cause of adult cancer deaths. For example, the let-7 miRNA family, among the first miRNAs discovered, suppresses the growth of non-small cell lung cancer (NSCLC). Several other miRNAs have been shown to regulate the response of lung cancer cells to anticancer agents, such as the microtubule-targeting agent paclitaxel. These findings highlight the potential application of miRNAs as the next generation of therapeutic agents, either alone or in combination with chemotherapeutic drugs. **Methods:** A high throughput screen with a library of 1,239 miRNA mimics was performed in NSCLC cell lines. Candidate miRNAs that both inhibited NSCLC cell growth and sensitized NSCLC cells to paclitaxel were validated by short- and long-term assays for cell viability. The targets of candidate miRNAs were identified by expression profiling following miRNA transfection, validated by luciferase reporter assay for direct and specific interactions, and confirmed by downstream functional assays. **Results:** We have shown that miR-195 not only inhibits the growth of NSCLC cells but also synergizes with paclitaxel. Transfection of miR-195 into NSCLC cells results in G1 phase arrest by targeting CCND3, leads to apoptosis and reduction of cell migration and invasion by targeting BIRC5 and synergizes with paclitaxel by regulating CHEK1. Clinical data from The Cancer Genome Atlas demonstrates that miR-195 is significantl down-regulated in lung cancer tumors compared to adjacent normal tissues and that its down-regulation in lung cancer patients is associated with worse survival. Intriguingly, miR-195 up-regulates the oncogene MYC, inhibition of which sensitizes NSCLC cells to miR-195. Myc, which is reported to transcriptionally repress let-7c, also represses miR-195. Additionally, we have demonstrated that let-7c is inhibited by miR-195 and in turn, inhibits miR-195. **Conclusion:** We identified miR-195 as a tumor suppressor and chemotherapy sensitizer in NSCLC cells mediated by its repression of CCND3, BIRC5 and CHEK1. We also uncovered a feedback regulation network among miR-195, Myc and let-7c in NSCLC cells. The ability of miR-195 and MYC to regulate each other not only indicates a mechanism by which NSCLC cells can become resistant to miR-195 but also suggests a potential for miR-195 to be oncogenic under specific conditions. The mutual inhibition between miR-195 and let-7c suggests an interesting regulatory loop between cancer-related miRNAs that deserves further study.

53 **CPRIT Grantee Poster Session A**

Development of a Chromatographic Screening Approach for Metabolomic Profilin *P. Lorenzi, The University of Texas M.D. Anderson Cancer Center; T. Horvath, The University of Texas M.D. Anderson Cancer Center; M. Pontikos, The University of Texas M.D. Anderson Cancer Center; D. Hawke, The University of Texas M.D. Anderson Cancer Center; J. Weinstein, The University of Texas M.D. Anderson Cancer Center*

Introduction: Metabolomics, as a platform, is comprised of a comprehensive set of analytical and informatics tools that can be used to assess the degree of metabolic perturbation present in diseases such as cancer. Many metabolites in the known metabolome are polar molecules that are suitable for analysis under HILIC chromatographic conditions. Here we present a targeted LC-MS/MS screening method that is used to perform a bottom-up assessment of chromatographic figures of merit for a subset of biologically-relevant metabolites under a variety of HILIC conditions. The data gleaned from these targeted screens is used to select suitable chromatographic conditions for individual metabolites, or complete metabolic classes of molecules for use in either targeted or untargeted analysis. **Methods:** We used an Agilent 6460 triple quadrupole mass spectrometer with Agilent 1290 quaternary UHPLC system. Three different columns were screened: ZIC-pHILIC, ZIC-cHILIC, and Kinetex HILIC. Mobile phases were: MPA: 95/5 acetonitrile/200 mM ammonium formate + 2.0% formic acid, MPB: 50/45/5 acetonitrile/water/200 mM ammonium formate + 2.0% formic acid. The following chromatographic figures of merit were determined: retention time (RT), retention factor (k'), theoretical plates (N), and peak asymmetry (As). We screened the following neat metabolite standards (~ 5 μg/mL for each): oxidized and reduced glutathione, AMP, ADP, ATP, NADH, NAD+, NADPH, NADP+, α-ketoglutarate, succinate, malate, citrate, isocitrate, pyruvate, lactate, asparagine, aspartate, glutamine, and glutamate. **Results:** Screening data generated for NAD+ is used to demonstrate the utility of this approach. For NAD+, the figures of merit for ZIC-pHILIC column (RT: 13.2 min; k': 13.3; As: 1.35; N: 33,400) were similar to those obtained with the ZIC-cHILIC column (RT: 13.9 min; k': 14.0; As: 2.42; N: 50,900), but the peak symmetry for both columns proved to be superior to that provided by the Kinetex HILIC column (RT: 12.1 min; k': 9.08; As: 5.08; N: 24,400) for this metabolite. **Conclusion:** In conclusion, we present a screening method that can be used to select suitable chromatographic conditions for individual metabolites, or complete metabolic classes of molecules. The method is applicable to targeted and non-targeted analysis of biologically-relevant metabolites.

54 **CPRIT Grantee Poster Session B**

Epigenetic Variation And Non-Coding Somatic Mutations In The Glioblastoma Genome *V. Iyer, The University of Texas at Austin; A .Hall, The University of Texas at Austin; Y. Ni, The University of Texas at Austin; A. Battenhouse, The University of Texas at Austin; M. Shpak, NeuroTexas Institute St. David's Medical Center; M. Cowperthwaite, NeuroTexas Institute St. David's Medical Center*

Introduction: Somatic mutations in oncogenes and tumor-suppressor genes are known drivers of cancer. However, another important driver of cancer is the disruption of gene regulatory programs due to alteration of the epigenetic landscape or somatic mutations in non-coding regions of the tumor genome. We have adopted a two-pronged approach to characterizing epigenetic variation at the level of chromatin structure and the impact of non-coding somatic mutations in cancer. **Methods:** First, we carried out whole-genome chromatin profiling for histone marks and transcription factor binding in primary tumors of glioblastoma multiforme (GBM), using chromatin immunoprecipitation and deep sequencing (ChIP-seq). In parallel, we used RNA-seq to quantify expression profile of all genes including non-coding RNAs. In a second approach, we used whole-genome sequencing data from The Cancer Genome Atlas (TCGA) and our ChIP-seq data, to identify non-coding somatic mutations in GBM as well as lower grade glioma and breast cancer. We identified expression quantitative trait loci (eQTL) in this data to associate non-coding somatic mutations and copy number changes with transcript level variation across the same tumors. We adjusted the genotype dosage according to the read coverage at each allele to obtain a more accurate, copy-number-adjusted genotype for each somatic variant. **Results:** Interestingly, although we could readily identify the known subtypes of GBM based on RNA expression, we found that there is epigenetic variation between patients that is not necessarily reflected in gene expression profiles We used a Hidden Markov Model applied to the epigenetic datasets to identify cis-regulatory elements such as promoters, enhancers, insulators and repressed chromatin. We defined active enhancers that were clearly associated with genes involved in brain cancer-related pathways, and showed enrichment for transcription factor binding motifs that were not previously known to be regulators in glioblastoma. Our copy-number-aware eQTL analysis revealed that copy-number alterations show much stronger associations with differential gene expression across tumors than non-coding somatic mutations. However, non-coding mutations frequently occurred in potential regulatory regions including promoters and other cis-regulatory elements. Clusters of non-coding somatic mutations were also detected, including at the previously known regulatory elements of TERT and other potential regulators like the polycomb complex oncogene BMI1. **Conclusion:** Our studies reveal considerable epigenetic and genetic heterogeneity across individual cancer genomes that is not well represented in standard genomic analyses of coding mutations and gene expression profiling in cancers

55 **CPRIT Grantee Poster Session A**

The Function of Achaete-Scute Homolog 1 (ASCL1) in Glioblastoma Multiforme *T. Vue, The University of Texas Southwestern Medical Center at Dallas; M. Borromeo, The University of Texas Southwestern Medical Center at Dallas; R. Kollipara, The University of Texas Southwestern Medical Center at Dallas; T. Mashimo, The University of Texas Southwestern Medical Center at Dallas; T. Smith, The University of Texas Southwestern Medical Center at Dallas; R. Bachoo, The University of Texas Southwestern Medical Center at Dallas; J. Johnson, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Glioblastoma multiforme (GBM) are incurable brain tumors that account for the majority of high-grade gliomas in the central nervous system (CNS). Currently, therapeutic approaches targeted at genomic alterations are ineffective in stopping these tumors and prognosis for GBM patients remains poor. Interestingly, many of the genes that are upregulated in GBMs are transcription factors that are normally expressed in neural progenitor or stem cells. One such factor is the proneural factor ASCL1, which plays a crucial role in cell fate specification differentiation, and progenitor cell maintenance. It is possible that the aberrant expression of ASCL1 in GBMs may bestow upon tumor cells a "stem cell-like" property and the ability to proliferate and develop resistance to chemotherapy and radiation. Indeed, ASCL1 has been shown to be required for the survival of lung cancers with neuroendocrine features. Yet, to date the direct contribution and requirement of ASCL1 in GBM development and progression in vivo remains unclear. M**ethods:** In this study, we assess tumor generation in a genetic mouse model (TP53;NF1 loss) of GBM in the presence and absence of ASCL1. We use immunohistochemistry, ChIP-seq and RNA-seq to determine the expression and function of ASCL1 in GBM. **Results:** We show that ASCL1 is expressed in early stage as well as terminal stage brain tumors of a GBM mouse model. In these tumors, ASCL1 is co-expressed with OLIG2, SOX2, and Ki67, suggesting progenitor-like identity. Analysis of ASCL1 ChIP-seq shows that ASCL1 binds to about 9,800 sites within the genome associated with genes known to regulate diverse biological processes such as those involved in neural stem cell maintenance, inhibition of neural stem cell differentiation, and cellular response to vascular endothelial growth factor (VEGF). Conditional knock-out of ASCL1 (ASCL1-CKO) in the GBM mouse model significantly improved the survival time of the mice. Uniquely, RNA-seq gene expression profiling revealed that ASCL1+ tumors of the GBM mouse model have a gene signature resembling the proneural human GBM subtype, whereas ASCL1 mutant tumors resemble the gene signature of the mesenchymal GBM subtype. **Conclusion:** Collectively, these findings demonstrate a critical role for ASCL1, a single factor, in regulating GBM development and subtype in vivo.

56 **CPRIT Grantee Poster Session B**

Delineating Tumor Evolution in Metastatic Colorectal Cancer Using Single Cell Sequencing *M. Leung, The University of Texas M.D. Anderson Cancer Center; Y. Wang, The University of Texas M.D. Anderson Cancer Center; C. Kim, The University of Texas M.D. Anderson Cancer Center; R. Gao, The University of Texas M.D. Anderson Cancer Center; E. Sei, The University of Texas M.D. Anderson Cancer Center; D. Maru, The University of Texas M.D. Anderson Cancer Center; S . Kopetz, The University of Texas M.D. Anderson Cancer Center; N .Navin, The University of Texas M.D. Anderson Cancer Center*

Introduction: Colorectal cancer (CRC) remains to be a detrimental disease worldwide because of our incomplete understanding on metastasis and intratumor heterogeneity. While TCGA studies have used next-generation sequencing (NGS) to characterize mutations in primary CRC tumors, few studies have identified mutations in metastatic tumors. The central problem is that conventional NGS technologies are limited to reporting genomic information on million of cells, and therefore cannot resolve genetic intratumor heterogeneity and identify subclones that play an important role in metastasis. **Methods:** To investigate genomic heterogeneity in CRC and trace metastatic lineage, we have developed a highly-multiplexed single cell DNA sequencing method that combines flow sorting of single nuclei, time-limited multiple-displacement-amplification low-input library preparation, library barcoding, targeted capture and NGS. Specificall , we perform targeted sequencing of a 200-cancer-gene panel using single cells from the primary tumor and liver metastasis of a CRC patient. **Results:** We sequenced 45 tumor cells from each primary and metastatic tumor, as well matched populations of tumor cells. Our data achieve 85% coverage breadth and 100x coverage depth on average. We have identified nonsynonymous mutations that are shared in both primary and metastatic tumors. We have also found a small portion of mutations that are only present in the liver metastasis, which may play an important role in metastatic dissemination. We used the single cell data to construct phylogenetic trees, which identified subpopulations at both organ sites. **Conclusion:** By sequencing single cells from primary and metastatic tumors, we resolve intratumor heterogeneity and identify subclones and mutations involved in metastasis in CRC patients. We found that this CRC patient followed a late-dissemination model, in which the primary tumor evolved for a long period of time before disseminating to remote tissue. These tools have higher sensitivity compared to bulk sequencing methods, and allow us to identify potential targets for therapy to inhibit the metastatic dissemination of tumor cells.

57 **CPRIT Grantee Poster Session A**

D2-hydroxyglutarate Dehydrogenase Regulates alpha-ketoglutarate Levels, Dioxygenase Function and Epigenetic Remodeling by Modulating Mitochondrial IDH2 *A. Lin, The University of Texas Health Science Center at San Antonio; S. Abbas, The University of Texas Health Science Center at San Antonio; S .Kim, The University of Texas Health Science Center at San Antonio; D .Rakheja, The University of Texas Southwestern Medical Center at Dallas; J . Sudderth, The University of Texas Southwestern Medical Center at Dallas; X. Gao, The University of Texas Health Science Center at San Antonio; A. Hinck, The University of Texas Health Science Center at San Antonio; S. Weintraub, The University of Texas Health Science Center at San Antonio; R. DeBerardinis, The University of Texas Southwestern Medical Center at Dallas; P. Dahia, The University of Texas Health Science Center at San Antonio; R. Aguiar, The University of Texas Health Science Center at San Antonio*

Introduction: Isocitrate dehydrogenases (IDH) catalyze the conversion of isocitrate to alpha-ketoglutarate (α-KG). Mutant IDH1/2 creates a neomorphic enzyme that reduces a-KG to the structurally related D2-hydroxyglutarate (D2-HG). This metabolic deregulation impinges on α-KG-dependent dioxygenases modifying DNA/histone methylation and HIF1α hydroxylation/stability. These observations highlight the need for a tight control in the interconversion of D2-HG to a-KG, which is mediated by D2-hydroxyglutarate dehydrogenase (D2HGDH). Still, the putative ability of D2HGDH to promote an oncogenic metabolic deregulation remains underexplored, an especially relevant issue in tumors associated with epigenetic remodeling, such as diffuse large B cell lymphoma (DLBCL). M**ethods:** Target resequencing of the D2HGDH gene in 150 DLBCLs; mass-spectrometry-based examination of the metabolic profile of D2HGDH wild-type and mutant cells; quantification of DNA/histone methylation and HIF1α hydroxylation in cell models of D2HGDH gain- and loss-of-function; characterization of cytosolic and mitochondrial IDH activity in the context of D2HGDH dysfunction; genetic modulation of IDH2 in various D2HGDH contexts **Results:** We discovered somatic, truncating and missense, heterozygous D2HGDH mutations in a subset of DLCBLs. Detailed metabolic, enzymatic and cellular examination defined these variants as loss-of-function, resulting primarily in a significant decrease in the cellular levels of α-KG, not massive accumulation of D2-HG. Further, we found that subtle modulation of D2HGDH levels in HEK-293 or DLBCL cell lines significantly influenced histone/DNA methylation and HIF1a hydroxylation consistent with changes in the activity of α-KG-dependent dioxygenases, including histone demethylases, TET DNA 5-mC hydroxylases and prolyl hydroxylases. Importantly, we discovered that D2HGDH meaningfully contributes to the cellular pool of α-KG by regulating IDH activity in the mitochondria, but not in the cytosol. Specificall , D2HGDH controls IDH2, but not IDH1, transcription, a feature that can be recapitulated by cell-permeable synthetic α-KG. Accordingly, genetic modulation of IDH2 levels rescued the effects of D2HGDH on histone/DNA methylation and HIF1a hydroxylation. Lastly, we found that the D2HGDH status define two subsets of DLBCL with distinct epigenetic remodeling profiles **Conclusion:** Together, these findings link D2HGDH to epigenetic remodeling in DLBCL and indicate that this enzyme is not simply a guardian against the cellular accumulation of toxic D2-HG. Instead, our data suggest that by regulating IDH2, D2HGDH is an important player in the generation of a-KG, a metabolite that coordinates epigenetic plasticity in various model systems and influences malignant behavior, longevity and stem cell maintenance. Our results further expose the intricacies of mitochondrial metabolism in physiologic and neoplastic settings, and inform on the pathogenesis of diseases associated with D2HGDH deficienc .

58 **CPRIT Grantee Poster Session B**

RUVBL1 and RUVBL2 are Prognostic of Patient Outcome and Potential Therapeutic Targets in Non-small Cell Lung Cancer *P. Yenerall, The University of Texas Southwestern Medical Center at Dallas; R. Carstens, The University of Texas Southwestern Medical Center at Dallas; K. Huffman, The University of Texas Southwestern Medical Center at Dallas; L. Girard, The University of Texas Southwestern Medical Center at Dallas; J. Canales, The University of Texas M.D. Anderson Cancer Center; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; D. Mangelsdorf, The University of Texas Southwestern Medical Center at Dallas; J. Minna, The University of Texas Southwestern Medical Center at Dallas; R. Kittler, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Non-small cell lung cancer (NSCLC) is a highly lethal and heterogenous disease with a dearth of molecularly targeted agents. As a better understanding of chromatin biology emerges, it is apparent that chromatin remodelers may be potential theraputic targets in multiple cancer types. Here, we investigate the role of two chromatin remodelers, RUVBL1 and RUVBL2 (collectively RUVBL1/2), in NSCLC. **Methods:** Expression of RUVBL1/2 mRNA levels in NSCLC patients was downloaded from TCGA. The association of RUVBL1/2 mRNA levels with patient prognosis was performed using KMplot.com. To interrogate the functional role of RUVBL1/2, RUVBL1/2 was depleted in 24 NSCLC cell lines and 2 models of normal bronchial epithelial cells using endoribonuclease-prepared siRNAs and siRNAs. Flow cytometry using propidium iodine staining was performed to measure the percent of cells in each stage of the cell cycle following RUVBL1/2 depletion. RNA-seq after RUVBL1/2 depletion was performed using the TruSeq Stranded mRNA Library Prep Kit and sequenced on an Illumina Hiseq 2000. Reads were mapped, transcripts assembled, and differential transcripts called using Tophat, Cufflinks and Cuffdiff. ChIP for RUVBL2 was performed and then DNA was sequenced using the TruSeq ChIP Sample Prep Kit. This library was sequenced on an Illumina Hiseq 2000 and reads were mapped with bowtie2 with island calling by SICER. **Results:** RUVBL1/2 are overexpessed in NSCLC compared to normal tissue (n=224) , and high expression of RUVBL1/2 correlates with poor patient prognosis (n=697). Depletion of RUVBL1/2 in 24 NSCLC cell lines resulted in a range of growth inhibitory phenotypes, with normal cells being among the most resistant. This phenotype can be rescued by expression of an RNAi-resistant cDNA. Sensitivity to depletion did not correlate with any single molecular marker. Cell cycle analysis showed that RUVBL1/2 depletion can result in a G2/M arrest. RNA-seq following RUVBL1/2 depletion showed enrichment for gene sets involved in DNA replication, cell cycle, mTOR signaling, Notch signaling and DNA repair. Integration of this data with ChIP-seq for RUVBL2 revealed that RUVBL2 may directly regulate genes related to the cell cycle and Notch signaling. **Conclusion:** RUVBL1/2 are overexpressed and prognostic of patient outcome in NSCLC. In addition, many NSCLC cell lines are critically dependent upon expression of these genes for cell cycle progression. This dependenacy may be due to RUVBL1/2's direct regulation of genes invovled in the cell cycle, as well as genes involved in Notch signaling. Because RUVBL1/2 generally require ATPase activity for their functions, RUVBL1/2 may represent novel therapeutic targets.

59 **CPRIT Grantee Poster Session A**

Deregulated Cyclin E Expression Mediates Resistance to Aromatase Inhibitors in Estrogen Receptor Positive Postmenopausal Breast Cancer *I. Doostan, The University of Texas M.D. Anderson Cancer Center; C. Karakas, The University of Texas M.D. Anderson Cancer Center; S. Moulder, The University of Texas M.D. Anderson Cancer Center; K. Hunt, The University of Texas M.D. Anderson Cancer Center; K. Keyomarsi, The University of Texas M.D. Anderson Cancer Center*

Introduction: Aromatase Inhibitors (AIs) are the first line endocrine therapy for post-menopausal breast cancer patients with tumors that express estrogen receptor (ER+). These medications decrease estrogen levels by blocking the activity of aromatase enzyme, the main source of estrogen production in postmenopausal women. However, not all patients respond to AIs initially and among those who respond many develop resistance with unknown biology. One mechanism by which resistance to AI can develop is through the deregulation of G1 to S transition of the cell cycle. Cyclin E is a key regulator of G1/S transition and it is deregulated in many types of cancer including ER+ breast cancer. One mechanism of deregulation of cyclin E is the generation of low molecular weight isoforms (LMW-E) by elastase class of serine proteases. LMW-E is specifically expressed in tumor cells leading to enhancement of G1 to S transition, higher tumorigenicity in vivo and poor survival in patients. Here we show that deregulated expression of cyclin E pathway, through the generation of LMW-E can mediate resistance to AIs. **Methods:** Clinical data were collected from a cohort of seventy patients that were treated with AIs at MD Anderson. Tissue samples after neo-adjuvant AI treatment were stained for cyclin E to identify LMW-E expressing tumors. In addition, we generated overexpression model systems of cyclin E and LMW-E in aromatase overexpressing MCF7 and T47D (ER+) cell lines and subjected them to proliferation, cell cycle and western blot analysis following AI treatment. Lastly, we have established a xenograft model of AI treatment by injecting LMW-E overexpressing cells to mammary fat pad of ovariectomized mice in order to examine tumor response to AIs. **Results:** Patients with LMW-E expression show diminished early response to neoadjuvant AIs according to tumor size change. In vitro model system shows that AIs inhibit proliferation, induce G1 arrest of the cell cycle and decrease G1 regulators of cell cycle such as CDK2, pCDK2 and Rb protein. These effects were rescued only when LMW-E was overexpressed suggesting that LMW-E can bypass the AI growth inhibitory effect. Our in vivo model shows that LMW-E expressing tumors are not responsive to AI treatment. Taken together, these results suggest that LMW-E mediates resistance to AIs. **Conclusion:** This study shows that expression of LMW-E in breast cancer can reverse the activity of AIs and provides rationale for the use of CDK2 inhibitors to inhibit the cyclin E pathway in patients with LMW-E expressing tumors.

60 **CPRIT Grantee Poster Session B**

Quantitative Nuclease Protection Assay from Archival FFPE Discovers Systematic Downregulation of Nuclear Receptors in Lung Cancer *K. Huffman, The University of Texas Southwestern Medical Center at Dallas; R. Carstens, The University of Texas Southwestern Medical Center at Dallas; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; J. Minna, The University of Texas Southwestern Medical Center at Dallas; D. Mangelsdorf, The University of Texas Southwestern Medical Center at Dallas; H. Erickson, The University of Texas M.D. Anderson Cancer Center; M. Schwartz, High Throuput Genomics, Inc.; W. John, High Throughput Genomics, Inc.; L. John, High Throughput Genomics, Inc.; B. Kerns, High Throughput Genomics, Inc.; S. Fuqua, Baylor College of Medicine*

Introduction: Most clinically annotated tumor samples available worldwide are archived as Formalin-Fixed Paraffin-Embedded (FFPE) blocks that are difficult to study quantitatively using common RNA expression techniques. We utilized a quantitative nuclease protection assay (qNPATM, HighThroughPut Genomics) to examine expression of the 48-member nuclear receptor superfamily (NRs), and 72 closely associated co-regulators (CoRegs) from FFPE blocks comprised of 230 resected Non-Small Cell Lung Cancer (NSCLC) tumor-normal matched samples. NRs and CoRegs are known to play key regulatory roles in all aspects of developmental and homeostatic biology, and their connection to cancer has been well-established in several tumor types. M**ethods:** Initially, development of the qNPA assay required identification of endogenous control (EC) genes to establish FFPE sample quality and allow comparison of gene expression across samples. We identified 92 potential EC genes through data mining from 1,600 microarray datasets from various cancer types. We ran qNPA assays for these 92 genes using 889 FFPE samples with >98% passing quality control. We selected the five “best EC genes” (CTBP1, RSPA, OAZ1, EEF2, and RSP19) based on their levels of expression, variation, and number of detection failures to serve as “housekeepers” for data normalization. Correlation of qNPA expression from FFPE and fresh-frozen samples from the same cell lines were excellent (Ravg = 0.90). Furthermore, we compared qNPA expression data from a large panel of cell lines with other expression platforms, including qRT-PCR and microarray, and found good correlation (Ravg >0.7). **Results:** We analyzed 460 FFPE NSCLC tumor/normal matched samples for NR/CoReg expression using qNPA and found a subset of 9 NRs (PPARg, NR4A1, NR4A2, NR4A3, NR5A2, RARb, MR, AR and NR2F1) which have consistently lower expression in tumor samples versus matched normal. Data analysis using The Cancer Genome Atlas (TCGA) and 14 other publically available NSCLC studies confirmed this observation. Retrospective analysis of a 2,347-sample NSCLC meta-dataset identified a significant overall survival benefi (HR: 0.39-0.67, $p < 0.0001$) for patients who retained a higher average expression of these 9 NRs. Moreover, multivariate analysis revealed an association between loss of NR expression and increasing tumor stage, suggesting loss of NRs might have functional relevance during tumor progression **Conclusion:** Using qNPA technology, we developed a well-controlled, quantitative assay to detect NR and CoReg expression in FFPE specimens. Unexpectedly, we observed the systematic down-regulation of 9 NRs which are related to survival benefits and disease stage, and may have important functional roles in lung cancer.

61 **CPRIT Grantee**
Poster Session A

Telomerase Regulation in Human T Lymphocytes *E. Huang, The University of Texas Southwestern Medical Center at Dallas; E. Tedone, The University of Texas Southwestern Medical Center at Dallas; C . Cornelius, The University of Texas Southwestern Medical Center at Dallas; W. Wright, The University of Texas Southwestern Medical Center at Dallas; J. Shay, The University of Texas Southwestern Medical Center at Dallas*

Introduction: In most human tissues, except some rare proliferating stem-like cells, telomerase activity is usually undetectable. Previous reports have shown that mitogen stimulated T lymphocytes transiently turn on telomerase activity that may reduce the rate of telomere loss during rapid proliferation. However, telomerase activation is transient in T-cells as opposed to cancer cells, and is only maintained for a few days even with continual mitogen stimulation. After approximately 3 days of stimulation, telomerase activity greatly decreased and eventually T-cells stop proliferating. With increased human age, T-lymphocytes show progressive telomere shortening. While almost all cancer cells activate telomerase, it does not turn off, and cells achieve unlimited proliferation and telomere do not further shorten. Currently how telomerase activation is regulated in normal cells (such as in T lymphocytes) and how this regulation is hijacked by cancer cells are unclear. **Methods:** Stimulated by anti-CD3/CD28 beads, T cells can be activated and transiently show telomerase activity. Using the novel ddTRAP technique, we are able to measure and monitor telomerase activity in a more accurate and quantitative manner. **Results:** Recently, our lab has reported evidence that hTERT alternative splicing is a potential mechanism for telomerase regulation. In our recent studies, we found that changes of telomerase activity after T lymphocyte stimulation corresponds with the expression shifts of several hTERT splicing variants, so by 3 days the ratio of catalytically active telomerase (splicing variant 5-9) is increased compared to the non-functional splice variants (minus alpha and minus beta). This observation further emphasizes the potential role of hTERT alternative splicing in telomerase regulation in normal T lymphocytes. **Conclusion:** Taking advantage of the T lymphocytes stimulation model, we aim to study telomerase regulatory mechanisms in normal cells. The elucidation of how telomerase is regulated reversibly in primary proliferating transiently amplifying cells may facilitate our understanding of the potential mechanism(s) that cancer cells use to maintain telomerase activation.

62 **CPRIT Grantee**
Poster Session B

Mechanisms Underlying the Tumor Suppressive Role of PTPN12 in Triple-Negative Breast Cancer *N. Neill, Baylor College of Medicine; T. Sun, Baylor College of Medicine; T. Westbrook, Baylor College of Medicine*

Introduction: Triple-negative breast cancer (TNBC) is a common and aggressive subtype of breast cancer that is newly diagnosed in approximately 220,000 women annually. TNBC is refractory to current targeted therapies and despite recent efforts to characterize TNBC genomes and epigenomes, a major barrier to developing TNBC therapies is the paucity in our understanding of the molecular drivers of TNBC. Identifying the signaling networks whose dysregulation drives TNBC would have enormous impact on our understanding of the disease and how we treat afflicted patients. **Methods:** Using a forward genetic screen, we recently identified the tyrosine phosphatase PTPN12 as a tumor suppressor in TNBC (Sun, Cell 2012). Our preliminary data indicate PTPN12 is compromised in many epithelial cancers, including more than 70% of TNBCs, making it the second most frequently inactivated tumor suppressor in TNBC (behind p53). Loss of endogenous PTPN12 leads to transformation of human mammary epithelial cells (HMECs) and mammary carcinogenesis in mouse models. Moreover, restoring PTPN12 function dramatically impairs tumor progression and metastasis in TNBCs. These and other studies suggest PTPN12 functions as a suppressor of human TNBC. **Results:** Our objectives are to elucidate the mechanisms by which PTPN12 suppresses human TNBC and discover new vulnerabilities of PTPN12-deficient TNBCs that can be used as therapeutic entrypoints. To define such vulnerabilities, we performed unbiased genetic screens in PTPN12-overexpressing and PTPN12-deficient TNBC models to identify genes that regulate tumor survival and proliferation in a PTPN12-dependent manner. **Conclusion:** By combining this genetic-interaction profiling with quantitative proteomics data, we have identified several cellular processes that are dysregulated in PTPN12-deficient cancers and selectively required to support the growth of these tumors. We are currently exploring these pathways as novel therapeutic entrypoints that can be exploited to treat patients with TNBC.

63 **CPRIT Grantee**
Poster Session A

Pericytes Expressing Foxd1 Are Recruited as Cancer Associated Fibroblasts in Lung Adenocarcinoma *E. Best, The University of Texas M.D. Anderson Cancer Center; N. Bota- Rabassedas, The University of Texas M.D. Anderson Cancer Center; J. Canales, The University of Texas M.D. Anderson Cancer Center; J. Kurie, The University of Texas M.D. Anderson Cancer Center*

Introduction: More patients will die each year of lung cancer than colon, breast, and prostate cancers combined [American Cancer Society]. Lung adenocarcinoma is a complex disease with about half of patients presenting with no apparent dominant driver mutation which is reflected in the overall survival rate of 5% at 10 years. Cancer Associated Fibroblasts (CAFs) are implicated as important for tumor growth and progression [Bissell & Hines, 2011; Kalluri & Zeisberg, 2006], but the fibroblasts that give rise to CAFs are still unknown. Recently, Foxd1 + lung progenitors have been implicated to give rise to pericytes (endothelial-associated fibroblasts) and contribute to fibrosis upon bleomycin injury [Hung, et al., 2013]. We hypothesize that CAFs may be sourced from progenitor-capable lung pericytes which once recruited, facilitate neoangiogenesis and ultimately allow indirectly for tumor extravasation. M**ethods:** Mice with the KRASLA1 gene [described Johnson et al., 2001] develop lung adenocarcinoma that follows human pathological stages. Mice were bred to achieve labeled and non-labeled tumors: KRASLA1; Foxd1Wildtype; Rs26R-tdTomatoflox/+ (Control Tumor) or KRASLA1; Foxd1cre; Rs26R-tdTomatoflox/+ (Foxd1 Labeled Tumor). Animals were aged 12 months for tumor establishment. The number of tdTomato + cells in normal and tumor tissue was quantified. Total tumor lesions and tumor burden were also noted. Microscopically, tissue slides were assessed for recruitment of tdTomato + cells to tumor area, pericyte coverage, CAF marker co-localization with tdTomato +, neoangiogenesis, proliferation, and MMPs. All tissue areas and immunostaining were validated by a Pathologist. **Results:** Foxd1 expression via tdTomato was detected in the lungs as expected via FACS sorting and immunofluorescence. Mice have been bred, are viable, and will be sacrificed in one month's time (≈12 months). Preliminary (macroscopic) results suggest that there may be an infiltratio of Foxd1+ cells to tumor lesions, but testing is still ongoing to determine infiltration and if sufficient criteria are met to validate Foxd1+ cells as a source of CAFs in KRAS mouse model of Lung Adenocarcinoma. **Conclusion:** Foxd1+ progenitor cells are normally short lived during

development and give rise to pericytes in the lung [Hung, et al., 2013], but preliminary data suggest that Foxd1+ cells may contribute to "reactive stroma". It has been thought before that the stromal fibroblasts "activated" to proliferate in the context of fibrosis may also represent "reactive stroma" in tumor burdens [Ronnov-Jessen, et al., 1993 & 1996]; therefore, it is of interest whether Foxd1+ cells could contribute to "reactive stroma" or CAFs in Lung Adenocarcinoma.

64 **CPRIT Grantee**
Poster Session B

mir-130b Targets Arhgap1 Increasing Cdc42 Activity and Metastatic Potential in Ewing Sarcoma Cells *L. Satterfield, Baylor College of Medicine; L. Kurenbekova, Baylor College of Medicine; R. Shuck, Baylor College of Medicine; L. Donehower, Baylor College of Medicine; J. Yustein, Baylor College of Medicine*

Introduction: Ewing's sarcoma (EWS) is the most common bone tumor in the pediatric population. While significant progress has been made towards increasing patient outcomes, our success in eradicating this malignancy is significantly less than most other pediatric malignancies. One reason for continued treatment failure is due to high risk disease states such as the presence of metastasis. Metastasis is of the utmost clinical relevance as it accounts for more than 90% of solid tumor deaths. Improvements in treatment regimens have been stagnant over the past 20-30 years most likely due to our lack of understanding their molecular pathogenesis. Overall survival rates are approximately 65-70% for localized disease and less than 20% for aggressive, disseminated states. Thus, the clinical need to understand high risk disease, such as metastasis is significant in order to develop novel therapeutics. Recently, miRNAs have been implicated in numerous cancers; however the functional role of miRNAs in sarcomas is limited. M**ethods:** We used quantitative PCR to profile miRNAs that are differntially expressed. Western blot analysis and ELISA were used to assess to assess the activation of Cdc42 and subsequent signaling cascades. Xenograft studies were also performed to assess the in vivo significance of miR-130b overexpression. Luciferase assays were used to identify miRNA targets and Chromatin Immunoprecipitations were performed to assess transcription factor binding. **Results:** We identified a mechanism whereby miR-130b was overexpressed in Ewing Sarcoma cell lines and patient samples. Subsequent microarray analysis identified that miR-130b targets Cdc42GAP. We confirmed that Cdc42GAP is novel target of miR-130b and is downregulated in Ewing Sarcoma. Downregulation of Cdc42GAP leads to activation of Cdc42 and the downstream effector PAK1. Furthermore, we identified a signaling axis whereby miR-130b overexpression eads to activation of Cdc42 and the AP-1 transcription factor an in turn AP-1 binds to the miR-130b promoter to regulate it's expression. **Conclusion:** Taken together, these results suggest that miR-130b and the AP-1 transcription factor form a novel signaling axis to promote the aggessive, metastatic states of Ewing Sarcoma.

65 **CPRIT Grantee**
Poster Session A

The TMEM127 Tumor Suppressor Is a Component of the Lysosomal mTOR Activation Complex and Modulates Autophagy *Y. Deng, The University of Texas Health Science Center at San Antonio; Y. Qin, The University of Texas Health Science Center at San Antonio; S. Srikantan, The University of Texas Health Science Center at San Antonio; P. Dahia, The University of Texas Health Science Center at San Antonio*

Introduction: We previously identified TMEM127 as a tumor suppressor gene in endocrine and renal cancers. We reported that TMEM127 associates with endo/lysosomal membranes, and its depletion or absence leads to disrupted endosomal progression and increased mTOR signaling. The mTOR pathway is aberrantly activated in multiple cancers, and understanding its regulation can provide potential new avenues for therapeutic targeting. Here we examined the interaction of TMEM127 with the lysosome, a site of mTOR activation, and its effect on autophagy, a lysosomal-based catabolic process that is dependent on mTOR. **Methods:** We developed a Tmem127 KO mouse model using a LoxP/Cre system targeting Tmem127 under a generically-expressed promoter (CMV-Cre). Mouse Embryonic Fibroblasts (MEFs) were obtained from E13.5 of heterozygous matings. Hela and HEK293T cells overexpressing GFP-TMEM127 or with TMEM127 knockdown (KD) were also used. Immunoprecipitation, western blot and confocal microscopy were carried out following standard protocols. Images were quantifie using ImageJ, Columbus or Metamorph. **Results:** We observed amino acid-dependent colocalization between GFP-TMEM127 and lysosomal marker LAMP1. Moreover, we found that Tmem127KO MEFs have increased size, number and perinuclear distribution of lysosome and increased expression of lysosomal genes without affecting lysosomal pH. These data suggest TMEM127 impinges on lysosome activity. Next, we examined whether TMEM127 participates in the lysosomal complex required for mTOR activation. We found TMEM127 associates with two members of this complex, LAMTOR1 and v-ATPaseV0d1, and this requires LAMTOR1 localization to the lysosomal membrane. Expression of multiple components of the LAMTOR-vATPase complex is increased in Tmem127KO cells, as is the fraction of lysosome-associated mTOR, suggesting that TMEM127 modifies the composition, stability and/or activity of the mTOR-related lysosomal complex. Next, we investigated autophagy, a nutrient-dependent lysosomal process inhibited by mTOR. We found that in HeLa cells TMEM127 colocalizes with the autophagy marker LC3 in a nutrient-dependent manner. In contrast, starvation-induced autophagy was reduced in TMEM127-KD cells. Furthermore, these cells showed increased phosphorylation of the autophagy initiator ULK1. Reduced autophagy and ULK1 phosphorylation were rescued by the mTOR inhibitor rapamycin, suggesting that impaired autophagy of TMEM127-deficient cells is mTOR-dependent. **Conclusion:** Our data demonstrate that TMEM127 is a lysosomal protein and a functional component of the lysosomal-centered mTOR signaling machinery. TMEM127 loss inhibits autophagy in an mTOR-dependent manner. Further studies addressing the mechanisms of TMEM127 actions on this complex should provide insights on the regulation of mTOR at the lysosome. This work sheds light on a novel component of the lysosomal signaling system that is involved in mTOR regulation.

66 **CPRIT Grantee Poster Session B**

Transient Exposure to Tamoxifen Prevents Mammary Tumorigenesis by Inducing Ptpn5 in p53 null Mouse Mammary Gland **M. Palaniappan, Baylor College of Medicine; D. Edwards, Baylor College of Medicine; D. Medina, Baylor College of Medicine; O. Conneely, Baylor College of Medicine**

Introduction: The tumor suppressor gene p53 is frequently mutated in human breast cancer and is a marker for poor prognosis and resistance to chemotherapy. Transplantation of p53 null mouse mammary epithelium into syngeneic wild-type mice leads to development of spontaneous mammary tumors that recapitulate many of the phenotypic, molecular and genetic features of human breast cancer. Short term exposure to the antiestrogen, tamoxifen, prior to tumor development confers robust protection against tumor development. Using this mouse model, we seek to elucidate the molecular mechanisms underlying tamoxifen-dependent tumor prevention. **Methods:** Mammary epithelium from p53 null mice was transplanted into the cleared fat pad of syngeneic wild type Balb/c mice. At 8 weeks after transplantation mice received a tamoxifen (5mg) or sham pellet subcutaneously for 13 weeks. At 4 weeks after tamoxifen removal, all mice were injected with 17β-estradiol (100ug) for 8 h, and mammary epithelial cells (MECs) were isolated for global gene expression analysis. Whole mount and immunohistochemical staining of mammary glands were also performed to assess mammary gland morphology and proliferation. To test the functional contribution of a candidate tamoxifen regulated gene (PTPN5) to mammary tumorigenesis, MDA-MB-231 human breast cancer cells were transduced with an inducible lentivirus expressing PTPN5 and its effects on cell proliferation and growth factor signaling in vitro as well as tumor development in a mouse xenograft model were examined. **Results:** Transient exposure to tamoxifen led to a persistent reduction in mammary ductal side-branching and epithelial cell proliferation after tamoxifen withdrawal. Tamoxifen exposure also led to persistent changes in expression of a subset (245) of estrogen regulated gene signatures in MECs. Among these were the protein tyrosine phosphatase, non-receptor type 5 (Ptpn5) which was persistently upregulated in tamoxifen exposed MECs. Functional analysis of the role of PTPN5 in breast cancer cells showed that PTPN5 inhibited EGF-dependent activation of the MAPK pathways. PTPN5 potently induced apoptosis and repressed long-term colony formation of MDA-MB-231 cells in vitro and suppressed tumor growth in a xenograft model. C**onclusion:** Transient exposure to tamoxifen led to a persistent reduction in mammary gland side branching morphogenesis and MEC proliferation which was associated with differential expression of a subset of estrogen regulated target genes in MECs. PTPN5, a novel upregulated gene in tamoxifen exposed MECs, was found to negatively regulate EGF signaling and inhibits growth of breast cancer cells. Thus, PTPN5 may contribute to tamoxifen-dependent tumor prevention.

67 **CPRIT Grantee Poster Session A**

Therapeutic potential of DNA2 inhibition in treatment of cancers
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Introduction: Cancer cells due to their hyper-proliferation state and high rate of DNA replication are more vulnerable to killing by DNA damaging agents. The ability of cancer cells to repair DNA damage can interfere with these therapies. Thus to increase the efficacy of treatments they are often used in combination with inhibitors of DNA repair pathways. DNA2 is an evolutionary conserved nuclease/helicase with functions in DNA replication and repair. Results from our group show that DNA2 nuclease/helicase is needed for tolerating replication stress and DNA damage in cancer cells. Also, DNA2 is overexpressed in a broad spectrum of cancers including breast cancer, ovarian cancer, lung cancer, prostate cancer, glioblastoma, pancreatic cancer etc. Further, partial depletion of DNA2 reduces tumorigenicity of breast cancer cells raising the possibility that DNA2 might be a drug target in cancer therapy. **Methods:** Here we developed a sensitive and robust high-throughput assay with triple labeled DNA substrates for screening for DNA2 inhibitors. The screen was performed with yeast DNA2 nuclease and the top nuclease inhibitors were confirmed with purified human DNA2. Further these inhibitors are tested in vitro for their ability to inhibit DNA resection, which is the firs step of DNA repair. **Results:** Using this screening procedure we screened ≈ 50,000 compounds and found two very specific inhibitors of DNA2 nuclease. These small molecule inhibitors of DNA2 nuclease can inhibit the growth of cancer cells and have the ability of selectively targeting cells with oncogene-induced replication stress by reducing DNA2-mediated DNA repair capacity. These inhibitors also successfully inhibited DNA end resection and homologus recombination in both, cell-based assay and DNA resection in vitro. **Conclusion:** Together we identified first small molecule inhibitors of DNA2 nuclease and demonstrated their potential as a promising novel class of anticancer agents.

68 **CPRIT Grantee Poster Session B**

Regulation of HIF-2 by Synthetic and Endogenous Ligands
R. Bruick, The University of Texas Southwestern Medical Center at Dallas; H. Wang, The University of Texas Southwestern Medical Center at Dallas; A. Khadilkar, The University of Texas Southwestern Medical Center at Dallas; Y. Chen, The University of Texas Southwestern Medical Center at Dallas; J. MacMillan, The University of Texas Southwestern Medical Center at Dallas

Introduction: Hypoxia Inducible Factors (HIFs) are key transcriptional regulators of hundreds of genes involved in biological processes such as anaerobic metabolism, oxygen delivery, and angiogenesis. Consequently, HIFs often play key roles in the progression of a number of diseases in which oxygen availability is compromised, notably tumorigenesis. We have previously characterized the structure/function relationships of individual HIF domains that are important for maintaining transcriptionally active HIF complexes. Though transcription factors have often been considered "undruggable", we found a large cavity within the hydrophobic core of the HIF-2α PAS-B domain that provides a unique foothold for small molecule regulation. We've identified artificial ligands that can occupy this pocket and induce structural and functional changes that antagonize HIF-2 activity. Despite the high sequence identity shared between HIF-2α and HIF-1α, these ligands are highly selective and do not affect HIF-1 function. We hypothesize that this cavity may be exploited by endogenous ligands that selectively regulate HIF-2α function *in vivo*, contributing to HIF-2 mediated responses under both physiological and pathophysiological settings such as cancer. **Methods:** In support of this hypothesis, we pursuing two lines of research. First, we've initiated purification of candidate endogenous metabolites from animal tissues using a radiolabeled ligand competition assay. A variety of genetic and pharmacological approaches are being used to manipulate the abundance of the metabolites to establish HIF-2 selective relationships. In addition, a new mouse model has been generated through introduction of a specifi mutation within the endogenous *EPAS1* gene locus that partially fills the ligand cavity in the HIF-2α PAS-B domain and renders the homozygous knock-in mouse insensitive to antagonists. **Results:** Manipulation of the ligand occupancy state of HIF-2α results in changes in HIF-2 target gene expression. In addition to being an ideal control animal to assess on-target effects of HIF-2 inhibitors, the knock-in mice demonstrate phenotypes in HIF-2 dependent responses under stress that may stem from the inability

of this variant to recognize endogenous metabolites. **Conclusion:** Both efforts take advantage of our uniquely-coordinated molecular, chemical, biophysical, and physiological approaches to characterize and exploit these novel mechanisms linking cellular metabolism to HIF regulation. Together these studies have advanced our understanding of HIF isoform regulation, particularly as a reflection of cellular metabolism - critical considerations for future therapeutic applications targeting this pathway.

69 **CPRIT Grantee**
Poster Session A

Meiotic Protein HORMAD1 Modulates DNA Damage in NSCLC

B. Nichols, The University of Texas Southwestern Medical Center at Dallas; E. McMillan, The University of Texas Southwestern Medical Center at Dallas; M. White, The University of Texas Southwestern Medical Center at Dallas; A. Whitehurst, The University of Texas Southwestern Medical Center at Dallas

Introduction: Cancer Testis Antigens (CTAs) are genes whose expression is generally restricted to the testes but aberrantly reactivated in tumors. As evidenced by loss of fertility in genetically modified mouse models, six of these CTAs (HORMAD1, HORMAD2, TEX15, SPO11, SYCP1, SYCE1) are essential for proper meiotic recombination by facilitating DNA double-strand breaks, interhomolog crossover, and subsequent repair. Analyzing the expression patterns for these meiotic CTAs in NSCLC revealed that HORMAD1 displays a striking bimodal distribution pattern in both tumor-derived cell lines and patient tumors, with high expression correlating with high mutational burden. **Methods:** To test whether HORMAD1 is capable of altering DNA repair dynamics in NSCLC, HORMAD1 was stably introduced into a HORMAD1 null NSCLC line using lentiviral mediated transduction. Conversely, HORMAD1 expression was stably depleted in a NSCLC with high endogenous HORMAD1 expression using shRNA technology. Effects on cell viability were tested using colony formation assays and DNA damage was assessed by measuring 53BP1 foci and H2AX phosphorylation. **Results:** HORMAD1 depletion does not alter levels of 53BP1 foci nor phosphorylation of H2AX. On the other hand, ectopic expression of HORMAD1 decreases anchorage-independent growth and significantly increases the average number of DNA double-strand breaks per cell. However, these phenotypes were only evident after prolonged culturing, suggesting HORMAD1 increasingly alters DNA repair dynamics over time. **Conclusion:** These findings suggest that tumor cells may engage meiotic regulators, such as HORMAD1, to modulate DNA damage and promote tumorigenesis. Future endeavors aim to determine the molecular mechanism for HORMAD1 in DNA damage response pathways, the relevance of these findings in vivo, and whether HORMAD1 may serve as a drug target for enhancing response to current cytotoxic therapies.

70 **CPRIT Grantee**
Poster Session B

Drug Repositioning in Ovarian Cancer through Systematically Investigation of Crosstalk with Platelet

J. Sheng, The Methodist Hospital Research Institute; M. Haemmerle, The University of Texas M.D. Anderson Cancer Center; F. Li, The Methodist Hospital Research Institute; A. Sood, The University of Texas M.D. Anderson Cancer Center; S. Wong, The Methodist Hospital Research Institute

Introduction: Ovarian cancer ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system. About 21,290 women will receive a new diagnosis of ovarian cancer and about 14,180 women will die from it (American Cancer Society). Highly elevated platelet levels fuel tumor growth and reduce the survival of ovarian cancer patients. Previous studies only focus on specifi proteins and downstream pathways in platelet and cancer cells. Here, we systematically investigated crosstalk between platelet and ovarian cancer cells and present a communication network that is associated with patient survival rate. We also try to repositioning drugs targeting on this network which may be effective in treating ovarian cancer. **Methods:** RNA-seq data of platelets from healthy controls and ovarian cancer patient as well as microarray data of HeyA8 cell line with/without platelet treatment were collected from Dr Sood's Lab in MD Anderson cancer center. DESeq was applied for RNA-seq analysis. T test followed by BH correction was applied to find differentially expressed genes (DEGs) from microarray data. Up-regulated ligands in platelet cells and downstream activated pathways in cancer cells were identified using CCCExplorer. Pathway enrichment analysis was done using DAVID. RNAseq V2 and clinical data of ovarian cancer patients from TCGA were downloaded and the patients were clustered to different groups based on the expression level of the pathway genes. Survival analysis was performed to check if the genes in the crosstalk network were associated with patients' survival rate. To find potential drugs that target on the genes in the pathways, we apply connectivity map method to repositioning over 1000 drugs/compounds. The top ranked drugs were selected and literature survey was done to find potential e fective drugs. **Results:** Seven ligand-receptor pairs and 5 pathways were identified as key candidates for platelet-tumor crosstalk. Several pathways including DNA replication, cell cycle were up-regulated in cancer cells after co-culture with platelet. Hierarchical clustering showed two groups for TCGA patients. Survival analysis showed significan difference in survival rate between the two groups. Two out of ten top ranked drugs were reported to be efficient in treating ovarian cancer. **Conclusion:** Our study provide a comprehensive crosstalk network between platelet and ovarian cancer, which may lead to dysfunctions in several downstream pathways in cancer. Genes in the network were associated with patients' survival rate. Drugs that target on this network may be used alone or combined with other drugs to treat ovarian cancer.

71 **CPRIT Grantee Poster Session A**

Viral Deployment of Bacterial Effectors Reveal Molecular Vulnerabilities in Human Cancer *L. Reddick, The University of Texas Southwestern Medical Center at Dallas; P. Woida, The University of Texas Southwestern Medical Center at Dallas; G. Cox, The University of Texas Southwestern Medical Center at Dallas; M. Peyton, The University of Texas Southwestern Medical Center at Dallas; E. McMillan, The University of Texas Southwestern Medical Center at Dallas; M . White, The University of Texas Southwestern Medical Center at Dallas; J .Minna, The University of Texas Southwestern Medical Center at Dallas; N. Alto, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Great emphasis has been made to understand and describe both adult and childhood cancer etiology in terms of the specific genetic mutations that drive proliferative growth, replication, and metastasis. As a result, improved outcomes have been achieved for many of the major adulthood and childhood cancers, however drug resistance remains a major problem. Thus, the discovery and identification of new therapeutic targets is needed to augment traditional small molecule approaches. In order to address this global problem in cancer biology, we have embarked on an unconventional and asymmetric approach in which we utilized highly evolved bacterial toxins (called effectors) to probe human cancers for systematic weaknesses. *Hence, we have leveraged one disease-causing agent to identify points of therapeutic intervention in another.* **Methods:** cDNA of Type III and Type IV effectors from various Gram-negative bacterial pathogens was cloned into a Lentiviral vector in-frame and C-terminal to an eGFP tag. Thus, we produced 93 unique Lentiviruses that each encoded a different bacterial effector gene and applied these viruses to cancer cell lines pair-wise in 96-well format with an n=4 to NSCLC, neuroblastoma, rhabdomyosarcoma, colorectal, prostate, and hematologic cancers. Cell viability assays were performed, analyzed by Robust Z-score, and bacterial effectors that reduced cancer cell viability more than 2 standard deviations from the population were considered hits. We followed up on these hits with both in vitro and in vivo analyses to confirm the growth inhibitory phenotype and to understand the mechanism of the anti-cancer activity. **Results:** We generated 'bacterial effector protein sensitivity fingerprints for all cancer cell lines tested. Interestingly, clades began to emerge, indicating that certain cancer cell lines shared common oncogenic addiction pathways. Two effector proteins in particular stood out: YopH from *Yersinia pseudotuberculosis* and OspI from *Shigella flexner* , which were capable of growth limiting EGFR-driven cancers and several neuroblastomas, respectively. Ectopic expression of YopH in EGFR-driven NSCLC in mouse lung xenografts was sufficient to reduce tumor volume and extend the survival of mice compared to control. **Conclusion:** 1. Bacterial effectors can serve as probes to identify molecular vulnerabilities in cancer. 2. YopH growth limits all EGFR-driven cancers tested in our screen. 3. Ectopic expression of YopH in xenografted EGFR tumors in vivo reduces tumor volume and extends survival. 4. OspI growth limited most neuroblastomas, and ablating the eukaryotic target of OspI, Ubc13, growth limits SK-N-DZ, revealing a new therapeutic target in neuroblastoma.

72 **CPRIT Grantee Poster Session B**

Accounting for Inter-tumor Heterogeneity Using a Sample-specific Error Model Improves Sensitivity and Specificity in Mutation Calling for Sequencing Data *Y. Fan, The University of Texas M.D. Anderson Cancer Center; L. Xi, Baylor College of Medicine; D. Hughes, Baylor College of Medicine; J. Zhang, The University of Texas M.D. Anderson Cancer Center; J. Zhang, The University of Texas M.D. Anderson Cancer Center; A. Futreal, The University of Texas M.D. Anderson Cancer Center; D. Wheeler, Baylor College of Medicine; W. Wang, The University of Texas M.D. Anderson Cancer Center*

Introduction: The detection of somatic point mutations is a key component of cancer genomics research that has been developing rapidly since next-generation sequencing (NGS) technology revealed its potential for describing the genetic alterations in cancer. As the NGS expense continues to decrease, the need to thoroughly interrogate the cancer genome spurs the migration from the whole exome sequencing (WES) to the whole genome sequencing (WGS). A critical challenge accompanying this migration is the rigorous requirement of specificit . In addition, the sequencing depth decreases to 30-60X for WGS data resulting in a lower signal-to-noise ratio making mutation-calling more difficult. Another nontrivial difficulty is the influence of tumor heterogeneity commonly found in samples, due to the presence of both normal cells and tumor subclones. **Methods:** Here we present MuSE, a novel approach to mutation calling based on a Markov model for molecular evolution, which models the evolution of the reference allele to the allelic composition of the matched tumor and normal at each nucleotide position. We further adopt a sample-specific error model to identify cutoffs dynamically, reflecting the fact that tumor heterogeneity varies among samples, to improve overall accuracy. **Results:** We evaluated the performance of MuSE using the virtual-tumor benchmarking approach, and validated MuSE through the participation in the ICGC-TCGA DREAM Mutation Calling challenge and the ICGC PanCancer Analysis of Whole Genomes (PCAWGs) project both of which provided independent experimental validation. We also applied MuSE to analyze the WES data of chromophobe renal cell carcinoma (KICH), adrenocortical carcinoma (ACC) and liver hepatocellular carcinoma (LIHC) all of which are part of TCGA project. MuSE performed on par with or better than best-in-breed mutation callers. The tier-based variant call sets helped evaluate the behavior of low allele fraction mutations and understand the effect of inter-sample heterogeneities. **Conclusion:** In summary, we presented a mutation caller, MuSE, based upon the somatic evolution estimation. Our caller accounted for the inter-tumor heterogeneity by building a sample-specific error model that further improved the sensitivity and specificit . We demonstrated the reliable performance of MuSE, a good balance of sensitivity and specificit , using various types of data. Even though the satisfactory performance of MuSE, we still would like to argue that it is the trend to incorporate the call sets from multiple callers in the future NGS analyses.

73 **CPRIT Grantee Poster Session A**

Enhancer Transcription Reveals Novel Gene Regulatory Networks In Breast Cancer *H. Franco, The University of Texas Southwestern Medical Center at Dallas; A. Nagari, The University of Texas Southwestern Medical Center at Dallas; Y. Xi, Baylor College of Medicine; W. Li, The University of Texas M.D. Anderson Cancer Center; D. Richardson, The University of Texas M.D. Anderson Cancer Center; K. Tanaka, The University of Texas M.D. Anderson Cancer Center; J. Li, University of Houston; M. Barton, The University of Texas M.D. Anderson Cancer Center; X. Shi, The University of Texas M.D. Anderson Cancer Center; K. Keyomarsi, The University of Texas M.D. Anderson Cancer Center; M. Bedford, The University of Texas M.D. Anderson Cancer Center; W. Li, Baylor College of Medicine; S. Dent, The University of Texas M.D. Anderson Cancer Center; W. Kraus, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Enhancer transcription is a defining feature of active enhancers. We and others have shown that enhancers that produce transcripts (so called “eRNAs”) are more likely to (1) be associated with active chromatin marks, such as H3K4me1 and K3K27ac, (2) loop to target gene promoters, and (3) be associated with target gene activation. Thus, enhancer transcription is a good predictor of active enhancers. In this regard, we have shown that enhancer transcription can be used in the absence of any other genomic information to predict enhancers. **Methods:** We have used Global Run-On coupled deep sequencing (GRO-seq) in 14 different breast cancer cell lines representing the five distinct molecular subtypes of breast cancer, coupled with a computational pipeline that we have developed, to predict enhancers based solely on enhancer transcription. **Results:** We found both common and unique sites of enhancer transcription across the cell lines. In addition, we observed that enhancer transcription correlates with nearest gene transcription. Unsupervised hierarchical clustering of enhancer transcription was sufficient to segregate the breast cancer cell lines into their specifi molecular subtypes. Transcription factor motif analysis performed at the sites of enhancer transcription identified transcription factors that may be important for the transcriptional programs of each cell type. Transcription factors whose motifs were uniquely enriched in a specific cell type were observed to be bound at the enhancers using locus-specific ChIP-qPCR assay. siRNA-mediated knockdown of the cognate transcription factors reduced enhancer transcription and cell proliferation in those cell types. The GRO-seq data were integrated with ChIP-seq data for several histone modifications typically enriched at promoters, gene bodies, enhancers, and repressive regions of the genome. The results from these analyses provide additional support for our enhancer identificatio pipeline. **Conclusion:** We have used a novel computational pipeline to perform an unbiased enhancer prediction analysis across 14 different breast cancer cells based solely on enhancer transcription. Our analyses have revealed novel gene regulatory networks that underlie breast cancer subtype-specific biology and highlighted potential targets for therapeutic intervention.

74 **CPRIT Grantee Poster Session B**

MiR-130b Sensitize Ovarian Cancer to Cisplatin and BH3-mimetics by Inducing TAp63 and Its Downstream Gene Bim *Y. Pan, University of Houston; A. Hernandez-Herrera, University of Houston; A. Benham, University of Houston; A. Venkatanarayan, The University of Texas M.D. Anderson Cancer Center; C. Chan, University of California; A . Rowat, University of California; K. Rajapakshe, Baylor College of Medicine; S .Duvvuri, University of Houston; C. Coarfa, Baylor College of Medicine; E. Flores, The University of Texas M.D. Anderson Cancer Center; P. Gunaratne, University of Houston*

Introduction: Ovarian cancer is the most lethal gynecological malignancy in women, which presents as an advanced stage aggressive cancer with widespread metastases of multi-cellular spheroids highly resistant to chemotherapy. Over 96% of high-grade serous ovarian cancer (HGSOC) and 50% of all cancer types carry mutations in p53 gene. **Methods:** Ovarian cancer lines HEYA8 (p53-wild type) and OVCAR8 (p53-mutant) were transiently transfected with miR-130b mimics. Transfected cells were tested by a scratch-wound migration assay and a Matrigel invasion assay. After cisplatin or ABT-737 drug treatment, the percentage of live, apoptotic and necrotic cells was analyzed by FACS. The effect of miR-130b overexpression in genes from the p53 pathway and p53-related genes were assayed by q-PCR and Western blot. The magnetic levitation system was used to generate three dimensional (3D) cell culture models to examine the impact of miR-130b on spheroid formation. To assess the clinical significance, we integrated microRNA-seq and RNA-seq data from the TCGA ovarian cancer project with clinical outcomes. **Results:** Here we present a tumor suppressor microRNA-130b, which can significantly attenuate cellular migration/invasion and spheroid formation in ovarian cancer cells. We found that miR-130b augmented wild-type p53 and circumvented mutant-p53 by activating the p53-family member TAp63 and its downstream gene Bim, while repressing ΔNp63, a competitive inhibitor of the p53/TAp63/TAp73 complex. Forced expression of TAp63 decreased ovarian cancer cell viability by 60-80%. Our data also suggested that miR-130b was able to sensitize ovarian cancer lines to conventional chemotherapeutic drug cisplatin, and to ABT-737 (BH3-mimetic), a novel small molecule drug in clinical trials for lymphoid malignancies, through inducing TAp63 and Bim. Increased expression of both TAp63 and miR-130b predict improved overall survival in ovarian cancer patients. **Conclusion:** We report that miR-130b is a clinically significant tumor suppressor miRNA that can overcome the effects of p53 mutations by inducing TAp63 and up-regulating BCL2L11 to increase cell death and attenuate migration/invasion. Because BH3 mimetics have shown promise in clinical trials and p63 is rarely mutated in human cancers, miR-130b and therapeutic agents that clinically activate the TAp63/Bim axis may be broadly applicable to many cancers.

75 **CPRIT Grantee Poster Session A**

MCT4 Defines a Glycolytic Subtype of Pancreatic Cancer with Poor Prognosis and Unique Metabolic Dependenies *G. Baek, The University of Texas Southwestern Medical Center at Dallas; A . Witkiewicz, The University of Texas Southwestern Medical Center at Dallas; E. Knudsen, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of cancer-related mortality, with a 5-year survival of only ~6%. While the prevailing genetic features of PDA (e.g. K-RAS) are monolithic in nature and actionable subtypes have yet to emerge, we have found that there are distinct metabolic features of PDA that can be selectively targeted. **Methods:** This work takes a multi-disciplinary approach incorporating the analysis of unique preclinical models in parallel with the detailed assessment of pancreatic cancer tissue to provide novel insights into the metabolic subytypes of disease and resultant vulnerabilities. **Results:** The Slc16A3 gene encoding the lactate transporter MCT4 is highly upregulated in PDA relative to other cancers and normal pancreatic tissue. Analysis of MCT4 in PDA cases (n=223) demonstrated an association with poor survival that remained significant in multivariate analyses. MCT4 attenuation compromised glycolytic flux with compensatory induction of oxidative phosphorylation and scavenging of metabolites by macropinocytosis and autophagy. In spite of these adaptations, MCT4 depletion induced cell death characterized by elevated reactive oxygen species and metabolic crisis. Cell death induced by MCT4-depletion was augmented by inhibition of compensatory pathways. In xenograft models, MCT4 had a significan impact on tumor metabolism and was required for rapid tumor growth. **Conclusion:** Together, these findings illustrate the metabolic diversity of PDA described by MCT4, delineate pathways through which this lactate transporter supports cancer growth, and demonstrate that PDA can be rationally targeted based on metabolic addictions.

76 **CPRIT Grantee Poster Session B**

Investigating the Role of Poly (Adp-Ribose) Polymerase-1 (Parp-1) in The Development and Progression of Small Cell Lung Cancer (Sclc) *S. Mukherjee, The University of Texas Health Science Center at Houston; R. Cardnell, The University of Texas M.D. Anderson Cancer Center; L. Byers, The University of Texas M.D. Anderson Cancer Center*

Introduction: Small cell lung cancer (SCLC) is a highly lethal malignancy characterized by rapid growth, early metastasis and poor prognosis. We previously reported that poly (ADP-ribose) polymerase 1 (PARP-1), a DNA repair protein, is dramatically overexpressed in SCLC cell lines and patient samples. The current understanding about the therapeutic efficacy of PARP inhibitors indicates that PARP inhibitors downregulates the expression of proteins in the DNA damage pathway. In SCLC, high expression of DNA damage proteins correlated with increased sensitivity to PARP inhibitors (Cardnell RJ, and Byers LA, CCR 2013, 13-1975). However, the biological effect of PARP inhibition on functions beyond DNA repair has not been elucidated yet. In this study, we want to investigate the "DNA repair independent" functions of PARP inhibitors. PARP-1's has been shown to limit pro-malignant processes such as vasculogenic mimicry by regulating EMT markers, sucha as E cadherin, Snail and vimentin. Based on this, I hypothesize that PARP inhibitors suppresses tumorigenic potential of the cells by (a) promoting DNA damage induced cell cycle arrest which sensitizes the cells for programmed cell death (apoptosis) (b) inhibiting "DNA repair independent" function of PARP such as EMT and metastasis by upregulating E-cadherin. **Methods:** SCLC cell lines were treated with PARP-1 inhibitors, BMN 673, AZD-2281 and ABT-888 for 72 hours. The effect of PARP-1 inhibitors on cell cycle and apoptosis was evaluated by propium iodide and Annexin V staining. The effect of PARP inhibitors on DNA damage was evaluated by Comet assay. Boyden chamber assay was used to study the effect of PARP-1 inhibition loss on cell migration. To investigate the effect of PARP-1 knockdown on downstream proteins, reverse phase protein array (RPPA) analysis and western blotting were performed using lysates from SCLC cell lines treated with PARP inhibitors. **Results:** PARP-1 inhibition leads to G2/M cell cycle arrest and induced apoptosis and necrosis in SCLC cell lines. PARP-1 inhibition resulted in decreased cell migration. Proteomic analyses show PARP-1 inhibition resulted in decreased expression of cell cycle proteins (e.g. p-CHK1, p-ATR), in agreement with our hypothesis. Further, proteomic profiling also showed upregulation of E-cadherin and downregulation of β-catenin after PARP inhibition. C**onclusion:** Our results strongly indicate that inhibition of PARP-1 in SCLC by resulted in suppressed cell proliferation and migration; with decreased expression of cell cycle and EMT related proteins suggesting a role for that PARP-1 play a role in maintaining cell cycle and EMT-induced metastasis.

77 **CPRIT Grantee Poster Session A**

Rotten to the Core: Tumor Immune Suppression and Immunotherapy Resistance Critically Requires Hypoxia *M. Curran, The University of Texas M.D. Anderson Cancer Center; M. Ai, The University of Texas M.D. Anderson Cancer Center; P. Budhani, The University of Texas M.D. Anderson Cancer Center; S. Balasubramanyam, The University of Texas Medical School at Houston*

Introduction: Tumor hypoxia predicts poor outcomes across all cancers and has long been recognized as a critical source of resistance to both chemotherapy and radiotherapy. Despite the success of T cell immune checkpoint blockade in treating melanoma, aggressive adenocarcinomas of the prostate and pancreas are largely resistant to CTLA-4 and PD-1 antibody therapy in the mouse and in man. We find that hypoxic zones of these tumors resist infiltration by T cells even in the context of robust infiltration of normoxic areas of the same tumor (e.g. in the context of T cell checkpoint blockade). Beyond this lack of accessibility to tumor-specific T cells, hypoxia drives the establishment of a highly interdependent network of immunosuppressive stromal cells. Among these, we find myeloid-derived suppressor cells and myofibroblasts to be the critical populations which act together to suppress T cell responses and mediate immunotherapy resistance. **Methods:** Tumor therapy and mechanistic studies were performed using the C57BL6 syngeneic prostate cancer cell line TRAMP-C2. The TRAMP transgenic mice were used as a spontaneous model of prostate cancer to validate findings from the the transplantable model. Mice were treated with the hypoxia-specific pro-drug Evofosfamide at 50mg/kg i.p. alone or in combination with anti-CTLA-4 (9H10, 100ug/injection) and anti-PD-1 (RMP1-14, 250ug/injection). **Results:** Using the hypoxia-specific prodrug Evofosfamide, we show that disruption of hypoxia in both transplantable and genetically-engineered murine models of prostate cancer sensitizes them to antibody blockade of CTLA-4 and PD-1. Loss of immune resistance is a consequence of re-oxygenation of hypoxia zones which results in 1) loss of myeloid suppressors, 2) reduced capacity to suppressively polarize new myeloid immigrants, and 3) loss of suppressive activation of myofibroblasts. Combination therapy results in robust infiltration of the tumor microenvironment by active CD8 T cells and control or elimination of established prostate cancer in both transplantable and spontaneous tumor models. **Conclusion:** This combination of hypoxia disruption and T cell checkpoint blockade has immense potential to render some of the most therapeutically resistant cancers sensitive to immunotherapy.

78 **CPRIT Grantee Poster Session B**

TANRIC: An Interactive Open Platform to Explore the Function of LncRNAs in Cancer *J. Li, The University of Texas M.D. Anderson Cancer Center; L. Han, The University of Texas M.D. Anderson Cancer Center; H. Liang, The University of Texas M.D. Anderson Cancer Center*

Introduction: Through decades of efforts, we now have a better understanding about genetic elements in our genomes: the human genome encodes not only ~20,000 protein-coding genes, but also an astounding number of transcriptionally active noncoding RNAs. Among noncoding RNAs, long non-coding RNAs (lncRNAs) have been increasingly recognized to play essential roles in tumorigenesis, representing a new focus in cancer research. Therefore, a central task in the cancer research community is to identify lncRNA components involved in tumorigenesis and elucidate their functions in the specific tumor contexts. However, efficient bioinformatics tools to systematically explore the function and underlying mechanisms of lncRNAs are still missing. To fill this important gap, we have developed **TANRIC** (*http://bioinformatics.mdanderson.org/main/TANRIC:Overview*), a user-friendly and open resource for interactive exploration of lncRNAs in cancer. **Methods:** TANRIC integrates lncRNA expression data with sample clinical data and cancer genomic data and provides a user-friendly interface consisting of six modules: Summary, Visualization, Download, My lncRNA, Analyze all lncRNAs and LncRNAs in cell lines. Comparing to other available lncRNA-focused bioinformatics resources, TANRIC has several unique features: (i) It interactively analyze lncRNAs of interest with other TCGA genomic/proteomic/epigenomic and clinical data both within a tumor type and across tumor types; (ii) it enables users to query expression profile of user-defined genomic regions; and (iii) it includes other non-TCGA large-scale cancer RNA-seq data, allowing researchers to independently validate a pattern of interest. **Results:** In this study, we demonstrated an abundance of lncRNAs with potential biomedical relevance: a large number of lncRNAs show differential expression between tumor and normal samples, among established tumor subtypes or in correlations with clinical variables; the expression levels of many lncRNAs show strong correlations with the molecular signatures of key therapeutic targets and biomarkers; and finall , the tumor subtypes defined by lncRNA expression profiles show extensive concordance with established tumor subtypes and provide prognostic value. **Conclusion:** Consistent with previous studies, our analysis reveals a large number of lncRNAs with potential biomedical significance but on an unprecedented scale. Importantly, we report that some lncRNAs show strong correlations with established therapeutic targets across tumor types. Although the correlations do not necessarily indicate cause-effect relationships, these results highlight the tremendous potential of lncRNAs as regulators of key therapeutic targets or a novel class of biomarkers. TANRIC, thus, represents a starting point for exploration of particular lncRNA species and for generation of testable hypotheses for further experimental investigation.

79 **CPRIT Grantee Poster Session A**

Methylation of Epidermal Growth Factor Receptor by Protein Arginine Methyltransferase 1 Regulates EGFR Signaling And Cetuximab Response *H. Liao, The University of Texas M.D. Anderson Cancer Center; M. Hung, The University of Texas M.D. Anderson Cancer Center*

Introduction: Protein modifications of epidermal growth factor receptor (EGFR) intracellular domain are well known regulators of EGFR functions whereas those of its extracellular domain remain relatively unexplored. **Methods: Results:** Here, we report that methylation at R198 and R200 of EGFR extracellular domain by protein arginine methyltransferase 1 (PRMT1) upregulates its binding to EGF and subsequent receptor dimerization and signaling activation. Methylation-defective EGFR mutant reduced tumor growth in mouse orthotopic xenograft model. Importantly, increased EGFR methylation sustains its signaling activation and cell proliferation in the presence of therapeutic EGFR monoclonal antibody, cetuximab. EGFR methylation level also correlates with higher recurrence rate after cetuximab treatment and poorer overall survival in colorectal cancer patients. **Conclusion:** These data suggest that R198/R200 methylation plays important role in regulating EGFR functionality and resistance to cetuximab treatment.

80 **CPRIT Grantee Poster Session B**

PRMT6 Overexpression Causes Global Loss Of DNA Methylation *N. Veland, The University of Texas M.D. Anderson Cancer Center; A .Di Lorenzo, The University of Texas M.D. Anderson Cancer Center; S. Rothbart, Van Andel Research Institute; B. Strahl, UNC School of Medicine; M. Bedford, The University of Texas M.D. Anderson Cancer Center; T. Chen, The University of Texas M.D. Anderson Cancer Center*

Introduction: DNA methylation is a major epigenetic modification, mostly restricted to CpG dinucleotides, and it is present in 60-80% of all CpG sites in mammals. DNA methylation is essential for development and plays crucial roles in regulation of gene expression and genomic integrity. Aberrant DNA methylation patterns are observed in cancer, where cells exhibit global hypomethylation and regional hypermethylation. The underlying mechanisms for this dysregulation are largely unknown. DNA methylation is maintained after DNA replication by DNMT1, which is recruited by its cofactor UHRF1 to methylate the new DNA. Moreover, UHRF1 binds to the histone H3 and this is reduced when arginine 2 of H3 (H3R2) is methylated, suggesting that H3R2 methylation may regulate DNA methylation by altering the recruitment of the UHRF1-DNMT1 complex. Interestingly, PRMT6, responsible of H3R2 methylation, is frequently overexpressed in cancer cells. Based on these observations, we hypothesize that PRMT6 acts as a negative regulator of DNMT1 and that abnormal H3R2 methylation caused by PRMT6 upregulation contributes to global DNA hypomethylation in cancer. **Methods:** *Cells:* We used human HEK 293 cells and mouse embryonic stem (mES) cells to overexpress PRMT6 and human MCF-7 breast cancer cells for PRMT6 knockdown. *DNA methylation analyses:* Southern blots were performed using DNA digested with methylation sensitive enzymes and specifi labeled probes. DNA dot blot was performed with specific antibody. **Results:** Using HEK 293 cells we detect that PRMT6 overexpression leads to increased H3R2 methylation, as well as disassociation of UHRF1 from chromatin. We generate PRMT6-expressing mES cells clones to determine whether PRMT6 regulates DNA methylation. Our results indicate that overexpression of PRMT6 wild-type, but not of enzymatically inactive mutant, results in global loss of DNA methylation in mES cells. To determine whether there is a correlation between PRMT6 overexpression and global DNA hypomethylation in cancer, we analyzed the total 5-methylcytosine (5mC) level in cancer cell lines with different PRMT6 expression levels. Among them, MCF-7 and LNCaP, show the

highest levels of PRMT6 expression, which correlates with the lowest levels of 5mC. To confirm the effect of PRMT6 on DNA methylation, we performed PRMT6 knockdown to determine whether DNA methylation can be rescued. Our results indicate that shRNA-mediated PRMT6 downregulation indeed results in increase in the total level of DNA methylation in MCF-7 cells. **Conclusion:** Together, these preliminary results suggest that DNMT1 recruitment to chromatin is modulated by H3R2 methylation. Upregulation of PRMT6 may contribute to global DNA hypomethylation in cancer.

81 **CPRIT Grantee**
Poster Session A

Chromosome stability dependent on mammalian DNA polymerase θ (POLQ) *K. McBride, The University of Texas M.D. Anderson Cancer Center; Y. Mu, The University of Texas M.D. Anderson Cancer Center; K. Takata, The University of Texas M.D. Anderson Cancer Center; M . Yousefzadeh, The University of Texas M.D. Anderson Cancer Center; S. Hensley, The University of Texas M.D. Anderson Cancer Center; M .Zelazowska, The University of Texas M.D. Anderson Cancer Center; J. Plummer, The University of Texas M.D. Anderson Cancer Center; T. Gong, The University of Texas M.D. Anderson Cancer Center; R. Wood, The University of Texas M.D. Anderson Cancer Center*

Introduction: Mammalian DNA polymeraseθ (POLQ) participates in a process of "alternative" end joining of DNA double-strand breaks (DSB), including those caused by ionizing radiation. We found that Polq-null murine cells are selectively hypersensitive to DNA damaging agents that cause direct DSBs, and we are investigating the corresponding sensitivity of human cell lines. In addition to exogeneous DNA damaging agents, DSBs are deliberately created in immunoglobulin (*Ig*) genes during the B lymphocyte antibody diversification process of class switch recombination (CSR). Both classic and alternative non-homologous end-joining pathways participate in CSR that leads to antibody isotype switching. However, aberrant repair can also lead to chromosome translocations between the Ig locus and non-Ig loci such as the oncogenic *Myc/IgH* translocation. Our hypothesis is that POLQ plays an important repair role in repair in the presence of DNA damaging agents and during immunoglobulin diversity. **Methods:** We preformed biochemical analysis on PolQ function in vitro and analyzed wild-type and Polq null cells. **Results:** We found that 10-20% of junctions normally contain an insertion of more then 2 nucleotides. These insertions have homology to sequences within the *IgH* switch-region suggesting nearby DNA is used for templating. In the absence of POLQ no insertions were detected indicating that POLQ was the primary pathway responsible for insertions. Biochemical experiments with purifie human POLQ protein revealed the mechanism generating the insertions during DNA end joining. POLQ has a unique ability to extend DNA from minimally paired primers, facilitated by a tight grasp on the primer-terminus. There was also a marked increase in *Myc/IgH* translocations in Polq-defective mice, showing that POLQ suppresses genomic instability and genome rearrangements. **Conclusion:** This work defines important roles and a mechanism for POLQ in maintaining genome stability, generating diversity, and controlling radiosensitivity. POLQ expression in several human tumors types is negatively correlated with outcome, so that suppression of POLQ-dependent end joining may be a strategy for enhancing the effectiveness of radiotherapy

82 **CPRIT Grantee**
Poster Session B

IKKβ is an IRF5 Kinase that Instigates Inflammatio *J. Ren, The University of Texas Southwestern Medical Center at Dallas; X. Chen, The University of Texas Southwestern Medical Center at Dallas; Z .Chen, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Inflammation affects tumorigenesis by regulating its promotion, progression and immune surveillance. Interferon regulatory factor 5 (IRF5), a critical mediator of immune cell development and inflammatory response, has recently emerged as a potent tumor suppressor. IRF5 can induce apoptosis, arrest cell cycle and also regulate host immunity against cancer cells. However, the signaling pathways by which IRF5 contributes to antitumor immunity remain poorly understood. **Methods: Results:** We report that the kinase IKKβ, which is known to regulate the Rel/nuclear factor kappa B (NF-κB) family of transcription factors, phosphorylates IRF5 at a specific serine residue, and that this phosphorylation is critical for IRF5 activation and cytokine production. Thus, IKKβ regulates two master transcription factors, NF-κB and IRF5, which coordinately control gene expression to mediate inflammatory responses. **Conclusion:** These results provide insights into the underlying relationship between tumorigenesis and host anti-tumor immunity and suggest new therapeutic targets for cancer treatment.

83 **CPRIT Grantee Poster Session A**

Telomerase Reactivation In The Lgr5+ Cells Rescues Stem Cell Depletion And Extends Lifespan Through Suppressing The ER/UPR Stress Pathway *D. Chakravarti, The University of Texas M.D. Anderson Cancer Center; B. Hu, The University of Texas M.D. Anderson Cancer Center; A. Wang, The University of Texas M.D. Anderson Cancer Center; X. Su, The University of Texas M.D. Anderson Cancer Center; K. Dunner Jr., The University of Texas M.D. Anderson Cancer Center; R .DePinho, The University of Texas M.D. Anderson Cancer Center*

Introduction: Telomere shortening has been correlated with several aging related pathogenesis of the intestine like Crohn's disease and ulcerative colitis. Inflammatory bowel disease has been established as a significant risk factor for developing colon cancer. GWA studies have revealed ER stress related proteins play important role in the initiation of such inflammatory bowel diseases. Late generation (G4) of the telomerase deficient mice exhibit symptoms of inflammatory bowel disease and neoplasia of both the small and the large intestine by 6 months of age compared to the early generation counterpart (G0) and can be an excellent model to study the progression of inflammatory bowel disease to colon cancer development. Closer examination revealed an increase in ER stress protein expression in the stem cell compartment leading to premature differentiation of the stem cells to a more progenitor like population. Telomerase reactivation reverses the phenotype and extends the lifespan of these mice. **Methods:** In order to study the role of telomerase activation in the intestinal compartment we crossed the late generation telomerase deficient LSL-mTert animals to the tamoxifen inducible Lgr5-EGFP CreERT^2 model. In this model we could reactivate telomerase at desired time specifically in the intestinal stem cell compartment. We characterized the intestines with immunohistochemical analyses and electron microscopy. With the help of beta galactosidase lineage tracing experiments and BrDU incorporation assay we determined the proliferation rate and the cell turn over rates in these animals. We performed RNAseq and qPCR analysis. We also isolated crypts and performed organoid cultures from early and late generation of the animals. **Results:** Unexpectedly we found that the intestinal crypts from the G4 mice showed neoplastic lesions and crypt degeneration accompanied by high degree of immune infiltrates. Young G4 animals exhibit elevated rate of apoptosis, cell turn over and proliferation in comparison to the G0 mice. RNA-seq indicated upregulation of the ER phagosomal pathway and immune pathways. Increase in ER stress was further confirmed by electron microscopic analysis, IHC and qPCR. Interestingly reactivation of telomerase specifically in the Lgr5+ stem cells suppressed the ER/UPR pathway and reduced stem cell proliferation and loss through differentiation. This also led to the increased lifespan of these animals. **Conclusion:** In conclusion telomerase reactivation preserves stem cell loss by reducing ER stress and thereby extends lifespan.

84 **CPRIT Grantee Poster Session B**

Insights into the Regulation of MRN(X) ATPase Activity *R. Deshpande, The University of Texas at Austin; J. Lee, The University of Texas at Austin; T. Paull, The University of Texas at Austin*

Introduction: The Mre11-Rad50-Nbs1 (XRS2) (MRN/X) complex is important for DNA double strand break (DSB) repair and cell cycle checkpoint activation. The MRN/X complex is also involved in processing of DSBs during meiosis and telomere maintenance. In the DSB processing MRN(X) carries out end tethering, initiation of 5' end resection and recruitment of downstream processing complexes. The nuclease activity of Mre11 is essential for processing meiotic DSBs but generally dispensable for DSBs with clean ends whereas ATPase activity of Rad50 is essential for MRN/X DNA repair function. Significant advances were made in understanding its role in DSB processing and signaling in recent years. The catalytic head of the MRN/X complex comprised of the Mre11 nuclease and Rad50 ATPase active sites undergoes dramatic conformational changes on binding to ATP. In presence of ATP, Rad50 dimerizes through its ATPase catalytic domains forming two functional ATPase sites. This ATP-bound "closed" state promotes binding to DNA, tethering DNA ends, and ATM activation. The Mre11 nucleolytic center is inaccessible for DNA in this ATP-bound closed conformation, however. Separation of ATPase domain following ATP hydrolysis is important for subsequent nuclease activity of Mre11 and other downstream processing complexes. The rate of ATP hydrolysis by Rad50 is low compared to other ATPases and ATP-bound state is generally stable. Here we investigate the regulation of ATP hydrolysis as well as the importance of the two functional active sites. **Methods:** We performed in vitro assays for ATP hydrolysis, nuclease activity and ATM kinase activation function of the human MRN complex. **Results:** Using MR/MRN(X) complexes from Pyrococcus furiosus, budding yeast and humans, we find that linear DNA stimulates ATP hydrolysis by Rad50 over ~20 fold. DNA with either 3' or 5' overhangs and blunt ends showed similar stimulation. For the human complex, Nbs1 was observed to improve ATP hydrolysis by the MR complex, increasing the hydrolysis by 2 fold. We co-expressed ATPase catalytic site mutations such that a Rad50 heterodimer will result in only one functional site. This dramatically reduced the ATPase activity of Rad50 dimer. This heterodimeric complex with one functional site was also unable to activate ATM kinase in our in vitro assays, indicating both ATPase sites need to be functional. **Conclusion:** Our data shows that Rad50 ATPase activity is stimulated by linear dsDNA and functional ATPase sites are important MRN complex DNA repair function.

85 **CPRIT Grantee Poster Session A**

Pharmacokinetics and PET Imaging of novel tumor vascular targeting agents in Mice *S. Chiguru, The University of Texas Southwestern Medical Center at Dallas; R. Sharma, The University of Texas Southwestern Medical Center at Dallas; A. Schroit, The University of Texas Southwestern Medical Center at Dallas; E. Ward, Texas A&M University System Health Science Center; R. Mason, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Phosphatidylserine (PS) expressed on the luminal side of tumor vasculature has been identified as unique target for tumor therapy (Ran & Thorpe; Int J Radiat Oncol Biol Phys. 2002;54:1479-84). Tumor vascular targeting is attractive since local vascular damage may be associated with amplified downstream therapeutic efficac . Bavituximab, a first generation PS-targeting agent, is currently in phase 3 clinical trials. A goal of our CPRIT sponsored research is to modify mAbs to enhance targeting and improve their pharmacokinetics for potential diagnostic imaging. Here we evaluated a new tetramer of a PS-targeting antibody, 1N11-T, in mice with orthotopic breast tumors. **Methods:** Pharmacokinetic (PK), biodistribution and PET imaging investigations compared ^{125} or ^{124}I-labeled 1N11-T with ^{125} or ^{124}I-labeled bivalent wild type antibody, 1N11-WT. Initial PK studies used male SCID mice injected IV with ^{125}I-labeled 1N11-WT or 1N11-T (5 mice per group; 70-80 μCi/10 μg/mouse). To evaluate uptake in specific tissues, female SCID mice bearing orthotopically implanted MDA-MB-231 tumors (diameter ~5 mm) were injected i.v. with ^{125}I-labeled 1N11-WT or 1N11-T (3 mice per group; 80-90 μCi/60 μg/mouse) and analyzed at 24 and 48 hours. For the PET study, 1N11-T and 1N11-WT was directly labeled with Iodine-124 ($t_{1/2}$ = 4.18 days) yielding ^{124}I-1N11-T and ^{124}I-1N11-WT (RCY: 67%, RCP: >99% by ITLC, specific activity: 1.3 μCi/μg). Tumor bearing mice were injected with the radiolabeled antibodies (80-90μCi, 60μg antibody/mouse) and PET/CT was performed after 24 and 48 hours. After whole body imaging, tumor, the major organs, and blood were collected for direct comparative analysis. **Results:** Both whole body radioactivity and blood samples indicated more rapid clearance of the tetramer (half-life ~12 vs ~60 hours for 1N11-WT). Biodistribution indicated that all organs showed much lower uptake of tetramer, although the tumor to blood ratio was improved at 48 hours with values of 0.62 and 0.48 for 1N11-T and 1N11-WT, respectively. PET/CT data acquired at 24 and 48 hours post-injection revealed a tumor to heart signal ratio of 0.5 at both 24 and 48 hours for 1N11-WT and 0.7 and 1.0 for 1N11-T. **Conclusion:** ^{124}I-1N11-T showed more rapid vascular clearance and therefore better tumor contrast than the ^{124}I-1N11-WT. These results demonstrate proof of principle for successful PET imaging of mAb targeting orthotopic breast tumor in mice and serve as a foundation for examining additional PS targeting agents. Enhanced targeting may improve specificity and therapeutic efficac , while faster clearance may be crucial for optimizing potential diagnostic imaging agents.

86 **CPRIT Grantee Poster Session B**

NPSD4: New Player in the DNA Damage Response *E. Atkinson, The University of Texas M.D. Anderson Cancer Center; B. Wang, The University of Texas M.D. Anderson Cancer Center*

Introduction: Improper repair of damaged DNA or compromised repair signaling often leads to genomic instability, which is a hallmark of cancer. During the DNA damage response (DDR) signaling cascade, post-translational modifications play an important role in the recruitment and regulation of repair factors. For example, in response to damage, the ATM kinase transduces a signaling cascade by phosphorylating many DDR proteins. Another posttranslational modification, SUMO (Small Ubiquitin-like Modifier) has been shown to be important in DSB repair, with many repair proteins being SUMOylated. Through proteomic and mass spectrometry analyses of proteins involved in SUMOylation signaling induced by DNA damage, we have identified an uncharacterized protein that is recruited to sites of DNA damage that we have named New Player in SUMO dependent DNA damage repair 4 (NPSD4). NPSD4 has a potential ATM phosphorylation site and two SUMO interacting motifs (SIMs), making it a candidate SUMO regulated DDR protein. M**ethods:** We performed GST pulldown assays, mass spectrometry, and immunoprecipitation to identify NPSD4 interacting partners. We created point mutations in the SIMs and mutated the ATM phosphorylation site. We used live cell imaging to analyze NPSD4 recruitment to DNA damage sites and NPSD4 localization was evaluated by immunofluorescence. We generated NPSD4 knockdown cells and performed a DNA fiber assay to evaluate the effect of replication stress. **Results:** NPSD4 interacts with SUMO2/3 and this interaction requires the SIMs. NPSD4 forms nuclear foci that colocalize with PML nuclear bodies (NBs) and Alternative Lengthening of Telomeres (ALT) associated PML NBs, indicating that it may play a role in telomere maintenance in cancers. Nuclear foci formation is also SIM dependent. NPSD4 is recruited to DNA damage laser tracts but this recruitment is not dependent on the SIMs or the ATM phosphorylation. Following CPT induced replication stress, NPSD4 knockdown cells are defective in recovery from replication fork stalling. We have also confirmed NPSD4 interaction with proteins involved in DDR and replication. **Conclusion:** These results indicate that NPSD4 is an important player in the SUMO signaling involved DNA damage repair for maintaining genomic stability. As genomic instability contributes heavily to tumor development, the DDR must be better understood in order to develop treatments that specifically target tumor cells, capitalizing upon the defects they already exhibit in DDR and to understand how tumorigenesis occurs. Further study is needed to identify the mechanism by which NPSD4 functions in the maintenance of genomic stability.

87 **CPRIT Grantee Poster Session A**

A HER3-LncRNA-MST1 Module Links Hippo-YAP Pathway to Osteolytic Bone Metastasis *C. Li, The University of Texas M.D. Anderson Cancer Center; S. Wang, The University of Texas M.D. Anderson Cancer Center; Z. Xing, The University of Texas M.D. Anderson Cancer Center; A. Lin, The University of Texas M.D. Anderson Cancer Center; K. Liang, The University of Texas M.D. Anderson Cancer Center; J. Song, The University of Texas M.D. Anderson Cancer Center; Y. Zhang, The University of Texas M.D. Anderson Cancer Center; Q. Hu, The University of Texas M.D. Anderson Cancer Center; J. Yao, The University of Texas M.D. Anderson Cancer Center; P. Park, The University of Texas M.D. Anderson Cancer Center; D . Hawke, The University of Texas M.D. Anderson Cancer Center; H. Liang, The University of Texas M.D. Anderson Cancer Center; G. Gallick, The University of Texas M.D. Anderson Cancer Center; M. Hung, The University of Texas M.D. Anderson Cancer Center; C. Lin, The University of Texas M.D. Anderson Cancer Center; L. Yang, The University of Texas M.D. Anderson Cancer Center*

Introduction: Bone metastasis is a nearly universal feature of patients with advanced breast, lung, and prostate cancers. Considering the limited availability of known therapeutic targets as well as resistance to current targeted therapies, there is a serious need for the development of new anti-bone metastatic targets and agents in preclinical metastasis models. Despite the high degree of conservation of the Hippo-YAP pathways between Drosophila and mammals, the upstream signaling events of Hippo activation, particularly the regulation of the core kinase of the Hippo pathway, MST1/2, have yet to be explored. **Methods:** To identify potential long noncoding RNAs (lncRNAs) that might be involved in the regulation of the YAP signaling pathway, we screened the human siRNA library. To investigate the clinical relevance of the lncRNA, we searched the expression pattern in the TCGA database and examined the expression level in breast cancer and lung cancer tissues using RNAscope 2.0 technology. We examined the role of MAYA in primary osteoclast differentiation and in breast and lung cancer bone metastasis by using an experimental metastasis animal model. To identify the underlying mechanism of lncRNA involvement in Hippo signaling regulation, we performed an RNA pull-down assay followed by mass spectrometry to identify proteins that are potentially associated with lncRNA. **Results:** We identified a lncRNA, named MAYA (MNX1-AS1), that is required for YAP target gene activation and that is dramatically upregulated in human breast cancer and lung cancer tissues. We found that which suggests the potential role of MAYA in the bone metadstasis of breast cancer. Depletion of MAYA inhibited tumor cell-elicited osteoclast differentiation; the effects could be rescued by overexpression of MAYA or by the addition of recombinant CTGF. We also found that MAYA is required for bone metastasis of both breast and lung cancers in the mouse xenographic model. We also found that upon neuregulin stimulation, ROR1 phosphorylated HER3 at Tyr1307, independently of ERBB family members, and also phosphorylated LLGL2 (Tyr499); the phosphorylated LLGL2 resulted in the recruitment of both MAYA and MAYA-bound methyltransferase NSUN6 which methylates MST1, thus abolishing MST1 kinase activity and activating expression of YAP1 target genes. Importantly, high expression levels of phospho-HER3 (Tyr1307) and MAYA are dramatically correlated with cancer aggressiveness and poor patient outcomes. **Conclusion:** Our data comprehensively dissect the upstream signaling pathway that regulates MST1/2 in mammals, establishes the connection between the Hippo-YAP pathway and bone metastasis, and identifies promising therapeutic targets for breast and lung cancer bone metastasis.

88 **CPRIT Grantee Poster Session B**

Perfusion Correlated Heterogeneity in NSCLC Patient Glucose Metabolism *R. Lenkinski, The University of Texas Southwestern Medical Center at Dallas; C. Hensley, The University of Texas Southwestern Medical Center at Dallas; E. Jin, The University of Texas Southwestern Medical Center at Dallas; N. Lev-Cohain, Hadassah Medical Center; Q. Yuan, The University of Texas Southwestern Medical Center at Dallas; K. Kernstine, The University of Texas Southwestern Medical Center at Dallas; C. Malloy, The University of Texas Southwestern Medical Center at Dallas; R. DeBerardinis, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The results of CT screening of high risk subjects for lung cancer has prompted a great deal of interest in the biological characteristics of these tumors. The "Warburg Effect," an enhancement of conversion of glucose into lactate upon cellular transformation, is thought to be a hallmark of malignancy and the molecular basis of the clinical utility of FDG-PET to distinguish benign from malignant nodules. Our own preliminary studies into the imaging and metabolism of these tumors has shown intra and intertumoral heterogeneity. We undertook the present study to systematically investigate this heterogeneity both at the level of macroscopic imaging as well at the level of genomics, metabolomics as well as glucose metabolism using C-13 labelling. Here we report our findings on a preliminary cohort of patients. M**ethods:** We employed pre-operative imaging, primarily MRI using Dynamic Contrast Enhanced (DCE) MRI as a surrogate for perfusion and Diffusion Weighted MRI (DWI) as a surrogate for cellularity to determine the heterogeneity of each mass. In each patient we employed [U-^{13}C] glucose infusions prior to lobectomy, followed by metabolic analysis of tumor and non-cancerous lung. Tissue samples were resected in the operating room using the MR images as a guide for tissue procurement. The tissue samples were immediately frozen in liquid nitrogen for subsequent metabolic analysis. In each patient, resected tissue samples were analyzed for ^{13}C enrichment in metabolites in both oxidative and glycolytic pathways by the complementary methods of GC-MS and NMR. **Results:** We found that in this initial patient cohort, the tumor glycolytic enhancements relative to non-cancerous lung ("relative fractional enrichment" as determined by GC-MS) were highly variable in magnitude. Additionally, these glycolytic enhancements were directly correlated to glucose-derived TCA cycle activity (as assessed by citrate M+2 by GC-MS), potentially providing evidence that these tumors can "switch" models from oxidative glucose metabolism to glycolysis upon transformation. Surprisingly, glucose-derived TCA cycle activity, as assessed by both GC-MS fractional enrichment and NMR via glutamate C4, was inversely correlated with pre-operative perfusion assessed as time to and value of maximum contrast enhancement of the DCEMRI. Finally, we found GC-MS evidence for regional intratumoral heterogeneity in glucose metabolism. **Conclusion:** These results necessitate considerations of tumoral metabolic heterogeneity during experimental design in the fiel of cancer metabolism of basic and translational studies in primary human tumors. In human NSCLC, perfusion may be a significant determinant of both oxidative and non-oxidative glucose-derived metabolism.

89 **CPRIT Grantee**
Poster Session A

Role of Long Non-Coding RNAs in Breast Cancers: Identification, Characterization, and Determination of Molecular Functions
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Introduction: Long noncoding RNAs (lncRNAs) are emerging as key regulators of diverse cellular processes, but their roles in breast cancer biology are just beginning to be elucidated. Recent studies have suggested a key role for specific lncRNAs in regulating the expression of protein coding genes, ultimately controlling cell growth and development of cancer cells, but the mechanistic details of this regulation are not completely clear. Many fundamental questions about lncRNAs remain, such as the number of lncRNAs expressed in a given cell type, their role in controlling cellular functions, and how they control cell proliferation. **Methods:** In our current study, we have developed a novel genomic and computational pipeline for the discovery and annotation of lncRNAs that combines the power of global run-on sequencing (GRO-seq) to identify active transcription units across the genome with RNA-seq, which provides information about mature lncRNA structures. Further, we used "Guilt-by-association" analyses to predict the molecular functions of breast cancer-associated lncRNAs, which revealed likely roles in gene expression, DNA replication, and the cell cycle. Based on these analyses, we selected lncRNAs with a variety of properties (e.g., nuclear vs. cytoplasmic; estrogen-regulated vs. unregulated; relatively stable vs. unstable) for functional analyses. **Results:** The pipeline has yielded a comprehensive catalog of >1900 polyadenylated lncRNAs in MCF-7 cells, about half of which have not been annotated previously and about a quarter of which are estrogen-regulated. Analysis of RNA-seq data from samples representing 13 different cancer and normal tissue types, revealed that many lncRNAs are exclusively expressed in breast cancer cell lines. In functional assays, we have begun to explore the molecular functions of two estrogen-regulated lncRNAs, lncRNA152 (downregulated by estrogen) and lncRNA67 (upregulated by estrogen), in cell cycle control in breast cancer cells. The expression of both lncRNAs is elevated in breast cancers. siRNA-mediated knockdown of lncRNA152 or lncRNA67 inhibits the growth of ERα+ breast cancer cells. In addition, siRNAmediated knockdown of lncRNA152 or lncRNA67 alters the expression of a set of cell cycle control genes and promotes the accumulation of the cells in G1. The gene sets regulated by lncRNA152 or lncRNA67 are enriched for genes with binding sites for the cell cycle transcription factor E2F4 in their promoters. **Conclusion:** Collectively, our results support a model in which lncRNA152 controls the basal growth of breast cancer cells, while lncRNA67 controls estrogen-stimulated mitogenic growth.

91 **CPRIT Grantee**
Poster Session A

Single-Cell Biopsy And Characterization
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Introduction: Alterations in single-cell characteristics (e.g., gene expression profiles and biophysical features) can disrupt homeostasis leading to clonal cancer cell growth. Hidden heterogeneity within these tumors can be the source of malignant progression, recurrence and acquired resistance to therapy. In addition to examining cellular heterogeneity, developing non-invasive diagnostic and prognostic strategies using single-cell liquid biopsies will facilitate active monitoring of disease progression. To fill these needs, we have acquired single-cell isolation and analysis platforms and developed methodologies to interrogate single-cell genomic and phenotypic characteristics, which have been integrated in the Bioanalytics And Single-cell Core (BASiC) facility. **Methods:** Single cell isolation platforms in BASiC include immunofluorescence-micromanipulator workstations and a DEPArray unit, which can also be used for biomarker identification. As examples for single cell characterization technologies available at BASiC, the Biomark microfluidic PCR system is used for single-cell RNA expression and atomic force microscopy (AFM) for biophysical and cell surface expression patterns. **Results:** Three studies are highlighted here to illustrate the utility and versatility of the BASiC platforms for clinical sample analysis and translation studies. First, cellular heterogeneity of exfoliated cells in urine was examined to detect and characterize prostate tumor cells. Single-cell expression patterns revealed bimodal expression patterns in candidate genes with two distinct peaks compared to other unimodal genes, which showed a continuous Gaussian expression distribution in primary tumors. Second, we examined the effects of epidermal growth factor (EGF) on the induction of epithelial-mesenchymal-transition (EMT) in endometrial cancer cells, which was associated with increased invasiveness. AFM analysis revealed enhanced elasticity and decreased adhesiveness in EGF treated cells and a progressive decrease of the epithelial cell adhesion molecule (EpCAM) on the cell surface. Third, we have examined cell network communication through gap junction channels formed by connexins in endometriosis, which results in inflammatory and highly invasive endometriotic lesions in the pelvic cavity. Studies using the DEPArray and Biomark platforms demonstrated corresponding altered connexin expression profiles in primary endometrial single cells at the RNA and protein levels. These profiles corresponded with increased endometriotic invasiveness and aberrant gap junction cell network communication using in vitro assays. **Conclusion:** Our studies present examples for the utility of single cell analysis using our BASiC platforms in detecting and characterizing exfoliated cells in urine samples, characterization of EMT at the level of nanophysical characteristics of individual endometrial cancer cells, and examining gap junction networking in primary endometrial cells involved in endometriosis from laparoscopic biopsy.

92 **CPRIT Grantee Poster Session B**

Zebrafish Modeling of PAX3-FOXO1 Driven Rhabdomyosarcoma

G. Kendall, The University of Texas Southwestern Medical Center at Dallas; J. Amatruda, The University of Texas Southwestern Medical Center at Dallas

Introduction: Rhabdomyosarcoma is the most common soft tissue sarcoma in children, and is associated with a misregulation of skeletal muscle developmental pathways. Of the two histological subtypes, embryonal (ERMS) and alveolar (ARMS), ARMS is more aggressive and prone to metastases. The majority of ARMS cases have a defining t(1;13) or t(2;13) chromosomal translocation, which fuses the DNA binding domain of PAX7 or PAX3 with the transactivation domain of FOXO1, creating a transcriptionally active chimeric protein. Clinically, the presence of PAX3-FOXO1 predicts reduced overall survival, yet the underlying mechanisms for this are not clear. Here, we detail the development and study of PAX3-FOXO1 transgenic zebrafish model systems to characterize the underlying biology of ARMS and utilize this model for translational applications. **Methods:** We are using transgenic zebrafish models to interrogate how human PAX3-FOXO1 initiates and drives ARMS, including susceptible cell lineages, targeted signaling pathways, induced DNA mutations, and relevant cooperating mutations. **Results:** Using the UAS/GAL4 system we assessed developmental phenotypes of UAS driven PAX3-FOXO1 in 26 GAL4 transgenic zebrafish lines. We found that PAX3-FOXO1 inhibits somitogenesis or induces cyclopia depending on targeted cell type(s), and selected 10 GAL4 lines to determine each lineage's tumorigenic capacity. Resulting tumors will be characterized by high-resolution genomic analyses to facilitate cross-species comparison with human ARMS. In parallel, we found that mosaic expression of heterologous PAX3-FOXO1, but not PAX3, induces embryonic cyclopia, and is tumorigenic in adult zebrafish. Transient inhibition of early PAX3-FOXO1 expression eliminates cyclopia, and in preliminary experiments prevents tumors in adult zebrafish, indicating that the developmental timing of oncogene expression is likely critical to tumor formation. To identify these PAX3-FOXO1 modulated developmental pathways, we sorted cells expressing PAX3 or PAX3-FOXO1 from embryos, and found distinct gene expression signatures with new, potential PAX3-FOXO1 targets and implicated signaling pathways. **Conclusion:** By performing a cross-species comparative oncology analysis of zebrafish and human ARMS, this approach aims to pinpoint the most important ARMS genetic drivers, and the mechanisms by which they promote disease pathology. Overall, an ARMS zebrafish model with embryonic phenotypes that predict tumorigenesis has applications ranging from studying ARMS biology to translational in vivo drug discovery.

93 **CPRIT Grantee Poster Session A**

Twist1 Regulates Keratinocyte Stem Cell Proliferation and Is Required For Skin Tumor Formation

J. Srivastava, The University of Texas at Austin; J. DiGiovanni, The University of Texas at Austin

Introduction: Currently, little is known about the role that Twist1 plays in epithelial carcinogenesis. Twist1 is a basic helix-loop-helix transcription factor and is known to play a role in tumor progression through regulation of genes involved in epithelial-mesenchymal transition. Twist1 is a known transcriptional target of Stat3. In previous studies, Stat3 was shown to play a role in skin tumor progression in the two-stage skin carcinogenesis model, at least in part, by regulating the levels of Twist1. Studies using keratinocyte-specific knockout (KO) of Twist1 indicate that Twist1 regulates keratinocyte proliferation during skin tumor promotion. **Methods:** In order to investigate the role of Twist1 on skin tumor progression, Western blotting, polymerase chain reaction, cellular imaging and cell cycle analysis were used to analyze tissue and cells collected from BK5.Cre x Twist1$^{flox/fl}$ mice. **Results:** Western blot analysis of lysates from both Twist1 deficient mouse primary keratinocytes and from epidermis isolated from BK5.Cre x Twist1$^{flox/fl}$ mice indicated that Twist1 KO leads to reduced levels of the cell cycle proteins Cyclin E1, E2F1, and Cdk2 and increased expression of p21 following treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Moreover, Twist1 KO in keratinocytes impeded cell cycle progression by reducing the number of cells that advanced to S-phase. Further analyses demonstrated that Twist1 regulates the levels of p21 in keratinocytes through a mechanism that involves stabilization of nuclear p53 in both primary keratinocyte and in vivo models. Keratinocyte specific deletion of Twist1 in vivo suppressed epidermal proliferation induced by TPA treatment compared to that observed in wild-type (WT) mice. Furthermore, keratinocyte specific deletion of Twist1 *in vivo* led to a significant reduction in the number of label-retaining cells as well as the number of α6-integrin^{+}/CD34^{+} cells in the hair follicles of untreated mice. A two-stage skin carcinogenesis experiment showed that BK5.Cre x Twist1$^{flox/fl}$ mice have significantly reduced tumor development as observed by decreased tumor multiplicity and delayed latency compared to WT mice. Furthermore, tumors that developed in BK5.Cre x Twist1^{flox} ^{flo} mice have significantly reduced size compared to tumors on W mice. Initial experiments utilizing UVB as a promoting agent further validate the role of Twist1 as a regulator of cellular proliferation during UVB-induced skin tumor promotion. Specificall , BK5.Cre x Twist1$^{flox/fl}$ mice exhibited heightened sensitivity to UVB-induced apoptosis as compared to WT mice. **Conclusion:** These findings suggest that Twist1 has a novel role in epithelial carcinogenesis by regulating proliferation and migration of keratinocytes and keratinocyte stem cells.

94 **CPRIT Grantee**
Poster Session B

Nuclear Proteolysis in Multiple Myeloma *A. Catic, Baylor College of Medicine*

Introduction: The recruitment of transcription factors to promoters and enhancers is a critical step in gene regulation. This interaction is dynamic to allow for continuous adjustment of expression. While the binding of transcription factors to DNA is a topic of intense investigation, relatively little is known about how these complexes are removed. The ubiquitin-proteasome system is the main pathway to eliminate nuclear proteins. We previously devised a method to detect genomic locations that are associated with protein turnover. **Methods:** Multiple myeloma, the second most common hematopoietic malignancy, has become a model disease for drugs which interfere with the ubiquitin-proteasome system through either blocking or facilitating protein elimination. Our research is focused on defining how proteolysis regulates transcription in this disease. The proteasome inhibitor Velcade, for instance, has become first-line treatment in myeloma. Yet, it is unknown how myeloma cells are killed by this drug and how it affects transcriptional dynamics. **Results:** We are utilizing next generation sequencing to identify sites of nuclear protein turnover and to isolate transcription factors that may qualify as more specific targets for treatment compared to blunt proteasome inhibition. Our findings reveal that certain transcription factors are particularly sensitive to Velcade. These involve regulators involved in metabolism and cell growth. We are currently validating the mechanism of their degradation and the impact this has on myeloma proliferation. **Conclusion:** This research project will contribute to our understanding of genomic and epigenomic dynamics in multiple myeloma. With the focus on gene programs that are continuously adapting or changing, we seek to unlock new targets for molecular therapy.

95 **CPRIT Grantee**
Poster Session A

Identification of siRNAs That Have Selective Toxicity for Non-Small Cell Lung Cancer (NSCLC) *R. Carstens, The University of Texas Southwestern Medical Center at Dallas; L. Li, The University of Texas Southwestern Medical Center at Dallas; K. Huffman, The University of Texas Southwestern Medical Center at Dallas; S. Wei, The University of Texas Southwestern Medical Center at Dallas; B. Posner, The University of Texas Southwestern Medical Center at Dallas; D. Mangelsdorf, The University of Texas Southwestern Medical Center at Dallas; J. Minna, The University of Texas Southwestern Medical Center at Dallas*

Introduction: We designed and implemented a user-friendly small scale siRNA functional screen to identify new therapeutic targets that kill lung cancer cell lines but not normal human bronchial epithelial cells (HBECs). **Methods:** We tested a "mini-library" of siRNA pools (4 siRNAs per pool) targeting the 48 nuclear receptor superfamily (NRs), and 72 of their associated co-regulators (CoRegs) (120 genes, 480 unique siRNAs). Transfection conditions were optimized for all cell lines and the siRNA pools (Qiagen) were tested for their ability to inhibit the growth of 64 NSCLC lines without affecting 6 different immortalized normal HBECs. To validate results, we tested an independent "esiRNA" mini-library targeting the 120 genes. All screen results (9 replicates per cell line) were highly reproducible (r = 0.86). **Results:** We followed-up by identifying siRNA pools that selectively killed lung tumor lines without killing HBECs and noted that response phenotypes varied among the different tumor lines. Detailed validation studies demonstrated that while the target in each case was being knocked down, the survival/growth phenotype was not related to this knockdown. Observations include: inability to "rescue" the phenotype with a mutated "C911" siRNA control (detects on-target hits); lack of phenotypic correlation between esiRNA and siRNA library results (both knocked down the intended gene); and expression studies showing that with each siRNA transfection, more than 150 genes were down-regulated (including intended target). We identified 15 siRNA pools that killed subsets of tumor lines but did not kill HBECs and tested the 60 individual siRNAs from these pools on 22 lung cancer lines. For each siRNA, we also tested two "mutated" versions of that siRNA; one that would allow identification of on-target effects (C911 control) and one identifying potential miR-like or "seed sequence" effects (CSeed). Of the 60 individual siRNAs, 25% killed at least some lung cancer cells, while 9% C911 controls and 45% CSeed controls "rescued" the slow-growth/ kill phenotype. C**onclusion:** Although we initiated this project looking for vulnerabilities targeting NRs or associated CoRegs, we have identified a large panel of siRNAs which kill a subset of lung cancers but not normal lung epithelial cells. In many cases, these effects stem from "off target" miR-like seed sequence effect. Moreover, the lung cancers exhibit a large degree of heterogeneity in their response to these siRNAs. Regardless of target gene(s), these siRNAs represent new specific therapies for lung cancer.

96 **CPRIT Grantee**
Poster Session B

The Novel Role of 11β-Hydroxysteroid Dehydrogenases in Non-Melanoma Skin Cancer *A. Mancha-Ramirez, The University of Texas Health Science Center at San Antonio; H. Liang, The University of Texas Health Science Center at San Antonio; J. Junco, Baylor College of Medicine; T. Slaga, The University of Texas Health Science Center at San Antonio*

Introduction: Glucocorticoids (GCs) are used as a treatment for an array of cancers, with their main anti-cancer mechanism of action being GR-mediated mechanisms triggering cell death. Isozymes 11β-hydroxysteroid dehydrogenase 1 and 2 (11βHSD1/11βHSD2) elicit their effect on GCs at the prereceptor level by controlling the activation or deactivation of GCs in a tissue-specific manner. Significant evidence suggests that 11βHSD2 may play a role in the multifactorial process of tumorigenesis, as many studies have shown that it is expressed in tumors and malignant cells, and not expressed in the respective normal counterparts. Despite the growing body of evidence supporting a role for 11βHSDs in various different types of cancer, its role in skin cancer development and progression has not yet been evaluated. As topically administered GCs are a mainstay in the treatment of non-melanoma skin cancer (NMSC), we aim to determine the roles of 11βHSD1 and 11βHSD2 in regulating GCs during non-melanoma skin cancer development. **Methods:** In vitro experiments were carried out utilizing anchorage-independent transformation assays, 18β-Glycyrrhetinic acid (GA) and tumor promoting compound TPA drug treatments, western blotting, and transient transfection (electroporation). For in vivo work, FVB mice were subjected to both chemically and UVB induced carcinogenesis and epidermal tissue samples were collected for western blot analysis. **Results:** Our in vitro studies thus far show considerable evidence that 11βHSD2 is increased in TPA treated pre-neoplastic P+ cells as well as in basal levels of transformed RT101 cells. Additionally, sulfiredoxin (SRX), a well-established transformation biomarker in this model, is also increased with TPA treatment. Preliminary experiments with potential HSD2 inhibitor, glycyrrhetinic acid (GA), have shown inhibition of HSD2 expression and TPA-induced colony formation in soft agar. Results from two pilot in vivo studies, one utilizing chemically-induced carcinogenesis and the other utilizing UV-induced, suggest the same trend as seen in in vitro studies, where 11βHSD2 is increased with TPA or UVB treatment vs. untreated counterparts. **Conclusion:** These experiments suggest that the role of 11βHSDs in NMSC may be

of significant importance. Currently, 11βHSD2 knockdown experiments are underway to determine the integral role of these enzymes in the transformation of NMSC. We are also performing activity assays to determine if there is a corresponding increase in 11βHSD2 activity in cancer tissues vs. normal counterparts. Modulation of cell-specifi 11βHSD2 expression and activity can be extremely efficacious as it may diminish GC resistance and even serve as a potential target to aid in prevention of skin cancer development and progression.

97 **CPRIT Grantee**
Poster Session A

Engineering of a Human Asparagine-degrading Enzyme for Cancer Treatment *G. Agnello, The University of Texas at Austin; C. Chrysostomou, The University of Texas at Austin; E. Stone, The University of Texas at Austin; G. Georgiou, The University of Texas at Austin*

Introduction: Bacterial asparaginases (ASNases) are a front-line chemotherapy for acute lymphoblastic leukemia (ALL). These enzymes catalyze the deamination of L-Asparagine (L-Asn) and L-Glutamine, and ALL cells are highly sensitive to L-Asn depletion due to low levels of Asparagine sythetase (ASNS). Despite the demonstrated benefit of ASNases on ALL survival, these drugs can have significant side effects which limit their use. Allergic reactions and antibody formation can limit dosing and efficac , and often lead to change in the ASNase form used. Human proteins are less immunogenic than their heterologous counterparts as natural tolerance prevents recognition by the immune system. We previously reported the bacterial expression and enzymatic characterization of the human asparaginase-like protein 1 (hASRGL1), a mammalian enzyme exhibiting beta-aspartyl peptidase activity as well as ASNase activity (Cantor, Stone et al. 2009). **Methods:** To optimize the catalytic and pharmacological properties of hASRGL1 we implemented random mutagenesis strategies followed by a competitive culture screening method and Next-Generation Sequencing (NGS) analysis. **Results:** Assessment of *in vitro* cytotoxicity in truly ASNS negative ALL cell lines, revealed an IC_{50} of ~2-10nM depending on the cell line, the hASRGL1 variant used and duration of treatment. The results from these *in vitro* studies will allow defining the dosage of enzyme therapeutics to test in *in vivo* mouse xenograft models to evaluate the ability of hASRGL1 to prevent the growth of human tumors. **Conclusion:** We expect that the catalytic and pharmacological properties of hASRGL1, combined with low immunogenicity will provide a superior chemotherapeutic agent for the treatment of L-Asn-dependent tumors.

98 **CPRIT Grantee**
Poster Session B

Musashi1-SERBP1: New Partners In Glioblastoma Development *P. Araujo, The University of Texas Health Science Center at San Antonio; L. Penalva, The University of Texas Health Science Center at San Antonio*

Introduction: Musashi1 (MSI1) is a highly conserved RNA-binding protein (RBP) expressed during embryonic development as well as in adult stem cells and precursors. In the context of tumorigenesis, Msi1 has emerged as a key oncogenic factor in numerous solid tumors, including glioblastoma (GBM). Recently, we conducted a global analysis of MSI1 target genes and determined the main routes by which it influences GBM phenotypes. Our findings suggest that MSI1 is a central regulator of cell adhesion pathways which contribute to GBM by influencing cell adhesion, morphology, migration, and invasion. **Methods: Results:** Next, we sought to identify potential Msi1 partners via yeast-two hybrid screening. The top candidate identified was Serpine1 mRNA-binding protein (SERBP1). We later validated MSI1-SERBP1 interaction using pull-down and Bio-ID approaches and showed that MSI1 first RNA-binding domain and C-terminal region are responsible for the interaction with SERBP1. Likewise MSI1, SERBP1 is overexpressed in several types of cancer, including GBM. Interestingly, SERBP1 also controls biological processes important for GBM development. Upon SERBP1 knockdown in GBM cells, we observed changes in the cell cycle, an increase in apoptosis, as well a reduction in proliferation, migration, and viability. We hypothesize that Msi1 and SERBP1 are part of a RNA operon that, together, co-regulate the expression of mRNAs important for glioblastoma development. In fact, we have already established that MSI1 and SERBP1 co-regulate Serpine1, PDGFRα and EGFR genes. CLIP analysis will be performed to determine the extension of this partnership. Lastly, we have gathered some evidence that MSI1 and SERBP1 expression is co-regulated. RNA expression data from GBM samples deposited into The Cancer Genome Atlas (TCGA) showed a positive correlation of Msi1 and SERBP1 expression. We also determined that both Msi1 and SERBP1 are repressed by two tumor suppressor microRNAs, miR-128 and miR-137. Funding support: CPRIT RP 140105. **Conclusion:**

99 **CPRIT Grantee**
Poster Session A

Study of a Chromatin Remodeler in Medulloblastoma Progression and Metastasis *V. Gopalakrishnan, The University of Texas M.D. Anderson Cancer Center; R. Tao, The University of Texas M.D. Anderson Cancer Center; T. Dobson, The University of Texas M.D. Anderson Cancer Center; S. Shaik, The University of Texas M.D. Anderson Cancer Center; R. Hatcher, The University of Texas M.D. Anderson Cancer Center; E. Busschers, The University of Texas M.D. Anderson Cancer Center; P. Taylor, The University of Texas M.D. Anderson Cancer Center; J. Fangusaro, Northwestern University; R. Lulla, Northwestern University; S. Goldman, Northwestern University; V. Rajaram, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Medulloblastoma (MB) is a poorly differentiated cerebellar tumor and the most common pediatric brain malignancy. Current treatments including surgery, radiation and chemotherapy have led to improved short-term survival in approximately 75% of patients. Unfortunately, a number of patients recur and or have metastatic disease, and cannot be cured. The goal of our research is to understand the underlying molecular mechanisms and identify novel drivers of MB metastasis. We have focused on studying the role of the chromatin remodeler called Repressor Element-1 Silencing Transcription Factor (REST), a known regulator of neurogenesis, in promoting MB metastasis. **Methods:** The contribution of REST to MB metastasis was evaluated using our novel genetically engineered mouse model in which REST transgene (Tg) expression can be conditionally elevated in cerebellar granule neuron progenitors (GNPs-the cells of origin of a subset of MB) in the context of constitutive sonic hedgehog-expression-RTS mice. Results were validated in xenograft models of isogenic high-REST and low-REST MB cells. Kaplan Meier curves were generated to measure survival and tumor latency. Tumor cell proliferation was studied by neurosphere assay in vitro and immunohistochemistry (IHC) in vivo. Tumor cell migration and invasion was assessed by scratch tests, trans-well migration assays and IHC. Tumor vasculature was evaluated by tube formation assay as well as IHC. Tumor cell signaling was evaluated by PCR, Western blotting and chromatin immunoprecipiattion assays. **Results:** Following transgene induction, RTS exhibited a sharply decreased survival (10-40 days) compared to mice with REST Tg (RT) expression alone (4-6 months) or mice with constitutive Shh activation (Ptch+/-) (6-9 months).

Hemotoxylin-eosin staining and IHC of brain sections from RTS mice revealed significant leptomeningeal disease (metastasis), associated with infiltrative and highly vascularized tumors compared to Ptch+/- mice. Interestingly, RT mice also exhibited increased vasculature in the cerebellum and deranged GNP migration. Furthermore RTS tumors showed highly proliferative and poorly differentiated tumors compared to tumors in Ptch+/-. These findings were validated by in vitro assays using purified GNPs and high-REST and low-REST MB cells. Further molecular analyses revealed REST-dependent activation of the PI3kinase/Akt signaling cascade, a known mediator of metastasis in RTS tumors. **Conclusion:** Together, our data are the first to demonstrate a role for the REST chromatin-remodeling complex in promoting MB progression and metastasis and in activation of Akt signaling. Our studies also lay the foundation for targeting the REST complex for the treatment of metastatic MB.

100 **CPRIT Grantee Poster Session B**

Control of Overall Stem Cell Numbers in a Living Epithelial Bilayer *E. Sumner, The University of Texas M.D. Anderson Cancer Center; G. Eisenhoffer, The University of Texas M.D. Anderson Cancer Center*

Introduction: Epithelial bilayers provide a protective coating for all organs of the body. In order to maintain barrier function, cell renewal and turnover must involve similar numbers of cell division and death. Extrusion aids in this process by removing excess or defective cells from the tissue and closing the gap left behind to prevent a loss of barrier function or piling up of cells. Yet, how extrusion promotes replacement of lost cells is not well understood. Alterations in cell turnover can underlie human diseases, including cancer. Here we investigate maintenance of cell numbers and the ability to sustain a functional barrier by perturbing the stem cell population in a living epithelial tissue. **Methods:** Zebrafis have a bilayered epithelium that is molecularly and organizationally similar to epithelial coating of mammalian organs. We identified a GAL4 enhancer trap line expressed in a subset of basal epithelial cells, allowing us to visualize specific cells and target cells for apoptosis. An assay for selective ablation of a subset of p63 positive stem cells was developed using a genetically encoded enzyme to induce DNA damage only when the corresponding prodrug is added. Chemical inhibitors were used to interrogate the damage response when molecular pathways regulating extrusion, division, or death are altered. Apoptosis and proliferation were then quantified during the damage response and the return to homeostasis. **Results:** Live imaging and fixed tissue analysis revealed elimination of apoptotic stem cells by extrusion through the apical epidermis. Cell movement was observed in the basal layer to accommodate intra and interlayer migration to accommodate extrusion events. Interestingly, loss of these cells promotes proliferation of remaining stem cells. Transcriptional analysis identified molecular pathways associated with observed cell death and division. Current studies are focused on validation of these molecular targets and identification of inhibitors that perturb extrusion or compensatory proliferation. **Conclusion:** We have identified novel cell and molecular mechanisms used to eliminate and replace epithelial stem cells. A similar concept of targeted cell death has been studied in clinic where a delivered transgene induces apoptosis when a prodrug is added. Yet, it is poorly understood how cells targeted for death are removed or what the impact is on tissue function. Our approach allows for visualization and experimental manipulation of distinct cell types within a living epidermis. Understanding cellular movements and molecular pathways guiding cell removal and replacement will help elucidate how disruption of homeostatic cell turnover may lead to cancer formation and progression.

101 **CPRIT Grantee Poster Session A**

ER Chaperone BiP and its Regulatory Protein FicD – New Cancer Targets? *K. Stefanius, The University of Texas Southwestern Medical Center at Dallas; K. Orth, The University of Texas Southwestern Medical Center at Dallas*

Introduction: BiP/GRP78 is an ER chaperone protein with a central regulatory role in ER function and cellular homeostasis. Bip mediates protein folding and assembly of ER proteins and regulates transmembrane ER stress response proteins that control apoptotic and survival signaling in response to ER stress via the unfolded protein response (UPR). We have recently discovered that the protein FicD may play an essential role in regulating BiP activity through AMPylation, which could also regulate cell homeostasis. In normal cells this balance is well maintained, but in cancer cells BiP is inappropriately turned on and the UPR is constitutively active. Our goal is to study the role of BiP and its regulatory protein FicD function and elucidate their effects on UPR related signaling pathways and cancer cell proliferation. We hypothesize that BiP has unique role in cancer development and FicD regulation of BiP also contributes to cancer development. We predict that by manipulating these interactions we may identify new targets for cancer therapeutics. **Methods:** Using quantitative PCR, protein biochemistry, and molecular imaging techniques we have determined the gene expression, catalytic activity, and cellular localization of Bip and FicD with and without chemical stress induction in cancer cells and compared these results to primary adult dermal fibroblasts. **Results:** Our current results indicate that cancer cells have increased levels and abnormal localization of BiP without the induction of ER stress. Furthermore, induction of the UPR results in changes in BiP and FicD expression levels as well as surprising changes in the subcellular localization of BiP and FicD. Interestingly, these changes to BiP and FicD localization are not observed in non-cancer cell lines. **Conclusion:** Our discoveries may reveal an important role for BiP and FicD in tumor cell proliferation. It will be especially interesting to further study the role of the observed abnormal localization of BiP and FicD in tumor cell growth. Elevated FicD levels in certain cancer cell lines indicate that FicD may have a unique role in regulating BiP activity in these cell types. However, more detailed studies are needed to dissect the functions and interactions of BiP and FicD. These results may prove to be new and essential mechanism used by malignant cells to regulate UPR. This work is especially significant because by elucidating the roles of BiP and

FicD in UPR regulation we may uncover novel anti-cancer targets for manipulating cancer cell growth by switching from pro-survival state to pro-apoptotic state.

102 **CPRIT Grantee**
Poster Session B

Determining the Metabolic Changes by siRNA Treatment for Ovarian Cancer Using Nuclear Magnetic Resonance *J. Lee, The University of Texas M.D. Anderson Cancer Center; N. Millward, The University of Texas M.D. Anderson Cancer Center; R. Rupaimoole, The University of Texas M.D. Anderson Cancer Center; A. Sood, The University of Texas M.D. Anderson Cancer Center; P. Bhattacharya, The University of Texas M.D. Anderson Cancer Center*

Introduction: More than two thirds of all diagnosed cases of ovarian cancer occur after the disease has progressed in advanced stages. In these days many novel treatments have been explored for curing ovarian cancer. One of fast developing treatments is using RNA interference. This research is focused on determining metabolic biomarkers for early diagnosis and developing new treatment (siRNA) for ovarian cancer using high resolution nuclear magnetic resonance (NMR) which is a powerful metabolomics tool to measure the metabolic changes as the response of treatment and access the efficacy of therapy in ovarian cancer. **Methods:** Human ovarian cancer cells (SKOV8) were grown and treated by two different siRNAs (Treatment A and Treatment B). Three groups of control and treated cells were processed through sophisticated metabolomics sample prepartion procedures. All extracted metabolites from biological samples was preapared in a deuterium oxide with NMR standard solution including buffer including 1 mM (DSS-d6) and 5 mM potassium phosphate pH 7.5. In all samples, 1D 1H proton spectroscopy was performed with water suppression on a 500 MHz Bruker Avance III HD NMR equipped with a cyroprobe. Spectral resonances of in vitro and in vivo metabolites were determined using Chenomx NMR Profile . The quantification of NMR data were then analyzed using the MestReNova software program (line broadening 0.5 Hz, baseline correction, manually phased, and referenced to the DSS- d6 peak at 0.00 ppm). The ratio of the integration of each metabolite over the integration of DSS-d6 at 0 ppm was used to normalize the data. **Results:** We compared expression of measured metabolites from ovarian cancer cells and tissues after different siRNA treatments. Significant differences were found in expression of several metabolites such as lactate, alanine, acetate, glutamate and creatine in vitro due to the two different treatments which may point to the metabolic pathway these drugs target and their efficac . Also, treated animal tissues represented the significant metabolic changes of lactate, alanine, glutamate, glutamine and creatine. **Conclusion:** These results show high-resolution NMR can provide a comprehensive and complete overview of the entire metabolite compositions and distinguished efficacy of treatment for in vitro and in vivo samples. In conclusion, this NMR based metabolomics is a powerful tool to measure the metabolic changes to access tumor progression as well as the efficacy of new therapy and drug response in ovarian cancer. This method can help to investigate the effective metabolic pathway and target metabolites for better understanding and future drug discovery.

103 **CPRIT Grantee**
Poster Session A

Dissecting the role of PROTOCADHERIN 7 in lung cancer pathogenesis *X. Zhou, The University of Texas Southwestern Medical Center at Dallas; B. Updegraff, The University of Texas Southwestern Medical Center at Dallas; Y. Guo, The University of Texas Southwestern Medical Center at Dallas; M. Peyton, The University of Texas Southwestern Medical Center at Dallas; L. Girard, The University of Texas Southwestern Medical Center at Dallas; J. Larsen, The University of Texas Southwestern Medical Center at Dallas; X .Xie, The University of Texas Southwestern Medical Center at Dallas; J .Canales, The University of Texas M.D. Anderson Cancer Center; P. Villalobos, The University of Texas M.D. Anderson Cancer Center; C. Behrens, The University of Texas M.D. Anderson Cancer Center; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; J .Minna, The University of Texas Southwestern Medical Center at Dallas; K .O'Donnell, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Given the limited effectiveness of current treatments for non-small cell lung cancer (NSCLC), there is a great need to identify new driver genes that participate in the pathogenesis of this malignancy, which may represent novel therapeutic targets. Lung cancer genome sequencing studies have identified a multitude of mutations and copy number alterations. Distinguishing driver mutations from passenger mutations represents a major challenge since the functional relevance of a gene to tumorigenesis often cannot be inferred from mutation status alone. This is particularly problematic in NSCLC due to the high mutagenic burden of these tumors. **Methods:** To address this problem, our laboratory has developed an *ex vivo* transposon mutagenesis strategy using the *Sleeping Beauty* transposon system to identify functionally relevant driver genes in cancer. We recently adapted this method for lung cancer gene discovery and identified *PROTOCADHERIN 7* (*PCDH7*) as a novel gene that promotes transformation of human bronchial epithelial cells (HBECs) *in vitro* and tumorigenesis *in vivo*. **Results:** *PCDH7* is of particular interest because it encodes a cell surface receptor that is frequently overexpressed in NSCLC and high expression strongly associates with poor survival of NSCLC patients. Our data demonstrate that overexpression of PCDH7 initiates a signal transduction cascade that potentiates KRAS-induced MAPK-ERK signaling. Moreover, inactivation of *PCDH7* using the CRISPR/Cas9 nuclease system reduced colony formation *in vitro* and tumorigenesis of human KRAS-mutant NSCLC

cells *in vivo*, and sensitized cells to MEK and ERK inhibitors. Current efforts are focused on dissecting the mechanisms of PCDH7-mediated transformation, which may reveal new opportunities to pursue this cell-surface receptor as a therapeutic target in NSCLC. **Conclusion:** These findings illustrate how combining cancer genomics with unbiased forward genetic approaches enables functional annotation of genes in human malignancies. These results also establish a critical role for PCDH7 in lung tumorigenesis and thus set the stage for future development of PCDH7-targeting molecules such as monoclonal antibodies.

104 **CPRIT Grantee Poster Session B**

A Novel Regulator of PTEN in Her2-Positive Breast Cancer

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Introduction: HER2+ breast cancer (BC) represents one of the poorest prognosis group. Trastuzumab (Herceptin, Roche-Genentech) is the firs targeted therapy for HER2+ BC. Trastuzumab significantly improves outcome in breast cancer patients, especially those with metastatic breast carcinomas (MBC). However, urgent needs to overcome trastuzumab resistance is needed because (1) modest overall response rate: 26% for single therapy and 40-60% for adjuvant therapy due to primary (inherent) resistance and (2) around 75% of patients receiving trastuzumab-based therapy will progress to metastatic disease after a year due to secondary (acquired) resistance. In HER2+ breast cancers, PTEN a tumor suppressor, is severely down-regulated and more importantly, the loss of PTEN confers the resistance to trastuzumab. Thus, through mechanisms that are not fully understood, PTEN protein expression is frequently down-regulated in BC despite the lack of PTEN gene mutation or promoter methylation. In contrast, restoration of PTEN expression suppressed tumor formation and progression and enhanced the anti-proliferative function of trastuzumab. Therefore, how to effectively restore PTEN expression and function in HER2+ breast cancer is a critical question to be addressed. M**ethods:** During the course of our investigation, we use a variety of techniques, biochemical methods (shRNA), to validate our in vitro findings in vivo using mice models, TCGA and proteomics approaches such as the reverse phase protein array (RPPA). **Results:** In this study, we identified a novel negative regulator of PTEN function that controlled PTEN stability in HER2+ breast cancer. We also demonstrated that proteasome inhibitors failed to prevent PTEN degradation following overexpression of its regulator in BC cells. In contrast, knockdown of its regulator, re-sensitizes resistant cells to trastuzumab treatment. **Conclusion:** Taken together, our findings reveal a novel mechanism by which PTEN is modulated through a proteolytic pathway in HER2+ breast cancer. Moreover, our results suggest that targeting this new regulator is a promising approach to restoring PTEN expression and function in breast cancer resistant to targeted therapy.

105 **CPRIT Grantee Poster Session A**

Computational Improvement in Cancer Cell Visualization by Cryo Electron Tomography

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Introduction: Cancer is projected to become the deadliest disease in the USA within ~15 years. Early diagnosis can improve prognosis and survival. Cryo electron tomography (cryoET) might help in detecting structural changes in cells of cancer patients. However, this requires visualizing cellular features unambiguously, with few artifacts and at high resolution. **Methods:** CryoET can directly visualize macromolecular complexes inside cells (without staining artifacts or fluorescent tags) at nanometer resolution. However, tiltseries image collection is limited to -60° to 60°, causing a "missing wedge" of data that results in smearing artifacts in tomograms. Also limiting cryoET is the lack of robust correction for the contrast transfer function (CTF) of the electron microscope. Lastly, high noise levels and low contrast make feature identification difficult and error prone. Although the contrast is typically improved by computational denoising (filtering), the tomographic reconstruction process introduces additional artifacts that can be accentuated when filters are applied inappropriately to the tomogram in 3-D. We propose that CTF correction and a series of pre-reconstruction filters applied to the 2-D images of a tiltseries can improve contrast and reduce artifacts, facilitating the accurate identification of subcellular features in 3-D tomograms of cells. **Results:** We developed computational tools for automated defocus measurement and CTF correction for tiltseries. Results on control data using macromolecular assemblies of known structure demonstrate successful CTF correction, pushing reconstructions from ~23Å to ~15Å resolution. Preliminary data also show the feasibility of defocus measurement and CTF fittin in tiltseries of platelets from ovarian cancer patients and other cells. A critical step in the structural characterization of cells by cryoET is the identification of features of interest in tomograms. As controls, our pre-reconstruction denoising (pre low-pass, high-pass and non-linear anisotropic filters) yields images that facilitate feature identification on tomograms of known macromolecular assemblies. Automated feature identification is 15-75% more similar to manual feature identificatio (ground truth) when we apply pre-reconstruction denoising, compared to when the current standard post-reconstruction denoising approach is used. Finally, we tested pre-denoising for tomograms of cancer patient platelets and other cells, finding that it helps to discriminate between different subcellular features, such as mitochondria and granules. Tests on automated segmentation of microtubules and membranes are ongoing. **Conclusion:** Our CTF correction tools for cryoET improve resolution and pre-denoising helps with identification of features in the final reconstructed tomogram. This can facilitate more accurate structural characterization of platelets from cancer patients and any other cancer-related systems visualized by cryoET.

106 **CPRIT Grantee Poster Session B**

A SNP in the 3'-untranslated region of FZD4 linked to lung cancer survival modulates miR204-mediated FZD4 transcript cleavage and expression *J. Lin, The University of Texas M.D. Anderson Cancer Center; R. Zandi, The University of Texas M.D. Anderson Cancer Center; J. Gu, The University of Texas M.D. Anderson Cancer Center; Y. Ye, The University of Texas M.D. Anderson Cancer Center; X. Wu, The University of Texas M.D. Anderson Cancer Center; J. Roth, The University of Texas M.D. Anderson Cancer Center; L. Ji, The University of Texas M.D. Anderson Cancer Center*

Introduction: Single nucleotide polymorphisms (SNPs) in miRNA genes and miRNA-associated pathways (miR-SNPs) are predicted to have significant effects on gene expression and cellular processes by disrupting miRNA biogenesis and modulating miRNA-mRNA interactions. We identified several germline SNPs at potential miRNA-binding sites in the 3' untranslated regions (3'UTRs) of genes in the wingless (Wnt) signaling pathway to be significantly associated with overall survival in NSCLC patients. SNP (rs713065 with a C allele) in the 3'UTR of the FZD4 gene in the Wnt signaling pathway displayed a significant association with decreased risk of death in early stage NSCLC patients. M**ethods:** We used a novel stem-loop array-reverse transcription-PCR (SLA-RT-PCR) assay to assess miRNA:target mRNA interaction at the specifi SNP locus. We determined the biological function and clinical relevance of this novel epidemiological FZD4-miR-SNP (rs713065) in NSCLC by identifying specific miRNAs that could directly interact with the FZD4-miR-SNP locus and assessing the effect of such a SNP and associated miRNAs on target gene regulation and NSCLC prognosis. **Results:** We detected the miRNA-mediated FZD4 mRNA cleavage and 3'-uridynation activities in FZD4-SNP (C, rs713065) allele-bearing NSCLC cells H1299 and H322, but not in in FZD4-WT (T) allele-containing A549 and normal human bronchial epithelial cells. A significant down regulation of GFP and Luciferase (Luc) gene and protein expression was detected in NSCLC cell lines using both GFP-SNP and Dual-Luc-SNP reporter systems. The presence of the FZD4-SNP in the 3'UTR also down-regulated the ectopic expression of the host FZD4 gene and protein and inhibited tumor colony formation and tumor cell mobility in NSCLC cells. We identified miR-204 as a potential miRNA candidate that differentially interacts with and targets allelic variants at FZD4-miR-SNP (rs713065) loci. Target mRNA cleavage was detected at the SNP C allele site but not at the WT-allele T site in H1299 cells after co-transfection with a GFP-FZD4-SNP reporter and miR-204 expression constructs by SLA-RT-PCR. A significant correlation of miR-204 with FZD4 gene expression was also detected in NSCLC cell lines and primary tumor specimens in various miRNA expression profilin databases. **Conclusion:** Our findings suggest that the miR-204 binding site at the SNP (rs713065) loci in the 3'UTR of FZD4 may influenc survival in NSCLC by modulating FZD4 expression and the Wnt/FZD4-signaling driven tumor cell proliferation and progression.

107 **CPRIT Grantee Poster Session A**

Cancer Cells Enter Dormancy after Cannibalizing the Mesenchymal Stem/Stromal Cells (MSCs) that Encapsulate Them in 3-D Cultures *T. Bartosh, Texas A&M University System Health Science Center; J. Beaver, Texas A&M University System Health Science Center; M. Ullah, Texas A&M University System Health Science Center; A. Mohammadipoor, Texas A&M University System Health Science Center; M. Curry, Texas A&M University System Health Science Center; S. Adams, Texas A&M University System Health Science Center; D .Prockop, Texas A&M University System Health Science Center*

Introduction: Cancers evolve through a history rich in selective micro-environmental pressures and interactions with tumor stromal cells including mesenchymal stem cells (MSC). Despite evidence that MSCs have tumor-tropic properties, the overall effect of MSCs on cancer development and progression is circumstantial and few studies have explored interactions between MSCs and cancer cells under duress. We previously showed that when prepared as spheroids in three-dimensional (3-D) hanging drop cultures, MSCs changed dramatically and upregulated expression of numerous stress-associated and tumor-suppressive factors including TRAIL, IL-24, and CD82. Since 3-D cultures often better mimic conditions in vivo by permitting appropriate cellular interactions, here we studied the effects of MSCs on cancer cells under stress in hanging drop cultures. M**ethods:** Human bone marrow-derived MSCs were obtained from the Center for Preparation and Distribution of Adult Stem Cells (http://medicine.tamhsc.edu/irm/msc-distribution.html). To evaluate effects of 'stress' on the interactions between MSCs and cancer cells in vitro, hanging drop co-cultures were prepared with MSCs and various cancer cell lines. Cell death, proliferation, and phenotype were assessed. The effects of MSCs on tumorigenesis were evaluated after injection of cancer cells obtained from 3-D cultures into immune-deficient mice. **Results:** Within hanging drop co-cultures of MSCs and breast cancer cells (MDA-MB-231), the MSCs did not directly promote apoptosis of the MDA-MB-231 cells despite expressing numerous anti-tumor factors. Instead we observed that after surrounding the cancer cells, the MSCs promoted formation of compact cancer spheroids and then disappeared 24-48 hours later. Further experiments revealed that the cancer cells in 3-D cultures internalized and degraded the MSCs, a process that resembled cell cannibalism/entosis but that was not appreciable in monolayer co-cultures or in 3-D co-cultures of MSCs with normal cells. Similar cell-in-cell structures were also observed when MSCs were suspended in hanging drops with a variety of other carcinomas. Following cannibalization of MSCs, breast cancer cells acquired a pro-inflammator phenotype with indications of enhanced E-M-T. Moreover, the resulting cancer cells displayed markedly delayed tumorigenicity after injection into the mammary fat pads of immune-deficient mice. **Conclusion:** The results indicated that cannibalization of MSCs by cancer cells was elevated under the stressful conditions of hanging drop cultures that resulted in dormancy of the cancer cells. This study provides new insight into the interactions of MSCs and cancer cells with the potential to establish new targets for cancer therapy.

108 **CPRIT Grantee Poster Session B**

Accurate Sequencing Via Single Molecule Reconstruction Reveals Subclonal Variation in Tumor and Normal Tissues *K. Covington, Baylor College of Medicine; E. Shinbrot, Baylor College of Medicine; M. Wang, Baylor College of Medicine; H. Doddapaneni, Baylor College of Medicine; D. Muzny, Baylor College of Medicine; J. Meyer, The University of Texas Southwestern Medical Center at Dallas; C. Pickering, The University of Texas M.D. Anderson Cancer Center; M. Frederick, The University of Texas M.D. Anderson Cancer Center; R. Gibbs, Baylor College of Medicine; D. Wheeler, Baylor College of Medicine*

Introduction: Tumors are extraordinarily heterogeneous with many potentially clinically relevant mutations present in minor sub-populations. A complete description of a patient's tumor genome somatic mutation burden should include events present in less than 1% of the cells, whereas most mutations discovered to day are at 10% or higher. However, the error rate of current sequencing approaches represents a barrier beyond which rare mutations may not be detected. Oral squamous cell carcinoma (OSCC) is a common form of head and neck cancer (95%) with rapidly increasing rates (50% in recent years). The most common risk factors include HPV infection, tobacco and alcohol exposure. In this study, we performed deep sequencing of 128 OSCC tumors with matched blood normal to analyze the mutations involved in this disease. M**ethods:** We adapted published proof-of-concept methodologies for deep sequencing using "duplex sequencing" to reconstruct single molecules of DNA. Duplex sequencing relies on first tagging individual DNA molecules with unique identifiers followed by amplification and sequencing. With this strategy we turn PCR duplicates into an advantage to repeatedly sample fragments, which can be traced to a single DNA molecule. Reads where condensed into "blocks" using custom software to reassemble sequencing reads into their original single molecule DNA fragment. Statistical analysis of mutation frequencies across the blocks enables filtering the data to arrive at mutations present in 1% of tumor samples. **Results:** Our examination of somatic mutations with 5% or greater allele fractions was consistent with other OSCC mutation studies. Our unique approach to deep sequencing allowed detection of somatic variants at with a 1% allele frequency. Due to our exceptional sensitivity, we were able to detect mutations in matched control blood at a rate of 29 (11-67, 15 non-silent) mutations per sample. Mutation rates were higher in tumor samples ($p < 2E-5$) with no difference in depth, consistent with the hypothesis that tumors have a higher rate of mutation than "normal" cells. The vast majority of mutations were observed in single reconstructed molecules of DNA. We were able to validate 65% of events detected in only single molecules of DNA and 100% of mutations detected in greater than one molecule. C**onclusion:** We conclude that we had not yet begun to saturate the mutations observed in tumor samples, indicating that there exists reservoir of genetic variation within the tumor population that could fuel the process of tumor evolution that might be uncovered by still deeper sequencing.

109 **CPRIT Grantee Poster Session A**

Automatic Quantification of Short Telomeres Ratio in Cancer and Aging-related Disease *N. Zhang, The University of Texas Southwestern Medical Center at Dallas; T. Lai, The University of Texas Southwestern Medical Center at Dallas; S. Han, The University of Texas Southwestern Medical Center at Dallas; J. Shay, The University of Texas Southwestern Medical Center at Dallas; G. Danuser, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Telomeres are repetitive nucleotide sequences (TTAGGG) capping the end of chromosomes, which can prevent the chromosomes ends from fusion and degradation. Studies have demonstrated that cellular senescence can be initiated by a single or small number of telomeres reaching to the critically short length within the cell. Thus, measurement of the load of short telomeres provides the critical knowledge to study in cancer and aging biology. Universal Single TElomere Length Analysis (STELA), a ligation and PCR-based method, allows for detection of critically short telomeres by small amount of genomic DNA. However, manually quantify the length and dynamics of each telomere from Universal STELA is error prone and time consuming. Thus, it is important to design a program that can effectively identify and analyze results of Universal STELA. Here we developed an algorithm which can automatically detect bands and calculate the ratio of the short telomeres based on Universal STELA gel sample. **Methods:** The input gel image is firstly converted to gray scale image and then normalized. A 2D matched filter kernel is designed based on the patterns of bands. The pre-processed image is convolved with such a filter kernel and a large response will be obtained if the there is a band. Then the pixels intensity of each column are projected down to generate intensity profile. 1D watershed algorithm is applied on the intensity profile to find significant peaks which denote the center of lanes. Based on that classification, we can crop out each single lane, plot its vertical intensity profile, and apply 1D watershed again to figure out the center of each band. **Results:** Here we describe an algorithm which can automatically detect the bands of a Universal STELA gel sample. With given short telomere length threshold, it can calculate the ratio of the short telomeres to the rest of telomeres. This algorithm confirm that there is induced shortening of the shortest telomeres in Chronic Lymphocytic Leukemia (CLL) tumor samples after drug treatment. It also gives the result in one minute that colon polyp has more short telomeres than normal colon tissue and lymphocyte based on Universal STELA gel analysis. **Conclusion:** This algorithm can automatically identify STELA gel bands and count the ratio of short telomeres to the rest of telomeres. The results are consistent with the previous manual identification and calculation outcomes. But using this algorithm makes high throughput analysis possible and can avoid artificial bias

110 **CPRIT Grantee**
Poster Session B

Superoxide dismutase mimetic GC4419 protects against radiation induced lung fibrosis, exhibits anti-tumor effects, and enhances radiation induced cell killing. *B. Sishc, The University of Texas Southwestern Medical Center at Dallas; D. Bloom, The University of Texas Southwestern Medical Center at Dallas; D. Ramnarain, The University of Texas Southwestern Medical Center at Dallas; S. Eluru, The University of Texas Southwestern Medical Center at Dallas; D . Saha, The University of Texas Southwestern Medical Center at Dallas; M .Story, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Superoxide dismutases (SOD) catalyze the conversion of superoxide ion into hydrogen peroxide, an intermediate step within a process that protects cells from oxidative stressors including ionizing radiation as well as being a carcinogenesis prevention mechanism. Here, we report evidence supporting novel anti-tumor properties of GC4419 (Galera Therapeutics, St. Louis, MO), a small molecule selective SOD mimetic that acts as a radiation protector. GC4419 is currently being evaluated in a Phase 1b/2a clinical trial for the prevention of oral mucositis in patients with head and neck cancer treated with radiation. **Methods:** In vitro work was conducted on a panel of molecularly well characterized non-small cell lung cancer lines. In vivo tumor growth delay (TGD) studies were carried out in athymic nu/nu mice (Charles River) using the H1299 tumor line in a subcutaneous xenograft model. **Results:** Consistent with the data in experimental radiation mucositis that formed the basis for that clinical study, we report here that pretreatment with GC4419 significantly reduced the fibrotic density of murine lung tissues exposed to 54 Gy X-rays at 24 weeks post exposure. Importantly, since tumor protection is always a concern when preventing normal tissue toxicity, we also report here that GC4419 treatment in vitro results in delayed cell proliferation and enhanced radiation and cisplatin induced cell killing in non-small cell lung cancer (NSCLC) cell lines. Furthermore, using H1299 cells in a tumor xenograft model, GC4419 treatment resulted in tumor growth delay (TGD) alone ($p = 0.0022$), and increased the efficacy of ionizing radiation ($p = 0.0198$) with a radiation dose enhancement factor (DEF) of 1.84, and also mildly enhanced the effects of cisplatin treatment. Furthermore, additional in vivo experiments demonstrate that the observed enhancement of radiation induced TGD with concomitant GC4419 treatment is most effective in a high dose per fraction setting relative to conventionally fractionated schemata. Ongoing in vitro work suggests that this enhanced TGD results from GC4419 altering cellular redox state and sensitizing hypoxic cells to radiation. **Conclusion:** Overall, these findings suggest that GC4419 not only displays clinical potential as a normal tissue radiation protector, but has the additional advantage of enhancing radiation therapy and tumor cell killing, making it an ideal candidate drug for expanding its current clinical trials.

111 **CPRIT Grantee**
Poster Session A

Mobilizing ERβ Antitumor Activity Through a Phosphotyrosine Switch *B. Yuan, The University of Texas Health Science Center at San Antonio; Y. Hu, The University of Texas Health Science Center at San Antonio; R. Li, The University of Texas Health Science Center at San Antonio*

Introduction: The two estrogen receptors, ERα and ERβ, mediate diverse effects of estrogen in multiple tissues. Despite considerable sequence homology, ERα and ERβ carry out non-redundant physiological functions. While ERα is critical for mediating estrogen-dependent proliferation during normal mammary gland development, ERβ is known to inhibit cell proliferation and promote differentiation in a number of tissues. In cancer development and progression, ERα has a well-established role in supporting estrogen-dependent breast tumor growth, whereas ERβ significantly attenuates cell proliferation and invasion in a number of cancer cell types including breast and prostate cancers. **Methods:** Co-IP assay; Real-Time RT-PCR; Chromatin Immunoprecipitation (ChIP); Xenograft Assay; Human Tissue Procurement and Analysis **Results:** We have shown that phosphorylation of a tyrosine residue (Y36) present in ERβ, but not in ERα, dictates ERβ-specific activation of transcription and is required for ERβ-dependent inhibition of cancer cell growth in culture and in murine xenografts. Additionally, the c-ABL tyrosine kinase and EYA2 phosphatase directly and diametrically controlled the phosphorylation status of Y36 and subsequent ERβ function. A nonphosphorylatable, transcriptionally active ERβ mutant retained antitumor activity but circumvented control by upstream regulators. Phosphorylation of Y36 was required for ERβ-mediated coactivator recruitment to ERβ target promoters. In human breast cancer samples, elevated phosphorylation of Y36 in ERβ correlated with high levels of c-ABL but low EYA2 levels. Furthermore, compared with total ERβ, the presence of phosphorylated Y36–specific ERβ was strongly associated with both disease-free and overall survival in patients with stage II and III disease. **Conclusion:** These data identify a signaling circuitry that regulates ERβ-specifi antitumor activity and has potential as both a prognostic tool and a molecular target for cancer therapy.

112 **CPRIT Grantee**
Poster Session B

Exploiting the Dependence of Wnt Signaling on Lipid Metabolic Processes to Achieve Anti-cancer Goals *R. Tuladhar, The University of Texas Southwestern Medical Center at Dallas; L. Lum, The University of Texas Southwestern Medical Center at Dallas; C. Chen, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The homeostatic renewal of tissues in multicellular organisms couples metabolic health at the single cell level to communal cell fate decision-making. The secreted fatty acylated Wnt signaling molecules coordinate cell fate decision-making in adult tissues by eliciting cell and non-cell autonomous transcriptional responses. Despite the importance of lipidation to Wnt protein function, our understanding of whether or not the nature of the lipid adduct influences Wnt function in tissue homeostasis and thus cancer is limited. M**ethods:** Using a combination of cell biological, biochemical, and chemical approaches, we have interrogated the molecular bases supporting fatty acyl donor selectivity of the Wnt acyltransferase Porcupine (Porcn), the target of a first in class compound currently in clinical testing as an anti-cancer agent. **Results:** We have observed conservation of Porcn selectivity for the monounsaturated fatty acid palmitoleate across metazoan phyla. The active site determinants that impose this fatty acyl donor selectivity likely contribute to the specificity of Porcn inhibitors. We have also demonstrated that Porcn can accommodate an isomer of palmitoleate suggesting that a diet high in trans fats could directly influence somatic stem cell health and cancerous growth. **Conclusion:** Our findings provide a molecular understanding of how Porcn inhibitors achieve selectivity and insights into how the interconnectivity of lipid metabolism and cell differentiation could be exploited for anti-cancer goals.

113 **CPRIT Grantee Poster Session A**

KDM4/JMJD2 histone demethylase inhibitors block prostate tumor growth by suppressing the expression of AR and BMYB-regulated genes *Z. Liu, The University of Texas Southwestern Medical Center at Dallas; L. Duan, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Histone lysine demethylase KDM4/JMJD2s are overexpressed in many human tumors including prostate cancer (PCa). KDM4s are co-activators of androgen receptor (AR) and thus potential therapeutic targets. However, the mechanism by which KDM4 proteins promote prostate tumorigenesis remains elusive and whether inhibition of KDM4 activities can be used as therapeutics remains to be established. M**ethods:** We screened a chemical library of inhibitors that previously shown to inhibit demethylase activities of KDM4 in order to identify compounds that have inhibitory effect on the growth of prostate cancer cell line LNCaP. We also use siRNA knockdown of KDM4B to inactivate KDM4B in LNCaP and PC3 cells. Expression profiling with microarrays was carried out to identify the common targets of KDM4B siRNA and KDM4B inhibitors. Xenografts derived from PC3 cells were used to evaluate the efficacy of inhibitor B3 in vivo. We also used human prostate tumors in ex vivo culture to test the utility of B3. **Results:** Here we report the anti-tumor growth effect and molecular mechanisms of three novel KDM4 inhibitors (A1, I9, and B3). These inhibitors repressed the transcription of both AR and BMYB-regulated genes. Compound B3 is highly selective for a variety of cancer cell lines including PC3 cells that lack AR. B3 inhibited the in vivo growth of tumors derived from injection of PC3 cells and ex vivo human PCa explants. We identified a previously unrecognized mechanism by which KDM4B activates the transcription of cell cycle regulator polo-like kinase 1 (PLK1). We show that B3 blocked the binding of KDM4B to the PLK1 promoter that is associated with upregulation of H3K9me3 at the promoter. C**onclusion:** Our studies suggested a potential mechanism-based therapeutic strategy for PCa and tumors with elevated KDM4B/PLK1 expression.

114 **CPRIT Grantee Poster Session B**

Cancer Proteomics and Metabolomics Core Facility *D. Edwards, Baylor College of Medicine; A. Sreekumar, Baylor College of Medicine; S. Huang, Baylor College of Medicine; S. Jung, Baylor College of Medicine; J. Qin, Baylor College of Medicine; N. Putluri, Baylor College of Medicine*

Introduction: Cancer development and progression involves not only alterations in genes, but also protein signaling pathways and metabolism that collectively drive the cancer phenotype. Our goal is to develop a combined Proteomics and Metabolomics Core Facility to assist cancer researchers with identifying novel therapeutic targets. M**ethods:** Three main technology platforms have been developed for support of projects of cancer researchers at BCM including 1) mass spectrometry proteomics, 2) reverse phase protein arrays (RPPA) and 3) mass spectrometry-based metabolomics. A standard operating procedure has been developed that includes a request for project (RFP) application that is reviewed and approved by an advisory committee for scientific merit and cancer relevance. This is followed by experimental design with Core leaders, sample preparation and submission protocols with quality control standards, analysis of samples by Core staff and a two tiered data analysis pipeline. Tier 1, as a package with laboratory analysis, includes data normalization, quality control assurances, statistics and graphical representation of data. Upon request, tier 2 data analysis includes higher level statistics, data integration, bioinformatics and interpretation. Investigators are charged a fee-for-service (65-70% of costs are subsidized by the CPRIT Core grant) that is used to continually advance technologies and add new core services. R**esults:** Metabolomics has developed methods for targeted analysis of > 500 metabolites by (MRM) MS, an isotopomer-based flux assay to trace glucose and glutamine through glycolysis and fatty acid synthesis pathways respectively, lipidomics (up to 105 lipids) and MS2-based unbiased metabolomic profiling. RPPA has expanded from an initial analysis of 160 proteins representative of major known oncogenic signaling pathways to over 220 targets that includes 75 phosphoproteins (as markers of protein activity) and > 145 total proteins to additional pathways such as autophagy, EMT, stress response and DNA damage, transcription factors and metabolic enzymes. MS proteomics includes IP-MS analysis of protein complexes and a label-free semi-quantitative method for profiling up to 6,000 proteins in cells. Over three years, the Core Facility has provided project support for 77 cancer researchers resulting in 35 publications (high impact journals such as Cell Metabolism, Cell Reports, Cancer Cell, Nature, JCI) and awarding of 47 new grants ($7.5M direct annual costs) to Core users requiring services of the Core in Specific Aims. C**onclusion:** The Core Facility is now set to integrate multi-omics data sets with the goal of providing new insights and identification of therapeutic targets not possible by single omics platforms.

115 **CPRIT Grantee Poster Session A**

FOXO3a a Tumor Suppressor Regulated by Estrogen Receptor β in Prostate *S. Chaurasiya, University of Houston; J. Gustafsson, University of Houston; A. Strom, University of Houston*

Introduction: The estrogen receptor β (ERβ) is expressed in the normal prostate but its expression is decreased during progression of prostate cancer indicating a tumor suppressive role. Recently we found that ERβ transcriptionally regulates the well-known tumor suppressor gene FOXO3a that have been shown to be reduced in many types of cancer during progression including prostate cancer. Furthermore, ERβ has been shown to be up regulated by PTEN in prostate explaining its reduction in PTEN mutated or deleted prostate cancer. **Methods:** Transient transfection of ERβ responsive element from FOXO3a gene linked to luciferase. Chromatin immunoprecipitation assay (ChIP). Endogenous biotin labeling of ERβ by transfection of biotin ligase and biotinylation consensus linked to the receptor. Pulldown using streptavidin magnetic beads followed by detection of interacting proteins. **Results:** We have recently shown that endogenous ERβ in the prostate cancer cell lines PC3 and LNCaP can be activated by ERβ specific agonists like DPN, 8β-VE2 and 3β-Adiol following stimulation of apoptosis through the mechanism of transcriptionally activating FOXO3a and subsequent up-regulation of PUMA leading to inhibition of Bcl2 caspase 9 activation and apoptosis. We have found one ERβ binding element inside the FOXO3a gene which is responsive to 3β-Adiol bound ERβ when placed in front of heterologous promoter followed by luciferase. We can show that ERα is recruited to this element in MCF-7 cells as well as ERβ expressed in PC3 cells. **Conclusion:** Since ERβ seems to be expressed at low but functional levels in prostate cancer it may be possible to treat prostate cancer with ERβ specific agonists. Detailed information of how the ERβ element in the FOXO3a gene functions i.e. 3β-Adiol specific co-regulator(s) and their structural requirements can help to design better tumor suppressive agonists for ERβ in prostate cancer.

116 **CPRIT Grantee**
Poster Session B

Chromosome 18 Copy Number Variation in Metastatic Prostate Cancer *K. Ashcraft, The University of Texas Health Science Center at San Antonio; T. Johnson-Pais, The University of Texas Health Science Center at San Antonio; R. Leach, The University of Texas Health Science Center at San Antonio*

Introduction: Prostate cancer is the second most common type of cancer in men behind skin cancer. According to the National Cancer Institute, there will be an estimated 220,000 new cases in 2015 with 27,000 deaths. Previous investigations into allelic imbalance on chromosome 18 in prostate cancer found regions of loss that significantly correlate with prostate cancer metastasis. The genes that are targeted by these chromosomal alterations, however, have remained elusive. Our objective is to confirm the association between the loss of chromosome 18 sequences and aggressive prostate cancer, while narrowing the critical regions of loss. Using this information, we propose to identify putative metastasis suppressor genes coded within these regions. **Methods:** Formalin-Fixed Parafin-Embedded (FFPE) primary prostate tumor samples from the UTHSCSA Genitourinary Biorepository were identifie that met the following criteria. The men were followed for a minimum of five years after prostatectomy with an outcome categorized as either No Evidence of Disease recurrence (NED) or Metastatic disease (MET). DNA was isolated from these samples, along with FFPE tissue from normal prostate as reference DNA for array comparative genomic hybridization (CGH). These DNAs were labeled with Cy3 and Cy5 and hybridized to a custom CGH array focused on chromosome 18 and other well-established copy number variations in prostate cancer designed using Agilent's SureDesign software. The slides were scanned and data was analyzed using Agilent's Cytogenomics software. **Results:** Currently, I have used array CGH to analyze 32 unique primary prostate cancer samples. Twenty-one have an outcome of NED, while the other 11 tumors are from men who developed METs. Eight of the samples have amplification of the Androgen receptor (AR), while one MET showed deletion of AR. 9 samples have deletions on chromosome 12 involving CDKN1B, a known prostate cancer gene. Alterations in chromosome 18 are common with 18 of the 32 samples harboring some deletion and 9 having amplifications. Of note, ESCO1 is amplified in six of the 32 samples. ESCO1 may play some role in TMPRSS:ERG rearrangements, and three of the six amplification coincide with TMPRSS:ERG genomic alteration. A previously unrelated gene to prostate cancer, ZNF516, located on chromosome 18, is deleted in three of the 32 samples. **Conclusion:** Preliminary results have highlighted two intriguing genes in ESCO1 and ZNF516 that may have some role in chromosome 18's influence on prostate cancer. More samples are being prepared to fully identify significant changes in copy number variation between METs and NEDs.

117 **CPRIT Grantee**
Poster Session A

Development of Biochemical Assays for Targeting Fructose-1,6-bisphosphate Aldolase and Application for High Throughput Compound Screening *A. Devkota, The University of Texas at Austin; G. Stancu, The University of Texas at Austin; G. Powis, Sanford Burnham Prebys Medical Discovery Institute; E. Cho, The University of Texas at Austin; K. Dalby, The University of Texas at Austin*

Introduction: A high rate of glycolysis is observed in a wide range of tumor cells making it an attractive pathway to control cancer growth. Aldolase catalyzes a key reaction of glycolysis and its expression has been reported to be significantly elevated relative to other glycolytic enzymes in a number of human tumor types. Deficiency of the Aldolase A (ALDOA) isoform is linked to myopathy and hemolytic anemia and its knockdown in cancer cells was associated with greater inhibition of cancer cell proliferation. We aimed to develop homogeneous methods to assay ADLOA activity by using coupled enzymatic assays and demonstrate their feasibility for high throughput compound screening. **Methods:** We designed two biochemical assays utilizing a coupled enzymatic reaction which converts Dihydroxyacetone phosphate (DAP), a product of ALDOA enzymatic reaction, to L-a-Glycerol phosphate (GP) in the presence of NADH. The depletion of NADH is then measured by addition of either luminescent or florescent detection reagents. Both assays were optimized and miniaturized in a 384 well format and achieved z' values over 0.65. Full description of assay optimization and data analysis are discussed. Its feasibility for high throughput screening using a fluorescence-base assay was validated on screening 65,000 compound libraries. **Results:** We found the luminescence-based assay provided wider dynamic range than fluorescence-based assay while it required two additional steps. Buffer compositions also affected assay kinetics and turnover time. We demonstrated both assays were robust and will facilitate high throughput screening of larger size libraries for the identification of small molecule inhibitors. **Conclusion:** By developing two simultaneous assay platforms for ALDOA, we provide researchers with a choice of using a luminescence-based assay for primary screening and a fluorescence-based assay for a confirmation screen (or vice-versa) and significantly contribute to the development of therapies for targeting ALDOA.

118 **CPRIT Grantee**
Poster Session B

Microarray and LC-MS/MS Profiling Identifies Cancer-Specific Signatures in the Sphingolipid Pathway for Acute Lymphoblastic Leukemia *D. Verlekar, Texas Tech University Health Sciences Center; H. Cho, Texas Tech University Health Sciences Center; M. Kang, Texas Tech University Health Sciences Center*

Introduction: Sphingolipids are essential structural components of cell membranes and have messenger functions that regulate growth, differentiation, apoptosis and senescence of cells. Ceramides are a group of the key intermediating lipids in the sphingolipid pathway. Six different isoforms of ceramide synthase (CERS1-CERS6) with varying substrate specificity generate ceramides of diverse chain lengths, each of which have differential roles in maintaining cellular homeostasis. Studies showed that expression levels of ceramide synthase homologs vary among different tissues. In normal tissues, CERS2, which generates ceramides with C24 acyl chain (C24-Cer), is highly expressed and has the widest tissue distribution while CerS5/CerS6 generates C16-Cer, and their expression is low and limited to specific tissues. In the current study, we investigated whether there are significant differences in sphingolipid profiles between pediatric cancer cells, including acute lymphoblastic leukemia (ALL) cells and normal cells. **Methods:** CERS2/5/6 mRNA expression levels in a NCI PPTP panel of 23 cell lines were determined using U133 Affymetrix microarray solutions. Sphingolipid levels in ALL cell lines and peripheral blood mono-nuclear cells (PBMC) were measured by HPLC/tandem mass spectrometry. CERS2/5/6 mRNA expression levels in ALL cell lines were determined by RT-PCR analysis. Statistical comparison between two groups (ALL and normal cells) was carried out using unpaired student's t-test with Welch's correction. **Results:** CERS5/6 mRNA expression levels, usually found to be low in comparison to CERS2 in normal cells, were significantly increased in the majority of NCI PPTP panel of 23 cell lines which comprise six different pediatric cancers. The ratio of C16-Cer to C24-Cer was significantly higher ($P<0.01$) in all 8 leukemia cell lines tested when compared to PBMC samples from 15 normal volunteers. mRNA expression of CERS5/6 was higher than CERS2 in 6 out of 7 ALL cell lines tested and corresponded to the high levels of C16-Cer in these samples. C16-Cer levels were lower in 2 sets of lymphoblastoids (representative of normal cells) in comparison to neuroblastoma cells that were established from the same patients. **Conclusion:** There are significant differences in the sphingolipid profiles between normal and cancer cells, including ALL.

Understanding these differences could enable uncovering of novel targets for ALL treatment. Future studies on determining the biological roles of various ceramides in cancer are warranted.

119 **CPRIT Grantee Poster Session B**

Tumor Suppresive Role of MicroRNA-34c in Osteosarcoma

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Introduction: MicroRNAs (miRNAs) have been implicated in diverse biological roles including development, cell proliferation, apoptosis and tumorogenesis. During tumor development, many miRNAs function as both tumor suppressors and oncogenes. The miR-34 , a tumor suppressive miRNA, is evolutionally conserved and directly regulated by p53 in response to DNA damage and oncogenic stress. Our previous study have shown a critical role of miR-34c during bone development and homeostasis by using osteoblast- specific gain of function miR-34c mice (*Col1a1 2.3 kb-miR34c*) by targeting multiple components of the Notch signaling pathway. We and others have shown that an osteoblast-specific Notch gain of function mouse model can stimulate proliferation of immature osteoblasts while inhibiting their differentiation into mature osteoblasts. This gain of function phenotype leads to the development of osteoblastic tumors and indeed, Notch signaling was also up-regulated in human osteosarcoma (OS) samples. Consistent with this observation, our transcriptional profiling of OS from p53 +/- mice exhibited elevated expression of Notch signaling, which leads to the proliferative and metastatic potential of OS. It has been also shown that genetic and epigenetic alterations in OS lead to decreased miR-34 expression levels. Here, we hypothesize that the miR-34 family plays a critical role in the negative regulation of Notch signaling in osteoblasts and exhibits a potential mechanism to modulate the proliferative effect of Notch in the committed osteoblast progenitors. Hence, perturbation of the crosstalk between miR-34s, p53, and Notch may contribute to the pathogenesis of OS. M**ethods:** To examine whether GOF miR-34c can affect OS formation, progression and metastasis, *Col1a1 2.3kb-miR34c* was crossed with *Col1a1 2.3kb Cre/+; p53 f/f* (osteoblast-specific loss of p53 allele). Clinical endpoints include survival, hind limb paralysis, tumor progression and metastases. We have performed xenografts using the hOS cell line 143b ($p53^{-/-}$) stably expressing miR-34c (143b-34c) with proper controls, and examined whether GOF of miR-34c affected OS progression *in vivo*. The putative targets of miR-34c, such as *Snap23* and *Arhgap35,* involved in lung metastasis are tested. **Results:** In our genetic mouse model of *Col1a1 2.3kb-miR34c; Col1a1 2.3kb Cre/+; p53 f/f* we have found that GOF miR-34c mice have increased survival rate compared to *Col1a1 2.3kb Cre/+; p53 f/f* mice. Furthermore, xenograft studies using hOS cell line 143b-34c shows regression of tumor growth compared to 143b-controls. **Conclusion:** Overall, these results suggest that miR-34c plays a tumor suppressive role in osteosarcoma. The underlying molecular mechanism of tumor suppressive function of miR-34c is currently being pursued.

120 **CPRIT Grantee Poster Session B**

The Germinal Center B-Cell Subtype of Diffuse Large B-Cell Lymphoma uses Tonic B-Cell Receptor Signaling

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Introduction: Targeting BCR signaling with the BTK inhibitor ibrutinib is clinically effective against most B-cell lymphomas, including the activated B-cell (ABC) subtype of diffuse large B-cell lymphoma (DLBCL), but not the germinal center B-cell (GCB) subtype. We addressed these questions: why is the BCR active in DLBCL, and how does it signal in GCB-DLBCL? M**ethods:** We used CRISPR/Cas9 technology to modify selected genes by knockout (KO) or homologous recombination-mediated knock-in (KI). KI was used to express a fluorescent protein instead of the targeted gene (KI/KO) or as a marker of genomic modification **Results:** In GCB lines (OCI-Ly7 and OCI-Ly19) and ABC lines (U2932 and HBL-1), we simultaneously replaced the hypervariable region (HVR) exons of both immunoglobulin heavy (IgH) and light chains (IgL) with HVR sequences from normal B cells recognizing tetanus toxoid (TT). The TT-BCR maintained growth of GCB lines, indicating that they use "tonic", antigen-independent BCR signaling. Other features of tonic signaling were confirmed in more GCB lines: 1) the toxicity of BCR KO was rescued by PTEN KO or expression of constitutively active AKT (mAKT); and 2) KO of SYK or CD19, or truncation or ITAM mutation of the cytoplasmic tail of CD79A, none of which affect surface BCR levels, were as toxic as BCR KO but were non-toxic in BCR/PTEN double-KO cells. In contrast, the TT-BCR was as growth-slowing as BCR KO to the ABC line U2932, and substantially toxic to HBL-1, indicating that BCR signaling is self antigen-dependent in ABC-DLBCL. Tonic signaling by the TT-BCR provided a detectable benefit (as compared to BCR KO) in PTEN-expressing HBL-1, whereas there was no difference between

TT-HVR BCR and BCR KO in PTEN-deficient U2932. The presumed self-antigen in ABC lines seems to be cell line-specific, since HVRs from ABC lines TMD8 and HBL-1 did not rescue growth of U2932. Several findings suggested the clinical potential of targeting tonic BCR signaling in DLBCL: 1) clinical trial-stage inhibitors of SYK (P505-15) and PI3K (idelalisib) were toxic to GCB lines (less so with PTEN KO); 2) GCB lines (6/8) were sensitized by BCR KO to an in vitro CHOP-like regimen; 3) P505-15 or idelalisib sensitized GCB lines (3/3) to CHOP in vitro; and 4) evidence of tonic signaling in ABC line HBL-1 after removing antigen-driven signaling by HVR replacement. **Conclusion:** Antigen-independent BCR tonic signaling activates PI3K/AKT in GCB-DLBCL, adds to the antigen-dependent signaling in ABC-DLBCL, and is a potential therapeutic target.

122 **CPRIT Grantee**
Poster Session B

Investigating The Crosstalk Between Calcium And WNK-OSR1/SPAK Osmotic-Stress Signaling Pathways *A. Lorente-Rodriguez, The University of Texas Southwestern Medical Center at Dallas; M. Cobb, The University of Texas Southwestern Medical Center at Dallas*

Introduction: WNK [with no lysine (K)] protein kinases are ubiquitous and their canonical function is to regulate ion homeostasis through phosphorylation and activation of the downstream kinases OSR1 and SPAK, which phosphorylate and modulate the activity of several plasma membrane ion transporters. This pathway has described roles in hypertension, schizophrenia and autism. Recently, we published that this pathway regulates angiogenesis and we have found mutations in pathway components present in several cancers. We are characterizing novel pathway inhibitors with potential use in the clinic. The aim of this study is to characterize the crosstalk between calcium and WNK-pathway signaling. Published studies using different model systems have described interactions between calcium and WNK-pathway signaling. We showed that WNK1 phosphorylates synaptotagmin 2 decreasing its affinity for calcium, which is required for its role in vesicle exocytosis. A separate study showed WNK1 is regulated by intracellular calcium ($[Ca^{2+}]_i$) by an undescribed mechanism. We submitted a study showing that short-term FK506 treatment, an inhibitor of calcineurin (calcium/calmodulin[Ca^{2+}/CaM]–dependent phosphatase), potentiates WNK signaling and decreases Akt phosphorylation in HeLa cells and isolated mouse glomeruli. Inhibition of Akt signaling phenocopied FK506 suggesting calcineurin regulates the WNK-pathway through Akt. Interestingly, two recent reports show that calcium and Ca^{2+}/CaM directly regulate WNK4 activity. M**ethods:** My goal is to use a single model system (mouse insulinoma Min6 cells) to determine the crosstalk between calcium signaling and the WNK1-pathway using endogenous proteins. Using fluorescence-reporter calcium assays, kinase assays and immunoblotting I will address: whether calcium signaling is modulated by osmotic stress (WNK-pathway activating stimulus); if so, whether calcium signaling regulates the WNK1-pathway; whether the WNK1-pathway feeds back to modulate calcium signaling; whether WNK-pathway inhibitors influenc this crosstalk; and whether WNK1 kinase activity is modulated by calcium or Ca^{2+}/CaM. **Results:** I found that hypertonic stress causes a biphasic increase in $[Ca^{2+}]_i$: first characterized by an acute sharp increase in $[Ca^{2+}]_i$ followed by a quick recovery; and then characterized by a slow and steady increase in $[Ca2^{+}]_i$. Inhibitor studies suggest OSR1/SPAK regulate the acute and quick recovery phases. Severe hypotonic stress caused a sharp decrease in $[Ca^{2+}]_i$ followed by a slow recovery. OSR1/SPAK inhibition in this condition caused a dramatic sharp and sustained increase in $[Ca^{2+}]_i$. C**onclusion:** Changes in osmotic pressure have well described effects in cellular morphology and volume, and fluctuations in volume modulate the cytosolic ionic strength. Here I am rigorously characterizing the crosstalk between calcium and WNK1 signaling pathways in Min6 cells.

123 **CPRIT Grantee**
Poster Session A

Using CRISPR/Cas9 Technology in the 129 Inbred Mouse Model to Study Testicular Germ Cell Tumor Susceptibility *D. Lanza, Baylor College of Medicine; E. Dawson, Baylor College of Medicine; J. Heaney, Baylor College of Medicine*

Introduction: The 129 inbred mouse strain is the only strain of mice to develop spontaneous testicular germ cell tumors (TGCTs). The frequency of tumors in 129 males can be increased or decreased using genetic modifiers, which interact with 129-specific susceptibility genes to influenc tumor incidence. Human genome-wide association studies (GWAS) have identified over 20 TGCT susceptibility loci in humans. Two of these loci contain genes (KITLG and DMRT1) that were first characterized as modifiers of TGCT incidence in 129 mice, which highlights the similarity between mouse and human TGCT genetic susceptibility and pathogenesis. Based on these findings, we propose that 129 inbred mice can serve as a platform for validation and functional characterization of additional genetic associations first identified by human GWAS. Furthermore, gene expression studies between low and high tumor risk mouse strains can provide a novel source of additional genes to test for potential modification of tumor susceptibility. **Methods:** Using genes identifie by human GWAS and next generation sequencing, exonic deletions were created by non-homologous end joining targeting critical exons by CRISPR/Cas9 technology. Critical exons for deletion were selected based on targeting strategies devised for the International Knockout Mouse Consortium. Genotyping of CRISPR-targeted mice were done by standard PCR to visualize deletion products, which were subsequently Sanger sequenced after TA cloning. High resolution melt analysis was performed to screen for off-target mutagenesis and select F1 mice without additional mutagenesis at non-targeted alleles. **Results:** To overcome the reproductive difficulties of using 129 mice, guide RNAs for three genes were pooled together in a single injection (Cyp26B1, Prdm14, and Dazl). Genes selected for pooling are not on the same chromosome and do not functionally interact. Founders were obtained with exon deletions for all three genes targeted, including founders with successful targeting for two genes in the same mouse. Founders were backcrossed to wild-type 129 mice and deletion alleles were successfully transmitted to the progeny. Tumor surveys are underway to determine the effect of the specific gene knockout on TGCT susceptibility in 129 mice. Once susceptibility genes are characterized using knockout lines, the CRISPR/Cas9 system will be

employed to knock-in human TGCT-associated polymorphisms into the 129 mouse genome to identify disease causing mutations. **Conclusion:** Our data highlight both the ease of CRISPR/Cas9 technology to create knockout animals on a specific strain, without the need for backcrossing, and the usefulness to study complex human diseases in easily accessible mouse models.

124 **CPRIT Grantee Poster Session B**

MAPK4 Induces AR Expression and Activation in Prostate Cancer *T. Shen, Baylor College of Medicine; W. Wang, Baylor College of Medicine; F. Yang, Baylor College of Medicine*

Introduction: Prostate cancer (PCa) is the most common cancer and the second leading cause of death from cancer in men in the United States. Androgen receptor (AR) signaling plays pivotal roles in the development and homeostasis of prostate tissue as well as in PCa progression. Androgen-deprivation therapy (ADT) remains as major standard treatment for advanced and metastatic PCa. However, most initially regressed PCa will relapse and progress to the lethal castration-resistance PCa (CRPC). Most CRPCs maintain a functional AR that is re-activated in the presence of castration level of ligands and/or AR antagonists. A common clinical observation of AR reactivation in CRPC is linked to direct modulation of AR mRNA or protein level. However, currently little is known about the adaptive molecular mechanism of AR overexpression in CRPC. In our preliminary study, MAPK4, an atypical mitogen activated protein kinase, was discovered to strongly induce AR expression and activation in PCa cells. MAPK4 expression is also strongly correlated with AR expression and activity in human CRPC tissues. Therefore, we hypothesize that MAPK4 activates AR and promotes CRPC and that MAPK4 may be a novel therapeutic target for CRPC. **Methods:** MAPK4 mRNA or protein level was surveyed in multiple PCa cells. MAPK4 expression is correlated with AR expression. 3-5 of independent small hairpin RNAs were used to stably knock down MAPK4 in PCa cells with high endogenous MAPK4 (MAPK-high). In addition, MAPK4 was stably expressed in PCa cells with low endogenous MAPK4 (MAPK-low) using virus-based expression system. AR signaling was examined by Western Blotting and Real Time-PCR. Cell proliferation and anchorage independent growth in vitro were characterized by MTT assay and soft agar assay. **Results:** Knockdown of MAPK4 in MAPK4-high PCa cells reduces AR mRNA and protein levels, inhibits AR activation (the expression of AR target genes including PSA and TMPRSS2), inhibits cancer cells proliferation and anchorage-independent growth. Overexpression of MAPK4 in MAPK4-low PCa cells induces AR expression and activation, as well as promotes cancer cell proliferation. Knockdown of MAPK4 reduces GATA2 level and overexpression of MAPK4 induces GATA2 level. Knockdown of GATA2 reduces AR level and activation. **Conclusion:** We conclude that MAPK4 induces GATA2 activation to promote AR expression and activation in PCa cells. MAPK4-GATA2-AR signaling axis may provide a plausible mechanism for AR re-activation in CRPC and this signaling axis emerges as a valuable therapeutic target for CRPC.

125 **CPRIT Grantee Poster Session A**

Beta Oxidation of Octanoate by Diverse Human Primary and Metastatic Brain Malignancies in an Orthotopic Transplant Model *K. Pichumani, The University of Texas Southwestern Medical Center at Dallas; T. Mashimo, The University of Texas Southwestern Medical Center at Dallas; R. DeBerardinis, The University of Texas Southwestern Medical Center at Dallas; C. Malloy, The University of Texas Southwestern Medical Center at Dallas; B. Mickey, The University of Texas Southwestern Medical Center at Dallas; E. Maher, The University of Texas Southwestern Medical Center at Dallas; R. Bachoo, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Brain tumors in situ are exposed to fatty acids, lactate, carbohydrates and other substrates for energy production, yet the overwhelming majority of studies focus on conversion of glucose to lactate in cultured cells. There is growing evidence that cells lines conditioned to culture may not reflect metabolism in situ, and some tumors appear capable of oxidizing substrates other than glucose. Furthermore, the enzymes for β-oxidation of fatty acids are present in some malignancies. In this study, our previously described human orthotopic tumor (HOT) glioblastoma GBM mouse models were studied by ^{13}C NMR isotopomer analysis to assess whether β-oxidation is active. Since animals (and patients) with advanced malignancies are often undernourished, the effects of hepatic gluconeogenesis on ^{13}C-labeling in plasma glucose, also available to the tumor, was evaluated. **Methods:** All studies were performed with approval of the local Institutional Review Board and Animal Care Committee. Six individual HOT models, all isocitrate dehydrogenase wild type, were used. Expression analysis for the common driver GBM mutations, c-Met, EGFR, P53 and PDGFRα, was performed. Three metastatic cancers were studied; breast cancer, adenocarcinoma, and endometrial cancer. MRI was used to monitor tumor growth. Under general anesthesia [U-^{13}C] octanoate (220mM, 2.5uL/min) was infused intravenously for 150 min. Liver and brain were rapidly dissected. The liver was freeze-clamped and the tumor was dissected. Tumor and surrounding brain were freeze-clamped separately. All tissue was extracted and the soluble fractions were studied by high resolution ^{13}C and ^{1}H NMR spectroscopy. Histology showed that > 95% of the cells in the tumor mass were malignant. **Results:** The lactate methyl resonance from tumor and surrounding brain was < 5% ^{13}C (from the ^{1}H spectrum) and of this, ~20% was [2,3-^{13}C] lactate (from ^{13}C spectrum).

Therefore, <1% of the pyruvate pool in the brain was [2,3-^{13}C] pyruvate derived from ^{13}C-labeled glucose from hepatic gluconeogenesis. Spin-coupled multiplets were observed in glutamine or glutamate in all tumors and in all non-tumor bearing brain. The C4 resonance of glutamine was dominated by [4,5-^{13}C]glutamine from octanoate and about 14% of the signal in C4 was derived from [3,4,5-^{13}C]glutamine. Isotopomer analysis showed that ~25% of acetyl-CoA was derived from infused octanoate; the remainder was derived from unlabeled substrates. Surprisingly, all tumors, regardless of origin, had preserved capacity to oxidize octanoate. **Conclusion:** In mice with orthotopic transplants of diverse human brain malignancies, beta oxidation is active in both surrounding brain and tumor.

126 **CPRIT Grantee Poster Session B**

MiRNA Markers that Predict Therapeutic Outcomes of Post-operative Radiotherapy in High-risk HNSCC *L. Ding, The University of Texas Southwestern Medical Center at Dallas; H. Tang, The University of Texas Southwestern Medical Center at Dallas; N. Karanam, The University of Texas Southwestern Medical Center at Dallas; T. Hwang, The University of Texas Southwestern Medical Center at Dallas; U. Giri, The University of Texas M.D. Anderson Cancer Center; J. Heymach, The University of Texas M.D. Anderson Cancer Center; M. Story, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Advanced squamous cell carcinomas of the head and neck (HNSCC) have high mortality rate even after complex and aggressive therapeutic regimens. Data from recent clinical trial of RTOG-0234 indicated a 5-year disease-free survival rate of about 50% after combined therapy. Distal metastasis and local-regional recurrence are the major causes of treatment failure. It is indicated that individualized selection of patients based on biomarkers would be ideal for determining the therapeutic regimens and improve the treatment outcome. We have performed miRNA profiling using a cohort of high-risk HNSCC patients and developed a miRNA signature that predicts clinical outcome of post-operative radiotherapy (PORT). **Methods:** Frozen HNSCC specimens were obtained from 118 patients treated by PORT at M.D. Anderson Cancer Center. All patients were considered to be at high-risk for recurrence by clinical and pathological criteria. They were further categorized by outcomes to PORT treatment, including 53 patients who responded to PORT (no evidence of disease, (NED)), 25 recurred locally (LR), 23 had distal metastasis (DM) and 17 with both LR and DM. Exiqon 7th-generation miRNA arrays were used for miRNA profiling. The dataset was randomly divided into a training set with 75 patients and a test set with 43 patients. Within the training set, a 36-miRNA signature was selected by using two-way ANOVA to identify miRNAs differentially expressed in DM or LR samples (FDR < 0.2 and fold change > 1.5), and by removing low-impact miRNAs to the initial classification models (relative importance < 0.1). Two classifiers were constructed using RandomForest models in the training set to classify DM and LR respectively. **Results:** Using the 36-miRNA signature, both classifiers resulted in reasonable prediction accuracy with AUC = 0.77 for DM prediction and AUC = 0.7 for LR in the test set. The combined high risk of being predicted as DM or LR resulted in significant decrease of patients' survival. Many of the miRNAs from the 36-miNA signature, such as miR-125a, miR-374b, miR-28, miR-615-5p and ebv-miR-BART9, are known to be associated with tumor progression and metastasis. Our data indicated that miR-551b-3p facilitates HNSCC cell migration and invasion by enhancing autophagy. We are in process to validate our signature in independent miRNA dataset, Such as TCGA miRNA-seq data for HNSCC. **Conclusion:** Our data demonstrated the potential of miRNAs as biomarkers that predict therapeutic outcomes and suggested the mechanistic studies of miRNA markers will lead to novel pathway discovery and further improvement of cancer treatment.

127 **CPRIT Grantee Poster Session A**

Genome-wide Modulatory Network Involved in Epithelial-mesenchymal Transition is Associated with Three-dimensional Chromatin Organization *X. Li, The University of Texas Medical Branch at Galveston; M. Zhu, The University of Texas Medical Branch at Galveston; T. Bing, The University of Texas Medical Branch at Galveston; J. Yang, The University of Texas Medical Branch at Galveston; B. Fongang, The University of Texas Medical Branch at Galveston; D. Homouz, The University of Texas Medical Branch at Galveston; A. Brasier, The University of Texas Medical Branch at Galveston; A. Kudlicki, The University of Texas Medical Branch at Galveston*

Introduction: Epithelial-mesenchymal Transition (EMT) is a cell de-differentiation process involved in embrogenesis, would healing and cancer metastasis, where epithelial cells are transformed to mesenchymal cells. This process involves epigenetic and transcriptional programming by rewired network of TFs and histone modifiers, which activated or depressed TFs in a target gene specific way. Nuclear subcompartments may form specialized transcriptional hubs or factories, comprise an important layer of TFs activity modulation during EMT. Previously, we computationally identified the potential master regulators of type EMT process, by comparing type EMT perturbed genes with the target genes obtained from ChIP-Seq or ChIP-chip data based on hypergeometric distribution tests. Here, we characterize the regulatory and modulatory network of EMT in a 3D chromatin organization context. **Methods:** A modulator (M) is a protein or gene modifies a TF activity by binding or indirect interacting with the TF in a target gene (TG) unique way. An M can enhance, attenuate, or invert a TF activity in activating or inhibiting the TG expression. All modulators-TF-TG triplets of the EMT perturbed genes comprise a modulatory network. The effect of a modulator and transcription factor on a target gene expression is inferred by a probabilistic model with interactive term, based on a high compendium of gene expression profiles, expO. The chromatin subcompartment data were downloaded from GEO (GSE63525). **Results:** Genes from different nuclear compartment have differential functions. The A1 subcompartment genes are enriched in chromatin organization, cell cycle, translation, protein transport and DNA damage response process. A2 subcompartment genes are enriched in RNA and ncRNA processing, cell cycle and innate immune process. B compartment genes are enriched in development associated processes. After mapping the modules of the

EMT modulatory network into the nuclear subcompartments, we found that different network modules have distinct subcompartments distribution and involved in more loops. Up and down regulated genes in EMT are distributed in different compartments. The target genes of TFs identifie from ChIP-Seq are not randomly distributed in nuclear subcompartments, while TGs identified from motif enrichment only surprisingly did not show the same non-random distribution. **Conclusion:** The results indicate that the chromatin organization and accessibility play a great role in TF activity and modulatory network of EMT. The modules of the modulatory network are organized in a form of nuclear compartments in the nucleus. Currently, we are developing novel methods to refine, evaluate and characterize, nuclear compartment and chromatin state boundary regions.

128 **CPRIT Grantee Poster Session B**

The Tumor Suppressor DEAR1 Negatively Regulates TGFBeta-Driven EMT in Breast Cancer *A. Killary, The University of Texas M.D. Anderson Cancer Center; S. Balasenthil, The University of Texas M.D. Anderson Cancer Center; N. Chen, The University of Texas M.D. Anderson Cancer Center*

Introduction: Ductal carcinoma in situ (DCIS) is an early, preinvasive form of breast cancer which, if untreated, progresses to invasive ductal carcinoma (IDC) in approximately 40% of cases. Predictive and prognostic markers are critically needed to stratify DCIS with a heightened risk of progression to IDC for which more aggressive surveillance and treatment might be warranted, as well as individuals with favorable prognosis, who might be spared rigorous therapeutic regimens. Our laboratory has discovered a novel TRIM family member DEAR1 (Ductal Epithelium Associated Ring Chromosome 1, annotated as TRIM62) that is mutated and homozygously deleted in breast cancer. DEAR1 expression is downregulated in DCIS lesions, suggesting its role in early breast cancer. Targeted disruption of Dear1 in the mouse results in late onset adenocarcinomas of multiple tissues, including mammary adenocarcinoma, as well as lymphoma and sarcoma. DEAR1 is thus a novel tumor suppressor, the mutation or loss of function of which, may play an important role in DCIS to IDC progression. M**ethods:** shRNA lentiviral stable knockdown of DEAR1 was performed in human mammary epithelial cells to examine epithelial-mesenchymal transition (EMT), thought to be the earliest step in migration and invasion from the primary tumor. Wound assays, anoikis resistance, migration and invasion assays were performed as well as expression studies. Ubiquitination assays and in vitro and in vivo binding assays were also performed. **Results:** DEAR1 loss of function in human mammary epithelial cells in the presence of TGFβ results in loss of polarity, failure of acinar morphogenesis, upregulation of EMT markers, anoikis resistance, migration and invasion. DEAR1 blocks TGFβ-SMAD3 signaling by binding to and promoting the ubiquitination of SMAD3, the major effector of TGFβ-induced EMT. DEAR1 loss increases levels of SMAD3 downstream effectors, SNAI1 and SNAI2, with genetic alteration of DEAR1/SNAI2 serving as prognostic markers of poor survival in a large cohort of invasive breast cancers. C**onclusion:** Thus, DEAR1 functions as a breast cancer tumor suppressor and master regulator of TGFβ-driven EMT, suggesting that DEAR1 mutation/loss could be important in DCIS to IDC transition. DEAR1 also controls polarity and tissue architecture in mammary epithelial cells. Experiments are underway to determine if DEAR1 is a critical regulator of polarity and signaling from the microenvironment in breast cancer which should result in a novel paradigm for the regulation of both EMT and polarity as well as novel therapeutic treatments aimed at the pathways regulated by DEAR1 to prevent IDC progression.

129 **CPRIT Grantee Poster Session A**

Multiphoton/Confocal Spectroscopy in Cancer Research at TTUHSC *Q. Smith, Texas Tech University Health Science Center at Amarillo; R. Samala, Texas Tech University Health Science Center at Amarillo; H. Thorsheim, Texas Tech University Health Science Center at Amarillo*

Introduction: Confocal/multiphoton microscopy fills an important niche in the research at the Texas Tech University Health Sciences Center (TTUHSC), allowing fluorescent imaging at high resolution in three dimensions in living tissues and cell preparations. The Nikon A1R-MP laser scanning confocal system was purchased with a combination of CPRIT (RP110786) and institutional funds. This technology has opened up a broad range of novel cancer blood flow and cell migration studies utilizing the high speed resolving power that the A1R-MP multiphoton microscope uniquely allows. **Methods:** Multiphoton microscopy was used to localize and quantify cells of interest in living tissues in vivo and in cryopreserved slices of tissue. Fluorescently tagged cells were also used to track movement, and activity of cancer cells across the body and the roles of selected proteins in transvascular transport. Finally, vessels and tumors were imaged ex vivo or in vivo to characterize vascular growth patterns in tumors and healthy tissues and the effects of various treatments on these patterns. **Results:** Investigations of brain metastases, mammospheres of breast cancer, and renal microfibroblasts all benefited from use of the Nikon A1R-MP microscope. Dissemination of cancer cells across vascular endothelial, lymphatic, and blood-brain barrier tumors were all studied. The instrument demonstrated the separate roles of vascular trapping, transvascular transcytosis, and migration within the tissue interstitial space. Multiple grant applications were submitted based upon the obtained data. The instrument has enhanced the cancer research of our institution and has also resulted in recruitment of faculty with specialty in multiphoton-based cancer research. **Conclusion:** The use of confocal and multiphoton microscopy has become the standard of excellence for research at TTUHSC, and the resulting high quality images that can be produced with this system have greatly enhanced publications and grant applications.

130 **CPRIT Grantee**
Poster Session B

HSD11B1-Generated Glucocorticoids Fuel Ovarian Cancer Growth and Lipid Metabolism *P. Dorniak, The University of Texas M.D. Anderson Cancer Center; A. Sidalaghatta Nagaraja, The University of Texas M.D. Anderson Cancer Center; C. Ivan, The University of Texas M.D. Anderson Cancer Center; P. Ram, The University of Texas M.D. Anderson Cancer Center; A. Sood, The University of Texas M.D. Anderson Cancer Center*

Introduction: Ovarian cancer (OvCa) is the leading cause of mortality among women in developed countries. It is now well recognized that the bio-behavioral factors, such as chronic stress, depression and low social support, promote ovarian cancer progression. Effects of stress are mediated by cortisol, a glucocorticoid hormone released by the hypothalamic–pituitary–adrenal (HPA) axis in response to environmental stimuli. Emerging clinical evidence indicates that disrupted circadian cortisol rhythms and elevated nocturnal cortisol are commonly observed in OvCa patients before the primary treatment, but remain normalized in those who do not develop recurrent disease within one year after surgery. In our preliminary study, we assessed the expression of HPA axis-associated genes in our OvCa orthotropic mouse model and found that chronic stress stimulates tumoral expression of *HSD11B1* encoding the rate-limiting enzymes important for cortisol generation. Therefore, we hypothesize that ovarian tumor disrupts diurnal cortisol rhythms by continual ectopic cortisol activation and asynchronous HPA axis stimulation and these effects are augmented by bio-behavioral stress. **Methods:** To examine the biological role of HSD11B1-derived glucocorticoids in ovarian cancer, we used both *in vitro* and *in vivo* approach followed by comprehensive analyses of TCGA data sets. **Results:** First, we examined *HSD11B1* mRNA abundance and HSD11B1 activity in the panel of OvCa cells and found that ES2, HEY8, OVCAR3, OVCAR432 and SKOV3 cancer cells generate cortisol from cortisone, inactive glucocorticoid precursor that is readily available in the blood. Next, we found that *HSD11B1* loss-of-function reduced viability and motility of HEY8A and SKOV3 *in vitro*. Our *in vivo* studies with SKOV3ip1 and HEY8A demonstrate, that DOPC-encapsulated *HSD11B1* siRNA reduced tumor growth and metastasis in our restrained stress ovarian cancer orthotropic mouse model and that was associated with reduced glucocorticoids level in the blood. Detailed assessment of tumor samples revealed that HSD11B1-regenerated glucocorticoids regulate lipid droplets accumulation. Our *in vitro* studies demonstrate that cortisol affects lipid metabolism and these effects are mediated via *LPIN3* that encodes the key enzyme in glycerolipid biosynthesis. Furthermore, analysis of TCGA data sets indicated that high *LPIN3* expression is correlated with poor overall survival of OvCa patients. **Conclusion:** Results of these studies provide novel insight in glucocorticoid action in OvCa cell and suggest that novel therapeutic strategies should be developed to mitigate glucocorticoid-mediated stress effects in OvCa patients.

131 **CPRIT Grantee**
Poster Session A

Identification of MSI and POLE Mutations in Colorectal and Endometrial Tumors Using the Texas Cancer Research Biobank *E. Shinbrot, Baylor College of Medicine; K. Covington, Baylor College of Medicine; N. Dewal, Baylor College of Medicine; M. Gingras, Baylor College of Medicine; R. Gibbs, Baylor College of Medicine; L. Becnel, Baylor College of Medicine; M. Ittmann, Baylor College of Medicine; D. Wheeler, Baylor College of Medicine*

Introduction: Endometrial (EC) and Colorectal (CRC) cancer are two of the most common cancers. Both of these cancers contain high mutation frequency subtypes caused by either microsatellite instability (MSI) or mutations in POLE. MSI is characterized by elevated mutation frequencies especially at regions consisting of mono or di nucleotide repeats (homopolymer regions), thought to be caused by defective mismatch repair genes, while POLE mutant tumors show elevated C>A mutations in a TCT context caused by defects in the exonuclease domain of POLE. The identification of MSI is important for prognosis and potential treatment options. M**ethods:** In this study we examined 38 CRC and 19 EC tumors using an ampliseq gene panel that we designed to allow identification of MSI and POLE tumors in both CRC and EC. We examined the number of insertions and deletions at homopolymer sites to arrive at an HP score per tumor. POLE mutations were examined using traditional variant calling approaches. **Results:** We identified 7 tumors with microsatellite instability, all from CRC tumors. In addition, we identified one POLE P286R mutated tumor from EC. We are further characterizing the mutations in these tumors. C**onclusion:** We have developed a method for identifying MSI from targeted sequence data. Our current panel is able to simultaneously identify POLE-mutation status and MSI.

132 **CPRIT Grantee**
Poster Session B

JNK2 Oligomerization Regulates its Activation Through Non-Canonical Pathways *T. Kaoud, The University of Texas at Austin; A. Riggs, The University of Texas at Austin; K. Dalby, The University of Texas at Austin*

Introduction: C-Jun N-terminal kinases (JNKs) regulate various cellular functions, their activation is known to be mediated cooperatively by both MKK4 and MKK7. Uniquely, c-Jun N-terminal kinase 2 (JNK2) isoform has been reported to be constitutively activated in glial tumor cell lines and human glioblastoma models. **Methods:** Herein, we investigated the regulation of JNK2 self-activation in vitro in both cell-free and cell-based experiments. **Results:** The light scattering analysis suggested that unphosphorylated recombinant JNK2α2 exists in solution as a mixture of monomers, dimers and tetramers. JNK2α2 self-phosphorylates more rapidly in vitro when assayed at low concentration leading to its activation. However, at a slightly higher concentration, the formation of a tetramer is favored, whose ability to self-phosphorylate is suppressed. Similarly, HEK293T cells that were transfected with varying amounts of pcDNA-JNK2α2 showed that an increased concentration of JNK2α2 (54 kDa) could suppress its self-activation in mammalian cells. Inactive DFG-out conformation of JNK2 that stabilized by F170R mutation has triggered the existence of inactive JNK2 tetramer and when HEK293 cells were treated by 50 μM BIRB796 (as inducer of DFG-out conformation of JNK2), we were able to cross link the tetramer in the cell lysate using DSS (disuccinimidyl suberate), suggesting that JNK2 tetramer auto-inhibition may return to favoring the DFG-out conformation. C**onclusion:** Oligomerization of JNK2 regulates its activation through non-canonical (by self-activation) pathways.

133 **CPRIT Grantee**
Poster Session A

Si-2 is a novel SRC-3 inhibitor and successfully inhibits TNBC progression *X. Song, Baylor College of Medicine; C. Zhang, Baylor College of Medicine; J. Chen, Baylor College of Medicine; M. Zhao, Baylor College of Medicine; D. Lonard, Baylor College of Medicine; B. O'Malley, Baylor College of Medicine; J. Wang, Baylor College of Medicine*

Introduction: Triple negative breast cancer (TNBC) is one of most malignant disease among all types of breast cancer. Since the absence of hormone receptors and HER2, TNBC patients do not respond to any FDA approved hormone therapies and HER2 targeted therapies, which work well on none-TNBC cancers. To identify novel molecular targets and develop corresponding inhibitors is always highly demanded for TNBC treatment **Methods:** The SRC-3 inhibitors was screened out by pBIND based high-throughput screening. Si-2 was chemically synthetized in our lab. The tumor inhibition function of Si-2 was tested in an TNBC orthotopic mouse model. **Results:** We found Si-2 specifically and significantly down-regulate SRC-3 expression level. Si-2 inhibits TNBC cell proliferation with IC50 at low nM range, but does not bring obvious cytotoxicity on Hepatocyte cells. We also demonstrated that Si-2 effectively reduce TNBC tumor growth rate in an orthotopic TNBC mouse model. Moreover, the cardiotoxicity of Si-2 was not detectable. **Conclusion:** For TNBC treatment, Si-2 is considered as a promising drug, which is featured with high specificity to SRC-3, significant toxicity to TNBC tumor cells, and non-detectable cardiotoxicity.

134 **CPRIT Grantee**
Poster Session B

Role of Stromal DDR-1 in Obesity associated Breast cancer *K. Gupta, The University of Texas Health Science Center at San Antonio; R. Li, The University of Texas Health Science Center at San Antonio; B .Yuan, The University of Texas Health Science Center at San Antonio*

Introduction: Breast Cancer is a highly heterogeneous disorder. It has been reported that women with high mammographic density have 2-6 fold higher risk of developing breast cancer and, changes of tumor stroma are a predictive of patient survival. Adipose cells are one of the major cell types in the stromal compartment of breast and have an active role in tumor cell survival and progression. Studies have shown mechanical forces as the key regulators of the mammary gland phenotype. These mechanical cues are transduced through the discoidin domain receptor-1 (DDR-1), which forms a part of the arsenal of cell surface receptors that mediate tumor cell-collagen interactions. DDR-1 expression has been found to be upregulated in several tumors models, and is known to play a role in tumor epithelial cell adhesion and migration. However, very less is known about the role of DDR-1 in the tumor stromal compartment. Therefore, we aim to investigate the role of stromal DDR-1 in the development and progression of breast cancer. **Methods:** DDR-1 Wildtype (WT) and Knockout (KO) mice will be injected with highly aggressive M-Wnt cells (Wnt activated mesenchymal cell line, derived from MMTV-Wnt-mouse mammary tumors). Tumor growth will be monitored for 5-6 weeks and tumor volume will be estimated. To elucidate the mechanisms, we aim to delineate the effects of stromal DDR-1 on proliferation and migration of M-Wnt cells using conditioned media from the mice primary adipose stromal cells. Since advanced tumors are fibrotic, tumor sections obtained from WT and KO mice will be analyzed using second harmonic generation imaging (SHGI) for the differential expression of collagen fibers, respectively. Furthermore, we will collect blood plasma from these tumor bearing DDR-1 WT and KO mice for cytokine profiling to understand the role of stromal DDR-1 in the overall tumor physiology. A cohort of obese mice (DDR-1 WT and KO) will be generated and similar experiments will be performed to get a deeper insight into the association between obesity and breast cancer. **Results:** We have observed a significant reduction in the weight and volume of tumors obtained from DDR-1 KO mice as compared to the WT mice. Our invitro data shows a significant reduction in the cell migration properties of M-Wnt cells when subjected to the conditioned media obtained from the primary adipose stromal cells of DDR-1 KO mice as compared to that of the DDR-1 WT mice (further experiments ongoing). **Conclusion:** Stromal DDR-1 plays a role in breast tumor development.

135 **CPRIT Grantee**
Poster Session A

Biases and sequencing errors in the Illumina HiSeq X series of sequencing systems *X. Liao, Baylor College of Medicine; K .Covington, Baylor College of Medicine; D. Wheeler, Baylor College of Medicine*

Introduction: Illumina has broken down barriers in human genome sequencing and dramatically reduced the price to sequence a human genome with their next-generation sequencing (NGS) systems, in particular the HiSeq X series of sequencing systems. The HiSeq X Ten System, a set of 10 HiSeq X instruments, has broken the $1000 barrier for 30x coverage human whole-genome sequencing. Those instruments utilize paired-end sequencing technology, which enables both ends of DNA fragment to be sequenced, and generate forward reads and reverse reads, also called read one and read two. It is usually believed that the base quality of read one and read two are the same because of random sequencing error. Here weanalyzed tested to determine whether the sequencing errors distribute equally between read one and read two generated by HiSeq X Ten. **Methods:** We utilized the Illumina HiSeq X Ten System and went through 30x coverage of whole-genome sequencing for 259 lung cancer tumor and normal samples collecting from 125 subjects. We aligned the raw sequencing reads to the human reference genome version 19 (hg19), and identified all sequence variants, including single-nucleotide polymorphisms (SNPs), small insertions and deletions (INDELs). We analyzed and compared each of the variants in read one and read two, and tested to determine whether they distribute equally. **Results:** Our results shown that the distributions of variants are significantly different between read one and read two. We found that most of the variants appear more frequently in read two than in read one, and some of the variants even double in counts in read two. In contrast to random sequencing errors and equal error rates, these biases indicate that read two has higher sequencing error rates than read one. Furthermore, the raw sequencing reads from the HiSeq X series also show lower base quality than Illumina's previous platforms. **Conclusion:** The low base quality, and biased sequencing error rates between read one and read two definitely affect subsequent variant calling, and impact its accuracy. This leads to a concern particularly for cancer genome analysis, which requires high base quality and low sequencing error rate because of low mutation rates. Further conduction are needed to check whether these biases come from coding regions or non-coding regions.

136 **CPRIT Grantee Poster Session B**

Epigenetic analysis following combinatorial exposure to bisphenol A and genistein reveals their mammary cancer predesposition properties *R. Jadhav, The University of Texas Health Science Center at San Antonio; T. Huang, The University of Texas Health Science Center at San Antonio; V. Jin, The University of Texas Health Science Center at San Antonio; C. Lamartiniere, University of Alabama School of Medicine*

Introduction: Long term exposure to estrogenic compounds have been known to be responsible for development of breast cancers in adult females. Our earlier studies revealed this long term exposure to estrogen in breast cancer cell lines introduce epigenetic alterations that alter the expression of many tumor suppressors and oncogenes. Here we investigate prepubertal exposure to BPA and genistein which are both environmental estrogens, on rats followed by MBDCap-seq to look at long term alterations in DNA methylation. **Methods:** Prepubertal rats (postpartum days (PND) 2–20) were exposed through lactation via nursing dams treated orally with BPA and genistein. Mammary glands from 100 day old rats were then used to perform MBDCap-seq analysis. MBDCap-seq data was also collected from various breast cancer cell lines. Bioinformatics analysis on sequencing data focused on DNA methylation changes in the gene promoter regions. IPA network and pathway analysis were further implemented to identify candidate genes. **Results:** We identified many genes differentially methylated in the promoter regions after exposure to BPA and genistein. Specificall , BPA exposure resulted in hypo-methylation of *Bag1* and *Etva*, two genes with oncogenic properties and hyper-methylation of *Prss8* a tumor suppressor gene. On the other hand, exposure to genistein, resulted in hypermethylation of six oncogenes *Bag1*, *Hmgn5*, Igf2, *Kif26b*, *Pak2* and *Kdm1b* and hypo-methylation of the tumor suppressor *Prss8*. Moreover, in rats exposed to both BPA and genistein, we observed hyper-methylation of all of these oncogenes. **Conclusion:** DNA methylation analysis revealed that prepubartal BPA exposure may increase susceptibility to mammary cancer development by epigenetically silencing tumor suppressor genes and activating oncogenes. On the other hand, genistein exposure results in epigenetic silencing of oncogenes and activation of tumor suppressor genes indicating towards its chemoprevention properties. In contrast to single chemical exposure, combinatorial exposure to BPA and genistein showed similar epigenetic patterns as genistein exposure alone, as it resulted in epigenetic silencing of many oncogenes. Differential methylation of these genes further support the roles of BPA in mammary cancer development and genistein's chemoprevention properties. Our data also indicates that genistein could possibly be able to epigenetically suppress BPA predisposition for mammary cancer development.

137 **Poster Session A**

Molecular Predictors of Long-Term Survival in Glioblastoma multiforme Patients *J. Lu, NeuroTexas Institute, St. David's Medical Center; M. Cowperthwaite, NeuroTexas Institute St. David's Medical Center; M. Burnett, NeuroTexas Institute; M. Shpak, NeuroTexas Institute St. David's Medical Center*

Introduction: Median survival time following diagnosis with Glioblastoma multiforme (GBM) is approximately 14 months. However, approximately 10% of patients survive longer than 3 years. We performed integrative study of molecular data and clinical variables on these long-term survivors (LTSs) to identify biomarkers associated with improved GBM patient outcomes, and to assess the possible similarity of molecular characteristics between low grade glioma (LGG) and LTS GBM. **Methods:** We analyzed the relationship between multivariable molecular data and LTS in GBM patients from the Cancer Genome Atlas (TCGA), including somatic mutations, gene expression, DNA methylation, copy number variation (CNV) and microRNA (miRNA). The continuous survival time was dichotomized with a cutoff of 3 years. Due to the large number of genomic predictor variables, initial feature selection was performed on individual genomic measurements using Least Absolute Shrinkage Selection Operator (LASSO) logistic regression, and with univariate logistic regression on individual variables followed by Benjamini-Hochberg FDR correction on the p-values. Models constructed with combinations of clinical variables and one or more classes of genomic variables were evaluated using tenfold cross-validation with mean Area Under the Curve (AUC) as performance prediction. The relationship between GBM LTS and LGG patients was examined through principal component analysis (PCA) on gene expression. **Results:** The overall survival time of the 593 sample GBM cohort ranges from 0-10.6 years. 44 (9.6%) of the patients survived more than three years and were classified as LTS patients. In a regression model considering only clinical and demographical variables, age is the only significant predictor of LTS ($p = 1.99e-07$). Feature selection with Lasso logistic regression identified 13, 94, 43, 29, and 1 significan predictors from the point mutation, gene expression DNA methylation, CNV, and miRNA data sets, respectively. Individually, DNA methylation yielded the best prediction performance (AUC = 0.77±0.10). Combining age, miRNA with gene expression (AUC = 0.90±0.01) or DNA methylation (AUC = 0.90±0.02) significantly improved prediction accuracy. Inclusion of three or more genomic categories did not further improve the prediction performance. We found no evidence that GBM LTS had gene expression profiles similar to LGG in spite of improved prognosis. **Conclusion:** We identified individual and combinatorial molecular signatures associated with GBM LTS through integrative analysis of multidimensional genomic data. We showed that combining age with several genomic measurements improved the predictive accuracy of LTS. However, the molecular markers underlying longer survival time of GBM are distinct from LGG.

138 **Poster Session B**

Stable In Vitro Reprogramming of Lentiviral Vectors for Targeted Delivery of Genetic Cargo *N. Kasaraneni, Texas A&M University System Health Science Center; A. Chamoun-Emanuelli, Texas A&M University System Health Science Center; Z. Chen, Texas A&M University System Health Science Center*

Introduction: Progress in gene therapy has been hampered by the absence of a suitable delivery vector that is both easy to produce and delivers genetic payloads efficiently and specifically to the targeted disease cells. Cell-targeting proteins, primarily monoclonal antibodies, already exist in abundance but there is currently no robust and reproducibly effective way to functionalize viral vectors with these proteins. In the case of antibodies, non-covalent approaches to incorporate antibody onto a lentivirus surface leaves the linkage vulnerable to interference from serum immunoglobulins in immune-competent individuals. The overall goal of this work is to enable facile reprogramming of lentiviral vectors to deliver genetic payloads to specific cell types through in vitro covalent functionalization with cell-binding proteins. **Methods:** A disulfide bond-forming protein-protein pair, the N-terminal PDZ domain of InaD (PDZ1) and its penta-peptide ligand (TEFCA) from Drosophilia protein NorpA, are exploited to covalently attach a cell-binding protein to the surface of a lentivirus. PDZ1 is inserted into an extracellular loop in the binding-deficien fusion-competent Sindbis virus envelope protein to form Sind-PDZ1, and the TEFCA tag is conjugated to a model HER2-binding designed ankyrin repeat protein (DARPin) to form DARPin-TEFCA. Coincubation of Sind-PDZ1-pseudotyped lentivirus (Sind-PDZ1-pp) with DARPin-TEFCA leads to covalent functionalization of the lentivirus with the HER2-binding protein through a disulfide linkage. **Results:** Lentiviruses pseudotyped with the chimeric envelope protein Sind-PDZ1 were covalently functionalized with DARPin-TEFCA. A high titer of 6x106 IU/ml was obtained for DARPin-displaying Sind-PDZ1-pp in HER2+ SKOV3 cells. The transduction efficiency appears to be dependent on the cell surface expression level of HER2. The interaction of DARPin-TEFCA with Sind-PDZ1-pp was found to be irreversible under non-reducing conditions, with transduction efficiency remaining largely unchanged after prolonged dialysis (5 days) of DARPin-displaying virions in a 1000-fold volumetric excess of DARPin-free buffer. **Conclusion:** We developed an in vitro chemical biological approach for covalently conjugating a cell-binding protein to lentiviruses, thus providing a convenient new tool to potentially reprogram lentiviral vectors to deliver their genetic cargo to specific cell types in vivo. This work should expand the utility of lentiviruses as gene delivery vectors for targeted cancer therapy. Ongoing work in the lab aims to extend this strategy to functionalize lentiviruses with monoclonal antibodies and assess the ability of the reprogrammed lentiviral vectors to deliver functional genetic cargo to specific cell types in vivo

139 **Poster Session A**

The Effect of pH Dependence of Antibody-Antigen Interactions on Subcellular Trafficking Dynamics *S. Devanaboyina, Texas A&M University; S. Lynch, The University of Texas Southwestern Medical Center at Dallas; R. Ober, Texas A&M University; S. Ram, The University of Texas Southwestern Medical Center at Dallas; D . Kim, Texas A&M University; A. Puig-Canto, The University of Texas Southwestern Medical Center at Dallas; S. Breen, Medimmune; S. Kasturirangan, Medimmune; S. Fowler, Medimmune; L. Peng, Medimmune; H .Zhong, Medimmune; L. Jermutus, Medimmune; H. Wu, Medimmune; C. Webster, Medimmune; S. Ward, Texas A&M University; C. Gao, Medimmune*

Introduction: IL-6 is a pro-inflammatory cytokine involved in numerous autoimmune and chronic inflammatory diseases. IL-6 also plays a crucial role in development and progression of cancers like prostate, ovarian, and renal cell carcinomas and lymphoid malignancies. In these pathological scenarios there is a dramatic elevation of IL-6, prompting development of effective blockade of IL-6 signaling by antibodies specific for IL-6 or anti-IL-6R. However, antibody binding to antigens such as inflammator cytokines can extend the in vivo persistence of the targeted through a 'buffering' effect. By modulating the antibody-antigen interaction in a pH-dependent manner, i.e. binding to antigen with high affinity at neutral pH relative to acidic endosomal pH, this undesired 'buffering' effect can be ablated. The current study involves the use of advanced fluorescenc microscopy to define the subcellular trafficking processes, including endosomal sorting, of IL-6 specific antibodies that have different pH dependencies for binding to antigen in the pH range 6.0-7.4. M**ethods:** The pH-dependent anti-IL-6 antibodies were generated using phage display followed by histidine scanning and their binding to IL-6 at pH 6.0 and 7.4 was analyzed by surface plasmon resonance (BIAcore). Fixed cell microscopy was used to analyze the distribution of IL-6 in the presence of anti-IL-6 antibodies with different pH-dependencies. This was combined with multi-color, live-cell fluorescence microscopy to investigate the spatiotemporal dynamics of multiple fluorescent-tagged proteins (IL-6, anti-IL-6 antibodies, and FcRn) simultaneously. In vivo pharmacokinetic studies were performed in mice to analyze the serum clearance rates of IL-6 when co-injected with engineered anti-IL-6 antibodies. **Results:** As the affinity of the antibody:IL-6 interaction at pH 6.0 decreases, an increasing amount of antigen dissociates from FcRn-bound antibody in sorting endosomes. The dissociated IL-6 trafficks into lysosomes. In live cells, antibody-FcRn complexes segregate from sorting endosomes in tubulovesicular transport carriers (TCs) into the recycling pathway, and the extent of IL-6 association with TCs correlates with increasing affinit of antibody:IL-6 interaction at endosomal pH. In-vivo, IL-6 was rapidly cleared from serum when injected along with a pH-dependent antibody in comparison with a pH-independent anti-IL-6 antibody. C**onclusion:** Subcellular trafficking analyses of antigen in the presence of antibodies with a range of pH dependencies can be used to inform the dynamic behavior of targeted antigen within cells. Decreased binding of antibody to antigen at acidic pH results in reduced recycling of antigen/antibody complexes in TCs and increased lysosomal trafficking of antigen for degradation, thus decreasing the in vivo levels of targeted antigen.

140 **Poster Session B**

The Effect of Radiofrequency and Non-Radiofrequency Induced Hyperthermia on Endothelial Cell Permeability *J. Ho, Baylor College of Medicine; L. Vergara, Baylor College of Medicine; S. Corr, Baylor College of Medicine; M. Ware, Baylor College of Medicine; R. Serda, Baylor College of Medicine; S. Curley, Baylor College of Medicine*

Introduction: Radiofrequency-induced hyperthermia (RFH) is an established technique uniquely capable of heating deep tissues. Hyperthermia has been described to increase endothelial permeability[1], although the mechanism has not been elucidated. Investigating the effects of RFH on endothelium is particularly relevant due to recent use of RF in triggering and delivering nanoparticles to the tumor microenvironment[2]. The purpose of this study was to investigate the effect of RFH versus non-RFH (water bath[WB]) on endothelial cell permeability. M**ethods:** Human microvascular endothelial cells (HMVEC) were plated as a monolayer onto 3µm pore-size Transwell permeable supports in quadruplicate. Cells were treated with RF and WB to target temperatures of 37, 40, 43, and 46°C each. Relative permeability of the HMVEC monolayer was measured by placing FITC-dextran (40kDa MW) into the upper Transwell chamber of each sample for 1 hour, then sampling the bottom chamber for fluoresence intensity at 519nm with a plate reader. Treatment groups were analyzed with two-sample t-tests compared to control. Separately, HMVEC were plated onto glass coverslips and subjected to the same RF and WB treatments, and were immunofluorescently stained for VE-cadherin, F-actin, and nucleus with primary antibody, phalloidin, and DAPI, respectively. The slides were imaged with confocal microscopy. **Results:** Both RF and WB treatment groups had statistically decreased permeability as temperature increased as much as one-tenth of control ($p<0.002$), except for 46°C RF in which a 7.8-fold increase in permeability was measured ($p=0.0029$). This likely signified a threshold breakdown in cell-to-cell adhesion that was present in the RF-treated group but not WB, suggesting that RF enhances the thermal effect. On immunofluorescence HMVEC become morphologically globular with actin fiber breakdown with hyperthermia treatment. Cell-to-cell junctions are not compromised at 43°C and instead may be enhanced. **Conclusion:** Hyperthermia decreases HMVEC permeability in a temperature-dependent fashion, contrary to previous studies. With RF-hyperthermia, permeability greatly increases at a critical threshold temperature, possibly due to breakdown of cell-to-cell junctions. If hyperthermia increases tissue uptake of drugs and nanoparticles, it is not likely due to the direct effect of hyperthermia on endothelial cells, but perhaps due to another mechanism such as the local inflammatory response to hyperthermia and associated cyto ines.

141 **Poster Session A**

Phosphodiesterase 4B is an Actionable Proangiogenic Factor in B Cell Lymphoma *A. Suhasini, The University of Texas Health Science Center at San Antonio; L. Wang, The University of Texas Health Science Center at San Antonio; K. Holder, The University of Texas Health Science Center at San Antonio; A. Lin, The University of Texas Health Science Center at San Antonio; H. Bhatnagar, The University of Texas Health Science Center at San Antonio; A. Moritz, The University of Texas Health Science Center at San Antonio; R. Aguiar, The University of Texas Health Science Center at San Antonio*

Introduction: Diffuse large B cell lymphoma (DLBCL) is a common tumor with an approximately 60% cure rate. Acquiring basic biology data in specific priority areas may improve the outcome of DLBCL. One important knowledge gap concerns the interplay between lymphoma cells and the microenvironment, especially because angiogenesis is associated with poor outcome in DLBCLCyclic-AMP (cAMP) is a second messenger that in B cells exerts mostly negative effects. In these cells, cAMP signaling is terminated by phosphodiesterase 4 (PDE4). We identified PDE4B in an outcome prediction signature of DLBCL and showed that its inhibition had anti-lymphoma properties. cAMP activity is highly contextualized and it appears to also attenuate vessel development in non-neoplastic cell models. Thus, we speculated that high PDE4B expression/activity, by abrogating cAMP signaling, could modulate angiogenesis in DLBCL **Methods: Results:** Using cell lines, we found that cAMP suppressed VEGF levels in PDE4B-low but not in PDE4B-high DLBCLs. In human umbilical vein endothelial cell (HUVEC) tube formation assays, we showed that conditioned media from PDE4B-high DLBCLs were significantly more angiogenic than from PDE4B-low models. Further, ectopic expression of PDE4B blocked the anti-angiogenic properties of cAMP, whereas a siRNA-mediated PDE4B knockdown, or exposure to the FDA-approved PDE4 inhibitor Roflumilast, suppressed VEGF levels and vessel formation. Mechanistically, we demonstrated that cAMP suppresses PI3K/AKT activities to impose its anti-angiogenic properties. Next, we created a composite mouse where c-Myc-driven lymphomas develop in Pde4b-null or wild-type backgrounds. Remarkably, primary lymphomas from Eμ-Myc;Pde4b$^{-/-}$ mice displayed significantly lower microvessel density (MVD, quantified by anti-CD34 staining) than the lymphomas from Eμ-Myc;Pde4b+/+ mice (n= 19, $p<0.001$). The lymphomas originating in the Pde4b$^{-/-}$ background also displayed lower PI3K activity, AKT phosphorylation (n=13, $p<0.01$) and VEGF levels (n=18, $p=0.01$). Subsequently, we tested if pharmacological inhibition of PDE4 suppressed angiogenesis in vivo. Using adoptive transfer, we generated cohorts of isogenic Eμ-Myc-driven lymphoma-bearing mice (n=68), which were randomized to receive vehicle or Roflumilast (5mg/kd/day gavage). Lymphomas from Roflumilast-treated mice showed marked suppression of angiogenesis ($p=0.01$, for MVD of Roflumilas vs. vehicle groups), decrease in PI3K/AKT activity ($p=0.003$), and lower levels of VEGF ($p=0.005$). Also, mice receiving Roflumilast displayed a smaller tumor burden ($p<0.0001$) and improved survival ($p=0.01$). Lastly, we confirmed a significant direct correlation between PDE4B levels and microvessel density ($r=0.43$, $p=0.02$) in primary human DLBCLs (n=28). **Conclusion:** These data uncover a novel signaling cross-talk between lymphoma cells and the microenvironment that regulates angiogenesis in vivo, and point to PDE4 as actionable proangiogenic target in DLBCL.

142 **Poster Session B**

Impact of the Intracellular Microenvironment on NF-κB Signaling *M. Jones, University of North Texas; A. Wilson, University of North Texas*

Introduction: The signaling of the nuclear factor-κB (NF-κB) pathway is critical to the onset and progression of oncogensis. Although molecular networks of NF-κB signaling have been identified, how these molecular mechanisms respond to changes in the intracellular microenvironment (i.e., pH, ionic strength, temperature) remains elusive. **Methods:** Computer simulation and modeling techniques were employed to determine how perturbations to the intracellular ionic strength alter how NF-κB activating kinases interact with other proteins and recognize small molecules. Several protein kinases complexed with small molecule activators or inhibitors were examined in the active, inactive, and mutant states to correlate structure-property and structure-function relationships as a function of intracellular ionic strength. **Results:** Analyses of structure-activity and conformational-activity relationships indicate that the protein-protein interactions and the binding of small molecules are sensitive to changes in the ionic strength. Ligand binding pockets either compress or expand, affecting both local and distal intermolecular interactions. **Conclusion:** The changes within the intracellular microenvironment affect molecular mechanisms. By better understanding how the microenvironment modifies signaling mechanisms, specific and adaptable inhibitors can be designed.

143 **Poster Session A**

Subtype Specific Addiction of the Activated B Cell Subset of Diffuse Large B Cell Lymphoma to FOXP1 *J. Dekker, The University of Texas at Austin; D. Park, The University of Texas at Austin; A. Shaffer; H .Kohlhammer, NIH; W. Deng, The University of Texas at Austin; B .Lee, The University of Texas at Austin; G. Ippolito, The University of Texas at Austin; G. Georgiou, The University of Texas at Austin; V. Iyer, The University of Texas at Austin; L. Staudt, NIH; H. Tucker, The University of Texas at Austin*

Introduction: High expression of the FOXP1 transcription factor distinguishes the aggressive Activated B Cell (ABC) Diffuse Large B Cell Lymphoma (DLBCL) subtype from the more indolent Germinal Center B Cell (GCB) DLBCL subtype and is highly correlated with poor prognosis. A genetic or functional role for FOXP1 in lymphomagenesis, however, remained unknown. Here we report that sustained FOXP1 expression is vital for ABC-DLBCL cell-line survival, is involved in activation of multiple ABC-DLBCL hallmark pathways and repression of GCB-DLBCL pathways. FOXP1 target gene expression within a large cohort of primary DLBCL distinguishes ABC and GCB-DLBCL subtypes **Methods:** To determine whether FOXP1 expression is not solely a prognostic factor for DLBCL, we performed inducible shRNA-mediated FOXP1 knockdown (KD) in ABC-subtype cell lines to 1) determine whether cell viability was affected and 2) identify genome-wide expression changes by microarray. To identify direct transcriptional targets, we performed ChIP-seq on multiple ABC and GCB cell-lines. Bioinformatic analysis of FOXP1 targets was performed to identify involvement within known ABC or GCB-DLBCL pathways. Additionally, FOXP1 target gene expression was analyzed in both an expanded panel of DLBCL cell-lines and a cohort of 85 primary DLBCL tumors to determine whether these gene sets could segregate ABC and GCB-DLBCL subtypes. **Results:** FOXP1 KD resulted in near complete loss of viability in ABC cell-lines while GCB cell-lines were unaffected, indicating FOXP1 involvement in ABC-DLBCL biology. Target gene analysis identified FOXP1-mediated enforcement of several hallmarks deregulated in ABC-DLBCL, including the classical NF-κB pathway. In addition, FOXP1 repression of HLA class II expression may contribute to tumor surveillance failure in ABC-DLBCL. FOXP1 promoted gene expression that underlies differentiation of the GCB cell to the plasmablast—the suspected transition targeted by oncogenic transformation—via antagonizing pathways controlled by the GCB "master regulator", BCL6. FOXP1 target genes were highly correlated with FOXP1 expression in an expanded set of cell-lines as well as in primary DLBCL isolates, thereby allowing accurate segregation of the corresponding clinical subtypes of a large cohort of primary DLBCL isolates. **Conclusion:** Our observations establish FOXP1 as a central regulator of ABC-DLBCL survival and subtype distinction by direct and indirect transcriptional regulation of hallmark pathways that repress apoptosis and GCB cell identity while enforcing plasmablast identity and NF-κB signaling via the MYD88 and JAK-STAT pathways. We contend that further functional understanding of FOXP1 and other master TFs corrupted by ABC-DLBCL is essential for designing better prognostic and therapeutic approaches.

144 **Poster Session B**

The Structure of A Biologically Active Estrogen Receptor-Coactivator Complex on DNA *P. Yi, Baylor College of Medicine; Z. Wang, Baylor College of Medicine; Q. Feng, Baylor College of Medicine; G. Pintilie, Baylor College of Medicine; C. Foulds, Baylor College of Medicine; R. Lanz, Baylor College of Medicine; S. Ludtke, Baylor College of Medicine; M. Schmid, Baylor College of Medicine; W. Chiu, Baylor College of Medicine; B. O'Malley, Baylor College of Medicine*

Introduction: Estrogen receptor (ER) is a transcription factor critical for development, reproduction, metabolism and cancer. ER function hinges on its ability to recruit primary and secondary coactivators. No prior structural information exists on the full-length receptor-coactivator complex to complement pre-existing and sometimes controversial biochemical information. **Methods:** We use cryo-EM to determine the quaternary structure of an active complex of DNA-bound ERα, steroid receptor coactivator 3 (SRC-3) and a secondary coactivator (p300). **Results:** Identification of the protein components in this complex is aided by cryo-EM maps of p300 monoclonal antibodies bound to the complex and to isolated p300 itself, and of ERα monoclonal antibody bound to the complex. Further analysis identified two structurally similar densities interpreted as SRC-3 molecules, which bind to non-equivalent sites on one p300 and to a dimer of DNA-bound ERα. **Conclusion:** Our structural model is substantiated by biochemical experiments and multiple structure validation strategies, and suggests the following assembly mechanism for the complex: each of the two ligand-bound ERα monomers independently recruits one SRC-3 molecule via the transactivation domain of ERα; the two SRC-3s in turn bind to different regions of one p300 molecule through multiple contacts.

145 **Poster Session A**

Therapeutic Targeting Against GLI1 in Lung Squamous Cell Carcinoma *S. Kasiri, The University of Texas Southwestern Medical Center at Dallas; C. Shao, The University of Texas Southwestern Medical Center at Dallas; B. Chen, The University of Texas Southwestern Medical Center at Dallas; P. Yenerall, The University of Texas Southwestern Medical Center at Dallas; B. Timmons, The University of Texas Southwestern Medical Center at Dallas; P. Dospoy, The University of Texas Southwestern Medical Center at Dallas; S . Hight, The University of Texas Southwestern Medical Center at Dallas; A . Wilson, The University of Texas Southwestern Medical Center at Dallas; L. Girard, The University of Texas Southwestern Medical Center at Dallas; H .Tian, The University of Texas Southwestern Medical Center at Dallas; C. Behrens, The University of Texas M.D. Anderson Cancer Center; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; A. Gazdar, The University of Texas Southwestern Medical Center at Dallas; J. Kim, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The Hedgehog (Hh) signaling pathway is critical for embryonic development processes and its deregulation is implicated in a wide variety of tumor types. However, the role of the Hh signaling pathway in the initiation and growth of non-small cell lung cancer is largely unknown. The purpose of this study is to investigate the role of GLI1, a major Hh pathway transcription factor, in lung squamous cell carcinoma (SCC) and to test the therapeutic potential of targeting GLI1. M**ethods:** GLI1 expression in human SCC cell lines was evaluated by quantitative PCR and Western Blot. siRNA and shRNA of GLI1 in these cell lines were utilized in vitro and in vivo to test the requirement of GLI1 in tumor growth. Small molecule modulators of GLI1 were tested for their therapeutic potential. **Results:** We have demonstrated that GLI1has a critical role in SCC progression. GLI1 expression was significantly elevated in lung SCC compared to normal lung and lung adenocarcinoma patient specimens in several human genomic databases. Importantly, overexpression of GLI1 was correlated with poor overall survival in lung cancer patients. siRNA-mediated knock down of GLI1 in SCC cell lines decreased the expression of GLI1 target genes and caused a significant reduction in colony formation. Stable knock down of GLI1 in SCC cell lines caused a significant reduction in growth of xenograft tumors indicating the critical role of GLI1 in lung SCC progression. Inhibition or activation of SMO, an upstream component of Hh pathway, did not change GLI1expression level in SCC cell lines. However, inhibition of PI3K/AKT and MAPK signaling pathways down regulated GLI1 expression. These results suggested that GLI1 expression is dependent on PI3K/AKT and MAPK pathway activity rather than Hh ligand.Treatment with PI3K/mTOR inhibitor, or arsenic trioxide (ATO), a direct inhibitor of GLI proteins, significantl reduced GLI1 expression, proliferation, and clonogenicity in SCC cells. C**onclusion:** Our findings suggest that GLI1 is essential for lung SCC tumor progression. Furthermore, GLI1 expression in SCC is independent of Hh pathway ligand action and dependent on MAPK and PI3K pathway activity. Direct inhibition of GLI1 by repurposing ATO in combination with a PI3K inhibitor may represent a novel therapeutic strategy for lung SCC.

146 **Poster Session B**

Posaconazole, A Second-Generation Triazole Antifungal Drug, Inhibits the Hedgehog Signaling Pathway and Progression of Basal Cell Carcinoma *B. Chen, The University of Texas Southwestern Medical Center at Dallas; V. Trang, The University of Texas Southwestern Medical Center at Dallas; A. Lee, Children's Hospital Oakland Research Institute; A. Wilson, The University of Texas Southwestern Medical Center at Dallas; N. Williams, The University of Texas Southwestern Medical Center at Dallas; E. Epstein, Children's Hospital Oakland Research Institute; J. Tang, Stanford University; J. Kim, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Basal cell carcinomas (BCC) are driven by aberrant Hedgehog (Hh) pathway activation. Inhibitors of the Hh pathway that are currently available or under clinical investigation all antagonize and bind to the same region of Smoothened (SMO). Tumor regrowth and therapy failure have been attributed to mutations in the binding site of these small molecule SMO antagonists. Itraconazole, an FDA-approved anti-fungal drug, potently inhibits the Hh pathway at a site distinct from clinically available SMO antagonists. However, itraconazole's clinical utility is limited by its numerous drug-drug interactions. Here we test posaconazole, a second generation FDA-approved triazole antifungal with greatly decreased drug-drug interactions, as an Hh pathway antagonist. **Methods:** We determined the potency and mechanisms of posaconazole action through the use of cell cultured signaling assays, cells with genetic perturbations of pathway components, flow cytometry, and confocal microscopy. Pharmacokinetics of posaconazole was tested in murine models. Allograft mouse models of BCC were used to test the *in vivo* anti-tumor efficacy of posaconazole. **Results:** Posaconazole potently inhibited the Hh pathway in vitro by a distinct mechanism from its antifungal action. It did not compete with cyclopamine and other known SMO antagonists for SMO binding and antagonized Hh pathway activation by inhibiting the accumulation of SMO in the primary cilium. Posaconazole also potentiated the inhibitory activity of cyclopamine and has robust activity against drug-resistant SMO mutants. *In vivo*, posaconazole inhibited the growth of Hh-dependent basal cell carcinoma in murine mouse models. **Conclusion:** Posaconazole is a potent inhibitor of the Hh pathway with demonstrated activity against drug-resistant SMO mutants in vitro and Hh-dependent BCC *in vivo*. As posaconazole inhibits the Hh pathway by a distinct mechanism from current small molecule SMO antagonists in clinical trials with proven long-term safety profile and decreased drug-drug interactions than itraconazole, our results suggest that posaconazole, alone or in combination with other Hh pathway antagonists, may be readily tested in clinical studies for the treatment of Hh-dependent cancers.

147 **Poster Session A**

Role of Nck in MMP14 Function and Localization in Invasive Breast Cancer *D. Morris, Texas A&M University; J. Popp, Texas A&M University; R. Barhoumi, Texas A&M University; H. Gibbs, Texas A&M University; A. Yeh, Texas A&M University; W. Porter, Texas A&M University; G. Rivera, Texas A&M University*

Introduction: While we know that malignant tumors are marked by the ability of cancer cells to invade their surrounding matrix, further research is needed to fully understand the cellular and molecular mechanisms involved. Invasion of tumor cells is driven by remodeling of the cytoskeleton, and particularly, the formation of actin-based structures called invadopodia. Invadopodia are capable of remodeling the extracellular matrix (ECM) using matrix metalloproteinases (MMP), most notably the cell surface-expressed MMP14. The adaptor protein Nck is an important regulator of actin remodeling that has been involved in invadopodia biogenesis. Using a combination of in vitro and in vivo models we identified an important role of Nck in breast carcinoma invasion through a mechanism that involves actin-dependent regulation of MMP14 at the cell surface. **Methods:** We used tools of molecular genetics to alter the levels of Nck in MDA-MB-231 invasive carcinoma cells. Invasiveness in vitro was determined using two-dimensional (2D) and 3D assays, such as Transwell® Matrigel® invasion assay, a spheroid invasion assay, and a fluorescent gelatin degradation assay. Tumorigenesis and metastasis were assessed using a xenograft model in nude mice. Advanced optical imaging, including total internal reflection fluorescence, confocal, and non-linear microscopy were used to probe cytoskeletal organization and signaling mechanisms. **Results:** Loss of Nck reduced invasiveness of breast carcinoma cell in gelatin degradation assays, quantitative immunofluorescence and live cell imaging techniques; this loss of Nck also inhibited primary tumor growth and metastasis **Conclusion:** Our results suggest that Nck-dependent actin remodeling modulates the spatiotemporal activation of Cdc42 and the subcellular distribution of MMP14.

148 **Poster Session B**

Desacetyl Nimbinene Inhibits Growth and Metastasis of Breast Cancer Cells Through Reactive Oxygen Species Mediated Mechanisms *A. Arumugam, Texas Tech University Health Science Center at El Paso; R. Subramani, Texas Tech University Health Science Center at El Paso; S. Nandy, Texas Tech University Health Science Center at El Paso; S. Powell, Texas Tech University Health Science Center at El Paso; M. Velazquez, Texas Tech University Health Science Center at El Paso; A. Orozco, Texas Tech University Health Science Center at El Paso; R. Lakshmanaswamy, Texas Tech University Health Science Center at Dallas*

Introduction: Accumulation of intracellular reactive oxygen species has been implicated in induction of apoptosis and regulation of key signaling molecules in cancer cells. Phytochemicals from indigenous medicinal plants are potent source of anticancer drugs as wells as potential inducers of reactive oxygen species (ROS). Neem (Azadirachta indica), is a medicinal plant used for the treatment of various diseases. One of the active ingredients of neem is desacetyl nimbinene (DAN), which has been shown to possess anticancer activity in our preliminary studies. In this study we investigated the anticancer mechanism of DAN against breast cancer growth and metastasis. **Methods:** Normal and breast cancer cell lines were used for the study. These cells were treated with different doses of DAN and analyzed for its effect on cell proliferation, induction of apoptosis, production of ROS, migration and invasion. Antioxidant enzymes superoxide dismutase 1 (SOD1) and SOD2 were over expressed in these cells to test the effect of DAN induced ROS generation on breast cancer growth. Western blot analyses of key survival and apoptotic protein markers were also performed to validate the anticancer effect of DAN. **Results:** Our data demonstrated that DAN inhibited the growth of breast cancer cells by inducing intracellular ROS generation. Further investigations revealed that DAN disrupted the mitochondrial membrane integrity leading to the loss of mitochondrial membrane potential, which lead to the mitochondria dependent apoptotic cell death. Increased phosphorylation of c-Jun-N-terminal kinase (JNK) and reduced phosphorylation of p38 were also observed in response to DAN treatment indicating the involvement of mitogen activated protein kinases (MAPKs) in ROS mediated apoptosis. Inhibition of ROS production by overexpressing antioxidant enzymes SOD1 and SOD2 reduced the DAN induced cytotoxicity. Additionally, DAN significantly inhibited migration and invasion of MDA MB 231 breast cancer cells. **Conclusion:** Overall, our data suggest that the induction of mitochondria-mediated apoptosis due to ROS accumulation is a major mechanism of action by which DAN exerts its anticancer effect on breast cancer cells.

149 **Poster Session A**

Insights Into the Mechanisms of the AMPK-dependent Activation of Eukaryotic Elongation Factor 2 Kinase *D. Giles, The University of Texas at Austin; C. Crittenden, The University of Texas at Austin; J. Brodbelt, The University of Texas at Austin; K. Dalby, The University of Texas at Austin*

Introduction: eEF-2K (eukaryotic elongation factor 2 kinase) acts to directly slow the rate of protein synthesis by phosphorylating elongation factor 2 (eEF-2) at Thr56, leading to a reduction in the affinity of eEF-2 for the ribosome. eEF-2K is dependent on calcium and calmodulin (CaM) for activity and is intricately regulated by multiple phosphorylations from upstream kinases. Activation of eEF-2K by AMP-dependent protein kinase (AMPK) is thought to enhance the ability of cancer cells to adapt to nutrient deprivation. However, the mechanisms by which AMPK activates eEF-2K have not yet been described. **Methods:** The objective here is to define the mechanism of eEF-2K activation by AMPK. It was previously found that AMPK phosphorylates eEF-2K directly at Ser398, and this event is thought to be responsible for the activation of eEF-2K by AMPK in cells. In vitro, AMPK phosphorylates eEF-2K at three sites, Ser78, Ser366 and Ser398, but only Ser398 is a target for AMPK in cells. Thus, Ser78 and Ser366 were mutated to alanine to lend a form of eEF-2K in which Ser398 is the only site that can be phosphorylated by AMPK (eEF-2K S78/S366A). To determine if phosphorylation of Ser398 could alter the activity of eEF-2K, we purified a form of eEF-2K S78/S366A stoichiometrically phosphorylated at Ser398 (eEF-2K S78/S366A-pS398). This phosphorylated eEF-2K was used in detailed biochemical studies to determine what effect phosphorylation of Ser398 has on the activity of eEF-2K in vitro. **Results:** We found that phosphorylation of eEF-2K at Ser398 does not significantly alter the maximal activity of eEF-2K or its catalytic efficiency for a peptide substrate. Moreover, Ser398 phosphorylation has no effect on the sensitivity or affinity of eEF-2K for calmodulin, which eEF-2K requires for activity. The autophosphorylation of eEF-2K at Thr348, which we previously determined is required for eEF-2K activity in vitro and in cells, was also unaffected by phosphorylation of Ser398. **Conclusion:** Our biochemical characterization of eEF-2K S78/S366A-pS398 reveals that phosphorylation of Ser398 by AMPK has no effect on any of the biochemical parameters of eEF-2K tested thus far. This suggests that if phosphorylation of Ser398 by AMPK does activate eEF-2K in cells, it does not do so by modulating the activity of eEF-2K directly. We hypothesize that the ability of AMPK to inhibit the mechanistic

target of rapamycin complex 1 (mTORC1) and thus relieve eEF-2K from inhibition by mTORC1-dependent kinases (such as p70S6K) may be primarily responsible for AMPK-dependent eEF-2K activation in cells.

150 **Poster Session B**

Hydrogen/Deuterium Exchange - Mass Spectrometry Reveals Conformational Changes between Human Phosphatase PP2Cα and a Catalytically Inactive Metal Binding Site Mutant *E. Gallagher, Baylor University; S. Debnath, National Cancer Institute, National Institutes of Health; S. Mazur, National Cancer Institute, National Institutes of Health; L. Miller Jenkins, National Cancer Institute, National Institutes of Health; S. Durell, National Cancer Institute, National Institutes of Health; E. Appella, National Cancer Institute, National Institutes of Health; J. Hudgens, Institute for Bioscience & Biotechnology Research, National Institute of Standards and Technology*

Introduction: PP2Cα (a member of the protein phosphatase M (PPM) family) is a human serine/threonine phosphatase that inactivates mitogen-activated protein kinase and activates p53 following cellular stress, resulting in cell cycle arrest, apoptosis, and decreased tumorigenicity. Members of the PPM family are characterized by their dependence on Mg2+ or Mn2+ for activity. Crystal structures show that the active site of the enzyme is a groove at one end of a β-sandwich that contains multiple, conserved aspartic acid residues that coordinate the divalent metals required for catalysis. Crystal structures illustrate the presence of two divalent metals with micromolar binding affinities; however, more recent studies indicate that a third metal ion with a millimolar binding affinity is present in the active site and required for enzymatic activity. Using hydrogen/deuterium exchange – mass spectrometry (HDX-MS) we probed PP2Cα and a mutant lacking the third metal binding site to examine how the absence of the third metal modifies the enzyme structure. **Methods:** Proteins were expressed in E. coli. For HDX-MS, proteins were diluted 1:5 in D2O buffer for specified times and quenched at pH 2.5 and 1ºC. Sample handling utilized a LEAP H/D-X PAL. Proteins were digested on immobilized-pepsin columns, trapped, separated, and analyzed on a Thermo LTQ/Orbitrap Elite. HDX Workbench was used to evaluate the D-content of peptides. **Results:** HDX-MS was used to study PP2Cα and a mutant with an aspartic acid to alanine mutation at residue 146. The D146A mutant lacks the third metal binding site and is not catalytically active, although it still binds phosphorylated substrates. Proteolytic digestion resulted in 94% and 83% sequence coverage for the wildtype and D146A mutant, respectively, with 71 identical peptides detected for both proteins. Deuterium uptake plots for both proteins showed that the mutant had a more rapid rate of deuterium uptake for peptides containing aspartic acid residues involved in forming the firs

and second metal binding sites. Additionally, peptides present in the Flap domain and the neighboring region had increased deuterium uptake in the mutant when compared to the wildtype. **Conclusion:** These data indicate that metal binding to the third binding site results in a more rigid active site structure since the deuterium uptake rates are slower in the wildtype enzyme. This work illustrates the solution phase conformation of the protein, adding to our understanding of how the third metal affects the active site structure and providing new insights in our understanding of the catalytic mechanism.

151 **Poster Session A**

Histone Deacetylases Positively Regulate Transcription through the Elongation Machinery *T. Kim, The University of Texas at Dallas*

Introduction: Induction of the elongation phase of transcription by RNA polymerase II is an important regulatory step in the expression of many genes. We have found that HDACs are involved in positively regulating this process, despite being components of several repressive complexes. HDAC inhibitors are a class of anti- cancer drugs that block the deacetylation of histone tails and other proteins. It was discovered over a decade ago that they repress the transcription of oncogenes necessary for tumor growth and survival that are transcribed at high levels in different types of tumors, but the mechanism remained unknown. **Methods:** We used global run-on sequencing (GRO-seq), which measures the level of nascent transcription of RNA across the genome, and chromatin immunoprecipitation sequencing (ChIP-seq) to look at how HDACs affect the distribution of proteins that affect transcription elongation genome-wide. We also explore how proteins that are involved in elongation, such as P-TEFb and NELF, contribute to the transcription elongation blockade induced by HDAC inhibitors. **Results:** We determined that heat shock protein 90 (Hsp90) activity is required for the elongation block after HDAC inhibitor (HDACI) treatment. HDACI change the binding of elongation factors, especially negative elongation factor (NELF), an Hsp90 client complex. HDACIs affect elongation upstream of the well-known positive transcription elongation factor b (P-TEFb). We determine that transcription elongation regulation by HDAC is not limited to transcription of genes, but to the synthesis of eRNAs as well. eRNAs are a mark active enhancers, and therefore effects on enhancer function are an additional way that HDAC inhibitors affect gene transcription. Thus, HDACs are required to maintain acetylation of proper sites that facilitate elongation factor binding and enhancer function that lead to transcriptional elongation. **Conclusion:** HDACs play a critical role in transcription elongation control. HDAC inhibitors cause a block in the elongation step of transcription by RNAP2 that represses many genes that are important for cancers. Our work highlights the importance of dynamic regulation of histone acetylation during transcription intitation and elongation. Our results suggest that deacetylation by classical HDACs post-initiation is likely an important step in inducing gene body transcription and implicates cycling of acetylation and deacetylation as important regulatory step in the process of transcription elongation.

152 **Poster Session B**

From Molecular to Phenotypic Heterogeneity: A Mechanistic-Based Approach to Prediction *M. Behar, The University of Texas at Austin; J. Webb, The University of Texas at Austin*

Introduction: Analysis of a single cancer sample may reveal thousands of mutations of which only a small subset drive the onset of the disease. Identifying driver mutations, vastly outnumbered by passenger mutations with no direct effect on the disease, is an important challenge that must be overcome for diagnostic and therapy if personalized medicine is to fulfill its promise. Current approaches for identifying relevant sources of molecular heterogeneity are based on mining large datasets in search of mutations statistically over-represented in specific diseases. Because of the large proportion of passenger mutations significant numbers of false positives ensue. These approaches not only cannot capture relevant non-genetic heterogeneity that may underlay fractional response or resistance to treatment, but also ignore knowledge about biological mechanisms that could a) improve accuracy and sensitivity because of "canalization" of redundant changes, and b) provide a biological causal basis for evaluating potential biomarkers or therapy targets. **Methods:** Here we use large scale computational models of synthetic signaling networks and data-mining approaches to identify principles for predicting how cell-to-cell variation in composition (molecular heterogeneity) translates into diversity at the signaling and gene expression levels that lead to phenotypic heterogeneity. **Results:** We show that some sources of molecular heterogeneity lead to dramatic qualitative shifts in signaling patterns whereas others produce smaller quantitative-only effects. Importantly, these phenotypic outcomes can, under some conditions, be predicted based on the functional regulatory role played by the heterogeneous components as well as the network architecture through a relatively simple set of rules. **Conclusion:** These results represent a firs step towards a conceptual framework for understanding the functional effects of molecular heterogeneity that may lead to novel approaches to treat and prevent diseases characterized by high cellular heterogeneity such as cancer.

153 **Poster Session A**

Analysis of Intercellular Nanoparticle Trafficking Through Tunneling Nanotubes in RAW Macrophages *J. Newton, Baylor College of Medicine; M. Pulikkathara, Baylor College of Medicine; S. Molavi, Baylor College of Medicine; M. Atsushi, Hitachi High Technologies America, Inc.; S. Curley, Baylor College of Medicine; L. Vergara, Baylor College of Medicine; R. Serda, Baylor College of Medicine*

Introduction: Coordination of cellular actions is critical for organs to function and for the immune system to protect the host from biological and foreign invaders. Extensive cellular bridges known as tunneling nanotubes (TNT) create networks between cells at distances, enabling rapid transmission of signals. Messages include chemical, electrical, mechanical, and the transfer of organelles and foreign objects, including synthetic nanomaterials. This study examines transfer of iron oxide and silica nanoparticles (NP) between RAW macrophages and looks at the influence of stress on rate of transfer. **Methods:** RAW macrophages adherent on Silicon Chip Specimen Supports (Ted Pella, Inc., Redding, CA, USA) were treated with silica or iron oxide NPs, and then cells were fixed with glutaraldehyde, dehydrated, and imaged by scanning electron microscopy at 1-10 kV, using a Hitachi SU8230 Scanning Electron Microscope equipped with an upper and lower secondary electron detector and a Bruker Quad detector. Energy dispersive spectra (EDS) was acquired at a working distance of 10 mm at 10 kV. The effect of heat and radiofrequency energy on transfer rates were determined by flow cytometry and imaged by electron and confocal microscopy. **Results:** RAW macrophages displayed extensive TNT networks under both control and stimulated conditions. Electron and confocal images of TNTs captured the transfer of iron oxide, polystyrene, and silica NPs between cells. While low kV imaging revealed surface bound NPs, high kV imaging detected intracellular NPs, with EDS confirming the elemental identity of the NPs. Neither stress induced by heat nor radiofrequency energy increased the rate of nanoparticle uptake or transfer between macrophages, supporting a high basal, non-stimulated role for material transfer between macrophages. **Conclusion:** RAW macrophages display high rates of NP transfer between cells. Electron microscopy is a powerful tool for studying both surface and intracellular dynamic events.

154 **Poster Session B**

Correlative Cryo-Electron Tomography and Fluorescence Light Microscopy for the Imaging of Breast Cancer Cell:Bone Cell Crosstalk *A. Muscarella, Baylor College of Medicine*

Introduction: The bone is the organ most frequently affected by breast cancer metastasis, and can serve as a reservoir of cells for further disease spread in addition to having its own pathology. While the mechanism of osteolytic bone metastases has been established, little is known about the early stage of bone metastasis in which disseminated tumor cells (DTCs) arrive in the bone, enter the bone marrow, and begin dividing again. The Zhang lab recently published that luminal breast cancer cells which grow from single cells to multicellular lesions are almost always in direct contact with osteoblast cells. This relationship, termed the "osteogenic niche" allows the cancer cells to grow and is mediated by heterotypic adherens junctions between the two cell types, activating mTOR signaling in the cancer cells. In order to further understand how cells make the initial contact with the niche cells, we set out to image the interaction process in real time. **Methods:** We utilized cryo-electron microscopy (cryoEM) and tomography (cryoET) as well as florescence light microscopy (FLM) to image metastatic breast cancer cells MCF7 both in the presence of osteoblasts and without. This was done in sequence on the same cells, allowing us to match high resolution electron microscopy data with the bigger-picture aspects of whole-cell florescence. **Results:** Using these techniques, we were able to observe a unique, undescribed membrane-extension phenotype in which the cancer cells project long, thin (20-40nm diameter) tubes of membrane across long distances (2-3 cell lengths, 40-60 μm). These tubes branch out and end with larger (0.5 -1 μm) spheroid blubs which we have termed "Balloon-Like Structures" or BLS. We have found while MCF7 exhibit these extensions occasionally in normal culture conditions, they are greatly enriched in the presence of osteoblast cells. Curiously, under cryoET these structure do not appear to contain actin or microtubules, and this was supported through the use of inhibitors. We observed that BLS have a propensity to grow towards and on top of osteoblasts, even if the main cancer main cell body is not nearby. Additionally there is a high local concentration of ribosomes where the two cell types touch, indicating a possible signaling component. C**onclusion:** When exposed to osteoblast cells, MCF7 exhibit long, cytoskeleton-free extensions across many cell lengths. These extensions end in small blubs of membrane about one micron across. We are working to characterize the functionality of these structures both in vivo and in vitro.

155 **Poster Session A**

CELF1 is a Central Node in a Post-Transcriptional Regulatory Program Underlying EMT in Breast Epithelial Cells *A. Chaudhury, Baylor College of Medicine; N. Kongchan, Baylor College of Medicine; J. Fachini, Baylor College of Medicine; L. Simon, Baylor College of Medicine; R. Mao, Baylor College of Medicine; T. Wang, Baylor College of Medicine; D. Rosen, Baylor College of Medicine; M. Ittmann, Baylor College of Medicine; S. Hilsenbeck, Baylor College of Medicine; C . Shaw, Baylor College of Medicine; J. Neilson, Baylor College of Medicine*

Introduction: Tumor metastasis is the foremost cause of cancer mortality. Current evidence overwhelmingly suggests that epithelial-mesenchymal transition (EMT) is one of the early manifestations of metastasis. For this reason, defining the molecular mechanisms that induce and drive EMT have long been the focus of intense investigation. The vast majority of these studies have primarily focused on transcriptional regulation associated with EMT. Yet, control over translation of mRNA via RNA binding proteins and microRNAs dictates which mRNA transcripts are translated under different contexts, often independent of transcript abundance, as evident by limited correlation between transcript abundance and corresponding protein expression. Here, we prospectively and functionally define a translational regulatory program underlying epithelial to mesenchymal transition in breast epithelial cells. **Methods:** Polyribosomal profilin was utilized to prospectively identify translational regulatory programs underlying EMT in MCF-10A cells. The 3' untranslated regions (UTRs) of impacted mRNA transcripts were computationally analyzed for common motifs. That these UTRs, and the common motif shared among them, confer translational regulation was established via reporter assay. Altered relative expression of mRNA and protein was assessed via qRT-PCR and immunoblot, respectively. Direct binding of CELF1 to the common motif was demonstrated by RNA immunoprecipitation/qRT-PCR analysis. The impact of misexpression of CELF1 and its downstream regulators in several cell lines and in orthotopic tumor progression assays was analyzed via standard transwell assays, luminescence, and histology. Expression of CELF1 in human primary breast tissue and primary tumors was assessed via immunohistochemistry. **Results:** Our analysis identified a set of functional drivers of EMT that are positively regulated at the translational level via a common GU-rich cis-element within the 3' UTR of their mRNA transcripts. These 3' UTRs confer translational regulation upon reporter genes in EMT programs unless the common GU-rich element is removed

via site-directed mutagenesis. The GU-rich elements are commonly bound by the CELF1 protein, itself highly induced upon initiation of EMT. CELF1 is both necessary and sufficient for cells to acquire mesenchymal characteristics in vitro and for metastatic colonization in a xenograft model. Examination of patient samples revealed marked increases in CELF1 protein expression in human breast cancer tissue as compared to normal adjacent control tissue. **Conclusion:** Cumulatively, our data present a distinctive model in which the CELF1 protein serves as a central node controlling the translation of several drivers of the mesenchymal phenotype and ultimately tumor metastasis.

156 **Poster Session B**

Overexpression of Exportin-5 (XPO5) Defines a Novel Subset of MicroRNA-Addicted Uterine Cancers with Poor Outcome

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Introduction: We have previously shown that the loss of miR-143 is a robust feature of uterine serous carcinoma (USC). However, the mechanisms by which miR-143 contributes to the growth and metastasis of USC are not currently known. **Methods:** Established bioinformatic algorithms were used to mine a database documenting patterns of microRNA and gene expression previously generated by our group using flash-frozen specimens of USC and healthy endometrium. Patterns of gene and microRNA expression were confirmed using an independent panel of specimens by rt-qPCR and/or Western blot. A tissue microarray containing representative specimens of uterine cancer was used to examine expression of putative miR-143 targets. Logistic regression models were used to assess relationships between clinical demographics, uterine cancer histology and expression scores for genes of interest. Gene expression was targeted in established USC cell lines using siRNAs. Proliferation and apoptosis were measured using MTS and Caspase 3/7 assays. **Results:** Bioinformatic analyses identified the nuclear transport receptor Exportin 5 (XPO5) as a key target regulated by miR-143 in USC. This observation was validated using multiple USC cell lines transfected with miR-143 mimics and 3-UTR assays. Immunohistochemical interrogation of our uterine cancer microarray revealed that robust expression of XPO5 was observed not only in specimens of USC (3.9 + 0.53; n=53), but also grade 3 endometrioid cancers (2.58 + 0.53; n=50), clear cell (2.22 + 0.66, n=18) and MMMT (4.13 + 0.90; n=21) Much lower levels of XPO5 expression were observed in well-differentiated endometrioid cancer (1.28 + 0.20; n=202). XPO5 expression was only infrequently observed at low levels in healthy endometrium (0.1+0.3, n=20). Logistic regression determined that expression of XPO5 correlated with survival (HR = 0.34; CI=0.15 to 0.80, p=0.01) as well as multiple other clinically adverse features of uterine cancer, including vascular invasion. Knockdown of XPO5 in UPSC-Ark1 and UPSC-Ark2 cells inhibited proliferation and enhanced apoptosis. Knockdown of XPO5 expression adversely impacted the expression of multiple oncogenic miRNA species expressed at low levels in both USC cell lines and specimens. **Conclusion:** Despite proposed roles for other downstream components of microRNA processing pathways as tumor suppressors in uterine and other human cancers, dysregulated expression of XPO5 identifie a subset of USC and potentially other high grade uterine cancers as microRNA addicted cancers with poor outcomes. Further work is needed to more precisely define the mechanisms by which miR-143-mediated XPO5 overexpression contributes to endometrial carcinogenesis.

157 **Poster Session B**

Cyclin Dependent Kinase 5 Regulates MicroRNA Secretion in Ovarian Cancer

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Introduction: Cancer cells have a remarkable ability to manipulate their surrounding environment to their own advantage. Secreted microRNAs (miRs) represent a newly discovered mechanism by which donor cells can influence the gene expression of recipient cells. Available evidence indicates that the release of miRs from cells into extracellular biofluid is a selective process that may correlate with malignancy. However, mechanisms regulating miR release and the specific role of secreted miRs in cancer initiation and progression remain unclear. Dissection of those mechanisms may provide stronger tools to study the physiologic functions of miRNA transfer and develop novel strategies for cancer treatment. **Methods: Results:** In this study, we performed a kinome siRNA library screen in a high grade ovarian cancer cell line to identify candidate molecules that regulate RNA secretion. Cyclin dependent kinase 5 (or CDK5) emerged as a top hit in our screen modulating microRNA secretion. CDK5 is a small serine/threonine cyclin-dependent kinase that is known for its regulatory role in synaptic vesicle release and endocrine hormone secretion. We found that CDK5 is abundantly expressed in a panel of ovarian cancer cell lines and its expression is elevated ($p<0.05$) in ovarian tumors as compared to normal ovarian epithelium. To study the functional implications of elevated CDK5 in ovarian cancer, we used siRNA to silence CDK5. Profiling of microRNAs showed global downregulation of extracellular microRNA levels in culture media of CDK5 siRNA treated cells as compared to controls. Concomitantly, several of those miRs were elevated in CDK5 siRNA treated cells. Silencing CDK5 in ovarian cancer cell lines resulted in decreased cell proliferation and decreased tumor burden and metastasis in an orthotopic model of ovarian cancer. Mechanistic studies revealed that CDK5 knockdown alters multivesicular body fusion with cell plasma membrane, reduces exosome release and thereby impairs microRNA secretion. **Conclusion:** CDK5 inhibition

provides a novel strategy for manipulating exosomal microRNA secretion in cancer.

158 **CPRIT Grantee**

Inferring Genome-Wide Interaction Networks Using the Phi-Mixing Coefficient, and Applications to Lung Cancer *N. Singh, The University of Texas at Dallas; M. Ahsen, IBM Thomas J. Watson Research Center; S. Mankala, Highmark Inc; H. Kim, Yonsei University College of Medicine; M. White, The University of Texas Southwestern Medical Center at Dallas; M. Vidyasagar, The University of Texas at Dallas*

Introduction: The behavior of cells is governed by complex interactions amongst genes and gene products. Diseases such as cancer have their origin in a departure of these interactions from their normal patterns. By comparing the interactions that are present in normal cells versus cancerous cells, or between cells manifesting different forms of cancer, it is possible to draw some conclusions about the triggers of cancer and/or potential therapeutic targets. Therefore, constructing gene interaction networks (GINs) from high-throughput gene expression data is an important and challenging problem in systems biology. Existing algorithms produce networks that either have undirected and unweighted edges, or else are constrained to contain no cycles, both of which are biologically unrealistic. In the present work we propose a new GIN inference algorithm that produces networks whose edges are weighted and directed, and are permitted to contain cycles. M**ethods:** The algorithm presented here makes use of a directionally sensitive measure of dependence between random variables, known as the phi-mixing coefficient; accordingly the algorithm is referred to as "phixer." Phixer algorithm computes phi-mixing coefficient between every gene pair thus resulting in a complete network, and then "prunes" the edges that does not satisfy data processing inequality. Finally, all edges that remain after pruning are compared against a threshold, and those whose weight is smaller than this threshold are considered as spurious and thus discarded. **Results:** We analyzed the outcomes of several experiments on lung cancer, and matched the predictions from the inferred networks with experimental results. Specificall , we inferred three networks (NSCLC, Neuro-endocrine NSCLC plus SCLC, and normal) from the gene expression measurements of 157 lung cancer and 59 normal cell lines; compared with the outcomes of siRNA screening of 19,000+ genes on 11 NSCLC cell lines; and analyzed data from a ChIP-Seq experiment to determine putative downstream targets of the lineage specific oncogenic transcription factor ASCL1. The inferred networks displayed a power law behavior between the degree of a node and the number of nodes with that degree. There was a strong correlation between the degree of a gene in the inferred NSCLC network and its essentiality for the survival of the cells. The inferred downstream neighborhood genes of ASCL1 in the SCLC network were significantl enriched by ChIP-Seq determined putative target genes, while no such enrichment was found in the inferred NSCLC network. **Conclusion:** Phixer algorithm was able to infer lung cancer GINs in an accurate and context specific manne .

159 **CPRIT Grantee**

Notch-Jagged Signaling can give rise to Clusters of Cells in a Hybrid Epithelial/Mesenchymal Phenotype *M. Jolly, Rice University; M. Boareto, Rice University; I. Tsarfaty, Tel Aviv University; E. Ben-Jacob, Rice University; H. Levine, Rice University; J. Onuchic, Rice University*

Introduction: Metastasis involves repeated cycles of Epithelial-to-Mesenchymal Transition (EMT) and its reverse MET. Cells can also undergo partial transitions to attain a hybrid epithelial/mesenchymal (E/M) phenotype that allows collective migration of cells as a cluster of circulating tumor cells (CTCs). These clusters can be apoptosis-resistant and be more metastatic than the cells that undergo a complete EMT and migrate solitarily. Elucidating the molecular players that can give rise to and/or maintain such clusters may inform anti-metastasis strategies. **Methods:** Here, we devise a mechanistic dynamical mathematical model to couple Notch-Delta-Jagged signaling with the core EMT circuit that acts as a three-way switch between the E, E/M and M phenotypes. We further incorporate the effect of inflammatory factors such as IL-6, TNF-α, and NF-kB that can increase the production of Jagged as well as inhibit that of Delta to understand how chronic inflammation affects formation of such clusters of hybrid E/M cells. **Results:** We demonstrate that while both Notch-Delta and Notch-Jagged signaling can induce EMT in a population of cells; only Notch-Jagged signaling, but not Notch-Delta signaling, can lead to the formation of clusters of hybrid epithelial/mesenchymal (E/M) cells. We further show that inflammation augments Notch-Jagged signaling thereby expanding the size of such clusters as well as increasing the population of Cancer Stem Cells (CSCs). The longer the pulse of inflammatory signals to a population of cells, the greater the increase in cluster size and potentially the CSCs. Consistently, such cluster formation and CSC expansion is restricted by Fringe, a glycosyltransferase that promotes Notch-Delta signaling instead of Notch-Jagged, and acts as a tumor-suppressor. **Conclusion:** Our results suggest that targeting Notch-Jagged signaling specifically can alleviate the formation of clusters of hybrid E/M cells and thereby mitigate metastatic risk of the Circulating Tumor Cells (CTCs). They also indicate how chronic inflammation, often a residue of many therapies, can be utilized by cancer to increase its cooperative behavior via Notch-Jagged signaling and eventually their metastatic potential. Our computational framework can be tailored to include additional signals such as p53 and hypoxia that affect this plasticity to gain stemness, providing a platform that can be useful in designing novel therapies.

160 **CPRIT Grantee**

SOX9-Mediated Transcriptional Reprogramming Drives Endocrine Resistance in ER+ Breast Cancer *J. Lei, Baylor College of Medicine; J. Shao, Washington University School of Medicine; S. Haricharan, Baylor College of Medicine; D. Chan, Baylor College of Medicine; M .Ellis, Baylor College of Medicine*

Introduction: More than 75% of diagnosed breast cancers express estrogen receptor (ER) alpha, which classifies these cases as ER+ and eligible for endocrine therapy. Despite the success of targeted therapies for estrogen receptor and estrogen synthesis, endocrine resistance results in the deaths of more than 40,000 women every year in the US. We propose to study transcriptional reprogramming as a molecular event that drives endocrine resistance in ER+ breast cancer. One target under investigation is SOX9, an embryonic transcription factor, which has critical roles in sex determination and cell cycle progression. SOX9 overexpression in the mammary gland has been demonstrated to increase tumorigenic and metastatic potential of breast epithelial cells. Preliminary data from our laboratory identified ER-fusion gene products that appeared to be causally associated with induced resistance in breast tumors. The main focus of this study was to explore the underlying biology of ER-fusion-mediated endocrine resistance. We hypothesize that SOX9-mediated reprogramming is responsible for driving endocrine resistance in ER+ breast cancer. **Methods:** We will use a luminal epithelial breast cancer cell line, T47D, as well as stably expressing ER-fusion T47Ds to test whether ER-fusion genes induce SOX9 activity. Cells will be cultured in media containing full serum or charcoal stripped serum devoid of estradiol. A human SOX9 promoter firefl luciferase reporter, along with Renilla luciferase reporter, will be co-transfected into cells 2 days after plating. 48h after transfection, SOX9 promoter activity will be assayed in the presence/absence of ER-fusion genes and estradiol in T47D cells. In addition, we will examine SOX9 transcript levels in tumors from patients with endocrine sensitive and endocrine resistant ER+ breast cancer. **Results:** Our data indicates that ER-fusion genes induce varying levels of SOX9 promoter activity. Interestingly, cells expressing “non-canonical” ER-fusion genes, which antagonize estrogen response element (ERE) activation of target genes, appeared to induce higher levels of SOX9 promoter activity than cells expressing “canonical” ER-fusions, which constitutively activate EREs. Moreover, patient tumor analyses revealed a trend of higher SOX9 transcript levels in individuals with endocrine resistant compared to endocrine responsive ER+ breast cancer. **Conclusion:** We conclude that SOX9 is a candidate driver of endocrine resistance in ER+ breast cancer. Future studies will identify additional candidate drivers of endocrine resistance and define their functional roles in ER+ breast cancer.

161 **CPRIT Grantee**

Modeling Gene Regulatory Network of Cancer Metabolism *L. Yu, Rice University; M. Lu, Rice University; J. Ma, Baylor College of Medicine; B. Kaipparettu, Baylor College of Medicine; E. Ben-Jacob, Rice University; J. Onuchic, Rice University*

Introduction: In general, cancer cells utilize glycolysis even in the presence of oxygen, referred as the Warburg effect. However, recent evidences suggest that mitochondrial oxidative phosphorylation is also present in cancer cells depending on their stage and microenvironment. It has been observed that cancer cells with mitochondrial oxidative phosphorylation are more aggressive and metastatic. Towards understanding the phenomenon, we aim to develop a theoretical framework to investigate the regulatory mechanism of the glycolytic and oxidative metabolic modes. **Methods:** From the literature, we constructed a network which includes both regulatory genes and metabolites that are involved in glycolysis and oxidative phosphorylation. We further reduced the network into a three-component core regulatory circuit, composed of HIF-1, AMPK and ROS. We thereafter performed computational modeling on the reduced circuit. First, we performed nullcline analysis to find all possible stable states that the circuit allows for parameters that correspond to normal and cancer cells respectively. Second, we used bifurcation analysis to evaluate the dynamic behavior of the circuit in response to the therapies that target metabolism. Finally, we evaluated the effectiveness of various therapeutic strategies by simulating the time course of the gene expressions. **Results:** From the modeling, we predicted that cancer cells have three stable states – the Warburg state (W: high HIF-1, low AMPK), the oxidative state (O: low HIF-1, high AMPK), and the hybrid state (W/O: high HIF-1, high AMPK). Yet, normal cells lack the hybrid phenotype because they usually have lower mitochondrial ROS production and higher HIF-1 degradation. We propose that the hybrid metabolism contributes to cancer metabolic plasticity, thus promoting metastasis and cancer cell proliferations. In addition, our analysis shows that therapies targeting glycolysis and oxidative phosphorylation are distinct in reducing cancer metabolic plasticity, which may explain their different efficac in cancer treatments. Moreover, we show that a combinatorial therapy that targets both glycolysis and oxidative respiration is more effective in reducing metabolic plasticity for cancer cells, which is consistent with some cancer treatment studies. **Conclusion:** Our model provides an insight on the interplay among the cancer metabolic pathways, the metabolic regulatory genes, and the metabolites. We propose that the ‘W/O’ hybrid metabolic state plays crucial roles in cancer progression and metastasis by promoting cancer metabolic plasticity. We also suggest that cancer treatment could be more effective by avoiding the hybrid metabolic state.

162 **CPRIT Grantee**

Noncoding RNA NORAD regulates genomic stability in human cells *S. Lee, The University of Texas Southwestern Medical Center at Dallas; F. Kopp, The University of Texas Southwestern Medical Center at Dallas; T. Chang, The University of Texas Southwestern Medical Center at Dallas; A. Sataluri, The University of Texas Southwestern Medical Center at Dallas; B. Chen, The University of Texas Southwestern Medical Center at Dallas; Y. Xie, The University of Texas Southwestern Medical Center at Dallas; J. Mendell, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The fidelity of chromosome segregation must be maintained at a high level to ensure the accurate transmission of genetic information as well as to avoid severe pathologic consequences. Chromosomal instatbiity (CIN), a phenotype characterized by the frequent gain or loss of chromosomes during mitosis, is a hallmark of cancer cells and is a key mechanism that contributes to gain- and loss-of-function of oncogenes and tumor suppressors. Long noncoding RNAs (lncRNAs) have emerged as regulators of diverse biological processes, yet their roles in the maintenance of genomic stability remain poorly understood. In a screen for human lncRNAs that are regulated by DNA damage, we identified a poorly characterized noncoding transcript that we termed Noncoding RNA Activated by DNA Damage (NORAD) that is essential for the maintenance of genomic stability in human cells. **Methods:** NORAD knockout cell lines were generated using TALEN-mediated homologous recombination. Chromosomes were enumerated using FISH. NORAD interacting proteins were identified by mass spectrometry following RNA affinity purification Genes regulated by NORAD and PUMILIO were identified by RNA-seq. **Results:** NORAD is ubiquitously expressed in human and mouse tissues and highly abundant, present at ~500-1000 copies/cell. Moreover, NORAD is strongly conserved among mammals with 65% nucleotide identity between human and mouse. To investigate functions of NORAD in human cells, TALENs were used to introduce a floxed transcriptional stop cassette into the endogenous locus. Remarkably, NORAD loss-of-function was sufficient to induce aneuploidy, with frequent tetraploidization. Depletion of NORAD with siRNA recapitulated these findings whereas Cre-mediated excision of the stop cassette reverted the chromosomal instability of NORAD–/– cells, confirming that this phenotype is due to NORAD loss. Mass spectrometry revealed interaction of NORAD with PUMILIO (PUM) proteins, which was confirmed by RIP-PCR and PAR-CLIP. Accordingly, NORAD harbors 15 conserved PUM binding sites, many more than expected by chance. RNA-seq of NORAD–/– and PUM-overexpressing cells revealed a common gene expression signature, indicative of PUM hyperactivity in NORAD–/– cells. Moreover, PUM overexpression phenocopied the chromosomal instability observed upon NORAD loss. Further analysis of RNA-seq data demonstrated that PUM represses a broad set of targets necessary for the maintenance of genomic stability, including key mitotic, DNA replication, and DNA repair factors. **Conclusion:** These findings introduce a new mechanism that regulates the activity of PUMILIO proteins, a deeply conserved and highly dosage-sensitive family of RNA binding proteins, and reveal unanticipated roles for a lncRNA and PUMILIO proteins in the maintenance of genomic stability in mammals.

163 **CPRIT Grantee**

The Role of Gene Fusions in Driving Resistance of Estrogen Receptor Positive Breast Cancers to Endocrine Therapy *M. Bainbridge, Baylor College of Medicine; S. Haricharan, Baylor College of Medicine; M. Rahmizadeh, Baylor College of Medicine; M .Ellis, Baylor College of Medicine*

Introduction: Approximately 20% of all breast cancer diagnoses, or 40,000 women a year, will be resistant to standard-of-care endocrine depletion therapy, despite the tumor being histologically ER+. Further, these tumors will be late stage, and potentially fatal, by the time they are identified as endocrine resistant. There are few effective therapies for endocrine resistant ER+ tumors and women with these tumors have overall survival comparable to those with ER- tumors. It is critical, therefore to investigate the underlying cause of resistance and identify such tumors early in order to treat patients effectively. Gene fusions have been found to underly disease characteristics in many different cancer types (e.g. BCR-ABL). In breast cancer, we previously showed that gene fusions between ER and multiple other genes can cause endocrine therapy resistance by varied mechanisms. A systematic study of gene fusions in druggable kinases associated with endocrine therapy resistance will allow the discovery of novel therapeutic targets in the endocrine therapy resistant, difficult to treat, subset of ER+ breast cancer. M**ethods:** RNA derived from tumor was reverse transcribed and sequenced by paired-end Illumina methodology. RNA was aligned to the whole genome using STAR in two-pass mode. Chimeric RNA was identified by reads that span exon-exon boundaries at canonical splice motifs. In-house software was developed to identify the phase of the resulting fusion transcript and annotate the function of the genes. **Results:** We identified seven kinases that underwent either promoter trap or mid-gene fusions in the endocrine therapy resistant subset of ER+ breast cancers. No such fusions were identified in endocrine therapy sensitive subset. These kinases show increased expression concomitant with the site of the fusion and 6/7 kinases identified in our dataset are also found to occur as gene fusion events in the TCGA dataset. Tumors with these kinase fusion events are associated with poor clinical outcome. Moreover, a larger fraction of ER+ tumors show perturbation of these kinases at the gene expression level, indicating a larger role for the kinases discovered in this screen. Interestingly, 2 of the genes, PAK1 and CHUK, are already targets for chemotherapy in other cancers. **Conclusion:** The impact of this investigation will be to identify druggable gene fusion events that occur in the endocrine therapy resistant subset of ER+ breast cancer. Moreover, identification of these gene fusion events can serve as an indicator of those kinases that induce endocrine therapy resistance.

164 **CPRIT Grantee**

Kinome Analysis to rationalize targeting HER2, DDR1 and FOXC1 breast cancer mutations *S. Kavuri, Baylor College of Medicine; M .Ellis, Baylor College of Medicine; S. Haricharan, Baylor College of Medicine*

Introduction: Massively parallel sequencing of breast cancer has revealed many mutations that contribute to disease progression but translating this knowledge into successful treatments is a major clinical challenge. This is often because we do not understand the precise biochemical effects of each mutation and therefore targeted interventions are not possible or are unsuccessful. We have therefore chosen to broadly explore how these mutations alter kinase biology and how tumors become resistant to drugs that should effectively treat tumors that harbor mutant kinase activity. An important recent technical development that we adopt in this proposal is the use of multiplexed kinase inhibitor beads (MIB) coupled with mass spectrometry (MIB/MS). By coupling kinase inhibitors with a variety of specificities to sepharose beads and profiling the proteins that are captured by MS we can broadly study kinase activity. This approach is called "active kinomics" because the drug beads capture kinases in their active state. **Methods:** We will focus our study on three examples of genes that are recurrently point-mutated in ER+ breast cancer. In our studies of HER2 mutations, we focus on kinome reprogramming. We will therefore use MIB/MS to identify the kinome remodeling events that propel drug resistance. The two other kinases we will focus on are, Discoidin domain receptor 1 (DDR1) and Forkhead box family of transcriptional factor1 (FOXC1) mutations. We have shown that DDR1 and FOXC1 mutations predict poor clinical outcome and in the case of FOXC1 specifically distant rather than local recurrence. We will therefore study how specific poor prognosis DDR1 and FOXC1 mutations, discovered in our Komen Promise Grant sequencing analysis, reprogram the kinome as a prelude to developing an effective therapeutic approach. **Results:** Our findings demonstrate that somatic mutations in HER2, DDR1 and FOXC1 may drive endocrine resistance through EMT and/or poor outcome in endocrine therapy refractory tumors. The oncogenic HER2 mutations were able to induce epithelial-mesenchymal transition (EMT), when cultured in reconstituted basement membrane or upon exposure to low-dose TGFβ. The induction of EMT was dependent on several signaling axes, including SRC-STAT3, NF-κB and MAPK-FOSL1- ZEB1. Anchorage-independent growth was also dependent on these same signaling axes, and on TWIST and TFAP2A. **Conclusion:** Somatic mutations in HER2, DDR1 and FOXC1 are drivers are poor outcome mediated by kinome reprogramming. By targeting key nodes of the adaptive kinome response, identified by MIB/MS we will overcome treatment resistance and improve outcomes for patients with tumors that harbor these mutations.

165 **CPRIT Grantee**

Altering Stem Cell Self-renewal in Cancer by Re-wiring Cell-of-origin Epigenomic Features *L. Zhang, The University of Texas Southwestern Medical Center at Dallas; J. Zheng, The University of Texas Southwestern Medical Center at Dallas; X. Wu, The University of Texas Southwestern Medical Center at Dallas; X. Kang, The University of Texas Southwestern Medical Center at Dallas; X. Wang, The University of Texas Southwestern Medical Center at Dallas; R. Tuladhar, The University of Texas Southwestern Medical Center at Dallas; L .Morlock, The University of Texas Southwestern Medical Center at Dallas; H .Yao, The University of Texas Southwestern Medical Center at Dallas; L .Huang, The University of Texas Southwestern Medical Center at Dallas; N. Williams, The University of Texas Southwestern Medical Center at Dallas; C. Chen, The University of Texas Southwestern Medical Center at Dallas; C. Zhang, The University of Texas Southwestern Medical Center at Dallas; L. Lum, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The epigenomic landscape of somatic stem cells is maintained throughout adult life and contributes to tissue-specifi properties such as cellular make-up and regenerative potential. **Methods:** As part of our effort to understand the mechanisms that support multipotency in adult stem cells, we have used an in vivo chemical strategy in mice to interrogate how epigenetic perturbations induced by the MLLAF9 fusion protein influences the machinery supporting self-renewal in normal hematopoietic and leukemic stem cells (LSCs). **Results:** Using this approach, we have identified de novo expression of the muscle homeobox protein Six1 in MLLAF9-transformed stem cells to be important to their self-renewal and subject to cell-autonomous Wnt signaling control. Six1 influences TCF/LEF transcriptional regulator activity and thus supports a novel signaling re-enforcement mechanism. **Conclusion:** Our study provides a rationale for pursuing additional de novo feed-forward cellular signals as high priority targets for countering epigenetically driven cancer stem cell self-renewal, and reveals the direct contribution of epigenomic features to the elaboration of tissue-specifi cell signaling circuitry.

166 **CPRIT Grantee**

LncRNA-mediated mTOR signaling activation in breast cancer progression *Q. Hu, The University of Texas M.D. Anderson Cancer Center; A. Lin, The University of Texas M.D. Anderson Cancer Center; K. Liang, The University of Texas M.D. Anderson Cancer Center; C. Lin, The University of Texas M.D. Anderson Cancer Center; L. Yang, The University of Texas M.D. Anderson Cancer Center*

Introduction: Triple-negative breast cancer (TNBC) is a heterogeneous breast cancer group. Treatment of patients with TNBC, lacking estrogen receptor (ER) and progesterone receptor (PR) expression as well as human epidermal growth factor receptor 2 (HER2) amplification, has been challenging due to the heterogeneity of the disease and the absence of well-defined molecular targets. Recently, more and more evidence has purported long noncoding RNA (lncRNA) as a new class of players involved in the development and progression of cancer. However, how lncRNA regulate the TNBC progression is not clear. **Methods:** Here, we surveyed the lncRNA expression profile in two pairs of TNBC patient tissues and adjacent normal counterparts, which identified 56 lncRNA candidates that are commonly up-regulated by more than 8 fold in TNBC samples compared to their normal counterparts. Through the further convinced, we found LOC14107 overexpression in TNBC cell lines and patient tissues. Through RNA pull-down, followed by mass spectrometry (MS) analysis, we identified that in vitro-transcribed biotinylated Loc14107 sense transcript associated specifically with raptor, a component of mTOC1 comlpex. **Results:** We examined the subcellular localization of LOC14107 by RNA fluorescence in situ hybridization (FISH) and qRT-PCR analyses on fractionated RNA, finding that the LOC14107 transcript is predominately localized in the cytoplasmic. When we knockdown LOC14107 in TNBC cell line, we found the mTOR signaling activation is inhibited, and when we overexpression it, we found the mTOR signaling activation is activated. To investigate the correlation between LOC14107 and human cancer, we detected the expression of LOC14107 in breast cancer tissue samples by RNAscope assay, finding that LOC14107 expression level is increased in malignant breast cancer tissues compared to normal breast tissues. By comparing RNAscope and immunohistological staining of phosphorylated p70S6K1 at Thr389 using same set of tissue, we observed strong correlation between the expression level of LCO14107 and p70S6K1 phosphorylation at Thr389. **Conclusion:** Our studies demonstrated the role of LOC14107 in promoting the mTOR signaling activation in TNBC. Utilization of drugs that inhibit mTOR signaling activation may increase the therapeutic benefit to TNBCs with LOC14107 overexpression, and potentially other malignancies.

168 **CPRIT Grantee**

Visualizing Cell Turnover During Epithelial Tissue Homeostasis And Repair *G. Eisenhoffer, The University of Texas M.D. Anderson Cancer Center; E. Sumner, The University of Texas M.D. Anderson Cancer Center; O. Ruiz, The University of Texas M.D. Anderson Cancer Center*

Introduction: Epithelial tissues provide an essential barrier for the organs they encase, and are also the primary sites of solid tumor formation. Changes in the balance between cell loss and division have been implicated in numerous human diseases, including cancer, yet how these two processes influence each other to regulate overall cell numbers within epithelia remain poorly understood. We have found that cell extrusion, a process used to eliminate cells from epithelia without disrupting barrier function, is key in driving cellular turnover. Here we investigate the role of extrusion in regulating cell turnover to control overall cell numbers in a living epithelial tissue. **Methods:** To investigate extrusion in a living epithelium, we identified a set of GAL4 enhancer trap lines that are expressed in discrete epithelial cell types in the developing zebrafish. When combined with UAS effector lines, our epithelial GAL4 lines provide the opportunity to visualize and track distinct cell types during imaging, observe subcellular cytoskeletal dynamics, and target cells for ablation. Here we have used time-lapse imaging to characterize cell turnover under normal physiological conditions and after damage. For these studies, we created an epithelial wounding assay that allows induction of death specifically in a subset of the surface keratinocytes. We then use live imaging to monitor extrusion and the response of the surrounding neighbors and basal stem cells. **Results:** Live imaging revealed that damaged cells underwent apoptosis and were rapidly eliminated by extrusion. This analysis also showed that some basal stem cells migrate to sites of excessive extrusion, delaminate and intercalate into the surface layer to maintain a functional barrier. Importantly, we found that increased cell extrusion drives compensatory proliferation to replace the lost cells. Transcriptional profiling at defined times during the repair process uncovered distinct molecular pathways associated with the observed cellular behaviors. We are now investigating the changes that occur when extrusion is perturbed and damaged cells are not properly eliminated. **Conclusion:** This study provides a high-resolution in vivo characterization of cell turnover in a living epithelial bilayer. Our new reagents and methods now allow the study specific subsets of epithelial cells and their dynamics during development, repair, or carcinogenesis and provide an in vivo alternative to study processes at resolutions typically found in cell culture. Together, this system will allow us to rapidly identify new mechanisms controlling tissue homoeostasis and the specifi alterations that lead to pathologies and cancer.

169 **CPRIT Grantee**

Serum miR152 as a novel prognostic biomarker and potential tumor suppressor for early stage non-small cell lung cancer *T. Oba, The University of Texas M.D. Anderson Cancer Center; Y. Ye, The University of Texas M.D. Anderson Cancer Center; J. Lin, The University of Texas M.D. Anderson Cancer Center; E. Gentile, The University of Texas M.D. Anderson Cancer Center; J. Wang, The University of Texas M.D. Anderson Cancer Center; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; J. Roth, The University of Texas M.D. Anderson Cancer Center; X. Wu, The University of Texas M.D. Anderson Cancer Center; L. Ji, The University of Texas M.D. Anderson Cancer Center*

Introduction: The cancer-derived miRNAs circulating in the bloodstream have displayed diverse correlations with tumor progression, invasion, immune modulation, metastasis, and therapeutic response and represent a new promising class of circulating biomarkers for cancer detection and prognosis. By determine changes in circulating miRNAs (c-miRNAs) in blood samples of a large population of non-small cell lung cancer (NSCLC) patients and analyzing their associated cellular expression, biological function, and clinical relevance in vitro, in vivo, and in clinical tissue specimens it will allow to assess the deregulation of tumor-derived c-miRNAs, identify molecular signatures with diagnostic and prognostic values, and develop novel c-miRNA biomarker-based therapeutics. **Methods:** Expression of about 100 candidate serum miRNAs was analyzed by a quantitative RT-PCR in 200 Caucasian, early stage lung cancer patients and 200 healthy controls to assess differential c-miRNA expression levels between cases and controls and determine risk of lung cancer. The miRNA expression profiling on microarrays were performed in 40 NSCLC cell lines and 250 primary tumor samples to determine the correlation of c-miRNA biomarkers with cellular miRNA expression and clinical characteristics. Effects of ectopic expression of selected c-miRNA markers or inhibition of their activities by synthetic miRNA inhibitors on tumor cell proliferation and tumor-cell induced clonogenesis were determined in NSCLC cell lines. **Results:** The serum miR152 exhibited significant differential expression between cases and controls (P = 1.64 X 10-6). The low level of serum miR152 expression was consistently associated with an increased risk of lung cancer, a higher chance of 5-year recurrence, and a lower rate of 5 year-survival. The down-regulated miR152 expression was also detected across NSCLC cell lines and primary tumors. Ectopic expression of miR-152 suppressed tumor cell proliferation and colony formation in NSCLC H1299, H460, and A549 cells transfected by miR-152 expression plasmids. Inhibition of miR-152 expression by miR-152 inhibitor promoted tumor cell proliferation in H1299 cells. An increased level of serum miR152 was detected with increased tumor volumes three weeks after tumor cell inoculation in human H1299 and H460 tumor xenograft mouse models by qRT-PCR assay, indicating its tumor cell origin. Overexpression of miR152 in H1299 cells significantly down-regulated the constitutive expression of DNMT1 target gene, suggesting its potential role in the epigenetic regulation of lung cancer pathogenesis. **Conclusion:** These results suggest that the miR-152 as a negative predictor for lung cancer risk, recurrence, and survival in early stage NSCLC function as a potential tumor suppressor miRNA by down-regulating epigenetic oncogenesis.

170 **CPRIT Grantee**

A novel cationic liquid crystalline nanoparticle for the delivery of synthetic RNAi-based therapeutics *E. Gentile, The University of Texas M.D. Anderson Cancer Center; T. Oba, The University of Texas M.D. Anderson Cancer Center; X. Cao, The University of Texas M.D. Anderson Cancer Center; J. Roth, The University of Texas M.D. Anderson Cancer Center; L. Ji, The University of Texas M.D. Anderson Cancer Center*

Introduction: The small interference RNA (RNAi)-based therapeutics have been used to silence expression of targeted pathological genes. However, short half-life, poor cellular uptake, and non-specific distribution of small RNAs call for the development of novel delivery systems to facilitate the use of RNAi as a new class of therapeutics. In this study, we are developing a novel cationic liquid crystalline nanoparticle (CLCN) with the microfluidic-facilitated encapsulation of hydrophilic biomolecules for efficiently delivering synthetic RNAi-based therapeutics including siRNAs, PNAs, and micro-RNA mimics. **Methods:** CLCNs were prepared by mixing under high speed homogenization a lipophilic phase made of glycerol monooleate, 1,2-dioleoyl-3–trimethylammoniumpropane, and 1,2-distearoyl-sn-glycero-3phosphoethanolamine-N-[amino-(polyethyleneglycol)-2000], with a hydrophilic phase contained a emulsifier/stabilizer such as Pluronic F127. The physicochemical characterization of CLCNs was conducted using a dynamic light scattering. Cytotoxic effects of the nanoparticles were tested in vitro on lung cancer and normal fibroblast and bronchial epithelial cells. The encapsulation efficiency and the siRNA condensation in the different nanoparticle formulations were evaluated. The cellular uptake and the transfection efficiency of fluorescent-labeled nanoparticles were studied in lung cancer cells using fluorescent imaging and flow cytometry analysis. **Results:** The physicochemical characterization analysis revealed that CLCNs from each formulation have small sizes between 100-200 nm. These CLCNs are very homogenous and stable nanoparticles as demonstrated by very low polydispersity index (PDI). The Zeta potential measurements confirmed the positive charge (25-35 mV) on the CLCN surface. The gel retardation assay and the encapsulation efficienc calculation indicated a high concentration of siRNAs was entrapped inside the nanoparticles. No cytotoxicity was detected in both lung cancer and normal cells treated with various concentrations of CLCNs (from 0.01 to 100 μM) by in vitro cell proliferation assay. All of these physicochemical characterization results suggested that these CLCNs are suitable for the delivery of small synthetic RNAi-based therapeutics in vitro and in vivo. **Conclusion:** Cationic liquid crystalline nanoparticles (CLCNs) are unique and advanced delivery systems for small molecule RNAi therapeutics. These nanoparticles can encapsulate high concentrations of hydrophilic and hydrophobic substances, due to the high internal surface area. The preparation method is very easy and CLCNs are biodegradable and not toxic on the normal and tumor cells.

171 **CPRIT Grantee**

A LncRNA-MEX3C-PTEN Module Links YAP Inactivation to Breast Cancer Metastasis *Y. Zhang, The University of Texas M.D. Anderson Cancer Center; L. Yang, The University of Texas M.D. Anderson Cancer Center*

Introduction: The Hippo-YAP pathway is critical for organ development, tumorigenesis, and most recently discovered, for epithelial-mesenchymal transition. However, how MST1/LATS1/YAP pathway is inactivated, especially in human cancer, remains unexplored. **Methods:** Here, we report the identification of the entire novel signaling pathway triggered by the E3 ligase MEX3C and PTEN to remove the phosphorylation of YAP1 and downstream gene transcription in a long non-coding RNA, LOC84856, dependent manner. **Results:** Mechanistically, upon 856 stimulation, MEX3C ubiquitinates PTEN at K66 and K80 by K27-linked ubiquitination. This modification of PTEN activates its phosphatase activity to one of its downstream target YAP1, which abolished the phosphorylation of YAP1, leading to inhibition of YAP1 target genes, including CTGF, which is critical for long distance metastasis, including bone and brain metastasis, in multiple cancer types. What's more, this regulation mechanism of 856 and expression level of 856 are regulated and correlated with Glucose stimulation. **Conclusion:** We have thus demonstrated the upstream signaling pathway for Hippo-YAP pathway inactivation and have revealed the relevance of lncRNA-dependent YAP transcriptional activation in human cancer bone and brain metastasis.

172 **CPRIT Grantee**

Proteomics/glycoproteomics of breast cancer cells with different clinicopathological features *R. Zhu, Texas Tech University; E. Song, Texas Tech University; L. Zacharias, Texas Tech University; S. Zhou, Texas Tech University; Y. Mechref, Texas Tech University*

Introduction: Breast cancer is one of the most-prevalent cancers among American women. The high metastatic property of breast cancer has been shown in clinical investigation. Among all the secondary cancers of breast cancer, brain metastatic breast cancer is one of the central issues in breast cancer research due to its high incidence and the low survival rate (ca. one year). In our previous work, N-glycomic profilin of six different breast cancers related cell lines revealed significan differences in N-glycomic profiles of these cells. Such differences suggest specific glycan signatures for each cell line. Herein, we investigated the proteomic/glycoproteomic profile of these six cancer cell lines, namely, MDA-MB-231, MDA-MB-231BR, MDA-MB-361, CRL-1620, HTB-131, and HTB-126. **Methods:** Breast cancer cell lines were cultured following ATCC protocol. After harvest, entire proteome of cells were extracted by 5% sodium deoxycholate solution. Equal amount of protein extracted form each sample, determined by BCA protein assay, was overnight tryptically digested. A 10-μg aliquot of protein digest of each sample was subjected to LC-MS/MS for proteomic profiling. In order to enrich the glycopeptides, the rest of tryptically digest samples were subjected to hydrophilic interaction liquid chromatography(HILIC). LC-MS/MS was acquired using LTQ Orbitrap Velos and TSQ Vantage mass spectrometers. In proteomics profiling and identification of glycosylation sites, LC-ESI-MS/MS data was searched using SwissProt database in MASCOT v2.4 while Scaffold Q+ was employed for spectral counts quantitation. According to spectral counts quantitation results, the significantly changed proteins were then confirmed by MRM LC-MS/MS experiment. In the glycoproteomic study, MS/MS spectra was identified using Glycomod or manually interpreted and quantified by extracted ion chromatogram. **Results:** According to the result of LC-MS/MS analyses, 1158 proteins were identified from 6 breast cancer cell lines at 0.1% protein FDR. Among all the identifie proteins, 772 proteins, which were identified in all three technical replicates of each biological sample, were further quantitatively evaluated using spectral counting quantitation. Based on an independent t-test strategy, 36 proteins shown the uniqueness of MDA-MB-231BR Cell Line compared to other breast cancer cell lines. These significant protein regulations were validated by LC-MRM-MS experiment. LC-MS/MS of

HILIC enriched and deglycosylated peptides permitted the identification of 876 deglycosylated peptides and 528 glycoproteins. LC-MS/MS of HILIC enriched glycopeptides, permitted the identification of 303 glycopeptides correlated to 104 glycosylation sites. **Conclusion:** The proteomic and glycoproteomic profiling of different breast cancer cell lines will prompt further investigation of their molecular biological features.

173 **CPRIT Grantee**

MIR-551a and MIR-551b-3p Target GLIPR2 and Promote Tumor Growth in High-Risk Head and Neck Cancer by Modulating Autophagy *M. Story, The University of Texas Southwestern Medical Center at Dallas; N. Karanam, The University of Texas Southwestern Medical Center at Dallas; L. Ding, The University of Texas Southwestern Medical Center at Dallas; H. Tang, The University of Texas Southwestern Medical Center at Dallas; T. Hwang, The University of Texas Southwestern Medical Center at Dallas; J. Heymach, The University of Texas M.D. Anderson Cancer Center; U. Giri, The University of Texas M.D. Anderson Cancer Center*

Introduction: Micro RNAs (miRNA) have been proposed as biomarkers in a variety of biological and medical conditions such as cancer and stress response. The advantages of miRNA molecules as biomarkers include stability within tissues and body fluids, and their potential use in non-invasive diagnosis and prognosis. We hypothesized that differential expression of miRNAs in head and neck aquamous cell carcinoma (HNSCC) play an important role in cancer progression and recurrence. **Methods:** miRNA expression profiling was performed on a panel of 118 head and neck squamous cell carcinoma (HNSCC) cancer patients treated with post-operative radiotherapy (PORT) to elucidate the molecular mechanism causing distant metastasis (DM) and local recurrence (LR). Forty one samples were found to have DM, while 53 responded to PORT with no evidence of disease (NED). We performed cell proliferation, migration and invasion assays using the HN5 and UMSCC-17B using head and neck cancer cell lines by transfection of either mimics or an inhibitors of miR-551a, miR-551b-3p or GLIPR2 siRNA or cDNA. Luciferase reporter assay was used to show that GLIPR2 is direct target of miRNA551a and miR-551b-3p. Western blotting on proteins associated with the respective signaling pathways was performed as was fluorescence imaging as validation of specific protein signaling. Kaplan Meier (KM) survival analysis as a function of the expression of specifi miRNA or proteins was also performed. **Results:** A comparative two-way ANOVA study of miRNA expression between DM and NED specimens identified 28 differentially expressed miRNAs (FDR < 0.2 and fold change > 1.5). MiRNAs 551a and 551b, which share the same seed sequence, were associated with the DM group and the expression of miR-551a and miR-551b were associated with poor patient survival. miR-551a and miR-551b-3p mimics promoted cell proliferation, migration and invasion while inhibitors had the opposite effect. GLIPR2 mRNA was identificed as the target of miRs 551a and 551b-3p. GLIPR2 is an important negative regulator of autophagy through the interaction with Beclin-1 and the use of miRNA mimics was capable of reducing GLIPR2 expression with a concommitant increase in autophagy. Using the TCGA HNSCC dataset, KM survival revealed increasing hazard ratios with GLIPR2 expression as disease stage increased. **Conclusion:** Our results suggest that miRs 551a and 551b-3p as well as GLIPR2 modulate autophagy and may play an important role in HNSCC disease progression. Furthermore, this axis may be responsible in part for therapeutic resistance via autophagy and they are potential therapeutic targets worthy of further exploration.

174 **CPRIT Grantee**

Comparative Transcriptomics Analysis of Different Breast Cancer Cell Lines to Reveal the Molecular Biological Mechanism of Breast Cancer Brain Metastasis *W. Peng, Texas Tech University; R. Zhu, Texas Tech University; S. Zhou, Texas Tech University; Y. Hu, Texas Tech University; K. Kottapalli, Texas Tech University; Y. Mechref, Texas Tech University*

Introduction: Recently, breast cancer brain metastasis has been recognized as one of the central issues in breast cancer research. One of the significant events of brain metastasis is the penetration of cancer cells through the blood-brain barrier. Elucidation of the process and pathway that mediate this step is expected to provide important clues for the development of more effective therapeutic options to inhibit breast cancer brain metastasis. Herein, we using transcriptome analysis to investigate the molecular mechanism of breast cancer brain metastasis. **Methods:** Six human breast cancer cell lines were incubated. Total RNA was extracted for the RNA-Seq. Illumina HiSeq 2500 was used for the transcriptome sequencing. Differential gene expression analysis was performed by QSeq software. Genes showing >2.0 fold change and p< 0.01 were picked up and mapped into biological pathways using Ingenuity Pathway Analysis software. Then transcripomics data were compared and analyzed to indicate the differences between cell lines. **Results:** Six cell lines, MDA-MB-231, MDA-MB-231BR, MDA-MB-361, HTB-22, HTB-131 and CRL-1620, were studied. Overall 1.25 billion paired end reads were generated. A more than 200G data set was processed for the differential gene expression analysis. Totally 14551 genes were differentially expressed in six cell lines compared with 231BR. According to the Gene Ontology (GO) analysis, cellular process and binding function are the most important biological process and molecular function, associated with differentially expressed genes. Pathway analysis showed that most of genes in the cell migration pathways were differentially expressed, such as pathways in cancer (53 gene involved), regulation of actin cytoskeleton (45 genes involved), MAPK signaling pathway (41 genes), focal adhesion (36 genes), VEGF signaling pathway (16 genes), cell-cell adhesion, etc. In addition, most of glycosylation genes were differentially expressed. **Conclusion:** MDA-MB-231 showed less differences than other cell lines compared with 231BR. This is expected since 231BR is a subline of MDA-MB-231. GO analysis showed that cellular process and binding function were the most important biological process and molecular function. Most differential expression genes were found associated with pathways in

cancer, focal adhesion, VEGF signaling and cell-cell adhesion pathways which contribute to the cell migration. Highly differential expression of glycosylation genes suggested the role of different glycan structures in assisting cancer cells penetration of blood-brain barrier. ST6GALNAC2, GMAT3, GAMT4 and FUT5 genes were down-regulated while ST6GALNAC5, POFUT1 and B4GALT6 genes were up-regulated in 231BR relative to the other cell lines. These genes are believed to be important in breast cancer brain metastasis.

175 **Oral Presentation**
CPRIT Grantee
Poster Session A

Diagnostic Utility of RNA Sequencing for the Detection of Pathogenic Fusion Genes in Childhood Sarcomas of Uncertain Diagnoses *V. Kumar, Baylor College of Medicine; A. Roy, Baylor College of Medicine; J. Reuther, Baylor College of Medicine; D .Parsons, Baylor College of Medicine; K. Covington, Baylor College of Medicine; H . Doddapaneni, Baylor College of Medicine; O. Hampton, Baylor College of Medicine; D. Muzny, Baylor College of Medicine; S. White, Baylor College of Medicine; N. Patel, Baylor College of Medicine; P .Sumazin, Baylor College of Medicine; D. Wheeler, Baylor College of Medicine; S. Plon, Baylor College of Medicine; D. Lopez-Terrada, Baylor College of Medicine*

Introduction: Fusion genes are well-recognized oncogenic drivers in cancers. We assessed the feasibility and yield of performing whole transcriptome paired-end sequencing (RNA-seq) in childhood bone and soft tissue sarcomas of uncertain diagnosis. **Methods:** A RNA-seq pipeline for detecting fusion genes was established and trained using a test set of 9 well-annotated soft tissue sarcomas with pathognomonic gene fusions. A validation cohort of an additional 25 soft tissue sarcomas or mesenchymal solid tumors of uncertain diagnosis were analyzed for gene fusions. Total RNA was extracted from fresh-frozen tumor tissue, and strand-specific, poly-A+ RNA-seq libraries were prepared for Illumina sequencing, generating ~73 million pair-end (2 x 100bp) reads/sample. Fusion transcripts were detected using deFuse (v.0.6.1). Predicted fusion genes were ranked on potential clinical utility using disease-specifi clinical guidelines (WHO), the COSMIC database, as well as published literature and categorized as Category 1 (pathognomonic fusion genes of established clinical utility), Category 2 (therapeutically targetable fusions or fusions activating targetable signaling pathway); Category 3 (fusions involving consensus cancer genes), and Category 4 (all other fusions). Predicted fusions in Categories 1 and 2 were validated using PCR. **Results:** A total of 6708 gene fusions (1316 interchromosomal, 5392 intrachromosomal fusions including 3740 predicted transcriptional read-through events) were identified, including 217 fusion genes predicted to retain the open reading frame (ORF). Analysis of the 217 ORF-retaining fusions revealed a median of 6 fusions per tumor (range 1-17). In the 9 sarcoma test set, RNA-seq analysis correctly identified 8 of 9 expected fusions (*EWSR1-WT1, 3 EWSR1-FLI1, EWSR1-ERG, PAX3-FOXO1, ASPSCR1-TFE3, BCOR-CCNB3*), while an expected *BCOR-CCNB3* fusion was detected but filtered. In four validation set cases in which only a single fusion partner was known from clinical testing (*EWSR1-, CIC-, EWSR1-, CIC-*), RNA-seq identified the reciprocal partner genes (*ETV1, FOXO4, FLI1, DUX4L4,* respectively). Eleven category 1 or 2 fusions were found in the 25 validation set tumors, including a *KIAA1549-BRAF* fusion in a chest wall sarcoma, a *MLL-MLLT10* fusion in a small round cell sarcoma, and a *FUS-CREB3L2* fusion in a sarcoma with myxoid background. These were validated by PCR, resulting in a total yield of 11/25 (44%) tumors harboring a Category 1 or 2 gene fusion. **Conclusion:** RNA-seq is a powerful tool for detecting fusions of clinical utility in sarcomas, and may be especially relevant for unbiased detection of therapeutic targets, as evidenced by the unexpected identification of targetable fusions in the analyzed sarcoma cohort.

176 **Oral Presentation**
CPRIT Grantee
Poster Session A

Applying Digital Holography Microscopy (DHM) to Label-Free Detection of Circulating Tumor Cells (CTCs) *C. Ahrens, Texas Tech University; D. Singh, Texas Tech University; W. Li, Texas Tech University; S. Vanapalli, Texas Tech University*

Introduction: Circulating tumor cells (CTCs) in blood flow have been recognized as a primary driver of cancer metastasis. Further, in clinical trials, CTC concentration in peripheral blood has been shown to correlate with patient prognosis and longitudinal treatment efficac . However progress in CTC-related basic and clinical research has been hindered because of their extremely low counts (1-10 CTCs in 1 billion blood cells), highly heterogeneous morphologies and molecular expression profile that depend on cancer type. We introduce microfluidic digital holography microscopy (DHM) applied to CTC detection as a potentially transformative technology that can process large volumes of blood in a short time and detect CTCs in a manner that is insensitive to cancer-dependent molecular signatures. **Methods:** Cancer cell lines were cultured using standard cell culture techniques and filtered through a 30 micron filte before characterization. Whole human blood and blood components were obtained from consenting healthy donors under IRB-approved protocols. White blood cells were isolated from whole blood using ACK Lysing Buffer (Life Technologies) and Ficoll-Paque (GE Healthcare Life Sciences) as directed by the manufacturers. Cells and blood components were flowed through a 1 mm by 1 mm PDMS microfluidic channel at varied flow rates. The inline-DHM setup consists of a He-Ne laser (10 mW, Thorlabs), spatial filter (10X objective and 25 μm pinhole) and an inverted microscope (IX71, Olympus). A sequence of holograms recorded by a CCD sensor was numerically reconstructed using optimized Matlab based processing to obtain 3D locations, sizes and diffraction patterns of each sample. **Results:** DHM successfully characterized suspended blood components including erythrocyte, leukocyte, platelet as well as representative prostate and breast cancer derived cell lines including PC3, LnCAP, MCF-7 and MDA-MB-231. The size distribution profile of each blood component as determined from 3D DHM reconstructions closely match reported literature values. In combination with distinct scattering intensity profiles, DHM holograms provide unique fingerprints to rapidly enumerate blood components. Finally, DHM-estimated cancer cell concentration is closely correlated with spiked cancer cell concentration. **Conclusion:** Preliminary results suggest that with optimized protocols red blood cells, leucocytes and tumor cells might be distinguished with respect to each other based on cell size, phase map, and refractive index. Further DHM images can capture cubic millimeters of sample volume at millisecond time scales, achieving clinically desirable high throughput blood processing.

177 **CPRIT Grantee Poster Session B**

Misexpression of Cyclin D1 in Embryonic Germ Cells Drives Testicular Germ Cell Tumorigenesis *E. Dawson, Baylor College of Medicine; D. Lanza, Baylor College of Medicine; S. Benton, Baylor College of Medicine; J. Richards, Baylor College of Medicine; J. Heaney, Baylor College of Medicine*

Introduction: Testicular germ cell tumors (TGCTs) are the most frequent solid tumor diagnosed in young men. In both mice and humans, TGCTs result from anomalies in embryonic germ cell development. In the 129 inbred mouse model of human TGCTs, these tumors arise during the same developmental period that male germ cells enter G0 mitotic arrest and female germ cells initiate meiosis (the mitotic:meiotic switch). Dysregulation of this switch associates with tumor initiation and involves three germ cell developmental abnormalities, namely delayed G0 mitotic arrest, retention of pluripotency, and aberrant expression of genes normally restricted to embryonic female and adult male germ cells. One such misexpressed gene, cyclin D1 (*Ccnd1*), is a known regulator of the G1-S phase cell cycle checkpoint and a potent oncogene in several tissues. Therefore, we hypothesized that misexpression of *Ccnd1* promotes a pro-proliferative program that drives TGCT initiation. **Methods:** To test whether components of the G1-S phase checkpoint associate with increased tumor risk, we compared a tumor-resistant strain, FVB/NJ (FVB), and two tumor-susceptible strains, the 129-Chr19$^{MOLF/Ei}$ chromosome substitution strain (M19; 80% affected) and the 129/SvImJ inbred strain (129; 8% affected). To assess the contribution of *Ccnd1* to tumor initiation, we obtained mice harboring a *Ccnd1KO* allele. The *Ccnd1KO* allele was then backcrossed onto the 129 inbred background for 10 generations to establish a congenic strain, and then transferred with crosses to the M19 background. All strains harbor a germ cell-specific GFP transgene for immunostaining experiments or fluorescence-activated cell sorting (FACS) followed by gene expression analyses. **Results:** We found that *Ccnd1* is aberrantly expressed in gonocytes that fail to enter G0 arrest during the mitotic:meiotic switch and is the only D-type cyclin misexpressed in male germ cells during this time period. We discovered that *Ccnd1* deficiency significantly reduced tumor incidence (P<0.001) and suppressed both the proliferation and pluripotency defects critical for tumor initiation. Loss of *Ccnd1* expression did not alter normal male somatic or germ cell development, implying that the mechanisms by which *Ccnd1* deficiency reduced tumor susceptibility were germ cell autonomous and specific to the process of tumor initiation. **Conclusion:** We conclude that misexpression of *Ccnd1* in male germ cells is a key component of a pro-proliferative program that disrupts the mitotic:meiotic switch and predisposes 129 inbred males to testicular teratocarcinogenesis. These experiments reveal the treatment potential of inhibiting CCND-kinase activity for patients with TGCTs, a strategy which will target cancer cells without significant adverse effects to normal tissue.

178 **CPRIT Grantee Poster Session A**

Elucidating Mechanisms of Oncogene-Induced Mutagenesis in Cancer *I. del Mundo, The University of Texas at Austin; S. Kerwin, Texas State University; K. Vasquez, The University of Texas at Austin*

Introduction: Genetic instability is a hallmark of cancer, and chromosomal alterations occur at common “hotspots” in the genome that co-localize with DNA sequences capable of adopting alternative DNA structures (non-B DNA, i.e. H- and G4-DNA). Non-B DNA-forming sequences have been recognized as an endogenous source of genetic instability. However, the initiating mechanisms of mutations are still unclear. Furthermore, in the human *c-MYC* gene, implicated in leukemia and lymphoma, there are multiple non-B DNA-forming sequences that may lead to its oncogenic potential. To demonstrate the extent to which one non-B DNA structure impacts the formation of another structure, so as to be responsible for the genetic instability also remains a challenge. We hypothesize that in a DNA sequence containing more than one non-B DNA-forming sequence; one tract may form a specific non-B DNA structure at a given condition and thus inhibit the formation of other non-B DNA structures within the region. With a properly identified structure, its interactions with repair proteins or drugs can be investigated. M**ethods:** Our study characterizes intramolecular oligonucleotides containing both tandem guanine (G) repeats (G4-forming) and H-DNA-forming sequences from a translocation breakpoint hotspot in the human *c-MYC* oncogene. We incorporated the fluorescent adenine analog, 2-aminopurine (2AP), into the oligonucleotides to probe structure formation in solution. We also use surface plasmon resonance (SPR) spectroscopy, to monitor the stability of the structure formed. Additionally, classical biophysical methods (circular dichroism (CD), gel electrophoresis and chemical footprinting analyses) were employed to support the findings from the novel 2AP and SPR experiments. **Results:** Under triplex-forming conditions, biophysical methods indicated the presence of intramolecular triplex structures. Substituting 2AP within the sequence and monitoring its basal and temperature-dependent fluorescence revealed that 2AP was base-stacked and within a secondary structure, consistent with triplex formation. Novel Biacore assays for DNA structural conformation are in progress. C**onclusion:** Results indicate that in triplex-forming conditions, the chromosomal breakpoint hotspot sequence of the human *c-MYC* gene formed an intramolecular triplex structure; whereas no definit results yet indicated that G4 formed under G4-forming conditions. Our results demonstrate that in multiple, overlapping non-B DNA-forming sequences, one structure formed in a given condition prevents the formation of another alternative DNA structure. The identified structure will be used for therapeutic drug screens and for protein-DNA interaction experiments. Thus, our findings will provide a therapeutic target and assist in the elucidation of the mechanisms underlying DNA structure-induced genetic instability.

179 **CPRIT Grantee Poster Session B**

Cancer Risk in Children with Birth Defects: A Population-Based Registry Linkage Study, 1996-2009 *P. Lupo, Baylor College of Medicine; H. Danysh, Baylor College of Medicine; M. Scheurer, Baylor College of Medicine; A. Sabo, Baylor College of Medicine; S. Plon, Baylor College of Medicine*

Introduction: Although a few genetic syndromes are known to increase cancer risk in children, the presence of a structural birth defect in the absence of these syndromes is increasingly being recognized as a risk factor for childhood cancer. To better understand associations of specifi defects and cancer types, we conducted a population-based assessment to avoid biases often present in studies relying on single clinics, referral centers, or treatment networks. **Methods:** We examined cancer risk in a population-based cohort of children with and without birth defects born between 1996 and 2009 by linking data from the Texas Birth Defects Registry, the Texas Cancer Registry, and birth records from the Texas Center for Health Statistics. Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) based on person-years at risk. We compared the risk of childhood cancer in infants born with and without specific types of birth defects. **Results:** We identifie a cohort of 5,275,792 live births for the study period. There were 184,698 infants with birth defects, and a total of 5,571 children with cancer. Overall, children with birth defects were 3-times more likely to be diagnosed with cancer compared to their unaffected contemporaries (HR=3.07, 95% CI: 2.80-3.37). This pattern was consistent when excluding those with known chromosomal abnormalities (HR=2.27, 95% CI: 1.98-2.60). The risk of childhood cancer was seen among most (55%) monitored birth defects including: biliary atresia (HR=13.20, 95% CI: 4.95-35.18); anophthalmia (HR=9.45, 95% CI: 1.33-67.13); and spina bifida (HR=7.59, 95% CI: 4.20-13.72). After excluding those with chromosomal abnormalities, childhood cancers that were more frequent among those with birth defects included: germ cell tumors (HR=3.94, 95% CI: 2.07-7.79); hepatic tumors (HR=9.65, 95% CI: 5.66-16.47); central nervous system tumors (HR=2.26, 95% CI: 1.69-3.03); and neuroblastoma (HR=2.53, 95% CI: 1.70-3.79). Bone tumors were the only group of childhood cancers that were not positively associated with birth defects (HR=0.68, 95% CI: 0.10-4.91). **Conclusion:** Consistent with prior studies, structural birth defects were associated with increased cancer risk in children. Pooling similar data from many regions will increase power to identify specific associations in order to inform molecular studies examining possible common developmental pathways in the etiologies of birth defects and cancer, and will provide information that can be translated into screening strategies for children at high risk of developing cancer.

180 **CPRIT Grantee Poster Session A**

Comparative Whole Genome Sequencing to Identify Candidate Somatic Driver Mutations of Li-Fraumeni Syndrome Sarcomagenesis in Humans and Mice *J. Wong, The University of Texas M.D. Anderson Cancer Center; L. Strong, The University of Texas M.D. Anderson Cancer Center; G. Lozano, The University of Texas M.D. Anderson Cancer Center; L. Bachinski, The University of Texas M.D. Anderson Cancer Center; R. Krahe, The University of Texas M.D. Anderson Cancer Center*

Introduction: Li-Fraumeni Syndrome (LFS) is a rare, clinically heterogeneous, inherited cancer predisposition syndrome that is associated with a broad tumor spectrum, including a high incidence of sarcomas. Although the majority of LFS families (>70%) have been linked to germline mutations in the tumor suppressor gene *TP53 (p53)*, variable penetrance across pedigrees and the development of sarcomas at a wide range of ages and sites suggests that while *p53* mutations alone may confer cancer predisposition, they are insufficient for sarcomagenesis. Typically, identification of somatic driver mutations is complicated by the concurrent acquisition of passenger events, including those driven and confounded by genome architecture. Given that *p53*-LFS patients start with an inherited predisposition of one mutant allele and have generally earlier cancer onset, LFS sarcomas may have fewer passenger mutations than sporadic sarcomas, improving our ability to identify tumor drivers. M**ethods:** To identify potential drivers for LFS sarcomagenesis, we used a comparative genomics approach, considering whole genome sequencing (WGS) and RNA-sequencing for normal/tumor samples from both humans and a mouse model of LFS. The "humanized" mouse model contains a germline *p53+/R172H* missense mutation (analogous to the human hot-spot *TP53 R175H* LFS mutation) and predisposes to a similarly broad tumor spectrum, including a high incidence of sarcomas. **Results:** The mouse WGS data show some recurrent somatic changes at the DNA-level including some genes with known human orthologs and demonstrated roles in human cancers. The human WGS data, consisting of two matched normal/tumor pairs from known p53 LFS carriers *(M133T, R175H)* show more minimal recurrence, both for a specific variant or any variant in the same gene. RNA-seq data from two other human normal/tumor pairs also suggest relatively minimal overlap at the variant and gene level, though some of this may be explained by different tumor types (myofibrosarcoma, liposarcoma). Integration of the data set to include all human and mouse samples revealed minimal overlap at the variant level, but potential recurrence at both the gene *(NF1, PTEN, PCDBH8)* and pathway levels (Notch, PTEN, p38 MAPK). **Conclusion:** Our overall results identify known players in tumorigenesis such as *NF1*, as well as more novel ones, including *PCDBH8*, and the p38 MAPK pathway, indicating the utility of comparative genomics of human cancers and their mouse models for the identification of somatic driver mutations

181 **CPRIT Grantee**
Poster Session B

Fecal Microbiota and Risk of Colorectal Tubular Adenoma *L. Jiao, Baylor College of Medicine; K. Royse, Baylor College of Medicine; J. Petrosino, Baylor College of Medicine; N. Ajami, Baylor College of Medicine; D. Hutchinson, Baylor College of Medicine; D. White, Baylor College of Medicine; D. Graham, Baylor College of Medicine; D. Rosen, Baylor College of Medicine; L. Chen, Baylor College of Medicine; A. Smith, Baylor College of Medicine; J. Uriostegui, Baylor College of Medicine; M. Wong, Baylor College of Medicine; J. Kramer, Baylor College of Medicine; S. Hilsenbeck, Baylor College of Medicine; M. Bondy, Baylor College of Medicine; H. El-Serag, Baylor College of Medicine*

Introduction: Most colorectal cancer arises from colorectal adenomas. Gut microbiota is important in maintaining intestinal homeostasis. Dysbiosis, in particular more abundant *Fusobacterium*, has been associated with increased risk of colorectal adenoma and cancer. However, there has been no comprehensive evaluation of the fecal microbiome in association with colorectal adenomas. **Methods:** We collected fecal samples before bowel preparation from seven men with tubular colorectal adenomas (cases) and seven men and one woman with normal appearing mucosa with no polyps or history of polyps (controls) in the endoscopy suite at the Michael E. DeBakey VA Medical Center between January 2015 and April 2015. Cases and controls were frequency-matched according to age, sex, ethnicity and body mass index. We extracted microbial DNA from fecal samples and amplified and sequenced the 16S V4 region on the Illumina MiSeq platform. We analyzed sequencing data using UPARSE and SILVA database for operational taxonomic unit (OTU) classification We calculated alpha and beta-diversity indices and conducted Weighted UniFrac principal coordinates analysis (PCoA). The difference between cases and controls was compared using the Mann-Whitney test for alpha-diversity and relative abundance and PERMANOVA for beta-diversity. **Results:** Proteobacteria, Bacteroidetes, and Firmicutes were the major phyla accounting for > 90% of microbiota. Differences in richness or evenness of fecal microbiota in cases and controls approached statistical significance (P = 0.08 for observed OTUs). We found increased abundance of the phylum *Actinobacteria* in cases compared with controls (1.39% vs. 0.16%, false discovery adjusted P value = 0.007). The mean relative abundance of genus *Fusobacteria* was 0.03% in cases and 0% in controls (P value = 0.38). Overall, weighted UniFrac PCoA indicated that the composition of the microbiota was not different between cases and controls (P value = 0.46). There were no significant differences in relative abundance at the genus level between cases and controls. **Conclusion:** In this pilot case-control study, we found that *Actinobacteria* was more abundant in fecal samples of cases with tubular adenoma than among controls with no polyps. Our ongoing study with greater statistical power is expected to confirm this finding, provide information on the higher relative abundance of *Fusobacteria* among cases by using a targeted approach, and define other dysbiotic conditions associated with adenomas

182 **Poster Session A**

Impact of Alcohol and Cigarette Smoking on Stress Physiology: An Investigation into Cancer Risk Behaviors in Rural African Americans *E. Obasi, University of Houston; L. Cavanagh, University of Houston; E. Ewane, University of Houston; S. Yan, University of Houston*

Introduction: Tobacco is the leading cause - and alcohol is the third leading cause - of preventable death and disability in the U.S. (Mokdad et al., 2004). In fact, one-third of all cancers are directly attributable to tobacco, and alcohol increases the risk for cancers of the mouth, pharynx, larynx, esophagus, liver, and breast (Boffetta & Hashibe, 2006). Moreover, alcohol and tobacco not only exert independent effects on cancer risk, but there is a synergistic effect for some cancers such as oral cancer (Blot et al., 1988; Ogden, 2005; Johnson, 2001). Taken together, it is important to investigate cancer-risk behaviors in rurally situated African Americans who suffer from stress and drug-related health disparities. The purpose of this study was to investigate the physiological impact of consuming alcohol and smoking cigarettes on cortisol and DHEA functioning, biomarkers previously linked to the Hypothalamic-Pituitary-Adrenal (HPA) axis. Having insight into the effects of drug use on stress physiology could provide an endophenotype for understanding cancer-risk behaviors in marginalized/underserved communities. **Methods:** A random sample of rural African American emerging adults (n = 84) between the ages of 18 and 23 (M = 20.1) completed a battery of assessments (incl., 90-day TLFB for alcohol and cigarette consumption) and provided six samples of saliva at specified times throughout the day. All participants gave informed consent, were financially compensated, and were debriefed. **Results:** Changes in cortisol and DHEA was positively coupled across the day ($p < .001$). Perceived stress ($p = .023$) and alcohol consumption ($p = .037$) had a significant inverse relationship with the cortisol awakening response (CAR). Participants tended to have more DHEA upon wakening as a function of smoking greater numbers of cigarettes ($p < .001$). Furthermore, the interaction between cigarette and alcohol use was associated with lower levels of DHEA upon wakening ($p < .001$). **Conclusion:** The CAR was blunted in individuals who reported higher levels of perceived stress and/or alcoholic consumption. The CAR was essentially obliterated in participants who reported higher levels of stress and alcohol consumption. While use of a single substance was related to higher DHEA and greater HPA activation (i.e., cigarettes), addition of a second substance (i.e., alcohol) shifted the individual toward the blunted arousal profile common within chronically stressed or challenged populations. These finding support the need to further investigate the relationship between comorbid substance use and physiological functioning that may be linked to known cancer-related health disparities in the African American community.

183 **CPRIT Grantee**
Poster Session B

Compact Open-MRI for Early Detection of Breast Cancer - New Magnet Technology *P. McIntyre, Texas A&M University; A. Sattarov, Texas A&M University*

Introduction: Breast cancer is a leading killer of women in America. Early detection of breast cancer dramatically enhances the prospect for cure. Mammography is a powerful method for early detection, but has limited effectiveness for patients with dense breasts. Dye-contrast breast MRI is effective for early detection in women with dense breasts, but conventionally it is performed in whole-body MRI systems for which the image cost is prohibitive for well patient screening. A new methodology has been developed to optimize the superconducting winding and flu return steel geometries for a compact open-MRI system for breast imaging. The goal of the project is to develop a 1.5 Tesla open-MRI system that is affordable for an image cost comparable to that of mammography and sufficiently compact that it can be staged in clinics and mobile units. **Methods:** The desired sensitive volume for dye-contrast breast imaging is a torus located in the top surface of a superconducting magnet so that, when a patient lies prone on the surface, her breasts are positioned in the homogeneous field region. In a conventional superconducting magnet the magnetic field diverges everywhere ouside the bore of the magnet, so that making homogeneous field in the end surface of the magnet would be impossible. In the Breast Imager an arrangement of structured coils is used, in which windings carry currents in both rotational senses. With suitable design methods it is then possible to cancel the divergence in a desired sensitive volume and produce homogeneous field. Novel mathematical algorithms have been developed to produce the ~ppm field homogeneity in this sensitive volume using a minimum quantity of superconductor and flux return steel. We have developed a structured cable suitable for the fabrication of the magnet windings for the structured coils. **Results:** The magnet design and the conceptual design for the complete Breast Imager is presented. Plans for construction of a scale-model winding is discussed. Collaboration is being developed with UT Southwestern Medical Center for follow-on design of a first prototype imager for first imaging studies. **Conclusion:** During the first year of the CPRIT-funded project, we have completed the magnetic design and optimization of a compact open-MRI magnet for breast imaging. Its size and projected cost give the prospect of reducing the cost of dye-contrast breast imaging so that it is affordable for well-patient screening.

in collaborations and welcome inquiries on application to different platforms, disease targets, or clinical applications.

184 **CPRIT Grantee**
Poster Session A

High Specificity Hybridization to Enable Reliable Detection of Rare DNA Biomarkers *D. Zhang, Rice University*

Introduction: Because the prognosis for disease-free survival worsens sharply as the stage at which cancer is detected becomes later, early detection is a critical component of reducing cancer mortalities (currently 40% in the US). Development of reliable DNA and RNA based molecular diagnostics for early detection of cancer can provide information about the growth rate of the cancer, as well as personalized guidance on therapies most likely to be effective for each patient. Pharmaceutical companies are actively developing third and fourth generation drugs, such as mutant-specific tyrosine kinase inhibitors, with drastically lower off-target effects and are considered as potential future front-line cancer treatments. This raises the potential of a future healthcare paradigm where systemic treatment can follow accurate molecular diagnosis--without diagnostic imaging to precisely locate small tumor masses. M**ethods:** Our group has developed competitive hybridization probe and primer systems that are highly sensitive to single-nucleotide variants. Unlike previous shotgun empirical approaches to primer design and optimization, we follow a uniquely knowledge-driven approach using kinetic simulations to guide the design of primers and probes. Our first-try experimental results are more than 30-fold better than prior art, even after multiple rounds of optimization. **Results:** We have applied our probe and primer systems to 44 most commonly observed cancer driver mutation sequences, and observed a median 890-fold affinity difference between these single-nucleotide mutations and their corresponding wildtype sequences, and this work has recently been published as the cover article of Nature Chemistry. We have subsequently applied our technology to 12 different point mutations from human genomic DNA extracted from 6 different cell lines. We are now validating our technology on a variety of platforms including quantitative PCR, barcoded RNA imaging (Nanostring), isothermal amplification and next-generation sequencing. We are initially interested in using our technology to enable early detection of lung cancer, and have started retrospective analysis of lung cancer patient samples (blood and saliva). **Conclusion:** Our next steps are to enable highly accurate quantitation of variant alleles at low frequencies (between 0.01% and 5%), and to improve multiplexing capabilities to allow simultaneous detection of up to 1000 rare mutations from a single 100ng DNA sample. We are interested

185 **CPRIT Grantee**
Poster Session B

The Characterization of CTC Subsets Related to Mechanisms of Breast Cancer Dormancy *D. Marchetti, The University of Texas M.D. Anderson Cancer Center; M. Vishnoi, Baylor College of Medicine; S. Peddibhotla, Baylor College of Medicine; W. Yin, Baylor College of Medicine; D. Hong, The University of Texas M.D. Anderson Cancer Center; A. Scamardo, The University of Texas M.D. Anderson Cancer Center*

Introduction: Uncovering phenotypes of patient-derived Circulating Tumor Cells (CTCs) offers the promise to dissect CTC heterogeneity in relation to metastatic competence, and to determine biomarkers of therapeutic utility for improved treatment. However, it is still unknown whether and how CTCs differ in their capacity to circulate while maintaining metastatic potential. Rates of CTC survival are highly variable, lasting less than a few hours in some patients but in the order of decades in others. This can lead to many questions for yet unexplored mechanisms of CTCs responsible for tumor dormancy. We hypothesized that breast cancer CTC subsets possessing markers of pluripotency avoid organ arrest with extreme efficiency by the concomitant presence of quiescence and stem cell properties; and that expression of urokinase plasminogen activator receptor (uPAR) and beta-1 integrin (β1int), two biomarkers known to be directly implicated in tumor cell dormancy, are relevant in controlling the recurrence of breast cancer brain metastasis (BCBM). **Methods:** We isolated CTC subsets not expressing the epithelial cell adhesion molecule (EpCAM-negative CTCs), and characterized these subsets using DEPArrayTM, a new CTC platform able to dissect CTC heterogeneity at a single-cell level, thus interrogating the smallest functional unit of cancer. **Results:** We captured EpCAM-negative/CD45-/CD44+/CD24- breast cancer CTC subsets that possessed combinatorial uPAR and β1int expression using multiparametric flow cytometry. Second, CTC subsets grew in vitro and were further characterized by DEPArrayTM. Markers expression was confirmed by confocal microscopy with subsets possessing a specific breast cancer gene profiling. Third, EpCAM-negative CTC subsets (uPAR+/β1int+ and uPAR-/β1int-) were interrogated for human embryonic stem cell markers by RT2 PCR arrays. Gene expression profiling was consistently distinct among uPAR+/β1int+ vs. uPAR-/β1int- CTC subsets and dependent upon patients' BCBM status: expression of genes implicated in cell cycle progression (e.g., CDK42, CDK1), angiogenesis (e.g., FGF-2),

and pluripotency (e.g., KLF4) was >30-fold higher than controls. Third, CTC subsets gene patterns isolated from patients with BCBM possessed RT2 profiles that were strikingly distinct from ones derived from patients with no BCBM. Of note, gene expression for RIF-1, a protein that counteracts actions of the breast cancer suppressor BRCA1, was highest (>50-fold) with distinct RIF-1 nuclear patterns in BCBM CTC subsets. C**onclusion:** We have linked EpCAM-negative uPAR/β1int CTC subsets and their properties to clinical BCBM. Deciphering further roles and targets of uPAR/β1int as key CTC biomarkers of dormancy vs. metastatic competence is relevant to elucidate the CTC mechanisms responsible for BCBM development and its timing.

186 **CPRIT Grantee**
Poster Session A

In Vivo Detection of 2-Hydroxyglutarate in Brain Tumors by Optimized 1H MR Spectroscopy at 7T *S. Ganji, The University of Texas Southwestern Medical Center at Dallas; Z. An, The University of Texas Southwestern Medical Center at Dallas; V. Tiwari, The University of Texas Southwestern Medical Center at Dallas; E. Pan, The University of Texas Southwestern Medical Center at Dallas; B. Mickey, The University of Texas Southwestern Medical Center at Dallas; E .Maher, The University of Texas Southwestern Medical Center at Dallas; C .Choi, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Following the discovery of 2-hydroxyglutarate (2HG) production in IDH-mutated gliomas, several studies reported noninvasive detection of this unprecedented imaging biomarker by 1H magnetic resonance spectroscopy (MRS) at 3T. Reliable detection of 2HG remains challenging because the MR signals from the J-coupled spins of 2HG are relatively small and extensively overlapped with the signals of other metabolites. High-field MRS benefits from signal gain and increased spectral resolution, especially in J-coupled resonances. Here we present 2HG detection by optimized 1H MRS at an ultra-high field 7T. **Methods:** To improve the spectral selectivity of 2HG against the neighboring signals from GABA, glutamate and glutamine, the echo time (TE) of point-resolved spectroscopy (PRESS) was optimized with numerical simulations and phantom test. This new 2HG MRS method was tested in 14 glioma patients in vivo. The data were analyzed with LCModel using numerically-calculated basis spectra. Metabolite levels were quantified with reference to water at 45 M. **Results:** At TE = 78 ms (TE1 = 58 ms and TE2 = 20 ms), the C4-proton resonance of 2HG at 2.25 ppm was temporally maximum and appeared as an inverted peak, as opposed to the signals of glutamate (2.35 ppm), glutamine (2.45 ppm) and GABA (2.29 ppm) resonances which all appeared as positive peaks. The PRESS TE = 78 ms offered improved discrimination of 2HG signal from the adjacent signals compared to short TE MRS at 7T. The new MRS method provided 2HG estimation with percentage standard deviation (%SD) less than 20% in all patients enrolled in the study. The 2HG concentration was estimated to be as high as 7.8 mM. The mean %SD was 8 ± 7 %. **Conclusion:** The optimized data acquisition scheme at 7T, along with the increased spectral resolution and higher sensitivity, offered in vivo detection of 2HG with precision in IDH-mutated gliomas.

187 **CPRIT Grantee**
Poster Session B

Translation of 2-Hydroxyglutarate MR Spectroscopy Into Clinics *S. Ganji, The University of Texas Southwestern Medical Center at Dallas; Z. An, The University of Texas Southwestern Medical Center at Dallas; V. Tiwari, The University of Texas Southwestern Medical Center at Dallas; E. Pan, The University of Texas Southwestern Medical Center at Dallas; B. Mickey, The University of Texas Southwestern Medical Center at Dallas; E. Maher, The University of Texas Southwestern Medical Center at Dallas; C. Choi, The University of Texas Southwestern Medical Center at Dallas*

Introduction: 2-Hydroxyglutarate (2HG) is the first imaging biomarker that is specific for IDH-mutated gliomas and therefore may have important implications for use in management of patients with brain tumors. Moreover, since IDH mutational status carries favorable prognosis, 2HG imaging can provide noninvasive diagnosis and prognosis. Our recent development of 1H magnetic resonance spectroscopy (MRS) in a “research” 3T scanner may offer an effective tool for noninvasive measurement of 2HG in IDH-mutated gliomas (Choi et al. Nat Med 2012). The potential impact of this imaging biomarker for patients can only be realized when the technique is widely available in clinical scanners at academic/community radiology centers. **Methods:** We have performed in-vivo 2HG evaluation in patients using our published 2HG MRS method in both clinical and research MR scanners, as part of the 2HG MRS translational project. Twenty-four glioma patients were recruited. Each subject underwent two examinations with the 2HG-optimized PRESS TE=97ms method; one in a clinical scanner and the other in a research scanner on same day. Two patients were scanned at 4 time points in both scanners, the total number of clinical/research scan pairs being 30. T2w-FLAIR images were used for identifying tumors. Spectral fitting was performed with LCModel software. Metabolite levels were quantified with reference to water at 45 M. **Results:** Of the 30 pairs of MRS data sets, 2 pairs of data had singlet linewidth larger than 0.1 ppm and thus were not proper for metabolite estimation. Excluding these 2 pairs, 28 pairs of data were included for subsequent statistical analysis. The concentrations of 2HG from the clinical and research scanners were found to be very similar (3.74±0.40 mM vs. 3.58±0.42 mM, p = 0.98). Small coefficien of variation (CV) (=0.17) of 2HG estimation further confirmed the high reproducibility of the 2HG MRS between the pairs of scans. Similarly, small CV was obtained for glutamate (0.17) and NAA+NAAG (0.14), indicative of good reproducibility between clinical and research scans. Choline, which is elevated in tumors, showed very small CV (0.09), depicting high confidence of reproducibility. **Conclusion:** The present study demonstrates that evaluation of 2HG across research and clinical scans using optimized PRESS is highly reproducible, suggestive of widespread dissemination of the 2HG MRS protocol for clinical use.

188 **CPRIT Grantee Poster Session A**

'Cytology-on-a-Chip' Based Sensors for Monitoring of Potentially Malignant Oral Lesions *T. Abram, Rice University; P. Floriano, The University of Texas M.D. Anderson Cancer Center; N. Christodoulides, Rice University; R. James, Rho, Inc.; A. Kerr, New York University College of Dentistry, Department of Oral and Maxillofacial Pathology, Radiology & Medicine; M. Thornhill, Academic Unit of Oral & Maxillofacial Medicine & Surgery, University of Sheffield School of Clinical Dentistry; S. Redding, The University of Texas Health Science Center at San Antonio; N. Vigneswaran, The University of Texas Health Science Center at Houston; P. Speight, Academic Unit of Oral & Maxillofacial Pathology, University of Sheffield School of Clinical Dentistry; J. Vick, Rho, Inc.; C. Murdoch, Academic Unit of Oral & Maxillofacial Medicine & Surgery, University of Sheffield School of Clinical Dentistry; A. Hegarty, Unit of Oral Medicine, Charles Clifford Dental Hospital, Sheffield Teaching Hospitals National Health Service Foundation Trust; J. Phelan, New York University College of Dentistry, Department of Oral and Maxillofacial Pathology, Radiology & Medicine; P. Corby, New York University College of Dentistry, Bluestone Center for Clinical Research; I. Khouly, New York University College of Dentistry, Bluestone Center for Clinical Research; J. Bouquot, The University of Texas Health Science Center at Houston; N. Demian, The University of Texas Health Science Center at Houston; Y. Weinstock, The University of Texas Health Science Center at Houston; S. Rowan, The University of Texas Health Science Center at San Antonio; C. Yeh, The University of Texas Health Science Center at San Antonio; H. McGuff, The University of Texas Health Science Center at San Antonio; F. Miller, The University of Texas Health Science Center at San Antonio; R. Raja, Rice University; J. McDevitt, New York University, Department of Biomaterials*

Introduction: Despite significant advances in surgical procedures and treatment, long-term prognosis for patients with oral cancer remains poor, with survival rates among the lowest for major cancers. However, when detected early, the prognosis for oral cancer patients is excellent. The current clinical practice paradigm for diagnosis of oral cancer relies almost exclusively on invasive tissue biopsies followed by expensive and time-consuming histopathological evaluation. Here, we demonstrate the utility of a new 'cytology-on-a-chip' approach combined with noninvasive sampling to afford a multi-parameter clinical decision tool for managing patients with "suspicious" lesions detected during an oral examination. **Methods:** Measurements from 714 prospectively recruited patients across 6 diagnostic categories (each confirmed by tissue biopsy and histopathology) were used to train and validate classification models developed using 1) random forests, 2) L2-regularized logistic regression (LASSO), and 3) logistic regression with "out of bag" bootstrapping. Over 200 measurements per patient related to biomarker expression and cellular morphology were recorded on a microfluidic platform designed to isolate and interrogate single cells from a brush cytology sample. By cataloging an average of 2,000 cells per patient, these efforts resulted in nearly 13 million indexed objects, each with over 200 unique measurements, arguably the largest well-qualified cytology database ever collected for prospectively recruited potentially malignant oral lesions (PMOL). **Results:** Primary LASSO logistic regression models differentiating "Low/ High risk" lesions yielded sensitivity/specificity of 85.3%/74.4% and 76.2%/75.2% for the training and validation models, respectively. On average, validation performance for random forests displayed a 2.62% drop in sensitivity (SD= 5.05%) and an increase of 3.86% for specificity (SD=1.94%), compared to a 7.01% sensitivity drop for LASSO (SD = 5.53%) and a corresponding increase of 3.26% in specificity (SD = 2.52%). At the higher end of the clinical spectrum, LASSO models (validation sens/spec/AUC = 77.1%/88.0%/0.883) and random forest models (validation sens/spec = 78.8%/70.8%) outperformed models distinguishing low grade lesions in terms of sensitivity, specificit , and AUC. Key parameters identifie in these models included cell circularity, Ki67 and EGFR expression, nuclear-cytoplasmic ratio, nuclear area, and cell area. **Conclusion:** We have demonstrated the utility of a new cytology-on-a-chip methodology that is capable of high-content single-cell analysis across nuclear and cellular morphometric and molecular biomarker expression measurements. Through the application of statistical machine learning algorithms, we have developed a classification model with validated and stable parameters with potential to serve as an adjunctive tool to assist in clinical decision making.

189 **CPRIT Grantee Poster Session B**

Mapping Oxygen Saturation in the Tumor Microenvironment via Photoacoustic-Ultrasonic Imaging *K. Dextraze, The University of Texas M.D. Anderson Cancer Center; N. Munoz-Gonzalez, The University of Texas M.D. Anderson Cancer Center; A. Heinmiller, VisualSonics; J. Bankson, The University of Texas M.D. Anderson Cancer Center; C. Kingsley, The University of Texas M.D. Anderson Cancer Center; K. Michel, The University of Texas M.D. Anderson Cancer Center; R. Avritscher, The University of Texas M.D. Anderson Cancer Center; R. Bouchard, The University of Texas M.D. Anderson Cancer Center*

Introduction: Oxygen saturation variations have been linked to tumor aggressiveness and poorer prognosis, when compared to normally oxygenated tumors. Therefore, by creating a high-resolution spatial map of oxygen saturation within the tumor, we may be able to identify regions of highest risk disease. These maps can improve guidance of tumor biopsies by sampling sampling poorly oxygenated regions and provide more information about the tumor's response to treatement. This study assessed the feasibility of using photoacoustic-ultrasonic (PAUS) imaging to map oxygen saturation at clinically relevant depths in an orthotopic rat model of hepatic cellular carcinoma. **Methods:** Three rats inoculated with an orthotopic model of human hepatocellular carcinoma (HCC) were assessed. Animals were sedated with isoflurane. To induce changes in oxygen saturation within the tissue, inhalation gas was switched from medical air to pure oxygen, at 10 minute intervals. Both ultrasound and PA imaging were performed using the Vevo LAZR system using a focused tunable laser source (VisualSonics, 15MHz transducer) and a custom-made, low-frequency array (15 MHz). Under medical air, we acquired 3D B-mode, 3D Doppler, 3D PA with 2 wavelengths (750 and 850 nm), 2D PA on a central slice of the tumor using all wavelengths (680-970 nm) and the OxyZated technique (750nm, 850nm). During gas transition, the OxyZated technique was applied. Under pure oxygen, the PA acquisitions were repeated, followed by perfusion contrast-enhanced ultrasound using a 150-microliter bolus injection esof MicroMarker microbubbles of microbubble contrast per animal (MicroMarker, VisualSonics). Analysis was performed using the VevoQC software, and customized MATLAB code. For each subject, the change in oxygen saturation was calculated via spectral unmixing, where the signal from each pixel was categorized as oxy-Hb, deoxy-Hb, or background. The PA images were coregistered to the non-linear contrast (perfusion) images to compare variations in oxygen saturation to perfusion variations. The PA oxygen saturation maps were also confirmed by comparing the PA images with histologic staining of the tumor (hematoxylin & eosin and pimonidazole). **Results:** We observed that decreased perfusion (from US contrast) and decreased vascularity (from H&E stain) correlated with low oxygen saturation (from PA). Through this study, we demonstrated that it is feasible to create oxygen saturation maps of in vivo tumors with sub-micron resolution. **Conclusion:** This study demonstrated that PAUS imaging is capable of characterizing oxygen saturation and tissue perfusion in a clinically-relevant orthotopic rat model of HCC. Such capabilities have potential for eventual clinical translation for diagnostic and treatment monitoring purposes.

190 **CPRIT Grantee Poster Session A**

Progress Report: TAMU–UT Southwestern Partnership for Breast Spectroscopy at 7 Tesla *S. Ogier, Texas A&M University; S. Wright, Texas A&M University*

Introduction: The recent surge in interest in *in vivo* NMR has created a need for receive array coils to improve the often-poor sensitivity of other nuclei. However, the development of these array coils has been slowed by the scarcity of multi-channel, multinuclear receivers. Multi-channel receivers are commonplace in MRI, but very few of these receivers are capable of operating over a broad enough bandwidth to accommodate nuclei other than ^{1}H. Our group is developing technology to enable the use of array coils for nuclei other than ^{1}H on the 7 Tesla MRI system at UTSW using frequency translation to adapt the manufacturer's narrow-band receivers to multinuclear use. This method works with a wide variety of nuclei and easily accommodates proton decoupling, a necessity for working with ^{13}C. **Methods:** Design, construction, and testing of the frequency translation system and radiofrequency coils is conducted at TAMU. Pulse sequence design and 7T experiments are conducted at UTSW. Frequency translation uses radiofrequency mixers to convert the received signal from the frequency of the X-nucleus to a frequency at or near the ^{1}H frequency. This conversion is performed after preamplificatio of the received signal to prevent degradation of the signal. In order to accommodate ^{1}H decoupling in ^{13}C spectroscopy, the received ^{13}C signal is mixed a hundred kilohertz away from the ^{1}H frequency. This prevents the powerful 1H decoupling signal from corrupting the ^{13}C spectrum. Two approaches are being considered. In one, only the received signal is mixed - the transmit signal path is left intact to simplify integration with the scanner. In order to maintain phase stability in this configuration, the LO is generated from the same 10 MHz reference used by the spectrometer, and the phase is reset by a trigger from the system before each acquisition. In the other, the RF pulse is transmitted at the 1H frequency and converted before amplification to the ^{13}C frequency. This requires more modification of the system, but fewer modifications to the pulse sequence. **Results:** A single frequency translation channel has been successfully tested on the 7T scanner at UTSW. A sixteen channel coil breast coil array and coil interface for 13C spectroscopy is under construction. **Conclusion:** Frequency translation can potentially provide the increased sensitivity of array coil receive to 7T scanners to enable multinuclear spectroscopy with higher sensitivity. This approach is being implemented on a commercial 7T scanner using custom made array coils, initially for 13C breast spectroscopy.

191 **CPRIT Grantee Poster Session B**

An Integrative Lasso-Based Model Containing Circulating Cytokines and Chemokines and Initial Metastatic Status Improves the Prognostic Prediction of Osteosarcoma Patients *A. Kelly, Baylor College of Medicine; R. Flores, Baylor College of Medicine; L. Perlaky, Baylor College of Medicine; L. Wang, Baylor College of Medicine; C .Lau, Baylor College of Medicine; C. Man, Baylor College of Medicine*

Introduction: Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents. Although only 20% of patients during diagnosis have detectable metastatic lesions using conventional imaging techniques, many later relapse, contributing to dismal outcomes. Unfortunately, no biomarkers other than initial metastatic status are currently used during patient diagnosis. Identification of molecular biomarkers that provide prognostic information beyond initial metastasis will facilitate better risk-stratification at diagnosis to determine the most appropriate treatment option to improve patient outcomes. Circulating cytokines/chemokines present an attractive opportunity for clinically used biomarkers since blood contains both tumor and host factors and only minimally invasive procedures are needed. Furthermore, chemokines and cytokines have been implicated in a host of pathogenic mechanisms in different solid tumors, indicating that discovery of biomarkers may lead to greater understanding of OS pathogenesis and identification of therapeutic targets. **Methods:** We employed Luminex bead assays to measure the concentrations of 76 cytokines/chemokines in a cohort of 233 patient serum samples from the Children's Oncology Group (COG). A Lasso-based CoxPH model with cross-validation feature selection was trained on a subset of this cohort (116) and tested on the remaining patient samples (117) to determine if a subset of proteins could give stronger predictive power with respect to overall survival than just metastasis alone. The selected model was that which minimized the partial likelihood deviance. The model was then validated on an independent cohort of 37 OS plasma samples collected from Texas Children's Hospital. **Results:** A Lasso model containing initial metastatic status and 11 chemokines/cytokines was trained on the initial COG cohort, which significantl outperformed metastatic status alone (LR p-value=0.009). A cutoff chosen using martingale residuals derived from training cohort was used to risk-stratify patients in the test COG cohort with respect to model output values, resulting in patients more significantly stratified than by metastasis alone in both overall (met-dx p-value=0.02, mod p-value=0.005) and event-free survival (met-dx p-value=0.002, mod p-value=4.4e-5). The model remained significant for overall survival when applied to the independent cohort (p=0.01). **Conclusion:** In conclusion, we have shown that a panel of chemokines/cytokines can add predictive power to the already used clinical covariate in OS prognosis and may have the potential to more effectively stratify patients for risk-based therapeutic options. Interestingly, all three ligands of one such therapeutic target, the CXCR3 axis, are present in our model, indicating that this axis may play a crucial role and intervention may be possible.

192 **CPRIT Grantee Poster Session A**

Performance on the National Quality Forum Colon Cancer Quality Metrics - How Are Hospitals Really Measuring Up? *M. Mason, Baylor College of Medicine; G. Chang, The University of Texas M.D. Anderson Cancer Center; Y. Sada, Baylor College of Medicine; H. Tran Cao, Baylor College of Medicine; D. Berger, Baylor College of Medicine; N. Massarweh, Baylor College of Medicine*

Introduction: The National Quality Forum (NQF) endorses adequate lymph node evaluation (ALNE), adjuvant chemotherapy (AC) administration, and AC within 4 months as quality metrics for colon cancer care. However, the current level of hospital performance on these measures and correlation across measures within hospitals are unknown. The study objectives were to evaluate trends in hospital NQF metric performance over time and to assess within hospital inter-metric correlation. **Methods:** This was a retrospective cohort study of surgically resected, stage I-III colon cancer patients using the National Cancer Data Base (2003-2009). Hospital compliance with three NQF metrics (≥12 LN pathologically-examined [ALNE]; AC administered to stage III patients; AC administered within 4 months of diagnosis for stage III patients <80 years [timely AC]) were calculated. Facilities were stratified into metric performance categories (very-low:0-25%; low:25-50%; high:50-75%; very-high:>75%]). Performance over time, metric correlation (stage III patients), and factors associated with increasing metric achievement were evaluated using the Cochrane-Armitage test, Cramer's V test, and multivariate multinomial regression, respectively. **Results:** Among 199,815 patients treated at 1449 hospitals, there were significant improvements in all three metrics individually (ALNE—52.5% 2003 vs. 84.4% 2009; AC administration—75.0% vs. 80.9%; timely AC—69.3% vs. 75.0%; trend test, p<0.001 for all) and proportion of patients who achieved all 3 metrics (42.5% vs 67.2%, trend test, p<0.001). Over time, the proportion of hospitals in the very-high performance category for each metric increased from 13.9% to 73.1% for ALNE (trend test, p<0.001), 56.6% to 64.1% for AC administration (trend test, p<0.001), and 46.6% to 53.8% for timely AC (trend test, p=0.014). The proportion of very-high performing hospitals on all three metrics increased over time (15.7% in 2003 vs 42.1% in 2009, p<0.001) with good correlation between the AC measures (V=0.84 in 2003 vs 0.80 in 2009), but poor correlation with ALNE (V<0.1). In a patient subgroup at low risk for postoperative complications, aggregate performance was better (20.7% vs 52.4%, p<0.001), but correlation with ALNE was poor (V≤0.1). Patient age, sex, comorbidity, and cancer program status were independently associated with number of metrics achieved. **Conclusion:** Despite significant national improvement on each NQF metric individually, only one-third of hospitals currently perform well on all three metrics collectively. This suggests quality improvement efforts directed at diseases benefiting from multimodality treatment could be more robust by shifting focus from individual components of care in isolation to the entire multidisciplinary care pathway and/or transitions from one treatment modality to the next.

193 **CPRIT Grantee Poster Session B**

Multimodal Optical Spectroscopy for Skin Cancer Diagnosis *A. Moy, The University of Texas at Austin; X. Feng, The University of Texas at Austin; M. Markey, The University of Texas at Austin; J. Reichenberg, The University of Texas at Austin; J. Tunnell, The University of Texas at Austin*

Introduction: Skin cancers are classified as either melanoma or non-melanoma skin cancer (NMSC). According to the Centers for Disease Control and Prevention, skin cancer is the most common form of cancer in the United States and is a recognized public health issue. It is estimated that 1 in 5 Americans will develop some form of skin cancer in their lifetime. Diagnosis of a suspicious skin lesion as skin cancer involves visual observation and subsequent biopsy by a dermatologist, followed by histological analysis by a dermatopathologist. Biopsies, which involve excision of the lesion or a piece of the lesion, are invasive, at times unnecessary, and are costly procedures (roughly $2.8B annually in the US). An unmet critical need exists to develop a non-invasive and inexpensive screening method that can eliminate the need for unnecessary biopsies. **Methods:** To address this need, our group has previously reported on the continued development of a multimodal spectroscopy (MMS) system towards the goal of an "optical biopsy" of skin. Our approach combines the optical modalities of Raman spectroscopy, fluorescence spectroscopy, and diffuse reflectance spectroscopy to collect comprehensive optical property information from suspicious skin lesions. A previous clinical study from our group, which utilized two separate systems for Raman spectroscopy and diffuse reflectance and fluorescence spectroscopy, demonstrated the potential of our approach. Subsequent efforts to combine the three optical modalities into a single instrument, along with a custom designed optical fiber probe to deliver and collect light for each modality in a single probe, were successful. The present MMS system, however, is large and difficul to transport, and is composed of expensive, customized research grade components that may not be optimal for a clinical environment. **Results:** We outline here our present efforts to build and design an updated MMS system composed of original equipment manufacturer components that will enable the system to become smaller, less expensive, and more clinic-friendly. We also present preliminary characterization results of a next generation optical probe with a simplified optical design. **Conclusion:** After completing our instrument build and performing characterization studies, the next step is the initiation of an extensive clinical study of the MMS system to characterize its performance in identifying skin cancers. We plan to acquire MMS data from 250 skin lesions, including melanoma, NMSC, precancerous and benign lesions using our updated clinic-friendly system.

194 **CPRIT Grantee Poster Session A**

Renal Clearable Luminescent Metal Nanoparticles: Molecular Nanoprobes for Cancer Imaging *J. Zheng, The University of Texas at Dallas*

Introduction: While inorganic nanoparticles with size-dependent material properties open up unprecedented opportunities for novel biomedical technologies, translation of these nanoparticles into clinical practices has been hampered by the potential toxicity resulted from their long-term nonspecific accumulation in healthy tissues. Emergence of renal clearable inorganic nanoparticles makes it possible to address this long-term challenge. **Methods:** In the past few years, we used glutathione, a tri-amino-acid peptide to stabilize 2~3nm gold nanoparticles, which can give different colored luminescence upon their valence states of gold atoms [1]. These glutathione coated gold nanoparticles (GS-AuNPs) have little interactions with serum proteins; and more impressively, they can be cleared from the body through kidneys with an efficiency of 10~100 times better than the same sized AuNPs [2] and exhibit unique molecular-like pharmacokinetics [3]. **Results:** By further modifying the surface chemistry, we found that these NPs can be successfully tuned to avidly target cancer cell membrane under mild acidic conditions (6.5 –5.3) even in the presence of serum proteins [4]. More recently, we found that they can passively target the MCF-7 breast cancer through enhanced permeability and retention (EPR) effect [5], which can be further enhanced through PEGylation [6]. The obtained renal clearable PEGylated AuNPs can target tumor at an efficiency of ~10%ID/g while have minimum accumulation in the liver and other organs. **Conclusion:** This new class of renal clearable AuNPs with high targeting efficiency and low toxicity holds great promise to address challenges in translation of nanomedicines into cancer imaging and therapy [7,8].

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195 **CPRIT Grantee Poster Session B**

Monitoring the Effects of Hypofractionated SBRT on a Human Lung Cancer Xenograft Rat Model Using Multiparametric MRI *H. Zhou, The University of Texas Southwestern Medical Center at Dallas; R. Denney, The University of Texas Southwestern Medical Center at Dallas; Z. Zhang, The University of Texas Southwestern Medical Center at Dallas; D. Saha, The University of Texas Southwestern Medical Center at Dallas; R. Mason, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Tumor hypoxia is an important biomarker affecting tumor treatment response especially for radiation therapy. Hypofractionated stereotactic body radiation therapy (SBRT) has attracted great attention in recent years and some promising results have been reported. Hypofractionated SBRT requires fewer patient visits and customized treatment planning has become a possibility. In this study, we applied blood oxygen level dependent (BOLD) and tissue oxygen level dependent (TOLD) MRI together with dynamic contrast enhanced (DCE) MRI to explore the longitudinal effects of hypofractionated SBRT on tumor re-oxygenation and development using human lung cancer xenografts in a subcutaneous rat model. **Methods:** A549 human lung cancer cells were implanted subcutaneously in the thigh of ten nude rats. MRI was performed at 4.7 T. Interleaved BOLD (multi-echo gradient echo) and TOLD (gradient echo) MRI were acquired with the intervention of an oxygen challenge (from air to 100% O2). DCE was performed with IV injection of gadolinium contrast. T1 maps were also acquired for pharmacokinetic analysis of DCE prior to contrast injection. MRI was performed on the day prior to, and 24 hours after radiation. Seven tumors received 12 Gy with oxygen breathing 30 minutes pre and during the irradiation, and three tumors received no radiation and served as control. Data were processed using Matlab. Percentage signal intensity changes (%DSI) of BOLD and TOLD and T1 and T2* maps were calculated. Initial area under the curve (IAUC), time-to-maximum (TTM) and slope were calculated from DCE. The Yankeelov model was used for the quantitative analysis to obtain Ktrans and ve. **Results:** Intra-tumor heterogeneity was seen on multi-parametric maps, especially in BOLD, T2* and DCE, which may indicate the special growth pattern of A549 subcutaneous tumors. Most tumors at baseline showed a positive BOLD signal response (%DSI) and increased T2* indicating increased oxygenation in response to oxygen challenge. Similar observations were found on control tumors 24 hours later. However, the irradiated tumors showed a significantl decreased T2* (-2.5±1.8ms) after 24 hours, which may imply decreased oxygenation. Muscle values from both irradiated and control tumors were also obtained for reference. DCE also revealed the heterogeneity in the tumor. **Conclusion:** Multi-parametric MRI revealed longitudinal changes in the tumor microenvironment. The changes in BOLD and TOLD may allow optimized timing of hypo-fractionated SBRT. Further exploration with more animals and different time points will be valuable to better understand the effect of radiation therapy and help optimize the interval of the treatments.

196 **CPRIT Grantee**
Poster Session A

LFSpro: a risk assessment tool to estimate TP53 mutation status in families with Li-Fraumeni Syndrome *G. Peng, The University of Texas M.D. Anderson Cancer Center; J. Li, The University of Texas M.D. Anderson Cancer Center; W. Wang, The University of Texas M.D. Anderson Cancer Center*

Introduction: The diverse cancer spectrum and incidence in Li-Fraumeni syndrome (LFS) and limitations of the clinical criteria make it difficult to accurately identify candidates for TP53 mutation testing. A more efficien prediction tool for TP53 mutation carrier identification, when followed by appropriate management and screening, may ultimately decrease mortality. **Methods:** We built LFSpro based on a Mendelian model, which estimates TP53 mutation probability through the Elston-Stewart algorithm. With independent validation data from 765 families (19,530 individuals), we evaluated the prediction performance of LFSpro using receiver operating characteristic (ROC) curves and observed to expected ratio (O/E) for TP53 mutation. **Results:** LFSpro predicted TP53 mutation carriers in (a) high-risk pediatric sarcoma cohort from MD Anderson Cancer Center in the US, O/E = 0.96 (95%CI: 0.74,1.20); area under the ROC curve (AUC) = 0.87 (95%CI: 0.77,0.95); and (b) population-based adult onset sarcoma cohort from the International Sarcoma Kindred Study (ISKS) in Australia, O/E = 0.79 (95%CI: 0.51, 1.21); AUC = 0.67 (95%CI: 0.57, 0.81). LFSpro outperformed the Chompret and classic criteria in the high-risk cohort and was comparable to the Chompret criteria in the ISKS cohort. Sensitivity analysis on de novo mutation rates showed that in both cohorts, a rate of 0.0005 gave LFSpro the best prediction performance. **Conclusion:** Family history of cancer evolves, and LFSpro is sensitive to mutation carriers in families newly presenting in high-risk clinics and in families followed for years. It is more broadly applicable than the current clinical criteria and may improve clinical management for families with LFS.

197 **CPRIT Grantee**
Poster Session B

Coupling Isothermal Amplificatio and Strand Exchange Reactions For Robust and Portable Cancer Biomarker Detection *Y. Jiang, The University of Texas at Austin; S. Bhadra, The University of Texas at Austin; B. Li, The University of Texas at Austin; Y. Du, The University of Texas at Austin; A. Nourani, The University of Texas at Austin; A .Ellington, The University of Texas at Austin*

Introduction: In many cases the early detection of cancer is critical to treatment. However, the detection of nucleic-acid-biomarkers frequently relies on a complex infrastructure for both amplification and analysis. In order to pave the way for home-testing, we propose to develop a handheld-isothermal-amplification assay with an unanmbiguous endpoint for known cancer biomarkers. In particular, loop-mediated isothermal amplification (LAMP) can be used to detect small numbers of amplicons, but currently has issues with non-specific amplification and hence false positive results. We have now solved these problems using the tools of nucleic acid computation. The LAMP concatamers that result from 'true' amplicons can be used to directly trigger a toehold-mediated Oligonucleotide-Strand-Displacement reaction (OSD) in real-time. In essence, OSD probes for LAMP function similarly to TaqMan-probes for qPCR, and in collaboration with our commercial partner Paratus Diagnostics should now allow the development of home-testing methods. **Methods:** OSD (a hemiduplex with a toehold of 10 to 12 bases) designed to probe target-specific loops which exclusively exist on true amplificatio products. The long strand of the hemiduplex specifically binds to a target sequence within a LAMP amplicon, leading to strand displacement of a shorter strand, which can participate in other reactions. Transduction into a fluorescent signal is achieved by having the long strand contain a fluorophore and the short strand contain a quencher. This is helpful for real-time monitoring and reaction optimization. However, by simply taking the same probe and coupling the long strand to magnetic beads and the short strand to the invertase, it has proven possible to directly transduce molecular analytes into glucose concentrations that can be directly read on a personal glucometer. **Results:** We have initially chosen melanoma biomarkers to test the specificity and robustness of LAMP-OSD. Melanoma is the most dangerous form skin cancer, and an early diagnostic may provide consumers with better opportunities to make decisions about treating skin lesions. We have succeeded in detecting as few as 200 copies of several melanoma biomarkers (NRP2, BRAF, and HELLS) using an off-the-shelf glucometer. Results with fluorescenc probes showed that LAMP-OSD demonstrated single-nucleotide-specificity for Yes/No detection of a melanoma BRAF allele (V600E) in the presence of 20-fold excess of the wild-type gene. **Conclusion:** The LAMP-OSD system we have developed has achieved sensitivities and specificities that rival much more complicated lab tests, such as qPCR. By coupling signal transduction to an existing point of care device it may prove possible to develop an early-warning, home-care cancer diagnostic.

198 **CPRIT Grantee**
Poster Session A

PIN prevention for prostate cancer management *A. Kumar, The University of Texas Health Science Center at San Antonio; G. Li, The University of Texas Health Science Center at San Antonio; R. Bedolla, The University of Texas Health Science Center at San Antonio; P .Rivas, The University of Texas Health Science Center at San Antonio; D .Thapa, The University of Texas Health Science Center at San Antonio; R. Glickman, The University of Texas Health Science Center at San Antonio; R. Reddick, The University of Texas Health Science Center at San Antonio; R. Ghosh, The University of Texas Health Science Center at San Antonio*

Introduction: Adaptation of cancer precursor lesions to grow and survive in the tumor microenvironment (TME) under conditions of stress (nutrient or hormone or oxygen limited) could contribute to their progression to invasive cancer. Published studies from our laboratory showed that resveratrol (RES), a compound from red wine and grapes reduced incidence of high-grade prostate intraepithelial lesions (HGPIN), putative precursor lesions of prostate in a preclinical animal model. We found that this inhibition occurred in part through activation of NAD+-dependent histone deacetylase SIRT1-mediated inhibition of mTORC1. Given that mTORC1 is also inhibited by AMPK, it suggests a possible link between SIRT1 and AMPK in prostate pathogenesis. **Methods:** We have examined the link between SIRT1/AMPK and mTORC1 using prostate cancer cell lines and preclinical animal model. **Results:** We observed that AMPK is activated in human prostate tumors and in HGPIN lesions from prostate specific PTEN knockout mice. Silencing AMPK decreases cell proliferation, anchorage-independent growth and causes distinct morphological changes indicative of epithelial to mesenchymal transition (EMT) and changes in mRNA expression of cytokines and growth factors in a cell specific manner in androgen insensitive prostate cancer cells. Furthermore, our results also suggest that AMPK contributes to progression of HGPIN lesions by preventing anoikis. We also assessed the impact of duration of RES intervention on HGPIN lesion development by carrying out interventions at 7, 11 and 28 weeks. We failed to see any difference in HGPIN incidence at 7 weeks (Kruskal-Wallis test, p=0.84) or at 11 weeks (p=0.07); however, we observed a significant dose-dependent effect at 28 weeks (p=0.037). Immunohistochemical analysis of the prostate from these animals showed prominent staining for pAMPK in all areas with intermittent smaller non-stained areas in the prostate

from control group of animals. On the other hand prominent staining was observed in a portion of the ducts that is bordered by non-stained areas in treated mice. Cumulative analysis of these data showed statistically significant decreased expression of pAMPK in RES intervention group compared to age-matched untreated group of animals. **Conclusion:** Taken together our results show that activation of AMPK is associated with prostate tumorigenesis and contributes to resistance to pro-death pathways in androgen insensitive prostate cancer cells.

199 **CPRIT Grantee**
Poster Session B

Electrochemical Genetic Testing of Hereditary Cancers with Multiplexing E-sensors *B. Lim, The University of Texas at Austin; Y. Du, The University of Texas at Austin; J. Sessler, The University of Texas at Austin; A. Ellington, The University of Texas at Austin*

Introduction: In order to overcome hereditary cancers, it is important to find inherited mutations on genes from individual patients. Thus far, many genetic testing process have been developed, however, most of them use a typical fluorescent signal as a sensing output. In recent years, electrochemical sensing methods have been developed to circumvent the short falls of fluorescent methods. In this research, we have attempted to develop electrochemical E-sensors (E-sensors) which are able to detect a few specific genes in the samples with multiplexing electrochemical probes for simultaneous sensing. **Methods:** We chose a ferrocene (Fc) as an electrochemical probe because it is a highly stable molecule and relatively easy to perturb its potential signals by simple chemical modifications By the introduction of different functional groups on Fc, we synthesized six Fc derivatives (called Fc1 to Fc6, respectively) as a nucleoside phosphoradmidite form to use in solid-phase oligonucleotide synthesizer. Following this, individual DNA probes of each Fc were synthesized from the synthesizer and immobilized on a gold surface electrode. The signal output was obtained as an oxidation potential from the immobilized Fc-DNA. **Results:** Synthesized ferrocene nucleosides showed diverse oxidation potentials as expected. The introduction of electron withdrawing groups (EWG) increased the potential, while electron donating groups (EDG) resulted in a decreased potential. However, only two Fc-attached DNAs showed clear signals from the probes. Based upon this data, we propose that some of the ferrocene probes were damaged during the process of chemical DNA synthesis and purification and some of them were damaged under the process. Therefore, we are trying to optimize the synthetic condition and obtain a diverse array of ferrocene modifie DNA. **Conclusion:** We designed a multiplexing electrochemically active E-sensors based on a potential diversity of the ferrocene moiety. Even though the modified bases showed a clear multiplexing in nucoelside level, they are not ready to use yet. Further studies will include optimization of our synthesis and purification, and performing real gene sensing assays to detect various tumor-related genes at ths same time.

200 **CPRIT Grantee**
Poster Session A

A Novel Simulation Environment for Hyperpolarized Magnetic Resonance Imaging of Cancer Metabolism *C. Walker, The University of Texas M.D. Anderson Cancer Center; J. Bankson, The University of Texas M.D. Anderson Cancer Center*

Introduction: Hyperpolarized (HP) MRI techniques have allowed real time detection of [13C]pyruvate to [13C]lactate conversion in-vivo which has been shown to correlate well with cancer presence, stage, and response to therapy. HP signal is non-renewable, thus relaxation processes and depletion of longitudinal magnetization during detection cause a permanent loss of signal and requires careful optimization to maximize data quality. In this work, we describe a novel simulation environment that couples pharmacokinetic modeling of substrate delivery with enzyme kinetics and basic spin physics, enabling systematic study of sequences and acquisition strategies using numerical HP phantoms. We used this environment to determine the accuracy of measurements made using a basic pulse-acquire sequence with a range of repetition times (TR) and excitation angles (FA). **Methods:** Custom software was developed using Matlab to process the Bloch equations coupled with kinetic models for enzyme activity and substrate delivery. In the case of a perfused system, pyruvate delivery was modeled by approximating vascular delivery. For the closed (non-perfused) system, pyruvate was assumed to be present at its maximum concentration at the start of data acquisition. Simulations of closed and perfused systems were performed for a pulse-acquire sequence with a range of FAs and TRs. Signal from the resulting dynamic FIDs were fit to muti-compartment models using a least squares algorithm. The resulting exchange constant for pyruvate to lactate were compared to the exchange constant assumed in the numerical phantom to determine the accuracy of the detection and modeling strategy. **Results:** For the closed system, measurements were accurate across a wide range of TRs and FAs. However, when very low metabolic conversion was assumed the accuracy at larger FA degraded. The accuracy of measurements using the perfused system was much more sensitive to FA and TR, yielding a smaller region of sequence parameters with accurate measurements. **Conclusion:** Using a novel Bloch simulator we were able to explore the effect of sequence parameters on the measured exchange rats. While the pulse acquire studies simulated were simplistic, the results show that sequence parameters can significantly impact results. The platform described was built to accommodate a wide range of biologic models and advanced pulse sequences. It is critical to understand the effect of sequence parameters and modeling assumptions on measurements the use of HP agents enters clinical trial. This system will be ideally suited to elucidate those relationships and guide investigators toward efficient and accurate use HP-MRI.

201 **CPRIT Grantee Poster Session B**

Minimally Invasive Diagnostic Methods for Screening and Surveillance of Oral Cancer in Patients with Fanconi Anemia

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Introduction: Fanconi Anemia (**FA**) is an autosomal recessive disorder caused by mutations of DNA repair genes. The risk of oral cancer (**OC**) among FA patients is 800-times higher than in the general population, occurring at younger ages. Hematopoietic stem cell transplantation (**HSCT**), which greatly extends the life-expectancy in FA patients further increases the risk of OC in these patients. Patients with FA cannot tolerate chemo-and radiation therapy; hence, early detection of OC is critical to improve survival. **Objective**: To determine the efficacy of autofluorescence visualization (**AFV**) using VELscope (Visually Enhanced Lesion scope) and a microfluidic nanochip-based brush cytology test (**MFNC-BT**) for surveillance of OC in FA patients. M**ethods:** Patients attending the Meeting for Adults with FA, held in Baltimore, Maryland in March 2014 underwent a conventional oral examination followed by AFV. Transepithelial brush samples of mucosal lesions suspected of being oral potentially malignant disorders (OPMD) were obtained using Orcellex® brush (Rovers Medical Devices, Oss, The Netherlands) and transported in ThinPrep® CytoLyt® for MFNC-BT assay. Brush biopsy samples of OPMD in FA patients were compared with site-matched brush biopsy samples of healthy volunteers. **Results:** A total of 28 FA patients (Age range: 18-61 yrs., M=9, F=19) participated in this study, of whom 13 have had HSCT. The majority of these lesions (89%) revealed loss of fluorescence (LOF+ve) with AFV. Nuclear area of lesional cells in FA patients (mean= 275px^2) is significantly larger ($p < 0.0001$) than cells (163px^2) of healthy volunteers. The median absolute deviation of DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) fluorescence intensity, a measure of nuclear DNA content, was significantly higher in OPMD cells of FA patients than healthy control cells ($p < 0.001$). The frequency of nuclear aberrations such as micronuclei, bi- and poly-nucleated cells was significantly higher in FA patient cells than healthy controls and significantly higher in LOF+ve compared to LOV-negative OPMD. Two samples exhibited significantly higher WBC (white blood cell) counts, an indicator of inflammation; both were from LOF+ve OPMD found in HSCT-recipient FA patients. C**onclusion:** FA patients who are HSCT recipients have increased prevalence of LOF+ve OPMD which show higher incidence of nuclear aberrations and inflammation. These finding are in line with recent reports that HSCT in FA patients increases their risk for oral cancer. Therefore, use of MFNC-BT in combination with AFV may improve the efficacy of conventional oral examination for long-term surveillance of OC in this high-risk patient population, while minimizing unwarranted scalpel biopsies.

202 **CPRIT Grantee Poster Session A**

Combining Large Area Metabolic Optical Imaging with Multiphoton Microscopy for Detection of Epithelial Neoplasia

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Introduction: Multiphoton Autofluorescence Microscopy (MPAM) and Second Harmonic Generation Microscopy (SHGM) are a promising imaging combination for revealing microstructural and spectroscopic indicators of neoplasia through depth imaging. However as microscopy, methods they provide limited field of view, which is not practical for large area surveillance. Fluorescence-based widefield imaging approaches have been developed to follow metabolic changes of suspicious tissues based on the cancer hallmark of the Warburg effect in which there is an increased rate of glucose metabolism in tumor cells. However, large area surveillance imaging methods such as widefield fluorescence cannot provide subsurface detail and while they are sensitive to neoplasia, specificity is lacking such as in the presence of inflammation. The objective of this study was to investigate the pairing of the microscopic and widefield approaches as a potential combination for identifying high-risk precancers and early cancer using an animal model for oral squamous cell carcinoma. M**ethods:** A fluorescent glucose analog 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) was used as a contrast agent for sites of increased metabolism in wide-field imaging, guiding imaging by high resolution MPAM-SHGM to assess morphological and spectral features. A chemically induced Golden Syrian Hamster model of precancer and oral cancer was used for these studies. 2-NBDG was applied to highlight areas of high glucose uptake and the entire buccal pouch of each hamster was imaged in vivo in a widefield setup using 470 nm excitation, with collection of 550 nm emission. Follow-up MPAM-SHGM was conducted on regions of interests (ROIs) to assess whether microscopy would reveal microscopic features associated with neoplasia to confirm or exclude 2-NBDG based findings Imaged sites were biopsied and processed, with hematoxylin and eosin stained sections graded by a pathologist. ROC analysis was conducted to determine specificity and sensitivity for detection of neoplasia. **Results:** 2-NBDG fluorescence was shown to significantly increase with conditions of dysplasia and OSCC compared to normal- an increase in fluorescenc was also observed in inflammation. MPAM-SHGM however were able to further differentiate normal and inflamed sites from neoplastic regions based on cytologic and layer based abnormalities that were correlated with grade of the imaged sites based on ROC analysis. **Conclusion:** Multimodal imaging combining large area fluorescence guidance with subsurface microscopic evaluation of neoplastic oral mucosa by MPAM-SHGM may present an alternative approach for development of a method for detection of epithelial neoplasia.

203 **CPRIT Grantee Poster Session B**

Blood-Based Biomarkers for Screening and Early Detection of Colorectal Neoplasia *R. Bresalier, The University of Texas M.D. Anderson Cancer Center; J. Byrd, The University of Texas M.D. Anderson Cancer Center; D. Chia, UCLA School of Medicine; Z. Feng, The University of Texas M.D. Anderson Cancer Center; D. Brenner, University of Michigan; S. Hanash, The University of Texas M.D. Anderson Cancer Center*

Introduction: Colorectal cancer (CRC) screening reduces mortality, but is costly and highly dependent on compliance in programmatic screening. Uptake of colonoscopic screening is especially poor among underserved populations (in Texas, approximately 53% of Caucasians ages 50 and older have undergone colonoscopy versus 40% in Blacks and Hispanics). Patient-friendly approaches to improve patient uptake, adherence and compliance are needed to achieve national screening goals. **Methods:** We developed a translational discovery and verification plan to optimize the clinical utility of a blood-based biomarker screening panel including the anchor biomarkers galectin-3 ligand (GAL-3 ligand), an aberrantly glycosylated form of haptoglobin produced by colon cancer cells and MAPRE1, an APC-binding protein alone and in combination with other markers, to detect colorectal adenocarcinoma (all stages) and high-risk precursor lesions (CPRIT Grant ID DP150059). We developed and validated a sensitive and specific serum-based ELISA assay for galectin-3 ligand. The assay is linear to 500pg with calibration curves validated for within and between day precision and reproducibility of analytic samples with a coefficient of variability of 15%. This assay was tested in a high quality test set from a multi-center cohort (Early Detection Research Network EDRN) comprised of 300 patients with CRC, adenomas, and endoscopically normal colons. **Results:** Galectin-3 ligand significantl (P<0.001) differentiated cancer versus normal for all stages of malignancy tested, including early stage (stages I+II, Area Under the ROC curve (AUC) = 0.70) and late stage (stages II + IV, AUC=0.77) cancers. We also validated this marker using a bead assay suitable for multiplex studies using the same EDRN specimens used in the original analysis with a plate-based ELISA. These findings confirmed that GAL-3 ligand effectively differentiated cancer versus normal independent of stage, as well as advanced adenomas (adenomas ≥ 1cm or with high grade dysplasia) versus normal (Normal vs All Cancers AUC =0.80; Normal vs Stages III + IV cancer AUC =0.84; Normal vs Stages I + II cancer AUC 0.76; Normal vs Advanced Adenomas AUC= 0.78). MAPRE1 was also significantly elevated in the blood of EDRN patients with CRC and high-risk adenomas compared to normal controls (p<0.001 for both comparisons), and the combined comparison of CEA and MAPRE1 gave an AUC of 0.79 for early stage cancers and 0.73 for advanced adenomas compared to normals. **Conclusion:** Galectin-3 ligand and MAPRE1 show promise as clinically relevant anchor biomarkers for the development of a multi-marker panel for early detection of colorectal neoplasia.

204 **CPRIT Grantee Poster Session A**

Exploring Non-invasive OE-MRI Responses and Radiotherapy Treatment Planning in Orthotopic Prostate Tumors *D. White, The University of Texas Southwestern Medical Center at Dallas; T. Chiu, The University of Texas Southwestern Medical Center at Dallas; S. Stojadinovic, The University of Texas Southwestern Medical Center at Dallas; P. Peschke, German Cancer Center; R. Mason, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The goal of this research is to evaluate oxygen enhanced MRI as a non-invasive prognostic imaging biomarker for radiation response. Previous investigations regarding the dynamics of tumor oxygenation have examined subcutaneous rat prostate tumors (*Magn Reson Med* 2014; 71(5):1863-1873). Little is known about such responses in orthotopic prostate tumors. Given that OE-MRI (semi-quantitative blood oxygen level dependent (BOLD) and tissue oxygen level dependent (TOLD) responses, as well as quantitative apparent transverse relaxation rate (R_2*) and longitudinal relaxation rate (R_1) responses) can be acquired non-invasively, we have now examined these methods with respect to hyperoxic gas challenge before radiotherapy treatment planning. **Methods:** Dunning R3327-AT1 syngeneic rat prostate tumors were implanted orthotopically into Copenhagen rats (n = 3, 0.2 cm^3, 1.1 cm^3, and 4.7 cm^3). OE-MRI was performed at 4.7 T. After OE-MRI, the planned tumor volume (PTV) was contoured on T_2-weighted MR images fused with the XRAD 225Cx (Precision X-Ray, North Branford, CT) onboard cone beam CT images. A Monte Carlo based treatment plan (n = 3) composed of 9 coplanar arcs in 40° increments was calculated for prescribed dose of 30 Gy in 1 fraction utilizing SmART-Plan (MAASTRO Radiotherapy Clinic). The plans were exported to XRAD 225Cx small animal platform for treatment delivery (n = 2, 0.2 cm^3 and 1.1 cm^3, breathing oxygen). **Results:** Pretherapy OE-MRI responses were (0.2 cm^3 – BOLD %ΔSI = 9.1±2.5%, TOLD %ΔSI = 2.8±2.5%, ΔR_1 = 0.0558±0.151s^{-1}, ΔR_2* = 4.1±15s^{-1}; 1.1 cm^3 – BOLD %ΔSI = 1.8±2.6%, TOLD %ΔSI = 0.14±1.6%, ΔR_1 = 0.0731±0.214s^{-1}, ΔR_2* = -0.3±7.2s^{-1}; 4.7 cm^3 – BOLD %ΔSI = -0.75±3.0%, TOLD %ΔSI = -0.24±2.3%, ΔR_1 = 0.279±0.133s^{-1}, ΔR_2* = 11.4±11.7s^{-1}). Radiotherapy treatment planning alone for the largest tumor (4.7 cm^3) took approximately an hour but the tumor was not irradiated. The plan revealed inefficient prostate tumor coverage. With improved beam coverage on the smaller tumors, planning and irradiation were accomplished within an hour for each tumor. **Conclusion:** Tumor targeting and irradiation were more efficiently achieved after OE-MRI in smaller orthotopic tumors. OE-MRI responses have been reported to provide predictive ability for assessing response to radiotherapy in subcutaneous AT1 tumors, suggesting patient stratification for radiotherapy treatment planning. BOLD and TOLD responses were smaller for larger tumors consistent with progressive hypoxiation. Our results indicate that we can achieve OE-MRI responses in orthotopic prostate tumors for evaluating future investigations in stratifying tumors based on tumor oxygenation using tumor control probability (TCP).

205 **CPRIT Grantee**
Poster Session B

Prediction and Detection of Breast Cancer Brain Metastasis through Integrative Glycomics/Glycoproteomics and Genomics Analyses *Y. Mechref, Texas Tech University; W. Peng, Texas Tech University; R. Zhu, Texas Tech University; S. Zhou, Texas Tech University; L. Zacharias, Texas Tech University*

Introduction: Recently, breast cancer brain metastasis has been recognized as one of the central issues in breast cancer research. One of the significant events of brain metastasis is the penetration of cancer cells through the blood-brain barrier. Elucidation of the process and pathway that mediate this step is expected to provide important clues for the development of more effective therapeutic options to inhibit breast cancer brain metastasis. Recently, increasing evidence suggests that the oligosaccharides of glycoproteins and the aberrant glycosylation patterns contribute to the cell invasion and cancer metastasis. We combined transcriptomic, proteomic, and N-glycoproteomic analysis to investigate the molecular mechanism of breast cancer brain metastasis. **Methods:** Six human breast cancer cell lines were investigated in this study. Proteins extracted from different breast cancer cells were subjected to proteomic and N-glycoproteomic analysis using LC-MS/MS. Differentially expressed proteins, N-glycans and N-glycopeptides were compared. Illumina HiSeq 2500 was used for the transcriptome sequencing. Differential gene expression analysis was performed by QSeq software. Genes were mapped into biological pathways using Ingenuity Pathway Analysis software. **Results:** MDA-MB-231BR, which has 100% metastasis frequency to the brain and has no tendency to invade other organs, is the subline of MDA-MB-231. 7071 unique peptides corresponding to 1158 proteins were identified at 0.79% peptide FDR and 0.1% protein FDR. Each cell line group was compared with MDA-MB-231BR using the normalized spectral counts value. Based on a student t-test, 374 proteins showed significantly over- or under-expression in at least one comparison. Additionally, LC-ESI-MS/MS was employed to analyze the N-glycan patterns. More than 50 structures were identified and quantitatively compared. N-glycans identified from MDA-MB-231 had the lower overall intensity. MDA-MB-231BR exhibited higher high-mannose and sialylated structure intensities than MDA-MB-231. In MDA-MB-361, sialylated structures were dominant while high mannose and fucosylated structures were not. The overexpression of sialylated N-glycans in MDA-MB-231BR suggests the role of sialylated glycans in assisting cancer cells to penetrate the blood-brain barrier. Transcriptome analysis (RNA-Seq) highlighted the molecular mechanisms underlying the fundamental differences between cancer cell lines. **Conclusion:** Sialylated, fucosylated, and high mannose glycan sutures were significantly overexpressed in 231BR relative to the majority of the cell lines analyzed. These findings were in total agreement with the genomic data related to the glycosylation genes. Additionally, 30 proteins were determined to be expressed at different levels in 231BR relative to other cell lines. These proteins were involved in different molecular functions, including transporter, molecular transducer transcription regulator, and structural regulate activities.

206 **CPRIT Grantee**
Poster Session A

Molecular Imaging of Mucin Expressing Colon Tumors Using Targeted Hyperpolarized Silicon Nanoparticles *J. Liu, Rice University; J. Hu, The University of Texas M.D. Anderson Cancer Center; J. Davis, The University of Texas M.D. Anderson Cancer Center; N. Whiting, The University of Texas M.D. Anderson Cancer Center; N. Millward, The University of Texas M.D. Anderson Cancer Center; D. Menter, The University of Texas M.D. Anderson Cancer Center; P .Bhattacharya, The University of Texas M.D. Anderson Cancer Center; D. Carson, Rice University; P. Constantinou, Rice University*

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer deaths in the U.S., largely due to limitations in early detection. Conventional clinical diagnostic techniques either pose risks of gastrointestinal perforation, or do not yield a sufficiently sensitive image-based detection of small tumors in early development. There is a need for non-radiologic methods to diagnose molecular changes in the gut including polyps, diverticulosis, and cancer using stable isotopes coupled with high-resolution MRI. A new, direct *in vivo* imaging modality has been developed using hyperpolarized ^{29}Si nuclei in silicon nanoparticles (SiNP) using MRI. The ^{29}Si hyperpolarization increases MR detection sensitivity of ~4-5 orders of magnitude with a characteristic decay time of ~50 minutes, thereby allowing a long (>1 hour) time window for diagnostic imaging. In parallel, we developed nanoparticle-based approaches to detect transmembrane mucin glycoproteins (including MUC1) overexpressed by many cancers. MUC1 is found on the epithelial cell surface in the normal colon lumen but is significantly enriched during cancer progression, correlating with tumor proliferation, invasiveness, metastasis, and poor prognosis. Thus the use of MUC1 targeted hyperpolarized ^{29}SiNPs following enteric delivery in conjunction with MRI may be a novel method for early diagnosis of colorectal abnormalities and cancer. M**ethods:** Various sizes of ^{29}SiNPs were used to characterize and optimize decay time, and surface functionality. ^{29}SiNPs were functionalized with antibodies/aptamers to target MUC1 and in vitro binding assays were developed to determine targeting efficienc . A novel MUC1 expressing human colon tumor is being established for orthotopic implantation into nude mice for use in non-invasive *in vivo* imaging studies. A MUC1 expressing transgenic mouse model is being developed concurrently for additional proof-of-principle testing. **Results:** SiNPs of various sizes (5 - 2000 nm) were hyperpolarized. All particles retain high MR sensitivity and long longitudinal relaxation times independent of surface functionalization showing promise for real-time MRI *in vivo*. Antibody-functionalized silicon particles exhibit specific binding to CRC cell lines *in vitro* and retain their targeting affinity post-hyperpolarization. Orthotopically implanted tumor cells have successfully engrafted and formed tumors in the colonic lumen of immunocompromised mice. C**onclusion:** Current results hold significant promise for employing antibody-functionalized silicon technology to identify specific molecular targets on CRC cells. In addition, this imaging platform can be extended to other cancers where mucins are overexpressed, including some of the most common cancers (lung, ovarian and pancreatic).

207 **CPRIT Grantee Poster Session B**

Oxygenation-Sensitive MRI for Targeting Hypoxia in Breast Cancer *D. Yang, The University of Texas Southwestern Medical Center at Dallas; J. Campbell, The University of Texas Southwestern Medical Center at Dallas; H. Zhou, The University of Texas Southwestern Medical Center at Dallas; Z. Zhang, The University of Texas Southwestern Medical Center at Dallas; R. Mason, The University of Texas Southwestern Medical Center at Dallas*

Introduction: A novel therapeutic strategy has been proposed targeting the hypoxic areas in breast tumors using bioreductively activatable prodrug conjugates. A prerequisite for developing this therapy is to characterize and identify rodent tumor models that exhibit well define levels of hypoxia. Herein, we apply three non-invasive MRI methods, namely, blood oxygen level dependent contrast (BOLD), tissue oxygen level dependent contrast (TOLD), and quantitative MR Oximetry (MOXI) to evaluate the hypoxic properties of the 4T1 breast cancer model in mice. **Methods:** Mouse 4T1 tumors were implanted into the frontal mammary fat pad in BALB/C mice. MRI BOLD, TOLD, and MOXI measurements were performed on anesthetized mice (2% isoflurane) at 4.7 T during oxygen gas breathing challenge. Responses in BOLD and TOLD signal intensity (SI), BOLD-derived R_2*, and MOXI-derived oxygen partial pressure (pO_2) were analyzed in regions representing tumor and upper back muscle, respectively. R**esults:** Under normal baseline air-breathing conditions, the temporal fluctuation in BOLD and TOLD signals during a 15-min time window was within 1.3% for both tumor and muscle. Baseline T_2*, T_2, and T_1 in tumor were significantly longer than those in muscle, respectively (differences between tumor and muscle: ΔT_2* = 2.3 ± 0.2 ms; ΔT_2 = 13 ± 1 ms; ΔT_1 = 20 ± 12 ms; $p < 0.01$ in all cases). Upon switching the inhaled gas to 100% O_2, in tumor, both BOLD and TOLD signals increased rapidly (within 5 min) and significantly (ΔSI_{BOLD} = 9.2 ± 1.4%; ΔSI_{TOLD} = 5.8 ± 2.0%; ΔT_2* = 0.55 ± 0.12 ms; $p < 0.01$ in all cases). In muscle, changes in BOLD and TOLD responses were not significant (ΔSI_{BOLD} = 3.0 ± 2.0%, p = 0.05; ΔSI_{TOLD} = 2.4 ± 1.2%, p = 0.01; ΔT_2* = 0.23 ± 0.18 ms, p = 0.08). The component data required for quantitative MOXI were obtained and analysis remains in progress. C**onclusion:** These results show the potential of combined BOLD, TOLD, and MOXI measurements for probing the oxygenation status in the 4T1 breast tumor model and detecting response to breathing gas challenges. They represent the first non-invasive assessment of hypoxia in 4T1 tumors and first time MOXI has been applied to mice. These results lay a foundation for the collaborative investigations with Drs. Pinney and Trawick (Baylor). Future studies will focus on further understanding, validation, and application of these methods in various breast cancer animal models.

208 **CPRIT Grantee Poster Session A**

Programmable Bio-Nano-Chip Platform (p-BNC): A multiplexed platform for early detection ovarian cancer biomarkers at the point-of-care *G. Simmons, Rice University; A. Simmons, The University of Texas M.D. Anderson Cancer Center; B. Shadfan, Rice University; K .Lu, The University of Texas M.D. Anderson Cancer Center; R. Bast, The University of Texas M.D. Anderson Cancer Center; J. McDevitt, Rice University*

Introduction: Point-of-care platforms have the potential to serve as cost-effective solutions for large scale screening for ovarian cancer. Ovarian cancer is a deadly gynecological malignancy, where 21,290 women will be diagnosed with this disease in 2015 and 14,180 women will die from this disease (U.S. Statistics, ACS). While no single biomarker is shed by all ovarian cancers, a panel of biomarkers comprising CA125, HE4, MMP-7 and CA 72-4 have shown strong promise for early detection of ovarian cancer. In this work, we have configured the programmable bio-nano-chip platform for multiplexed measurements of this biomarker panel, with the ultimate goal for a point-of-care diagnostic tool for ovarian cancer detection. **Methods:** The programmable bio-nano-chip (p-BNC) is a modular and multiplexable microfluidic, platform. The p-BNC serves as a miniaturized bead-based immunoassay system with a credit-card sized footprint. The platform integrates multiple lab-on-a-chip processes including automated sample metering, bubble and debris removal, reagent storage and waste disposal, thus permitting point-of-care analysis. In this work, we adapted the p-BNC for multiplexed measurements of four ovarian cancer biomarkers (CA125, HE4, MMP-7 and CA72-4). We assessed limits-of-detection, specificit , cross-reactivity, within- and between day-precision for the immunoassays on the p-BNC platform. We tested a 31 patient cohort encompassing early and late stage ovarian cancers along with benign and healthy controls. We also assessed longitudinal cases (n=2) and controls (n=4) from pre-diagnostic patients destined to develop ovarian cancer. **Results:** The multiplexed panel on the p-BNC demonstrated high specificit , low-cross reactivity, low limits of detection, while maintaining a short analysis time of 43 minutes suitable for point-of-care analysis. Day-to-day variability, critical for monitoring longitudinal biomarkers ranged between 5.4% to 10.5%, well below the biological variation of the four biomarkers. The concentrations correlated well (R2 = 0.71-0.93) for various biomarkers with established Luminex methods. The panel measured on the p-BNC achieved 68.7% sensitivity at 80% specificity for distinguishing ovarian cancer cases from controls. Additionally, longitudinal profiles of pre-diagnostic plasma were distinguishable between cases and controls. **Conclusion:** Taken together, the p-BNC shows strong promise as a diagnostic tool for large-scale screening by leveraging faster results and lower costs afforded by the p-BNC platform and the improvements in sensitivity from the multiplexed ovarian cancer panel.

209 **CPRIT Grantee**
Poster Session B

New [18F]-NHC-BF3 adducts as water stable radio-prosthetic groups for PET imaging *F. Gabbai, Texas A&M University; K .Chansaenpak, Texas A&M University; B. Vabre, Texas A&M University; Z. Li, University of North Carolina*

Introduction: Positron emission tomography (PET) is a fast growing cancer diagnostic technique that relies on the detection of radiolabelled compounds containing a positron emitting radionuclide. Owing to its nuclear properties and straightforward preparation by proton bombardment of [^{18}O]-water, fluorine-18 (^{18}F) has become the most widely used radionuclide in this area of science. Traditional approaches to 18F radiolabelling have often relied on the introduction of the isotope via formation of a covalent C—^{18}F bond. Typical examples of such agents include [^{18}F]-FDG (Fluorodeoxyglucose) which is a commonly used PET imaging agent. Because the half-life of ^{18}F is short ($t_{1/2}$ = 110 min), such agents must be synthesized just prior to injection in the patient. The syntheses of these compounds have been optimized but their implementation remains cumbersome, often requiring multiple steps and fairly sophisticated automations. **Methods:** To circumvent some of these limitations and make ^{18}F PET imaging a more widely accessible technique, we have become interested in harnessing the inherent high fluorophilicity of main group elements as a way to introduce ^{18}F into molecules of biological interest. In this presentation, we will show that the radiofluorination of N-heterocyclic carbene (NHC) boron trifluorid adducts affords novel [^{18}F]–positron emission tomography probes which resist hydrolytic fluoride release. **Results:** The labelling protocol relies on an ^{18}F–^{19}F isotopic exchange reaction promoted by the Lewis acid $SnCl_4$. Modification of the NHC backbone with a maleimide functionality provides access to a model peptide conjugate which shows no evidence of defluorination when imaged in vivo. This presentation will also include results obtained using group 15 elements as [^{18}F]–fluoride complexing agents. **Conclusion:**

210 **CPRIT Grantee**
Poster Session A

Multidimensional and High Throughput Microfluidic Phenotyping of Cancer Cells *S. Ahmmed, Texas Tech University; S. Vanapalli, Texas Tech University*

Introduction: From the primary site, circulating tumor cells are transported through blood vessels to produce secondary colony targets. Cell deformability plays an important role in regulating the squeezing and arrest of tumor cells in microcirculation. Likewise the adhesive interaction of tumor cells with microcapillary walls regulates their attachment and subsequent intravasation. Therefore phenotyping tumor cells with respect to cell deformability and adhesion properties might provide new insights into characterizing the heterogeneity in tumor cell population and their ability to metastasize. In this study, we introduce a new high throughput microfluidic device that uses flow-induced deformation of tumor cells in narrow microchannels to phenotype their deformability and adhesive properties. **Methods:** We engineer a new microfluidic device that contains a dozen constricted microchannels of diameter ranging from 10 – 30 mm. The diameters were chosen such that breast cancer cells (MCF-7 and MB231) are confined over a wide range, similar to in vivo. In addition, a microfluidic manifold was designed to induce pressure-driven flow across all the dozen channels enabling us to increase the throughput (100 cells/sec). Using high speed video-imaging and image processing, we measure four different parameters for each cell – entry time, passage time, deformation index and cell size. We evaluate how the multidimensional readouts vary with cytoskeletal drug interventions and antibody-based wall coatings. **Results:** We find that the very narrow channels (< 15 mm) allow us to quantify the entry time and passage time (or velocity) of tumor cells, which have been reported to be metrics of cell deformability and adhesive respectively. In the wider channels, we identify conditions where tumor cells undergo shear stress induced shape deformation. Thus, for the same cell population we correlate the entry time, passage time and shape deformation. We compare this multidimensional readouts for MCF-7 and MB231 cells and find differences. We also coat the channel walls with anti-EpCAM antibodies to identify differences in EpCAM expression of the two breast cancer cell lines. **Conclusion:** We engineered a novel microfluidic device that enables high throughput and multidimensional phenotyping of tumor cells. We anticipate this device to be useful in both cancer biology and fingerprinting cancer patient samples

211 **Poster Session B**

Potential Oral Cancer Salivary mRNA Biomarkers in Periodontitis Patients *Y. Cheng, Baylor College of Dentistry-TAMU Health Science Center; L. Jordan, Baylor College of Dentistry-TAMU Health Science Center; H. Chen, University of Toledo; D. Kang, Baylor College of Dentistry-TAMU Health Science Center; L. Oxford, ENT private practice; J. Plemons, Baylor College of Dentistry-TAMU Health Science Center; T. Rees, Baylor College of Dentistry-TAMU Health Science Center*

Introduction: Over 100 salivary constituents have been suggested as potential salivary biomarkers for oral squamous cell carcinoma (OSCC) detection, because they showed levels significantly different in OSCC patients compared to levels found in healthy controls. However, whether the levels of these potential biomarkers might also be affected by the presence of chronic periodontitis (CP), the most common oral inflammator disease, was not known. If inflammatory disease does affect levels of a potential biomarker to a degree that there is no significant difference from the levels found in OSCC patients, then use of that biomarker for clinical detection of OSCC would result in significant false positive rates—thereby greatly reducing its value as a non-invasive diagnostic adjunct. The purpose of this study was therefore to measure the levels of 7 previously-reported potential OSCC salivary mRNA biomarkers in patients with CP and compare them to levels found in OSCC patients and Healthy Controls. The 7 salivary mRNAs were: IL-8, IL-1β, dual specificity phosphatase 1 (DUSP1), H3 histone family 3A (H3F3A), ornithin decarboxylase antizyme 1 (OAZ1), S100 calcium-binding protein P (S100P), and spermidine/spermine N1-acetyltransferase 1 (SAT1). M**ethods:** Saliva samples were collected from a total of 105 human subjects from the following 4 study groups: OSCC; CPNS (periodontitis, moderate to severe degree, non-smokers); CPS (periodontitis, moderate to severe degree, smokers); and Healthy Controls. Levels of each mRNA in patient groups (OSCC or CP) relative to the Healthy Controls was determined by a pre-amplification RT-qPCR approach with nested gene-specific primers. Results were recorded and analyzed by Bio-Rad CFX96 Real-Time System. Mean fold changes between each pair of patient vs. control group were analyzed by the Mann-Whitney U test. **Results:** Only S100P showed significantly higher levels in OSCC patients compared to both CPNS patients (p= 0.003) and CPS patients (p=0.007), and to the Healthy Controls (p= 0.009). There was no significant difference in the levels of salivary IL-8, IL-1β, and DUSP1 mRNAs between the OSCC patients and the CPNS patients (p=0.510, 0.058, and 0.078, respectively); no significant difference in levels of salivary OAZ1 and SAT mRNAs between the OSCC patients and the CPS patients (p=0.318 and 0.764, respectively); and no significan difference in levels of the H3F3A mRNA between the OSCC patients and the Healthy Controls. C**onclusion:** Salivary S100P mRNA could be a reliable biomarker for OSCC detection, regardless of the presence of CP. CP would be a confounding factor for using the other 6 mRNAs.

212 **Poster Session A**

DermoScreen: A Smartphone Application for Screening Melanoma *K. Lancaster, University of Houston; T. Wadhawan, University of Houston; G. Zouridakis, University of Houston*

Introduction: In this study, we present DermoScreen, an enhanced application of our previously developed automated system for classificatio of melanocytic lesion as malignant or benign. The new system features a two-stage image analysis and a novel lesion asymmetry detection algorithm that improve overall classification performance. **Methods:** The system relies on the 7-point checklist and ABCD dermoscopy rules for determining the likelihood of a skin lesion being malignant. An image is converted to grayscale, preprocessed to remove noise, and then segmented to isolate the lesion from the surrounding healthy skin. Texture and structural features required for the dermoscopy rules are extracted using 3-level Haar wavelets and color histograms. These features are used to train support vector machines to assess the presence or absence of each feature. Lesion asymmetry is assessed using a new algorithm based on size functions (SF). The lesion is split into halves along its major and minor axes through its center of mass. Three SFs are calculated for each half, using measuring functions that focus the SF on the border, color variation, and mass distribution of the lesion. The Hausdorff distance between the SFs is used to determine lesion asymmetry on both axes. The extracted dermoscopic features are then used to calculate the 7-point score for the lesion. If the resulting score exceeds a threshold for malignancy, the lesion is classified as melanoma with a probability proportional to the score. Otherwise, the lesion is further assessed using the ABCD rule, and that assessment is used for final lesion classification. The entire system, from image capture to malignancy assessment, has been implemented on an iOS app and is deployed on the iPhone 6. **Results:** The system was evaluated using 15 melanomas and 54 benign lesions from a commercial library annotated by dermatologists. The 7-point checklist in the first stage classifier showed sensitivity of 87% and specificity of 78%, with 12 benign lesions incorrectly classified as melanoma and two melanomas classified as benign. The ABCD rule applied in the second stage correctly identified the two melanomas while incorrectly classifying one of the benign images, resulting in an overall system sensitivity and specificity of 100% and 76%, respectively. **Conclusion:** The proposed smartphone-based system can assist primary care physicians with lesion assessment during routine office visits to reduce unnecessary biopsies. It can also have a significan impact in underserved domestic areas and in developing countries, where specialized health-care infrastructure is limited.

213 **Poster Session B**

Detection of melanoma-associated DNA biomarkers using pregnancy test strips *Y. Du, The University of Texas at Austin; A. Pothukuchy, The University of Texas at Austin; J. Gollihar, The University of Texas at Austin; A. Ellington, The University of Texas at Austin*

Introduction: Modern personalized medicine relies on the identificatio of molecular markers that can identify, stage, and provide prognostic indicators for tumors, in order to ensure the timely selection and administration of appropriate therapies. Most melanomas follow a set progression profile, yet cases have steadily risen by 3-7% per year while mortality rates have increased by ~2%,1-3 indicating that this might be a particularly good area for public health and diagnostics innovations. This hypothesis is further bolstered by the fact that several genetic components that correlate with melanoma have been identified. **Methods:** In clinical practice, mutations that dispose an individual to cancer or that are indicative of cancer are frequently identified by molecular diagnostic assays, such as PCR. However, in the main these assays are impractical for point-of-care (POC) applications and for resource poor settings. In contrast, isothermal nucleic acid amplification technologies (IsoNAT) are a low-cost alternative that can be directly translated to POC diagnostics. **Results:** In particular, we have used Loop-Mediated Isothermal Amplificatio (LAMP) coupled with oligonucleotide strand displacement (OSD) probes to identify as 20 copies of the melanoma-associated nucleic acid biomarkers NRP2, HELLS and BRAF V600E.4 Building on this strategy, we are now attempting to convert LAMP-OSD outputs to a pregnancy test kit readout, thereby combining molecular diagnostics with a reliable off-the-shelf device. Our integrated approach relies on: i) isothermal amplification of melanoma RNA or genomic DNA; ii) sequence-specifi strand displacement of human chorionic gonadotrophin (hCG), which is normally found in the urine of pregnant women; and finally (iii) subsequent detection by commercially available and widely used pregnancy test strips. **Conclusion:** Based on our preliminary data, we anticipate a limit of detection of as few as 200 mRNAs, a number well within early, clinically relevant ranges for melanoma.

214 **Poster Session A**

Outreach Invitations for FIT and Colonoscopy Improve Colorectal Cancer Screening Rates: Results of a RCT in a Safety-net Health System *A. Singal, The University of Texas Southwestern Medical Center at Dallas; S. Gupta, Moores Cancer Center University of California San Diego; K. McCallister, The University of Texas Southwestern Medical Center at Dallas; J. Sanders, The University of Texas Southwestern Medical Center at Dallas; J. Tiro, The University of Texas Southwestern Medical Center at Dallas; W. Bishop, The University of Texas Southwestern Medical Center at Dallas; A. Loewen, The University of Texas Southwestern Medical Center at Dallas; C. Skinner, The University of Texas Southwestern Medical Center at Dallas; E. Halm, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Colorectal cancer (CRC) screening can reduce CRC incidence and mortality, but its effectiveness is limited by underuse, particularly among underserved populations. The optimal screening test and approach to increase CRC screening in safety-net health systems has yet to be defined. The aim of our study was to compare the effectiveness of FIT outreach and colonoscopy outreach to increase screening participation rates compared to usual visit-based care in a racially diverse and socioeconomically disadvantaged cohort of patients. **Methods:** Patients, aged 50-64 years who were not up-to-date with CRC screening, but used primary care services in community-based clinics of a large urban safety-net health system in the United States were randomized to mailed FIT outreach (1-sample FIT kit) (n=1200), mailed colonoscopy outreach (n=1200), or usual care with opportunistic visit-based screening (n=599). Patients who did not respond to outreach invitations within 2 weeks received a follow-up telephone reminder. Primary outcome was completion of FIT or colonoscopy within 12 months after randomization. We also examined time-to-screening completion in each outreach arm. We used an intent-to-screen principle for analyses. **Results:** Baseline patient characteristics across groups were similar; mean age was 55.9 years and 62% were women. The cohort was racially/ethnically diverse (48% Hispanic, 24% Black, and 23% White). Screening participation rates were significantl higher for FIT outreach (56.2%) and colonoscopy outreach (42.5%) than usual care (27.9%) ($p< 0.001$ for both). Screening participation rates with FIT outreach were significantly higher than colonoscopy outreach ($p< 0.001$). Most patients in both the FIT and colonoscopy outreach arms who completed screening did so as a direct result of outreach efforts (78.8% and 52.9% respectively). Visit-based screening accounted for 143 (21.2%) patients in the FIT outreach arm, with 119 undergoing FIT and 24 undergoing colonoscopy; however, visit-based screening accounted for nearly half (47.1%) of screening completion among those in the colonoscopy outreach arm, with 201 undergoing FIT and 39 undergoing colonoscopy. Among responders, FIT outreach had a higher proportion of "early responders" prior to telephone reminders (72.2% vs. 38.1%, $p< 0.001$), and shorter mean time to outreach response (50.1 ± 102.0 vs. 74.9 ± 122.7 days, $p< 0.001$) compared to colonoscopy outreach. **Conclusion:** Mailed outreach invitations can significantly increase CRC screening rates among underserved populations. Outreach with FIT was more effective than colonoscopy-based outreach to increase one-time screening participation. Further studies with longer follow-up are needed to compare effectiveness of FIT and colonoscopy outreach for promoting completion of the entire screening process.

215 **Poster Session B**

DNA Methylation and Hydroxymethylation Biomarker Discovery for Multiple Myeloma *L. Bennett, Baylor Research Institute*

Introduction: An overriding goal for the development of molecular biomarker panels is to empower physicians to stratify patients according to the characteristics of their disease, predict response to therapy and devise therapeutic strategies accordingly. DNA methylation is an ideal biomarker, as DNA is a stable molecule that can be isolated from a number of sources. To date, investigations of DNA methylation in multiple myeloma (MM) have used mid-resolution genome-wide technologies such as microarrays, giving an incomplete view of global methylation. Moreover, the extent of methylation intermediate modifications such as 5'-hydroxymethylation in the MM genome is unknown. Our goal is to interrogate the methylome and hydroxymethylome in MM cells at single base-pair resolution using Next-Generation Sequencing to discover biomarkers that associate with disease. Because myeloma patient samples are limited, we first optimized our protocols for small quantities of input DNA and DNA from formalin-fixed paraffin-embedde specimens. **Methods:** We used KMS and H929 MM cell lines and B-lymphoblastoid cell lines GM01056 and GM06990. We performed Reduced Representation Bisulfite Sequencing (RRBS) using the Ovation Ultralow Methyl-Seq Library System or the Ovation RRBS Methyl-Seq System (NuGEN, CA). To detect 5'-hydroxymethylcytosines and 5'-methylcytosines, we performed Reduced Representation Oxidative Bisulfite Sequencing (RRoxBS) and RRBS using the TrueMethyl-Seq Kit (cegx, UK). Sequencing was performed on the Illumina HiSeq 2500 system. For alignment to the Human Genome hg19 and to extract cytosine methylation information we used Bismark. Concordance of percentage cytosine methylation between samples was estimated using Pearson Correlation Coefficients. Differential methylation analyses were performed using MethylKit. **Results:** We comprehensively evaluated our RRBS protocol using 300ng and 30ng input DNA from freshly harvested cells and from cells that were formalin-fixed and paraffin-embedded to mimic archived pathological specimens. For all four cell lines, we observed ≥92% correlations between high quality DNA from freshly isolated cells and low quality DNA from FFPE cells even for the very low 30ng input. The distribution of differentially methylated CpGs with regard to their genic location plus the types of target genes was similar to that observed in other malignancies however further scrutiny is likely to reveal disease-specific modifications suitable as candidate biomarkers. **Conclusion:** We successfully performed RRBS from as little as 30ng of input DNA from FFPE and fresh cells. Our methods are therefore suitable to utilize archived specimens. We were also able to detect 5'-hydroxymethylated cytosines in myeloma and non-malignant B-cells.

216 **Poster Session A**

Ultrasound Imaging of Tumor Angiogenic Biomarkers *K. Hoyt, The University of Texas at Dallas*

Introduction: Monitoring response to treatment is a major challenge in the management of breast cancer. In the neoadjuvant setting, assessing tumor response to treatment prior to surgery including evaluation for pathologic response can provide prognostic information to help guide follow up care. The goals of personalized medicine are becoming increasingly important and include optimizing effective therapy for an individual patient, reducing drug associated morbidity, and reducing health care costs associated with overtreatment. **Methods:** Given a DCE-US image series, an ROI was manually segmented in Matlab after intensity thresholding and spatial filtering (noise suppression). A mean time-intensity curve (TIC) from this ROI was analyzed to compute global parametric perfusion measures (e.g., arrival time, TIN; time-to-peak enhancement, TPK; wash-in rate, SIN; wash-out rate, SOUT; peak enhancement, IPK; area under the curve, AUC). A binary maximum intensity image (MIP) was obtained and a series of morphologic processing operators were applied to remove shape imperfections of the tumor vessels. This binary image was skeletonized and analyzed locally to extract neovascular morphology features (e.g., vessel-to-tumor ratio, VR; number of bifurcation, NB; number of vessels, NV; vessel length, VL; vessel tortuosity, VT). All vascular trees were color-coded with spatial parametric perfusion estimates after analysis of local TICs and overlaid on US images to describe local tumor features. Preliminary tests were performed on data previously acquired from 6 breast cancer patients that received neoadjuvant treatment. Patients were scanned before and after contrast agent dosing (Definity) using a Philips iU22 US system and L9-3 transducer before therapy initiation and at 6, 12, 18, and 24 wk (using a nonlinear imaging mode). **Results:** Neoadjuvant therapy produced a change in tumor blood volume (AUC and IPK; 16.0% and 13.7%) and flow velocity (TPK, WIR and WOR; 70.5%, 39.5%, and 38.1%) before reductions in tumor size were observed. Also associated with tumor response at 24 wk, the number of vessels (25.8%) and branch points (46.1%) and vessel tortuosity (21.4%) decreased compared to baseline whereas vessel length increased (5.6%). Immunohistology revealed that all patients had a partial response to treatment. **Conclusion:** Preliminary results for measuring tumor perfusion and neovascular morphology features with ultrasound were promising and more research is warranted.

217 **CPRIT Grantee**

Spatial Characteristics of Large Intestine Mucosal Microbiota in Healthy Individuals *L. Jiao, Baylor College of Medicine; N. Ajami, Baylor College of Medicine; D. Hutchinson, Baylor College of Medicine; D. Graham, Baylor College of Medicine; Y. Shaib, Baylor College of Medicine; J. Uriostegui, Baylor College of Medicine; A. Smith, Baylor College of Medicine; L. Chen, Baylor College of Medicine; K. Royse, Baylor College of Medicine; D. White, Baylor College of Medicine; M . Wong, Baylor College of Medicine; R. Cole, Baylor College of Medicine; C. Hair, Baylor College of Medicine; J. Hou, Baylor College of Medicine; N. Husain, Baylor College of Medicine; M. Jarbrink-Sehgal, Baylor College of Medicine; F. Kanwal, Baylor College of Medicine; M. Velez, Baylor College of Medicine; H. El-Serag, Baylor College of Medicine; J. Petrosino, Baylor College of Medicine*

Introduction: Gut microbiota plays an important role in health and disease. However, our understanding of longitudinal spatial diversity of mucosal microbiota in the large intestine in healthy individuals is limited. **Methods:** We enrolled 23 individuals (age: 51-69 years old) who underwent colonoscopy at the Michael E. DeBakey VA Medical Center in Houston between July 2013 and April 2015. All individuals had adequate bowel preparation and were found to have grossly normal appearing colon mucosa during the procedure. We obtained a total of 132 snap frozen colonic mucosa biopsies from cecum, ascending, transverse, descending, and sigmoid colon, and rectum from each individual. We extracted microbial DNA from colonic mucosa and amplified and sequenced 16S rRNA V4 region using the Illumina MiSeq platform. Sequencing data were analyzed using UPARSE and SILVA database for operational taxonomic unit (OTU) classification. All samples were normalized to 2,708 reads. We calculated alpha-diversity indices (observed OTU and Shannon index) and beta-diversity indices (Weighted UniFrac principal coordinates analysis (PCoA) across the segments of the large intestine.. **Results:** Among 23 individuals, there was no difference in observed number of OTU (P = 0.48) nor Shannon index (P= 0.26) across different segments. Bacteroidetes (44.4%), Firmicutes (36.1%), and Proteobacteria (10.6%) accounted for > 90% of mucosal microbiota in all segments, followed by Verrucomicrobia, Fusobacterial, Actinobacteria, and other phyla. There were no significan differences in richness or evenness across the large intestine segments at the top five phyla or top 15 genera levels (P > 0.5). The PCoA indicated that there were no differences in composition or abundance of mucosal microbiota across the large intestine segments. However, each individual

had unique mucosal microbial community composition and structure as showed by PCoA plot (P < 0.001). C**onclusion:** This comprehensive evaluation of large intestine mucosal microbiota of 23 individual with normal colon showed that: 1) there was no significant variability in mucosal microbiota at OTU level across different segments of large intestine at population level; and 2) homogeneous mucosal microbiota was seen along the large intestine after bowel preparation. In consistent with previous findings, each individual has distinct mucosal bacterial characteristics. Intra-individual variabilty of gut microbiota along the large intestine will be examined.

218 **CPRIT Grantee**

Use of Fc-Engineered Antibodies as Clearing Agents to Increase Contrast During PET *R. Swiercz, Texas A&M University System Health Science Center; S. Chiguru, The University of Texas Southwestern Medical Center at Dallas; A. Tahmasbi, Texas A&M University; S .Ramezani, The University of Texas Southwestern Medical Center at Dallas; G. Hao, The University of Texas Southwestern Medical Center at Dallas; D. Challa, Texas A&M University System Health Science Center; M. Lewis, The University of Texas Southwestern Medical Center at Dallas; P. Kulkarni, The University of Texas Southwestern Medical Center at Dallas; X. Sun, The University of Texas Southwestern Medical Center at Dallas; R. Ober, Texas A&M University; R. Mason, The University of Texas Southwestern Medical Center at Dallas; E. Ward, Texas A&M University System Health Science Center*

Introduction: Antibodies have considerable promise as diagnostic imaging agents due to their high affinity and specificit . However, their long in vivo half-lives result in high background levels, poor contrast, and radiation exposure of normal tissue. These problems are exacerbated when the tumor burden is low. These undesirable characteristics also limit the use of radioconjugated antibodies in therapy. It is well established that the Fc receptor, FcRn, regulates the levels of antibodies of the IgG class in the body. We have described a class of engineered antibodies that bind with increased affinity through their Fc region to FcRn in the pH range of 6.0–7.4. These antibodies compete with endogenous, wild-type IgGs for binding to FcRn and, as such, increase their degradation. Antibodies of this class have been called Abdegs, for antibodies that enhance IgG degradation. We have previously demonstrated that Abdegs can be used to clear autoreactive antibodies and ameliorate disease in mouse models of autoimmunity. In the present study we tested the ability of these clearing agents to reduce background and improve contrast during PET. **Methods:** Mice bearing human epidermal growth factor receptor type 2 (HER2)-overexpressing tumors were injected with radiolabeled (125I, 124I) HER2-specific antibody (pertuzumab). Pertuzumab injection was followed 8 h later by the intravenous delivery of an Abdeg, or as controls, wild type IgG1 or PBS. Biodistribution analysis was performed at 24 and 48 h following 125I-radiolabeled pertuzumab injection (24 and 40 hours post-Abdeg delivery). PET/CT imaging was performed at 4, 24 and 48 h post 124I-radiolabeled pertuzumab delivery. **Results:** Pharmacokinetic and biodistribution analyses at 24 and 48 h following delivery of radiolabeled pertuzumab demonstrated that Abdeg delivery results in significantly lower levels of radiolabel in the whole body and major organs of tumor-bearing mice. In addition, tumor-to-blood ratios are approximately 3-fold higher in Abdeg-treated mice compared with control groups. Importantly, Abdeg treatment results in approximately 4-fold-higher contrast for the radiolabeled antibody during PET. **Conclusion:** Abdeg delivery leads to a rapid and tightly controlled reduction in systemic radiolabeled antibody levels. Our analyses demonstrate that this class of engineered antibodies holds considerable promise for use as a clearing agent to improve contrast in diagnostic imaging using PET.

219 **CPRIT Grantee**

Effects of Health Insurance on Tumor Stage, Treatment, and Survival in Large Cohorts of Patients with Breast and Colorectal Cancer *X. Du, The University of Texas Health Science Center at Houston; Y. Zhang, The University of Texas Health Science Center at Houston; L. Franzini, The University of Texas Health Science Center at Houston; W. Chan, The University of Texas Health Science Center at Houston; H. Xu, The University of Texas Health Science Center at Houston*

Introduction: Relationship between health insurance and delay in diagnosis, treatment rendered, and survival is less clear. There has been no population-based cancer study of these matters conducted in Texas. The purpose of this work was to examine the impact of health insurance status on the presentation of tumor stage at time of diagnosis, treatment rendered, and overall survival among adults ages >=20 years with breast or colorectal cancer. **Methods:** We identified 52,566 breast cancer patients and 34,316 colorectal cancer patients aged >=20 in 2007-2010 from Texas Cancer Registry. Exposure variable of primary interest was health insurance classified into private, uninsured, Medicaid, Medicare, military, and other/unknown. Treatment variables included the number of days to the first treatment, receipt of chemotherapy and cancer-directed surgery. Survival time was calculated from the date of diagnosis to the date of death or last follow-up (December 31, 2013). **Results:** Uninsured and Medicaid-insured breast cancer patients were more likely to be diagnosed at advanced stage disease (odds ratio: 1.47, 95% CI: 1.34-1.62; 1.42, 1.28-1.57, respectively) or receive chemotherapy (1.09, 0.99-1.21; 1.26, 1.13-1.39), and less likely to receive cancer-directed surgery (0.49, 0.44-0.55; 0.80, 0.70-0.91), compared with privately-insured patients after adjusting for socio-demographic and tumor characteristics,. The hazard ratio of mortality was 1.27 (95% CI: 1.13-1.44) for uninsured patients, 1.50 (1.33-1.69) for Medicaid-insured patients, and 1.30 (1.20-1.41) for Medicare-insured patients as compared with privately-insured patients with breast cancer, after adjusting for socio-demographic, tumor, and treatment factors. Similarly, colorectal cancer patients who were Medicaid-insured and Medicare-insured had an approximately 90% increased risk of death, and uninsured patients had a 42% higher risk of death. However, in patients younger than 65 years with breast cancer, the risk of mortality was not significantly higher for those who received chemotherapy or cancer-directed surgery in patients without insurance coverage compared with those with private health insurance. **Conclusion:** Lack of adequate

health insurance was an important determinant of having advanced stage at diagnosis and of receiving adequate treatment. The risk of mortality was significantly elevated in patients younger than 65 years without health insurance and in those 65 years or older with Medicare coverage alone who did not receive treatment compared with patients with private health insurance. However, broadening the access to health insurance alone would not eliminate all the disparities in cancer outcomes, although having patients to receive treatment regardless of health insurance types will significantly reduce the disparities in the risk of mortal ty.

220 **CPRIT Grantee**

Surgeon and Facility Variation in the Use of Minimally Invasive Breast Biopsy in Texas *N. Tamirisa, The University of Texas Medical Branch at Galveston; K. Sheffield, The University of Texas Medical Branch at Galveston; A. Parmar, The University of Texas Medical Branch at Galveston; C. Zimmerman, The University of Texas Medical Branch at Galveston; D. Adhikari, The University of Texas Medical Branch at Galveston; G. Vargas, Louisiana State University-Shreveport; F. Dimou, The University of Texas Medical Branch at Galveston; Y. Kuo, The University of Texas Medical Branch at Galveston; J. Goodwin, The University of Texas Medical Branch at Galveston; T. Riall, The University of Texas Medical Branch at Galveston*

Introduction: Minimally invasive breast biopsy (MIBB) rates remain well below the National Comprehensive Cancer Network (NCCN) guideline recommendations of more than 90% and vary across geographic areas. Our aim was to determine the variation in use attributable to the surgeon and facility and determine the patient, surgeon, and facility characteristics associated with the use of MIBB. **Methods:** We used 100% Texas Medicare claims data (2000–2008) to identify women older than 66 years with a breast biopsy (open or minimally invasive) and subsequent breast cancer diagnosis/operation within 1 year. The percentage of patients undergoing MIBB as the first diagnostic modality was estimated for each surgeon and facility. Three-level hierarchical generalized linear models (patients clustered within surgeons within facilities) were used to evaluate variation in MIBB use. **Results:** A total of 22,711 patients underwent a breast cancer operation by 1,226 surgeons at 525 facilities. MIBB was the initial diagnostic modality in 62.4% of cases. Only 7.0% of facilities and 12.9% of surgeons used MIBB for more than 90% of patients. In 3-level models adjusted for patient characteristics, the percentage of patients who received MIBB ranged from 7.5% to 96.0% across facilities (mean = 50.1%, median = 49.2%) and from 8.0% to 87.0% across surgeons (mean = 50.3%, median = 50.9%). The variance in MIBB use was attributable to facility (8.8%) and surgeon (15.4%) characteristics. Lower surgeon and facility volume, longer surgeon years in practice, and smaller facility bed size were associated with lower rates of MIBB use. **Conclusion:** We used multilevel hierarchical modeling techniques to demonstrate that a large amount of the observed variation in MIBB use was attributable to both surgeon and facility characteristics. In addition, we identified several surgeon and facility factors associated with low MIBB use. These data points can be used as specific targets for intervention to achieve MIBB rates of more than 90% for breast cancer patients in Texas.

221 **CPRIT Grantee**

TAMU–UT Southwestern Partnership for Breast Imaging and Spectroscopy at 7 Tesla *S. Cheshkov, The University of Texas Southwestern Medical Center at Dallas; I. Dimitrov, The University of Texas Southwestern Medical Center at Dallas; S. Seiler, The University of Texas Southwestern Medical Center at Dallas; S. Ogier, Texas A&M University; J. Rispoli, Texas A&M University; M. Wilcox, Texas A&M University; M. McDougall, Texas A&M University; S. Wright, Texas A&M University; C. Malloy, The University of Texas Southwestern Medical Center at Dallas*

Introduction: There is strong interest in improving diagnostic specificit for breast masses, understanding the factors predisposing to breast cancer, and assessing the response to therapy. One approach is advanced magnetic resonance imaging (MRI). Opportunities include monitoring glucose uptake in a mass by chemical exchange saturation transfer (CEST) imaging, investigating lipid composition via combined 1H/13C spectroscopy, and monitoring glucose metabolism by tracer 13C. Compared to standard clinical MR, high field MRI at 7T provides higher signal and chemical shift dispersion that is expected to dramatically improve metabolic imaging and enable probing tissue biochemistry in vivo. However, major technical barriers in 7T breast MRI include an increase in B1 inhomogeneity and in power deposition. Recognizing the challenges as well as exciting opportunities, Texas A&M and UTSW partnered to develop breast MRI at 7T. As a consequence of this collaboration and CPRIT funding, a number of enabling technologies are now available: 2-channel radiofrequency transmit, coils with uniform RF power across the breast, and the broadband 1H-decoupled 13C breast MRS capability. M**ethods:** Numerical simulations, RF engineering, and coil construction are performed at TAMU. Pulse sequence design, coil integration, validation and human studies are performed at UTSW on the 7T. The intent is to leverage the complementary strengths of both sites, expand our strong collaboration, and further develop metabolic breast imaging. **Results:** The work of our collaborative team - TAMU engineering group plus UTSW physicists and radiologists led to several recent developments. First, software for CEST and related methods has been implemented on the 7T. Second, the bilateral breast coil is being optimized for two-channel-transmit to increase contrast-to-noise for feasibility evaluation of glucose-CEST imaging in tumors. Third, we are addressing the sensitivity challenge posed by the lack of multiple 13C-receive channels by developing a 16-channel-receive-phased-array to acquire 13C data using the 1H-receive chain. We have established that combined ‘H/13C MRS improves lipid profiling. The next step is to determine composition, in women with high breast cancer risk and with confirmed malignancies. Finally, to directly investigate cancer metabolism, we will infuse 13C labelled glucose, detect its products e.g. 13C-lactate, 13C-bicarbonate, and analyze glycolysis to lactate and oxidation in the citric acid cycle. C**onclusion:** Our network of complementary expertise has demonstrated ability to construct advanced coils at TAMU and evaluate their use for clinical research at UTSW in collaboration with breast radiologists. We intend to advance cancer metabolic imaging at 7T by focusing on our strengths in RF engineering and metabolism.

222 **CPRIT Grantee**

Developing a Noninvasive Method for Measuring Biochemical and Structural Parameters of Skin Using Raman Spectroscopy *X. Feng, The University of Texas at Austin; A. Moy, The University of Texas at Austin; M. Markey, The University of Texas at Austin; J. Reichenberg, The University of Texas at Austin; J. Tunnell, The University of Texas at Austin*

Introduction: Despite being the most prevalent form of cancer, skin malignancies do not yet have a widely adopted screening tool other than visual inspection. Our group and others have demonstrated that Raman spectroscopy (RS), a noninvasive technique using light, is sensitive to changes in skin associated with dysplastic progression. However, current methods utilizing RS rely on statistically based algorithms to provide tissue classification and do not elucidate the underlying biophysical changes responsible for RS ability to provide an accurate diagnosis. The overall goal of this research is to develop a method to measure skin biophysical characteristics (biochemical and structural) using Raman spectroscopy. **Methods:** Our overall approach is to develop a linear model of skin Raman spectra that is based on individual basis spectra of primary skin constituents. In order to measure basis spectra, we have built a custom near-infrared confocal Raman microspectroscope system. The high resolution capability of the system allows us to extract spectroscopic features from individual tissue components in situ. The system allows co-registration with a confocal laser scanning microscope for imaging in real time. Raman spectra were collected from phantoms and skin samples. Phantoms were a mixture of chemicals with known Raman features. Skin samples were obtained from Mohs surgical biopsy specimens and sliced into thin sections. We also collected Raman micro-images and correlated them with histological images to develop a model of skin tissue Raman spectra. The spatial mapping of the constituents were reconstructed using multivariate statistics or least-squares fitting each data point in the image with basis spectra. **Results:** Using this system, we have recently obtained high quality Raman spectra for phantoms and human skin. The spatial mapping of the micro-images of the phantom is consistent with the true distribution of the chemicals. Micro-images of skin also showed strong areas of correlation with skin structural makeup. We have obtained a database of basis spectra from chemicals and morphological structures such as lipids, cell nucleus, collagen, etc., and then fit the basis spectra into the macroscopic Raman data using a linear combination model. **Conclusion:** Near-infrared RS can be used to develop a biophysical model of skin tissue. The fit coefficients provide insight into the chemical and structural makeup of the skin, and allow for determining skin diagnostic parameters similar to that a pathologist is familiar reading. Understanding the biophysical characteristics is essential in developing RS diagnostic decision schemes for fast skin cancer detection in vivo.

223 **CPRIT Grantee**

Examining Minimally Invasive Breast Biopsy Rates Among Physicians Using Social Network Analysis *F. Loresto, The University of Texas Medical Branch at Galveston; T. Riall, The University of Texas Medical Branch at Galveston; D. Adhikari, The University of Texas Medical Branch at Galveston; D. Jupiter, The University of Texas Medical Branch at Galveston; Y. Kuo, The University of Texas Medical Branch at Galveston*

Introduction: For patients with palpable breast masses, minimally invasive breast biopsy is the gold standard. MIBB offers several advantages over open biopsy, such as cost-effectiveness, lower rate of complications, and increased patient comfort. In 2009, the National Comprehensive Cancer Network established a target rate of 90% MIBB for breast biopsies; however, recent studies have demonstrated MIBB rates lower than this target rate. These studies showed that surgeon and facility variation in the use of MIBB was significant, with rates ranging from 7.5% to 96.0% across facilities, and 8.0% to 87% across surgeons. **Methods:** Social Network Analysis is a methodology that can further explore these variations by looking at physician networks. We wish to explore the networks of physicians involved in breast cancer care and the relation of network characteristics to MIBB rates. Limiting to patients who were treated for breast cancer, their physicians were identified. For 2010, we identified physicians for the following metropolitan areas: Houston, Austin, and the Rio Grande Valley (RGV). We crated a network using a shared patient model in which physicians are nodes and shared patients are ties. While physicians were identified based on treating breast cancer patients, ties were not limited to breast cancer patients. Using the fast greedy algorithm to determine highly connected sub-networks of physicians, communities were created. The rates of MIBB were compared across these communities. **Results:** Overall, the Houston network had 414 physicians with 86% MIBB rate, the Austin network had 203 physicians with 89% rate, and the RGV network had 96 physicians with 48%. In the Houston network, four communities with greater than 50 patients were identified with a range of 78% to 93% in MIBB rates. This rate difference was marginally significant ($p = 0.049$). In the Austin network, two communities with greater than 50 patients were identifie with 80% and 97% MIBB rates. This was significant ($p = 0.001$). Only one community with greater than 50 patients was identified in the RGV network. This community had an MIBB rate of 52.9%. **Conclusion:** Preliminary results suggests that there are differences within communities regarding the rate of MIBB use. Further exploring these subcommunities within these metropolitan areas may yield beneficial information in determining factors associated with the use of MIBB among physicians.

224

Outreach Invitations Improve Hepatocellular Carcinoma Surveillance Rates: Results Of A Randomized Controlled Trial In A Safety Net Health System *A. Singal, The University of Texas Southwestern Medical Center at Dallas; J. Tiro, The University of Texas Southwestern Medical Center at Dallas; K. McCallister, The University of Texas Southwestern Medical Center at Dallas; C. Mejias, The University of Texas Southwestern Medical Center at Dallas; L .Xuan, The University of Texas Southwestern Medical Center at Dallas; J .Marrero, The University of Texas Southwestern Medical Center at Dallas; E. Halm, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Hepatocellular carcinoma (HCC) surveillance is associated with early tumor detection and improved survival, but its effectiveness is limited by underuse, particularly among underserved populations. The aim of our study was to compare effectiveness of outreach and patient navigation strategies to increase HCC surveillance rates in a racially diverse and socioeconomically disadvantaged cohort of patients. **Methods:** Patients with documented or suspected cirrhosis at a large urban safety-net health system were randomized to receive mailed outreach invitations for surveillance ultrasonography, mailed outreach plus patient navigation, or usual care with opportunistic, visit-based screening. Documented cirrhosis was defined using ICD-9 codes for cirrhosis or cirrhosis-related complications, and suspected cirrhosis was defined as an AST to platelet ratio index (APRI) ≥ 1.5 in the presence of underlying liver disease. We excluded patients with Child C cirrhosis and significant comorbid conditions. The primary study outcome was completion of a surveillance ultrasound within 6 months of randomization. We used an intent-to-screen principle for analyses. We are randomizing 1800 patients in groups of 300 (i.e. 100 patients per arm), with results of the first two groups (n=600 patients) presented herein. **Results:** Baseline patient characteristics across the three groups were similar. Mean age was 55 years and 60% were men. The cohort was racially diverse with 36% Black, 35% Hispanic, and 28% White. Most patients (79%) had documented cirrhosis. Cirrhosis was due to HCV in 54%, alcohol in 19%, NASH in 15%, and HBV in 4%. Rates of scheduled ultrasounds were significantly higher in the outreach/navigation arm (34.0%) and outreach alone arm (26.5%) than usual care (11.5%; p<0.001 for both comparisons). Because of the back-log for getting ultrasounds completed in our safety-net health system, the proportion of ultrasounds completed in the first 2 months have lagged and are not yet different among the 3 arms (9.5% for usual care, 12.5% for outreach alone, and 13.5% for outreach/navigation; p=0.44). An additional 8.0%, 9.0%, and 7.0% of patients in the three arms, respectively, had a diagnostic ultrasound or contrast-enhanced CT/MRI imaging scheduled, so surveillance ultrasound was not required. Ultrasound scheduling was a direct result of outreach efforts in 32.8% of outreach alone patients and 52.6% of outreach/navigation patients. Patients with documented cirrhosis were significantly more likely to complete or schedule an ultrasound than those with suspected cirrhosis (30% vs. 18%, p=0.005). **Conclusion:** Outreach and patient navigation strategies can significantly increase HCC surveillance rates. Further follow-up data will be available for the meeting in November.

225 **CPRIT Grantee**

Quantitative Real-Time Imaging of Glutathione in Live Cells *J. Chen, Baylor College of Medicine; X. Jiang, Baylor College of Medicine; N. Cheng, Baylor College of Medicine; M. Wang, Baylor College of Medicine; J. Wang, Baylor College of Medicine*

Introduction: Oxidative stress is one of the major causes of cancer. Glutathione (GSH) is the most abundant antioxidant in eukaryote cells. Together with its oxidized partner (GSSG), GSH maintains the cellular redox homeostasis, regulates protein functions through S-glutathionylation, and acts as a signaling molecule to directly activate gene expression. All these important functions are regulated by the intracellular concentration and distribution of GSH. Up to date, most of the studies quantified global GSH levels using cell lysates or monochlorobimane (mBCl). These approaches are incapable of providing information about the dynamics of GSH concentration changes and crosstalk between GSH concentration differential in different cellular compartments. **Methods:** Taking advantage of the reversibility of Michael addition reactions, we synthesized the firs GSH probe that can monitor GSH real-time dynamics in living cells. We extensively validated our GSH probe in solution and in cells. **Results:** We developed the first fluorescent probe—ThiolQuant Green Real-Time (TQG-RT)—that enables quantitative real-time imaging of GSH in living cells and animals. We demonstrated that TQG-RT preferentially reacts with GSH under physiological conditions and the fluorescence of TQG-RT is insensitive to other environmental factors. TQG-RT responds to both increase and decrease of GSH levels within seconds. Furthermore, TQG-RT has a high quantum yield and can be applied for GSH live imaging using a concentration in the pM range. In addition, TQG-RT can quantitatively monitor the real-time GSH level changes in cells upon addition of exogenous H2O2 or physiological stimulation using growth factors, and be applied in hard-to-transfect cells, primary cells, and live animals. **Conclusion:** We have successfully developed the first molecular probe that can monitor GSH dynamics in living cells. We envision that our GSH probes will enable unprecedented opportunities to study GSH dynamics and revolutionize our understanding of the physiological and pathological roles of GSH in cells and organisms.

226 **CPRIT Grantee**
Poster Session B

Deep Sequencing identifies genetic variants associated with smoking abstinence *S. Shete, The University of Texas M.D. Anderson Cancer Center; R. Oum, The University of Texas M.D. Anderson Cancer Center; R. Yu, The University of Texas M.D. Anderson Cancer Center; J. Robinson, The University of Texas M.D. Anderson Cancer Center; D. Wetter, Rice University; A. Zheng, The University of Texas M.D. Anderson Cancer Center; D. Nielsen, Baylor College of Medicine; S. Scherer, Baylor College of Medicine; T. Kosten, Baylor College of Medicine; P. Cinciripini, The University of Texas M.D. Anderson Cancer Center*

Introduction: Over 40% of the 42.1 million American adults who still smoke make a serious cessation attempt each year but less than 3% of them maintain abstinence past 1 year. In this study, we aimed to identify genetic factors associated with abstinence in a large cohort of smokers that participated in similar smoking cessation clinical trials. **Methods:** To identify the role of rare and common genetic variants, we sequenced 54 targeted genes using Illumina Hi-seq. The short sequence reads were filtered by Casava and mapped to the reference genome using BWA to create a .bam file. Variants were called by Atlas-SNP2 (includes Atlas-Indel) to create a .vcf file and these in turn were annotated using the Cassandra pipeline. The average coverage of the target bases for the samples was 221x. A total of 37,945 variants were analyzed for this study. An exact logistic regression implemented in PLINK was used that adjusted for sex, age, ethnicity, and study. Analyses of gene-based rare variants were performed using the supervised Optimized Sequence Kernel Association Test. **Results:** The study included 592 smokers who were abstinent and 578 smokers who continued to smoke at the end of treatment from several clinical trials. About 43% of study participants were male and 58% were Caucasians. We identified 5 genes and one intergenic variant associated with smoking abstinence at chromosome 11 (HTR3B, P=4.0x10-14); Chromosome 15 (rs80210037, physical location=78727819, P=5.0x10-25; IREB2, P=8.5x10-11; HYKK, P=6.7x10-10; CHRNA5, P=4.6x10-8); and chromosome 19 (CYP2B6=, P=1.9x10-18). **Conclusion:** These findings underpin the role of genetics in smoking cessation studies.

227 **CPRIT Grantee**
Poster Session A

The Complexity Challenge In Visualizing Repurposed Clinical Data *J. Diaz Garelli, The University of Texas Health Science Center at Houston; E. Bernstam, The University of Texas Health Science Center at Houston; T. Johnson, The University of Texas Health Science Center at Houston*

Introduction: Clinical data reuse can direct the design of future clinical research. It is much easier and cheaper than traditional trials. Searching for associations is a very attractive use of large clinical datasets(1) but current tools can't handle their complexity; in particular their temporal nature(2). For example, weight and blood pressure are recorded as discrete measurements and analyzing them requires a large set of assumptions. Visualizations are useful here because they can present multiple aspects of the data by varying tools and assumptions(3). Thus, we hypothesized that visualization techniques may help dealing with the analytical complexity of association detection in repurposed clinical data. **Methods:** We used a real clinical dataset describing prednisone exposure, a widely prescribed corticosteroid medication, and patient weight over time, a continuous measure routinely recorded in patients and widely known to vary with exposure to prednisone. Our dataset was extracted from a production clinical data warehouse. It described weights over time for patients prescribed with prednisone, sex, age, and total cumulative prednisone milligrams prescribed. We visualized our data using EventFlow(4), a timeline similarity data visualization tool, for multiple assumptions: 1. percentage gains and loss, 2. weight gain/loss and 3. weight gain/loss grouped by exposure. This design innovates by using visualizations for discrete event data to represent sampled continuous variables and breaks the following frame: to distill useful information, data should be represented in their original form. **Results:** After data cleaning, the final dataset contained 93,617 records, spanning over 10 years, across 9,767 patients; 35.03% were male. Making minimal assumptions (i.e. percentage weight gain over time) yielded complex visualizations that were difficult to interpret. Gain/loss overviews were simpler, yet provided useful and precise information. Increasing complexity by adding covariate information (i.e. exposure) diluted the clarity of the effect shown. **Conclusion:** We found that making assumptions about the dataset's temporality and values reduced complexity but including covariates dramatically increases complexity. Visualizations proved useful in showing dataset flaws related to completeness and sparse sampling as well as finding simple associations. We recommend such techniques for preliminary dataset exploration and using alternate representations to handle complexity.

228 **CPRIT Grantee**
Poster Session B

HPV VISO: Encoding HPV Vaccine Education Content For Patient Knowledge-based Systems *D. Wang, The University of Texas at Austin; M. Amith, The University of Texas Health Science Center at Houston; C. Tao, The University of Texas Health Science Center at Houston*

Introduction: Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States and can cause many types of cancers. Although the HPV vaccine can prevent most HPV viral infections, vaccine coverage throughout the USA has been dismal. Current methods for patient education are primarily printed formats such as brochures and handouts which are difficult to navigate and query and do not serve parents and patients who would respond to knowledge-based efforts to increase vaccine uptake. We propose the use of an ontology or knowledge-based approach that stores patient-centered vaccination education information and is scalable and query-able in systems that can deliver rich vaccine information that is patient-friendly and bypass medical jargon. An ontology comprises of statements in a subject-verb-object format (triple) encoded in machine-level language, and are typically used for health care professionals. In addition, an ontology for patients opens opportunities for more interactive and precise delivery of vaccine information using human-friendly queries, where patients and parents receive direct and personalized answers. Using the developed Vaccine Information Statement Ontology (VISO) framework, our objective is to incorporate HPV knowledge into a patient language-friendly information knowledgebase. **Methods:** The information was gathered from various patient education resources, including the Vaccine Information Statements and sources by Paul Offit, a leading vaccine advocate. Using the conceptual classes already developed in the VISO framework, we extracted all relevant knowledge statements gathered from the sources and coded them using the web ontology language (OWL2) with Protégé. The ontology was then evaluated using the web-based tool, Semiotic Evaluation Management System (SEMS), which provides multi-faceted scoring criteria based on machine-readability, terminological meaningfulness, practical usefulness, and expert authority on a scale between 0 to 1 (highest). **Results:** Our draft ontology contains 285 factual statements, 137 classifications, and 45 relations among classifications. SEMS the overall score measured at 0.72, indicating relatively high quality for our draft. **Conclusion:** Attaining a functional

knowledgebase, we can potentially power the intelligence behind patient-centered interactive agents – mobile devices, kiosks, etc. – to improve patient vaccine literacy and address patient questions in situations that hinder interaction with their providers. Some of the challenges we faced in creating the ontology were incorporating some knowledge triples into Protégé in a concise and understandable way. Furthermore, including more HPV education information relevant for patients and incorporating feedback from subject matter experts will improve the overall quality of the ontology.

229 **CPRIT Grantee Poster Session A**

The Epidemiology of Cirrhosis in Hispanics in South Texas *J. Jiao, The University of Texas M.D. Anderson Cancer Center; G. Watt, The University of Texas Health Science Center at Houston; K. Vatcheva, The University of Texas Health Science Center at Houston; J. Pan, The University of Texas Medical School at Houston; J. McCormick, The University of Texas Health Science Center at Houston; S. Fisher-Hoch, The University of Texas Health Science Center at Houston; M. Fallon, The University of Texas Medical School at Houston; L. Beretta, The University of Texas M.D. Anderson Cancer Center*

Introduction: Liver cirrhosis is the main risk factor for hepatocellular carcinoma (HCC).HCC surveillance in cirrhotic patients is associated with significant improvement in early detection, access to curative therapy and overall survival. The highest incidence of HCC in the United States is among Hispanics in South Texas. Our aim, therefore, was to determine the prevalence of cirrhosis and associated factors in Hispanics in South Texas as a first step towards developing interventions to reduce the HCC disease burden in this population. **Methods:** We used a population-based Hispanic cohort from the US-Mexico Border region in Texas, the Cameron County Hispanic Cohort (CCHC). Clinical and demographic variables were extracted for 2466 subjects, and liver cirrhosis was estimated using aspartate transaminase (AST) to platelet ratio index (APRI) scores, which is calculated as (AST/upper-limit of normal)/platelet count X 100. Cutoffs of 2 and 1 were used to predict cirrhosis and cirrhosis/advanced fibrosis respectively. **Results:** The overall prevalence of cirrhosis in CCHC was 0.94%, which is nearly 4-fold higher than the national prevalence (0.27%) recently reported using the same approach (Scaglione S et al. *J Clin Gastroenterology*. in press). The highest prevalence (1.92%) was observed in the 25-34 age group, reaching 3.75% in males in this group. The overall prevalence of cirrhosis/advanced fibrosis was 3.54% and reached 4.65% in the 25-34 age group and 4.91% in the >65 age group. Cirrhotic and non-cirrhotic subjects differed significantly on HCV positivity and fasting insulin level. Subjects with cirrhosis/advanced fibrosis were more likely to be male, HCV positive, diabetic, with higher BMI and waist circumference, higher fasting glucose, insulin and triglyceride levels. Because patatin-like phospholipase domain-containing-3 (PNPLA3) gene SNPs (rs738409 and rs2281135) have been associated with elevated liver enzymes (Li Q et al. *Clin Invest Med.* 2012. 35:E237-45) and with cirrhosis (Trepo E et al. *Hepatology* 2014. 59:2170-7), we also analyzed these SNPs in CCHC participants. We identified both variants to be significantly correlated with elevated APRI scores. In ~20% of the subjects with high APRI scores, no known risk factor of cirrhosis (alcohol abuse, hepatitis virus, diabetes or PNPLA3 SNPs) was identified. **Conclusion:** The prevalence of cirrhosis and advanced fibrosis in Hispanics in South Texas is significantly higher than the national average, affecting in particular young males. In ~20% of the affected subjects, no known risk factor was identified. More research is needed to understand the unique pattern of cirrhosis and advanced fibrosis in this population and reduce this disease burden

230 **CPRIT Grantee Poster Session B**

Varenicline and Combined NRT for Initial Smoking Cessation and Rescue Treatment in Smokers: A Randomized Pilot Trial *P. Cinciripini, The University of Texas M.D. Anderson Cancer Center; J. Blalock, The University of Texas M.D. Anderson Cancer Center; J . Minnix, The University of Texas M.D. Anderson Cancer Center; M . Karam-Hage, The University of Texas M.D. Anderson Cancer Center; S. Shete, The University of Texas M.D. Anderson Cancer Center; C. Green, The University of Texas Medical School at Houston; D. Beneventi, The University of Texas M.D. Anderson Cancer Center; J .Ferguson, The University of Texas M.D. Anderson Cancer Center*

Introduction: Treatment of chronic, frequently relapsing illnesses often requires sequential decision-making to maximize the probability of subsequent treatment success, conditional upon what is known about patients and their responses to previous treatments. Interventions targeting smoking cessation and abstinence face two types of decisions: 1) choice of treatment initiation, 2) choice of subsequent treatments if initial attempts at cessation do not succeed. Many smoking cessation studies attempt to inform these decisions but fall short in two ways: 1) Such trials identify average effects of treatment without addressing potential heterogeneity in patient response, and 2) These trials fail to address questions regarding the most efficacious treatment strategies when patients fail to benefit from their initial treatments. **Methods:** We are conducting a novel Sequential Multiple Assignment Randomized Trial (SMART) in which we are randomizing 300 smokers to one of two initial treatment conditions: Nicotine Patch 21mg + ad lib Nicotine Lozenge 2mg, or Varenicline 2mg. Patients failing to achieve abstinence after 6 weeks of treatment will undergo re-randomization to: a) continuation of the same treatment; b) switching to the untried intervention, or c) augmentation of treatment with an additional 21mg patch or an increase in Varenicline dose from 2mg to 3mg. This permits estimates of the probability of benefi of switching versus augmentation versus a longer trial of the patient's current treatment, after they have failed at initially achieving abstinence. Furthermore, this study is recruiting smokers with and without psychiatric co-morbidities as well as those with varying levels of motivation to quit. **Results:** This clinical trial is ongoing, but after opening recruitment in May of 2015, we have randomized 34 study participants and are on pace to maximize our recruitment goals. We anticipate an accelerated recruitment schedule based on our relatively inclusive study criteria. **Conclusion:**

The novelty of this design is that it will allow us to dynamically evaluate "rescue" pharmacotherapy interventions for smokers who are initially unsuccessful in their attempt to quit. Moreover, we are evaluating the two active treatment combinations that are known to be the most effective for cessation, potentially maximizing success rates; and increasing our understanding of whether switch or augmentation medication approaches lead to better abstinence outcomes for those who fail in their cessation efforts during the acute phase of treatment. It is important to emphasize that the effectiveness of medication switching or augmentation as a rescue intervention has never been evaluated for smoking cessation.

231 **CPRIT Grantee**
Poster Session A

Evaluation of Two HPV Vaccination Educational Interventions for Hispanic Parents *M. Fernandez, The University of Texas Health Science Center at Houston; L. Savas, The University of Texas Health Science Center at Houston; E. Lipizzi, The University of Texas Health Science Center at Houston; A. Roncancio, The University of Texas Health Science Center at Houston; M. Khan, The University of Texas Health Science Center at Houston; N. Fernandez-Espada, The University of Texas Health Science Center at Houston; S. Vernon, The University of Texas Health Science Center at Houston*

Introduction: Hispanic women in Texas have among the highest rates of cervical cancer mortality in the country. This study examined the effectiveness of two educational interventions for parents to increase HPV vaccination among Hispanic girls. M**ethods:** Twenty-nine clinics were randomized to receive standard care or one of two lay health worker (LHW) delivered interventions: a print fotonovela or a tailored interactive multimedia intervention (TIMI) on an iPad which the parent used themselves. After baseline assessment, LHWs delivered the interventions to parents and data collectors completed follow-up assessments at 6 and 12 months following intervention. We assessed initiation at first follow-up and completion of the vaccine series by end of the study. We used hierarchical logistic regression to analyze data using both intent-to-treat and per protocol analyses. **Results:** Per protocol analyses (including parents who had completed follow-up or whose daughters vaccination status was confirmed through medical records review) showed significan differences in vaccine initiation at the 6 month follow-up: between TIMI and control (OR: 1.739, 95% CI: 1.048-2.886, p=0.032) and between fotonovela and control (OR: 1.770, 95% CI: 1.151-2.724, p=0.010). There were no statistically significant differences between TIMI and fotonovela (OR: 0.982 CI: 0.621-1.553, p=0.938). Percentages of vaccine uptake by group were as follows: TIMI (55%), fotonovela (48%), and Control (33.1%). We also conducted a more conservative intent-to-treat analyses in which the denominator is all those enrolled in the study including those not followed up; those not reached for follow-up were assumed to not have received the vaccine. Results showed significant differences in vaccine initiation at the 6-month follow-up: between TIMI vs control (OR: 1.452, 95% CI: 1.005-2.099, p=0.047), between fotonovela and control (OR: 1.589, 95% CI: 1.147-2.202, p=0.006). While we are still completing data collection for the 12 month follow-up, preliminary analysis indicate that completion of the vaccine series at the 12 month follow-up was higher among parents exposed to the TIMI vs. the control (OR: 2.507, 95% CI: 1.071-5.867, p=0.034). **Conclusion:** Findings showed that parents receiving either the fotonovela or the tailored tablet educational interventions were more likely to initiate HPV vaccination within 6 months than parents in the comparison group.

232 **CPRIT Grantee**
Poster Session B

Trends and Variations in Breast and Colorectal Cancer Incidence from 1995 to 2011: Comparative Study between Texas and SEER data *X. Du, The University of Texas Health Science Center at Houston; Z. Liu, The University of Texas Health Science Center at Houston; Y. Zhang, The University of Texas Health Science Center at Houston; L. Franzini, The University of Texas Health Science Center at Houston; J. Cormier, The University of Texas M.D. Anderson Cancer Center; W. Chan, The University of Texas Health Science Center at Houston; H .Xu, The University of Texas Health Science Center at Houston*

Introduction: No study has been conducted to compare the temporal trends of breast and colorectal cancer incidence in Texas with those of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) in the United States. This study aimed to conduct a parallel comparison between the Texas Cancer Registry and SEER on cancer incidence from 1995 to 2011. M**ethods:** We identified all women diagnosed with breast cancer and men and women diagnosed with colorectal cancer as their primary tumor in 1995-2011 from both SEER and TCR. In the 9 SEER registries, 328,142 patients with breast cancer and 224,511 patients with colorectal cancer were included. In Texas, 243,695 women with breast cancer and 155,551 patients with colorectal cancer were included. Annual incidence rates of breast and colorectal cancer cases per 100,000 persons were age-adjusted to the 2000 US standard population stratified by five age groups. The incidence rates were computed by age, sex, race, tumor stage, and tumor grade for breast and colorectal cancer separately. Poisson regression model with population size specific to demographic groups as offset variable were used to determine the association between incidence rates and potential risk factors. Covariates included age, sex, race, tumor stage, and tumor grade. The assumption of the Poisson model was examined by examining constant variance plots of the variables. No specific pattern was detected in the outputs indicating that the constant variance was valid. **Results:** Age-adjusted breast cancer incidence was 134.74 per 100,000 in Texas and 131.78 per 100,000 in SEER in 1995-2011, whereas age-adjusted colorectal cancer incidence was 50.52 per 100,000 in Texas and 49.44 per 100,000 in SEER. Breast cancer incidence increased from 1995 to 2001, decreased from 2002 to 2006, and then remained relatively stable from 2007 to 2011. For colorectal cancer, the incidence increased in 1995-1997, and then decreased continuously from 1998 to 2011 in Texas

and SEER areas. Incidence rates and relative risks by age, sex, and ethnicity were identical between Texas and SEER. C**onclusion:** Breast and colorectal cancer incidence trends from 1995 to 2011 were almost identical between the TCR and SEER areas. Breast cancer incidence increased in 1995-2001 and decreased afterwards, while colorectal cancer incidence decreased continuously from 1998 to 2011. The cancer risk also varied according to gender and race/ethnicity. Additional studies may be needed to explore smaller geographical areas within these registries and environmental factors associated with the changing incidence trends.

233 **CPRIT Grantee**
Poster Session A

Underuse of Surgical Resection Among Elderly Patients with Early-Stage Pancreatic Cancer *S. Giordano, The University of Texas M.D. Anderson Cancer Center; W. He, The University of Texas M.D. Anderson Cancer Center; W. Chan, The University of Texas Health Science Center at Houston; D. Lopez, The University of Texas System; S. Rachna, The University of Texas M.D. Anderson Cancer Center; H .Zhao, The University of Texas M.D. Anderson Cancer Center*

Introduction: Although surgery improves the health care quality and outcomes of patients with early-stage pancreatic cancer, these patients' operative resection rate has been historically low. We sought to identify factors that are associated with operative resection in this patient population. **Methods:** In this retrospective population-based study, we used Texas Cancer Registry-linked and Surveillance and Epidemiology End Results Program-linked Medicare data to study factors potentially associated with operative resection in patients age ≥66 years who had been diagnosed with localized pancreatic cancer between January 1, 2001, and December 31, 2009. Variables were assessed using multivariate logistic regression and Cox proportional hazards regression models. We used Kaplan-Meier analysis to assess the effect of operative resection on survival rate. **Results:** Of 1,501 patients with localized pancreatic cancer, only 340 (22.7%) underwent operation. Patients were more likely to undergo surgery if they were young, had small tumors, had low-grade tumors, and had nodal negativity (P < .05). Compared with patients who did not undergo surgery, patients who underwent surgery had a significantly higher 5-year overall survival rate (25.0 vs 2.3%; P < .0001) and had a higher median survival time (24.3 vs 5.8 months). **Conclusion:** The rate of operative resection of early-stage pancreatic cancer did not increase significantly from 2001 to 2009. Although we identified several variables associated with operative resection, why the percentage of patients with localized pancreatic cancer who undergo definitive surgery is so low remains unclea .

234 **CPRIT Grantee**
Poster Session B

Association Between Hispanic Ethnicity And Childhood Cancer Survival In Texas, 1995-2013 *E. Lopez Bertiery, The University of Texas Southwestern Medical Center at Dallas; L. Xuan, The University of Texas Southwestern Medical Center at Dallas; S. Pruitt, The University of Texas Southwestern Medical Center at Dallas*

Introduction: In 2014, 15,780 new cases of cancer were estimated to occur among children and adolescents in United States, and approximately 15% of those children were Hispanic. In prior research, Hispanic children with cancer have shown an increase risk of death compared to their White peers. However, data on the impact of Hispanic ethnicity on pediatric cancer outcomes is relatively scarce. The strongest evidence to date is in the field of pediatric leukemia (both acute lymphoblastic and acute myeloid leukemia) where Hispanic patients, compared to Whites, have lower survival. We evaluated the association of Hispanic ethnicity with mortality among children with all cancer types in Texas. M**ethods:** We conducted a retrospective analysis using 1995-2013 Texas Cancer Registry data, including all patients between 0 and 19 years of age diagnosed with a first primary malignancy. We examined differences between Hispanic and Non-Hispanic White patients in all-cause mortality. Differences in survival and 5-year survival rates were estimated using unadjusted Kaplan-Meier curves fitted separately by cancer type (leukemia, lymphoma, and solid tumors). For patients with all cancer types, multivariate Cox proportional hazard models controlled for patient age, sex, year of diagnosis, and cancer type and stage. **Results:** A total of 18,687 patients were identified (8,659 Hispanic and 10,028 Non-Hispanic white). In all, 27% had leukemia, 13% had lymphoma, and 59% had a solid tumor. In unadjusted Kaplan-Meier analysis, Hispanics with leukemia (p<.001) and solid tumors (p<.001) had higher risk of death, compared to non-Hispanic Whites. For Hispanics vs. Whites, respectively, five-year survival was as follows: leukemia (37.5% vs. 50.5%), lymphoma (62.0% vs 66.0%) and solid tumors (31.7% vs 45.2%). For patients of all cancer types combined, Hispanics (vs. Whites) had higher mortality after adjusting for all covariates (adjusted hazard ratio (aHR): 1.30; 95% CI: 1.22-1.37). C**onclusion:** Hispanic children with leukemia or solid tumors in Texas have higher risk of death, compared to Non-Hispanic Whites. Future research should seek to understand the reasons underlying this disparity and to develop interventions designed to improve survival among Hispanic children with cancer. Additional research will also explore the reasons behind the observed low survival rates.

235 **CPRIT Grantee**
Poster Session A

Utilizing Dynamic Proteomics Analysis to Determine Changes in the Targetome Affected by Nutrient Usage *M. Person, The University of Texas at Austin; S. Sowa, The University of Texas at Austin; A. Bui, The University of Texas at Austin; L. Contreras, The University of Texas at Austin*

Introduction: A major leap needed in studies of gene regulation is identification of cell-wide networks that activate "opportunistic phenotypes" that are developed during via stress-responses mechanism. An example of this is the invasion of bacteria into human cells, particularly during low-immunity periods (e.g. during chemotherapy and other cancer treatments). Two important aspects of this are: biofilm formation and adhesion systems. One common bacterial regulation pathway for modulation of these properties is the carbon storage regulator system (csr). The csr is present in many bacteria families that contribute to opportunistic infections. In Escherichia coli, one csr protein, CsrA, controls the expression of a wide variety of targets that affect metabolism, biofil formation, cell division, and cell morphology. While previous research has defined a subset of targets of the csr system, the full repertoire of targets has not been explored. This is especially true of the proteomic response of these cells when experiencing a new environment, like those encountered at the site of infection. **Methods:** E. coli were grown and subjected to an environmental stress, carbon starvation, to which the csr system is known to respond. Wild type and Csr mutant cells were harvested at multiple timepoints. The cells were lysed, then proteins extracted and digested with trypsin. After desalting, the digests were run by UPLC-MS/MS on a Thermo Orbitrap Elite using top 20 MS/MS data-dependent acquisition. Proteins were searched against the E. coli database using the Proteome Discover Sequest HT search algorithm and then validated using Scaffold. Normalized spectral counts were used for quantitation. **Results:** In this work, we identify the proteomic changes that occur as E. coli responds to an external stress and track these changes dynamically. This research identified new potential targets of the csr system and provides information about the extent to which targets are affected. We also provided more information about a large number of csr targets that had only loosely been characterized in the literature. Our data not only allows to determined targets, but to compare pattern to patterns of known targets allowing us to predict potential mechanisms of control. **Conclusion:** In combination, with other collected omics data we were able to define a picture of the

csr targetome and understand its control of disease-related properties in E. coli. These results will allow researchers to better understand the timescale at which bacterial proteins respond to a new environment such as one made available by chemotherapy or other cancer treatments.

236 **CPRIT Grantee**
Poster Session B

An Intervention to Change the Paradigm of Long-term Care for Cancer Survivors and Americans with Disabilities *Y. Tuchaai, The University of Texas Health Science Center at Houston; L. Frieden, The University of Texas Health Science Center at Houston*

Introduction: Cancer survivorship is rising as breakthroughs in medical sciences and treatments have increased the life expectancies of patients, but at a cost - these patients often become physically and/or mentally impaired, diminishing their performance of daily life activities, necessitating long-term care services. Survivors are considered disabled under the Americans with Disabilities Act (ADA), they are compounding the size of the aging and disability populations. Long-term care for disabled and elderly people is provided under two ideologically conflicting models: the Government-funded medical model where healthcare professionals direct care in institutions, and the independent living (IL) model where consumers direct their own care in their homes. IL yields greater consumer satisfaction. However, implementing IL in a society operating under a medical paradigm is challenging. An attractive low-cost intervention is required. **Methods:** This intervention proposes training of "IL Aides" through a short certificate program awarded by technical colleges, with online accreditation available. These aides are to be stationed at local Fire Departments in numbers proportional to the elderly and disability population in the area. Long-term care consumers would dial a proposed 1-800-ILHELP hotline and be connected to "on-call" IL Aides at the nearest fire station. The aide would arrive at the GPS location of the caller and provide consumer-directed care for the daily life activities they are unable to perform e.g dressing and undressing. This intervention would initially be funded by grants, but as the program and its success expands it should be integrated within Social Security. **Results:** The proposed IL model is based on research which combined the strengths of successful IL models in the Netherlands, Sweden and Japan and adapted these to integrate telecommunication. This "community medicine" paradigm in America is expected to yield widespread consumer satisfaction, following the trend observed abroad. Consumer growth is expected with the ADA prohibiting unjustified institutionalization, and Government support is expected to follow - it is significantly cheaper to fund long-term care outside expensive healthcare institutions, IL also boasts the welcomed prospect of job-creation. **Conclusion:** The IL model is an ideal long-term care solution for elderly and disabled people, including cancer survivors, who are mentally and medically able to live independently and direct the care they require. Today is the dawn of IL: 57 million Americans are disabled and with over half of the 76 million baby-boomers projected to have disability by 2020, the number of people requiring long-term care will surpass the number of beds in institutions.

237 **CPRIT Grantee**
Poster Session A

Lack of Improvement in Survival Rates for Women under 50 with Endometrial Cancer, 2000 to 2011 *A. Rodriguez, The University of Texas Medical Branch at Galveston; K. Schmeler, The University of Texas M.D. Anderson Cancer Center; Y. Kuo, The University of Texas Medical Branch at Galveston*

Introduction: In the United States (U.S.), endometrial cancer is the most commonly diagnosed gynecologic malignancy and will account for 54,870 new cases and 10,170 deaths in 2015. The incidence is expected to continue to rise with life expectancy, the epidemic of obesity and physical inactivity. The American Cancer Society has reported that, although death rates have remained stable for women aged 50 years and older between 2006 and 2010, they increased by 1.5% for women younger than 50. It is not clear if this is due to more aggressive histology or to differences in the treatment received. The purpose of this study is to understand how first course of treatment affects cancer-specific survival in women <50 diagnosed with endometrial cancer. **Methods:** Public use data from the Surveillance, Epidemiology, and End Results (SEER) Program was used. The study included 82,721 Hispanic, Asians/Pacifi Islander, non-Hispanic White and Black women diagnosed with primary, invasive endometrial cancer between 2000 and 2011. We assessed type of treatment using Cox proportional hazards models to determine survival disparity by age and stage. **Results:** Cancer-specific survival significantl improved for those aged ≥50 years with late stage, but did not improve for those <50. First course of treatment significantly affected cancer-specifi survival for endometrial cancer patients. Regardless of age, survival was greatly improved for late stage patients who received a combination of surgery and radiation (Hazard Ratio [HR] = 0.12 [95% Confidenc Interval {CI}= 0.47-0.78] and 0.64 [95% CI= 0.59–0.68]) compared to those who received total hysterectomy with removal of ovaries and tubes. However, the proportion of patients who received combination therapy decreased over time. The magnitude of decrease was larger in patients <50 than in those aged ≥50. Overall, about 20-40% of the difference in cancer-specific survival over time in patients aged <50 was explained by their initial treatment. **Conclusion:** Improvement in cancer-specifi survival was seen only in older women with late stage diagnosis. Despite improvements in diagnoses and treatments, the difference in age-specifi survival indicates that more should be done to understand why these rates are not improving for those <50.

238 **CPRIT Grantee Poster Session B**

Unplanned 30-Day Readmission after Medical and Surgical Hospitalizations in Patients with Gastrointestinal Cancer

J. Manzano, The University of Texas M.D. Anderson Cancer Center; R. Luo, The University of Texas M.D. Anderson Cancer Center; L. Elting, The University of Texas M.D. Anderson Cancer Center; M. Suarez-Almazor, The University of Texas M.D. Anderson Cancer Center

Introduction: Generalizable data on hospital readmissions among cancer patients are lacking. If readmission measures are to be applied to the cancer population, more information is needed about readmission patterns in cancer patients. To examine the patterns and predictors of 30-day readmission among elderly patients with GI cancer, we conducted a retrospective cohort study using linked Texas Cancer Registry and Medicare claims data from 2001 to 2009. **Methods:** Texas residents aged 66 years or older initially diagnosed with GI cancer between 2001 and 2007 who had uninterrupted Medicare Part A and Part B coverage throughout the period were studied. We determined the 30-day unplanned readmission rate after surgical and medical (i.e., nonsurgical) hospitalizations and factors associated with readmission (determined by multilevel analysis with hospitalizations nested within patients) in our cohort. **Results:** We analyzed 46,454 hospitalizations from 21,292 patients. The overall readmission rate was 17.4%, and the median time to readmission was 11 days. The readmission rate for medical hospitalizations (20.6%) was higher than for surgical hospitalizations (13.0%). Risk factors for readmission after both surgical and medical hospitalizations included distant disease (surgical: odds ratio [OR] 1.57, 95% confidence interval [CI] 1.38-1.78; medical: OR 1.62, 95% CI 1.47-1.78), high Charlson comorbidity index score (surgical: OR 1.80, 95% CI 1.54-2.11; medical: OR 1.54, 95% CI 1.39-1.71), emergency room visit within 30 days prior to index hospitalization (surgical: OR 1.67, 95% CI 1.42-1.97; medical: OR 1.30, 95% CI 1.18-1.43), and unplanned index hospitalization (surgical: OR 1.48, 95% CI 1.35-1.61; medical: OR 1.62, 95% CI 150-1.74). Additional risk factors for readmission after surgical hospitalizations included age ≥80 years (OR 1.16, 95% CI 1.02-1.32) and an intensive care unit stay (OR 1.14, 95% CI 1.04-1.25). **Conclusion:** Unplanned readmissions are common among elderly patients with GI cancer, especially after medical hospitalizations. Our findings emphasize the importance of transitions-of-care initiatives during hospitalization, care coordination and comorbidity management in this patient population. Knowledge of risk factors for readmission specific to medical and surgical hospitalizations can help determine which patients should receive targeted interventions to prevent readmission.

239 **Poster Session A**

Environmental Scan: HPV Vaccination in Texas Pediatric Care Settings

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Introduction: In response to the President's Cancer Panel Report outlining ways to accelerate vaccine uptake in youth, the National Cancer Institute issued a funding opportunity to NCI-designated cancer centers to conduct environmental scans to identify barriers and facilitators to HPV vaccination uptake in pediatric care settings. The University of Texas MD Anderson Cancer Center was one of the awardees. Goals included an assessment of best practices, areas to target for interventions and research, as well as a goal to strengthen collaboration among existing coalitions/stakeholders for enhanced prevention of HPV-related diseases. **Methods:** Primary and secondary data collection included a literature search, key informant interviews, and a web-based survey. Key informant interviews were conducted with various stakeholders including healthcare providers, researchers, public health departments and coalitions. The web-based survey was sent via email four times over a period of fiv weeks to those working in pediatric care settings including physicians, nurses, nurse practitioners, physician assistants, medical assistants and office administrators. The survey questions addressed barriers, facilitators, vaccination data management, communication of the vaccine recommendation, HPV education and vaccine stocking. **Results:** The data from the environmental scan showed that vaccination rates are higher where vaccine recommendations are bundled, an office champion is present, a whole office approach is used, and with the use of EHRs to track immunization status, reminders and recalls. Top reasons for refusal as noted by physicians are 1) believe child is too young (30.3%), 2) concerns about media portrayal of vaccine (19.5%), 3) lack of knowledge of HPV-associated diseases (16.9%) and 4) concerns about risky sexual behavior (15.9%). Barriers to uptake include the parental belief their child is at low risk for cancer (boys 62%/girls 40%) and safety concerns regarding the vaccine (boys 42%/girls 49%). **Conclusion:** Texas HPV vaccination rates lag far behind the 2020 Healthy People goal of 80 percent. Areas still in need of improvement are 1) knowledge among providers and parents of the diseases caused by HPV affecting boys and girls, 2) knowledge about the importance of vaccination at the optimum age of 11-12 with catch-up afterward, 3) how providers can strongly recommend the vaccine, 4) the use of electronic records systems and associated tools to track and boost uptake and 5) insurance and funding gaps. Research and interventions should focus on provider communication, education of parents and providers and better use of electronic office systems

240 **Poster Session B**

Correlates of Human Papillomavirus Vaccine Uptake among US Adolescent Males: National Immunization Survey-Teen, 2013 *M. Rahman, The University of Texas Medical Branch at Galveston; T .Laz, The University of Texas Medical Branch at Galveston; A. Berenson, The University of Texas Medical Branch at Galveston*

Introduction: The objective of this study was to examine the correlates of human papillomavirus (HPV) vaccine initiation and completion among a national sample of adolescent males two years after the routine recommendation that they receive the 3-dose HPV vaccine series. **Methods:** Provider-verified HPV vaccination information (N=9,554) among 13-17 year old males from the National Immunization Survey-Teen (NIS-Teen) 2013 study were analyzed. Weighted multivariable logistic regression analyses were performed to examine the correlates of HPV vaccine initiation (receipt of ≥1 dose) and completion (receipt of ≥3 doses). **Results:** Overall, weighted HPV vaccine initiation and completion rates were 34.6% and 13.9% among 13-17 year old US males. Weighted multivariable analyses observed that black (odds ratio (OR) 1.98, 95% confidence interval (CI) 1.49-2.64; OR 1.52, 95% CI 1.06-2.17) and Hispanic males (OR 2.42, 95% CI 1.83-3.20; OR 1.95, 95% CI 1.38-2.76), older age (OR 1.07, 95% CI 1.01-1.15; OR 1.10, 95% CI 1.01-1.20) and physician recommendation (8.71, 95% CI 7.20-10.55; OR 6.23, 95% CI 4.73-8.20) were associated with higher HPV vaccine initiation and completion. Further, adolescent males with household income below the poverty level, whose mother were not married, and who were residing in the Northeast and visited a health care provider in the past 12 months were more likely to initiate the vaccine. **Conclusion:** Provider recommendation was the strongest predictor for both HPV vaccine initiation and completion. Intervention programs targeted at physicians which focus on increasing their recommendations for the HPV vaccine may improve overall HPV vaccine uptake among US adolescent males. Racial disparities should be addressed as well.

241 **CPRIT Grantee Poster Session B**

Cancer Prevention Started Early: Evaluating the Lunch Policies of Child Care Centers in Texas *S. Mahajan, The University of Texas at Austin; C. Joyce, Southeast Missouri State University; D. Hoelscher, The University of Texas Health Science Center at Houston; M. Briley, The University of Texas at Austin; C. Roberts-Gray, Third Coast Research; C. Byrd-Williams, The University of Texas Health Science Center at Houston*

Introduction: A poor diet in early childhood has been associated with increased cancer risk in adulthood,[1] and preschool-aged children in the U.S. are not consuming the recommended amounts of fruits, vegetables and whole grains.[2] Over 24% of children in the U.S. under 5 years old attend child care centers,[3] and depending on the state, up to 40% of early childhood and education (ECE) centers may require parents to provide lunch.[4] Unfortunately, the majority of preschoolers' lunches sent from home do not meet dietary recommendations. The ECE setting can play a major role in the dietary intake of 3-5 year olds, and the aim of this study was to examine the information and guidance ECE centers give to parents about packed lunches. **Methods:** Parent handbooks and handouts were collected from 36 child care centers that require parents to pack lunch for their child in Austin, Houston, and San Antonio, TX as part of an NCI-funded study. Study staff conducted a content analysis to determine what information and guidance is provided to parents about food packed for lunch. Two staff independently coded the documents using the Wellness Child Care Assessment Tool (WellCCAT), a validated instrument that quantifies the comprehensiveness and strength of ECE nutrition policies. **Results:** Emergent categories from the content analysis included foods that were discouraged/prohibited (81%), food safety (53%), allergy considerations (33%), choking hazards (22%), ability to warm food (22%), and religious considerations (8%). Of twenty-three centers (64%) that provided suggestions of what to pack, ten (28%) either suggested or required both fruits and vegetables to be packed, and three (8%) suggested whole grains. Almost a third of centers provided guidance on the structure of the meal, such as suggesting a healthy lunch that includes all the food groups. Out of a 100-point scale, the total comprehensiveness of the lunch policies was 19.4 ± 10.8 and the total strength was 4.6 ± 6.9. **Conclusion:** This is the first study to our knowledge that explores the guidance that ECE centers give to parents when parents are asked to pack lunches for their preschool-aged children. There was vast variability, and low comprehensiveness and strength scores in lunch policies across the centers Results from this study show a possible area of improvement for ECE centers. Specific instructions and recommendations on how to create appropriate lunch guidelines may lead to a better diet among preschoolers, potentially reducing the risk of cancer throughout the lifespan.

242 **Poster Session B**

A Minimal Intervention to Promote Smoke-free Homes among 2-1-1- callers: Texas Gulf Coast Effectiveness Trial *P. Dolan Mullen, The University of Texas Health Science Center at Houston; L. Savas, The University of Texas Health Science Center at Houston; L. Bundy, Emory University Rollins School of Public Health; R. Haardörfer, Emory University Rollins School of Public Health; J. Monroy, The University of Texas Health Science Center at Houston; R. Williams, The University of North Carolina at Chapel Hill; M. Hovell, San Diego State University; M. Kreuter, Washington University in St. Louis; D. Jobe, United Way of Greater Houston 211 Texas/United Way HELPLINE ; M. Fernandez, The University of Texas Health Science Center at Houston; M. Kegler, Emory University Rollins School of Public Health*

Introduction: Rarely is adequate testing for effectiveness possible after an intervention's efficacy is established. With National Cancer Institute support, this report represents an exception, the 2nd effectiveness trial (after North Carolina) which tested Emory University's efficacious Smoke-free Homes intervention plus delivery support system delivered by trained 2-1-1 staff rather than university research staff as in the efficacy trial. The intervention, themed "some things are better outside" consisted of 3 mailings plus a coaching call. **Aim:** Test the effectiveness of Smoke-free Homes in partnership with the Texas Gulf Coast 2-1-1-call center in a catchment area with a large English-speaking Hispanic population. **Methods:** English-speaking callers living in smoking-discordant households (>1 smoker and >1 non-smoker) randomly assigned to intervention or control groups were followed by telephone at 3 and 6 months by interviewers blinded to group assignment. **Results:** 6.4% of callers were eligible; 81.4% consented and completed baseline measures (n=508). Trial participants were female (84%), smokers (71%), with children (68%), African American (65%), single (58%), and very low income (50%, <$10,000/yr). Hispanics (12%) and White, non-Hispanics (19%) comprised the other main racial/ethnic sub-groups. In the intervention group 71.8% received coaching calls. After 3 months 31.3% intervention group reported a full ban compared to 19.6% of the controls (intent-to-treat analysis, with non-respondents counted as failures, p=0.003). Full bans for subgroups varied, but did not differ statistically (p=0.52): African Americans (intervention=31.7%; control=18.5%), Hispanics (intervention=35.3%; control=28.6%), Whites (intervention=24.4%; control=23.3%). Baseline smokers and nonsmokers were equally likely to have a full ban (p=0.87). Among respondents (73%), mean days of home smoke exposure dropped significantly (p=0.004);

baseline smokers' mean cigarettes at 3 months approached significanc in regression analyses (p=0.08). We plan to present 6-month results, including process measures and moderator analyses. **Conclusion:** The intervention's effectiveness replicated in this region, including a new subgroup of English-speaking Hispanic callers, provided an important harm-reduction alternative for African American and Hispanic smokers. Reduction in home smoking also protects other family members, especially children and might increase likelihood of later complete cessation among smokers. Compared to the previous trials, fewer callers were eligible, partly because Hispanic smoking rates are lower and more callers refused to disclose their household smoking status. Research on wording/timing of a disclosure question might extend the reach of this intervention and bundling recruitment with other evidence-based interventions for 2-1-1 callers would increase efficiency of 2-1-1 staff time in catchment areas with lower rates of household smoking.

243 **Poster Session A**

Health Fair Effectiveness to Improve Breast and Cervical Cancer Screening *M. Rangel, The University of Texas Health Science Center at Houston; L. Gatus, The University of Texas Health Science Center at Houston; N. Heredia, The University of Texas Health Science Center at Houston; M. Fernandez, The University of Texas Health Science Center at Houston*

Introduction: Health fairs are widely used to increase cancer education and screenings in underserved, high risk populations. However, there is limited evidence on the effectiveness of special events such as health fairs to promote and receive recommended cancer screenings. We conducted this study to test the effectiveness of special events as part of a multi-site study that included a subgroup analysis on the Texas site. This presentation focuses on results based on two special events implemented by community-based organizations in Houston, Texas. **Methods:** We evaluated two health fairs delivered by Houston-based organizations, Dia de La Mujer Latina (DLM) and Lesbian Health Initiative (LHI). Both organizations provide cancer education and screening services for underserved high risk groups. Lay health workers recruited women 18 years of age and older in need of either a mammogram or Pap test at the health fairs and data collectors conducted in-person baseline surveys with interested participants. All women (100%) approached consented to participate and completed a baseline survey. Surveys measured participants' demographics, knowledge about cancer and screenings, cancer awareness, intentions to receive cancer screenings and screening behavior. Six months post-baseline women were contacted to complete a follow-up survey and self-reported mammogram or Pap test cancer screenings. **Results:** Among all participants who completed the baseline (68 from DLM and 41 from LHI), 56 were in need of a mammogram or Pap test. The majority of the participants were Spanish speakers (61%), older than 40 years of age (48%), and Hispanic (83%). Among the 56 participants in need of screening services, 37 completed follow-up survey (66%). Follow-up surveys captured self-reported breast and cervical cancer screenings. We verified reported screening through medical record reviews. Over half (54%) of participants at follow-up reported receiving cancer screening in the past 6 months. Participants who received a facilitated cancer screening during the health fair were more likely to complete their cancer screening (67%) within 6 months, compared to participants who received vouchers for free cancer screenings during the health fair (50%). The majority (82%) of participants at follow-up reported that attending the health fair was "very important" in their decision to getting cancer screening. **Conclusion:** Health fairs are a common strategy to increase breast and cervical cancer education and screening in the community and can be an effective approach to increasing screening among underserved women. More research is needed on improving the effectiveness of health fairs in increasing the screening behaviors.

244 **Poster Session B**

Formative Research for A Smoke-Free Homes (SFH) Intervention for Hispanic Texan Households. *L. Savas, The University of Texas Health Science Center at Houston; P. Dolan Mullen, The University of Texas Health Science Center at Houston; M. Hovell, San Diego State University; J. Cavazos, The University of Texas Health Science Center at Houston; C. Escoffery, Emory University; M. Kegler, Emory University Rollins School of Public Health; J. Jones, San Diego State University; J .Monroy, The University of Texas Health Science Center at Houston; M .Fernandez, The University of Texas Health Science Center at Houston*

Introduction: Although almost 1/3 of young Hispanic children are exposed to second-hand smoke in their homes, few tobacco-related interventions for Hispanics have focused on eliminating smoking in homes and vehicles. An efficacious English language Smoke-free Homes (SFH) intervention (3 sets of mailed materials and one coaching call) was tested with 2-1-1- callers in Atlanta and replicated in North Carolina and has great potential for cultural adaptation for Hispanics. **Aim:** To collect qualitative data to adapt the SFH intervention for Hispanics. **Methods:** Bicultural graduate students recruited and interviewed a convenience sample of Hispanic smokers (n= 10) and non-smokers (n= 13) who lived with at least one person in a Houston household with discordant smoking status. Independent reviewers analyzed transcripts' themes and household profiles produced using Atlas Ti. **Results:** Participants were predominantly female Spanish speakers with low education and low income. Themes regarding smoking in the home were respect for smokers, especially guests and heads of households; mixed English and Spanish spoken and read in multi-generational households; culturally appropriate, "non-confrontational" communication; and the meaning of a home smoking ban, i.e., enforcing a home ban was interpreted as asking a smoker to quit smoking. Participants consistently viewed protecting children's health as legitimate, but did not express a similar attitude towards protecting non-smoking adults. As with other groups, we found misconceptions about "safe" indoor smoking and strong approval of bans in public places. Potential storylines for print materials were: 1) non-smoking mother with a ban whose more acculturated teenage son smoked when she wasn't home, 2) a woman's strong ethic of respect for her smoking partner or parent who pays the rent; 3) smokers' and non-smokers' mutual respect for smoking bans to protect children, and 4) a non-smoker struggling with deference for smokers such as guests, elders,

and heads of household. **Conclusion:** A culturally adapted intervention targeting Hispanics households must accommodate generational differences in acculturation and power dynamics. Because most 2-1-1 callers are female, and Spanish speaking females are less commonly smokers, the cultural adaptation must help non-smoking Hispanic women overcome power dynamics to build consensus for a ban. Intervention methods may include modeling of respectful approaches to establish and enforce a household ban. English and Spanish mailed intervention materials are needed to reach all generations. This work informs next steps in the adaptation process, with delivery and evaluation of the culturally and linguistically targeted SFH intervention in partnership with Texas 2-1-1 call centers.

245 **Poster Session A**

Measurement of Motivation for HPV Vaccination in Safety-Net Clinic Populations *D. Denman, Southern Methodist University; A .Baldwin, Southern Methodist University; E. Marks, The University of Texas Southwestern Medical Center at Dallas; S. Lee, The University of Texas Southwestern Medical Center at Dallas; J. Tiro, The University of Texas Southwestern Medical Center at Dallas*

Introduction: According to Self-Determination Theory (Ryan & Deci, 2000), different types of motivation that underlie behavior lie on a continuum. The extent to which the motivation underlying behavior is self-determined (autonomously) or controlled (externally) influences its sustainability. This is particularly relevant for behaviors that must be repeatedly performed, such as completion of the human papillomavirus (HPV) vaccine series that requires three doses over 6 months. To date, no measures of motivation for HPV vaccination have been developed. **Methods:** As part of a larger study, parents (N=223) whose adolescents receive care at Parkland clinics, the safety-net system for Dallas County, completed a telephone questionnaire about HPV and the vaccine. We modified the Treatment Self-Regulation Questionnaire (Levesque et al., 2007) to assess parents' autonomous motivation (4 items; e.g., The reason you would get your child the vaccine is because you believe it is the best thing for your child), introjected motivation (2 items; e.g., The reason you would get your child the vaccine is because you would feel guilty or ashamed if you did not), and external motivation (2 items; e.g. The reason to get the vaccine is because you feel pressure from others to do so). Parents responded to the items in their preferred language (Spanish or English) using a strongly agree to strongly disagree scale. We used confirmatory factor analysis to test a three-factor measurement model. We also examined whether factor loadings and mean scale scores differed between Spanish- and English-speaking parents. **Results:** The three-factor model fit the data well (RMSEA=.04, CFI=.98, TLI=.96), and the scales' reliability were adequate (autonomous: α=.87; introjected: α=.72; external: α=.72). The factor loading strength for one item "The reason you would get your child the vaccine is because you want to take responsibility for your child's health," was stronger for Spanish- than English-speaking participants ($p<.05$); all others were equivalent. Spanish-speakers all reported higher levels of all three types of motivation (ps < .01). **Conclusion:** Findings support the use of three scales to measure motivation in HPV vaccination and suggest possible cultural differences in motivation. Measurement and clinical implications for both Spanish- and English-speaking populations will be discussed.

247 **Poster Session A**

Sensor-assisted Prevention of Dehydration in Head and Neck Cancer Patients Undergoing Radiation Therapy *S. Peterson, The University of Texas M.D. Anderson Cancer Center; E. Shinn, The University of Texas M.D. Anderson Cancer Center; A. Garden, The University of Texas M.D. Anderson Cancer Center; S. Shete, The University of Texas M.D. Anderson Cancer Center; C. Shen, The University of Texas M.D. Anderson Cancer Center; S. Martch, The University of Texas M.D. Anderson Cancer Center; E. Farcas, University of California-San Diego; K. Lin, University of California-San Diego; F. Raab, University of California-San Diego; N. Asomaning, The University of Texas M.D. Anderson Cancer Center; I. Christie, The University of Texas M.D. Anderson Cancer Center; V. Nandigam, The University of Texas M.D. Anderson Cancer Center; T. Chen, University of California-San Diego; Y. Yan, The University of Texas M.D. Anderson Cancer Center; D. Shojaie, The University of Texas M.D. Anderson Cancer Center; K. Patrick, University of California-San Diego; B. Beadle, The University of Texas M.D. Anderson Cancer Center*

Introduction: Early identification and mitigation of cancer therapy-induced complications can improve quality of life and reduce complications and health care costs. We are evaluating the efficacy of a system, called CYCORE (CYberinfrastructure for Comparative effectiveness Research), to systematically and accurately collect daily weight, blood pressure, pulse and symptom data from head and neck cancer patients during their 6 to 7-week radiation treatment regimen using home-based biometric and other sensors integrated with a cyber-physical system. Our study aims include: 1) evaluate CYCORE's efficacy in reducing hospitalization and emergency room visits related to dehydration in head and neck cancer patients undergoing radiation therapy; and, 2) evaluate the related impact on reducing costs related to treating dehydration. **Methods:** We are testing the efficacy of the CYCORE system to identify patients whose sensor-based data indicates that they are at increased risk for dehydration (i.e., reduced blood pressure, weight, increased pulse, and patient-reported outcomes including decreased food and fluid intake). Patients are recruited at initiation of radiation treatment, and are randomized to one of two arms: 1) standard care plus use of the CYCORE system, wherein biometric sensor data will be collected as well as patient reported outcomes (e.g., pain, nausea, dizziness, medication use, food/flui intake); or, 2) standard care. Outcomes include hospital and emergency room admissions, and related costs. **Results:** To date, we have recruited and enrolled 113 patients, yielding an 88.9% recruitment rate and a 90.3% retention rate. Patients randomized to the CYCORE system take daily home readings of weight, blood pressure and pulse Bluetooth enabled devices, and complete daily assessments of symptoms and other patient-reported outcomes using a mobile tablet. Patients' data are available for viewing and clinical decision-making via a Web-based interface by their physicians and other health care providers. Preliminary data from a prior feasibility study showed that 59% of head and neck cancer patients undergoing radiation showed physiologic signs of dehydration while using the CYCORE system during a two-week time period. Worse ratings of nausea and vomiting, which were among symptoms assessed on a daily basis using a mobile application, were significant predictors of dehydration risk. **Conclusion:** CYCORE may serve as one model for integrating mobile and sensor technologies during critical periods of cancer care that occur outside of the clinic setting, and may improve management of treatment side effects, quality of life, and greater satisfaction with care.

248 **Poster Session B**

Development of the Parkland-UT Southwestern Colonoscopy Reporting System *C. Skinner, The University of Texas Southwestern Medical Center at Dallas; S. Gupta, Moores Cancer Center University of California San Diego; E. Halm, The University of Texas Southwestern Medical Center at Dallas; S. Wright, Parkland Health & Hospital System; K. McCallister, The University of Texas Southwestern Medical Center at Dallas; W. Bishop, The University of Texas Southwestern Medical Center at Dallas; N. Santini, Parkland Health & Hospital System; C. Mayorga, The University of Texas Southwestern Medical Center at Dallas; D .Agrawal, The University of Texas Southwestern Medical Center at Dallas; B. Moran, The University of Texas Southwestern Medical Center at Dallas; J. Sanders, The University of Texas Southwestern Medical Center at Dallas; A. Singal, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Colorectal cancer screening begins a process whereby adenomatous polyps can be identified early and removed to reduce incidence and mortality. Effectiveness of this process is limited by suboptimal screening and surveillance among patients with prior polyps. Whereas more than one-third of patients with advanced adenomas fail to receive a follow-up colonoscopy within 5 years, overuse of surveillance colonoscopy has been documented in more than a quarter of patients with low-risk findings (e.g., non-adenomatous polyps). Reducing underuse and overuse of surveillance colonoscopy is a major focus of healthcare reform. Decision support tools have been developed to match colonoscopy findings to recommendations, but to date, none has generated tailored reports of recommendations to patients and their referring providers. **Methods:** In our urban, safety-net system, we developed and implemented the Parkland-UT Southwestern Colonoscopy Reporting System (CoRS), an electronic medical record-based pathology reporting system with tailored recommendations for patients and providers, to increase guideline-consistent surveillance recommendations and improve communication of recommendations to providers and patients. The system will also allow for tracking of under- and over-screening. CoRS was implemented in December 2013 for all colonoscopies that included a biopsy or polyp removal (673 of 1,775). In June 2014, we assessed use of the CoRS. We also surveyed the Parkland colonoscopists (n=18 ,100% response) to assess their perceptions of its acceptability and whether it improved guideline consistency of recommendations, and quality of results communication. **Results:** CoRS was used for 98.6% of colonoscopies, with biopsies during the first year. More than three quarters of colonoscopists agreed or strongly agreed that CoRS is easy to use (83%), provides guideline-based follow-up recommendations (89%), improves quality of Spanish-language letters (94%), and is something they would recommend for adoption at other institutions (78%). More than half agreed that the system led to improvement in the colorectal cancer screening practice (56%) and made their work easier (61%), with most of those who did not agree being neutral. **Conclusion:** Parkland-UT Southwestern CoRS provides a novel EMR-based tool that promotes guideline-based recommendations and improves communication to patients and providers.

249 **Poster Session A**

Qualitative Formative Research for the Development of an App to Increase Physical Activity in Cancer Survivors *M. Robertson, The University of Texas Health Science Center at Houston; E. Tsai, The University of Texas Health Science Center at Houston; M. Swartz, The University of Texas Medical Branch at Galveston; E. Lyons, The University of Texas Medical Branch at Galveston; M. Jones, MD Anderson; J. Song, The University of Texas Health Science Center at Houston; S. Srinivasan, MD Anderson; M. Cox, MD Anderson; S .Scruggs, MD Anderson; K. Basen-Engquist, The University of Texas M.D. Anderson Cancer Center*

Introduction: In 2012, an estimated 13.7 million cancer survivors were living in the U.S. By 2022 this number is expected to increase to 18 million. Despite improvements in treatment, cancer survivors still face: significant long-term challenges to physical functioning and mental health, an increased risk of chronic disease, and a significant risk of cancer recurrence. Physical activity (PA) has been associated with a reduced risk of these problems. Fitness mobile applications (apps) have been shown to facilitate adoption of PA in adults, however there is currently a limited number of apps designed to increase PA in cancer survivors. With the rise of smart phone ownership, apps are a promising tool to increase PA among cancer survivors. The aim of this study is to obtain this population's preferences for fitness app features and text messages for the development of an app to increase PA in cancer survivors. M**ethods:** Thirty five cancer survivors were recruited from MD Anderson and attended two rounds of focus group interviews (N=13). Participants completed questionnaires for demographic information, PA level, and technology use/access. A moderator asked open-ended questions and presented various app features in semi-structured interviews that were recorded and transcribed verbatim. Transcripts were coded independently by two coders using ATLAS.ti version 7 with theory-based codes determined *a priori*. Additionally, comments on presented app features were coded as positive, negative, mixed, or suggestion. Discrepancies in coding were resolved in a group discussion of the authors. All quotations for each code were aggregated and salient themes for participants' preferences were developed in an iterative process. **Results:** Participants were older (63.7 years±10.8), mostly female (69%), and mostly white (71%). Participants had been diagnosed with breast (60%), prostate (26%), colorectal (12%), and endometrial (9%) cancers. Approximately 69% owned/had frequent-access-to a phone with internet, and 41% were not sufficiently active. Themes for preferences obtained included: a casual tone, autonomy in goal selection, passive data collection, tailored feedback, access to role model narratives, video modelling of exercises, and information about PA. Specific app features that were well-received included: digital tracking, tailored messaging, video exercise instruction, and role model narratives. C**onclusion:** Participants indicated a preference for extensive data collection with minimal burden, and that this data be used to provide positive and concise feedback for goal setting/attainment. Video instruction and peer narratives would also be well-received. Further research is needed to confirm these findings with quantitative data in a pilot test when the app is developed.

250 **Poster Session B**

ADAPT Online: An Interactive Tool for Finding and Adapting Evidence-Based Cancer Control Interventions *M. Fernandez, The University of Texas Health Science Center at Houston; M. Hartman, The University of Texas Health Science Center at Houston; P. Dolan Mullen, The University of Texas Health Science Center at Houston; R .Wood, The University of Texas Health Science Center at Houston; C .Escoffery, Emory University; L. Bartholomew, The University of Texas Health Science Center at Houston*

Introduction: Authors have proposed numerous frameworks for adapting evidence-based interventions (EBIs) to fit the needs of new settings and/or populations. These models typically describe general processes with minimal guidance on completing specific adaptation tasks such as evaluating intervention fit, comparing the original intervention goals and products to needs of the new context and making adaptation decisions that avoid compromising intervention effectiveness. We have developed ADAPT Online, an interactive decision support tool for cancer control planners that walks users through steps of finding and adapting EBIs and provides tailored information and guidance for adaptation planning and implementation. **Methods:** With a Web development team, we operationalized the Intervention Mapping adaptation framework, "Int Map Adapt" from the upcoming 4th edition of the textbook. ADAPT Online guides users through an application of this framework, with tailored functionality, links, and expert advice; automatically producing graphics, to-do lists, and other products based on users' preferences, responses to questions, and information about the EBI. **Results:** The resulting program consists of 6 steps: 1) *A*nalyze the situation, 2) *D*iscover available interventions, 3-4) *A*dapt to fit your situation, 5) *P*ut into practice, and 6) *T*est your progress. Using algorithms, ADAPT Online generates a visual logic model of change (LMC) that organizes information from the new target community. The LMC describes potential intervention targets including the health promoting behavior(s) and/or environmental conditions and their determinants. ADAPT online also lists theoretical methods appropriate for influencing these determinants. This information is useful to precisely describe the goals of the adapted intervention and assist users in searching for interventions with good potential fit. ADAPT Online then guides the user through adaptation planning by comparing the LMC with the EBI. Earlier input is carried forward for use in later steps, e.g., the LMC is available for comparison with available EBIs and to guide decisions about what should change and what should stay the same. Moreover, the program assists planners in identifying core elements of the EBI and provides alerts when proposed changes risk compromising intervention effectiveness. Alpha testing is complete, and Beta testing will commence in September, followed by a trial set to launch in January. **Conclusion:** To increase the use of EBIs in practice, ADAPT Online is expected to enable community cancer control planners more easily and effectively to identify, assess fit, adapt, and implement EBIs. Conference attendees will have the opportunity to participate in the trial and adapt their own EBI.

251 **CPRIT Grantee**

Association of Insurance Status With Use of Immediate Breast Reconstruction in Women With Breast Cancer *B. Smith, The University of Texas M.D. Anderson Cancer Center; J. Jiang, The University of Texas M.D. Anderson Cancer Center; R. Jagsi, University of Michigan; S. Kronowitz, The University of Texas M.D. Anderson Cancer Center; S. Giordano, The University of Texas M.D. Anderson Cancer Center*

Introduction: Utilization patterns and predictors of breast reconstruction in Texas are unknown. We sought to determine the influence of health insurance coverage on use of immediate breast reconstruction for working-age women undergoing mastectomy for breast cancer in the state of Texas. **Methods:** We used two complementary databases, the Texas Cancer Registry-linked Medicaid database and the MarketScan private insurance database, to identify working age women treated with mastectomy for incident breast cancer from 2000-2007 in Texas. Logistic regression tested the association between Medicaid versus private insurance and receipt of immediate breast reconstruction, adjusting for patient, treatment, and sociodemographic covariates. Reimbursement for reconstruction, adjusted for inflation and reported in 2014 dollars, was estimated from claims. **Results:** Median age was 49.7 for the Medicaid cohort compared to 50.4 for the MarketScan cohort (P= 0.02). From 2000 to 2007, use of reconstruction increased significantly for patients in the MarketScan cohort (38.1% to 53.9%; Ptrend=0.009), but not those in the Medicaid cohort (10.5% to 16.6%; Ptrend=0.24). In total, 15.7% of patients in the Medicaid cohort underwent immediate reconstruction (n=213/1,360) compared to 50.7% (n=1,405/2,772) of patients in the MarketScan cohort (adjusted RR 3.09; 95% CI 2.78-3.40). Reimbursement for reconstruction was $3,167 (95% CI $2,512-$3,820) for patients in the Medicaid cohort compared to $15,432 (95% CI $14,030-$16,834) for patients in the MarketScan cohort. C**onclusion:** Type of insurance coverage is an important factor associated with receipt of immediate breast reconstruction. We postulate that the marked difference in reimbursement for reconstruction between Medicaid and private insurance creates a relative disincentive for plastic surgeons and hospitals to offer breast reconstruction to patients with Medicaid.

252 **CPRIT Grantee**

Improved Outcome of Breast Cancer Survivors Participating in a Multidisciplinary Survivorship Program in El Paso, TX *Z. Nahleh, Texas Tech University Health Science Center at El Paso; P. Rebecca, Texas Tech University Health Science Center at El Paso; R. Heydarian, Texas Tech University Health Science Center at El Paso; L. Sanchez, Texas Tech University Health Science Center at El Paso; D. Liss, Texas Tech University Health Science Center at El Paso; C. Ochoa, Texas Tech University Health Science Center at El Paso; I. Mallawaarachchi, Texas Tech University Health Science Center at El Paso; A. Dwivedi, Texas Tech University Health Science Center at El Paso*

Introduction: Breast Cancer (BC) survivors in El Paso, TX include a majority of Hispanics. We have previously reported that the majority of these survivors have decreased mental and physical health related Quality of Life (QOL) which limit normal social functioning. We sought to determine whether BC survivors would benefit from a multidisciplinary Breast Cancer Survivorship Program, using the following validated questionnaires: 1) Patient Health Questionnaire 9(PHQ9), 2) General Anxiety Disorder 7 (GAD 7), and SF36 QOL questionnaires. M**ethods:** After IRB approval, we recruited consecutive patients treated at our institution between October, 2013-October 2014, and who are within the first 5 years post-diagnosis with Stages I-III breast cancer . Survivors are surveyed for depression and generalized anxiety disorder and Qol at baseline, and every 3 months. Survivors receive personalized summary, dietary advice, in addition to in-depth psychological assessment and interventions using Mindfulness Based Stress Reduction. We used generalized estimating equations (GEE) analysis for comparing scores over the time period. **Results:** 94 patients were recruited. 90.4% were Hispanics. Mean age 54.4 years. Hispanic (90.4%), 30.8% of participants were younger than 50 years of age, distributed as Stage 1 BC (38.3%) , Stage 2 (42.6%), and stage 3 (19.2%) . 75% received chemotherapy ; and 71%% had hormone receptor positive BC and received endocrine therapy. Scores were available on 68 patients at 3-month, 63 patients at 6-month, 53 patients at 9-month, and 32 patients at 12-month follow ups. The scores at baseline were are as follows: PCS 45.25 representing the mean for the SF-36 QOL Physical Health , and the MCS 44.2 for Mental Health at baseline (both below the population norm of 50.0). Mean scores for GAD7 was 7.19 and for PHQ9 7.64, both abnormal (<5 on PHQ9 and GAD 7 are considered normal scores). All scores improved over the follow up period. At 12 months, - scores were as follows: PCS 46.03 ; MCS

45.62 ; GAD7 5.5 ; and PHQ9 5.28 . C**onclusion:** Breast cancer survivors in El Paso, TX are benefiting from participating in the new Breast Cancer Survivorship Program launched at the Texas Tech GBCC in El Paso, TX designed to address their unmet needs. Follow up is ongoing and is expected to continue to improve the outcome of these patients, empower them in their transition from cancer treatment to survivorship and lead to improved psychosocial adjustment and normal social functioning.

253 **CPRIT Grantee**

Prevalence of Comorbidity and Impact on Survival among Texas Medicare Beneficiaries Diagnosed with Breast, Lung, Colorectal, and Prostate Cancer *D. Zhang, The University of Texas Medical Branch at Galveston; J. Goodwin, The University of Texas Medical Branch at Galveston*

Introduction: Older cancer patients often have comorbid conditions prior to diagnosis. Prior studies have demonstrated that comorbidity at the time of cancer diagnosis influences treatment decisions and increases the patient's risk of death. However, less is known about the comorbidity rates and their effect on survival for Texas cancer patients diagnosed in recent years **Methods:** Texas Cancer Registry (TCR) -Medicare linked data was used to identify Texas Medicare beneficiaries age 66 and over diagnosed with cancer from 2001 to 2011. Medicare claims for inpatient, outpatient, and physician visits for the year before diagnosis were used to identify comorbid conditions. The Charlson Comorbidity Index was used to generate scores of no comorbidity (=0), low comorbidity (=1) and severe comorbidity (>2). Five-year survival was estimated using Kapan-Meier curves. **Results:** The rates of comorbidity were 40%, 72%, 44%, and 62%, respectively, for breast, lung, colorectal, and prostate cancer patients. The numbers of patients with comorbid conditions increase with age and stage of cancer. The 5-year death rate for breast cancer patients was 18% for those with no comorbidities, 29% for those with low morbidity, and 47% for those with severe comorbidity. The relative 5-year death rate for those with no comorbidity vs severe were: for lung cancer, 83% vs 90%; for colorectal cancer, 44% vs 68%; and for prostate cancer, 17% vs 47%. For those with a prior diagnosis of dementia, the death rate was 72% for breast cancer, 96% for lung cancer, 87% for colorectal cancer, and 76% for prostate cancer. After adjustment for age, race, and stage, the adjusted 5-year death rate was significant higher among cancer patients with comorbid conditions. The odds of death within five years of diagnosis for breast cancer patients with severe comorbidity was 2.48 (2.38, 2.60) compared to patients with no comorbidities. The odds was 1.53 (1.50, 1.56) for lung cancer, 1.96 (1.89, 2.02) for colorectal cancer, and 2.59 (2.49, 2.70) for prostate cancer. C**onclusion:** Texans on Medicare had significant high rates of comorbidity before being diagnosed with cancer. The comorbidity conditions were significantly associated with decreased overall survival and increased mortality. Further study is needed to address the patterns of treatment and care for Texas cancer patients with comorbidity.

254 **CPRIT Grantee**

Comparative Effectiveness of Chemotherapy versus Resection of the Primary Tumor as the Initial Treatment Modality in Stage IV Colorectal Cancer *H. Mehta, The University of Texas Medical Branch at Galveston; G. Vargas, Louisiana State University-Shreveport; N. Tamirisa, The University of Texas Medical Branch at Galveston; D. Adhikari, The University of Texas Medical Branch at Galveston; K. Brown, The University of Texas Medical Branch at Galveston; T. Riall, The University of Texas Medical Branch at Galveston*

Introduction: Since 2006, National Comprehensive Cancer Network (NCCN) guidelines recommend chemotherapy as the initial treatment modality for all patients with unresectable metastatic colorectal cancer. However, these recommendations are not based on level I data, and recent studies demonstrate that surgery is often used as the primary treatment modality. We aimed to determine trends in the use of chemotherapy as the initial treatment modality and evaluate the comparative effectiveness of chemotherapy versus resection of the primary tumor as the initial treatment modality with survival in patients presenting with stage IV colorectal cancer. **Methods:** We used the Surveillance, Epidemiology, and End Results (SEER)-Medicare data (2000-2011) for this cohort study. Patients ≥66 years presenting with stage IV colorectal cancer and no indication for urgent or emergent surgical resection were included in the cohort. The outcome was cancer-specific survival measured from the first date of diagnosis until death or up to two years. A multivariable Cox proportional hazards regression was used to evaluate the association between initial treatment modality and survival after adjusting for potential confounders. Instrumental variable analysis that controlled for unmeasured confounding was also used to determine the association of initial treatment modality with two-year survival. Percentage of use of chemotherapy as the initial treatment modality by health service area was used as the instrumental variable. **Results:** Of 6,368 patients who met inclusion criteria, 2,216 (34.8%) underwent chemotherapy and 4,152 (65.2%) underwent resection of the primary tumor as the initial treatment modality. The use of chemotherapy as the first treatment modality increased over time from 26.8% in 2001 to 46.9% in 2009 ($p<0.0001$). Cox regression analysis showed that chemotherapy as initial modality was associated with worse survival (HR, 1.20; 95% CI, 1.11-1.29). This association was no longer significant in instrumental variable analysis (HR, 0.77; 95% CI, 0.47-1.27) indicating no difference in survival between patients receiving chemotherapy versus resection of the primary tumor as the initial treatment modality, regardless of receipt of the second modality. **Conclusion:** While there is an increasing trend towards the use of chemotherapy as initial therapy, this approach remains underused based on current guidelines. The instrumental variable analysis found no difference between chemotherapy and resection of the primary tumor as the initial treatment modality for the two-year survival in patients with stage IV colorectal cancer. Given the high mortality associated with colorectal resection in this age group and the similar survival, a chemotherapy-firs approach should be used more frequently.

255 **CPRIT Grantee**

Variation in intensity and costs of end of life cancer care by payer and race in Texas *B. Guadagnolo, The University of Texas M.D. Anderson Cancer Center; K. Liao, The University of Texas M.D. Anderson Cancer Center; S. Giordano, The University of Texas M.D. Anderson Cancer Center; L. Elting, The University of Texas M.D. Anderson Cancer Center; Y. Shih, The University of Texas M.D. Anderson Cancer Center*

Introduction: Little is known about end of life care quality among patients dying of cancer in Texas. Almost 30% of Texas citizens receive tax-payer funded health care under the Medicare or Medicaid programs or both. The purpose of this study was to investigate end-of-life care for Medicaid, Medicare, and dually eligible beneficiaries dying of cancer in Texas. M**ethods:** We analyzed the Texas Cancer Registry (TCR)-Medicaid and TCR-Medicare linked databases' claims data for 69,572 patients dying of cancer in Texas from 2000-2008. We conducted regression models in adjusted analyses of cancer-directed and acute care and total costs of care (in 2014 dollars) in the last 30 days of life. **Results:** Medicaid patients were more likely to receive chemotherapy and radiation therapy. Medicaid patients were more likely to have >1 emergency room (ER) (OR=5.27, 95% CI: 4.76-5.84), and were less likely to enroll in hospice (OR=0.59, 95% CI: 0.55-0.63) than Medicare patients. Dually eligible beneficiarie were more likely to have >1 ER visit than Medicare-only beneficiarie (OR=1.19, 95% CI: 1.07-1.33). Black and Hispanic patients were more likely to experience > 1 ER visit and >1 hospitalization than whites. Costs were higher for non-white Medicare, Medicaid, and dually eligible patients compared to white Medicare enrollees. C**onclusion:** Variation in acute care utilization and costs by race and payer suggest efforts are needed to address palliative care coordination at the end of life for Medicaid and dually eligible beneficiaries and minority patients dying of cancer in Texas.

256 **CPRIT Grantee**

The Hispanic Paradox in Breast Cancer Survival: Impact of Patient Birthplace and Neighborhood Hispanic Density *S. Pruitt, The University of Texas Southwestern Medical Center at Dallas; J. Tiro, The University of Texas Southwestern Medical Center at Dallas; L. Xuan, The University of Texas Southwestern Medical Center at Dallas; S. Lee, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Despite a worse risk factor profile, Hispanic cancer patients often experience better survival outcomes compared to Whites. The reasons for this "Hispanic Paradox" are unclear and may reflect cultural advantages. We examined whether two proxies of culture—patient birthplace and neighborhood Hispanic density—moderate Hispanic-White differences in mortality among breast cancer patients. **Methods:** Using linked Texas Cancer Registry-U.S. Census data, we examined associations of patient ethnicity, birthplace, neighborhood percent Hispanic, and neighborhood poverty on all-cause and breast-cancer specific mortality among 149,616 non-Hispanic White (80.0%), Hispanic U.S.-born (16.1%), and Hispanic foreign-born (4.4%) adult women diagnosed with breast cancer, 1995-2009. Shared frailty Cox proportional hazard models (patients nested within census tracts) adjusted for age, diagnosis year, stage, grade, histology, urban/rural residence, and local area mammography capacity. **Results:** Results from adjusted models are as follows. Whites (vs. U.S.-born Hispanics) had *increased* all-cause (aHR: 1.11; 95% CI: 1.07-1.15) but *similar* (aHR: 1.04; 95% CI: 0.99-1.10) cause-specific mortality. Foreign-born (vs. U.S.-born) Hispanics had *increased* all-cause (aHR: 1.42; 95% CI: 1.35-1.49) and cause-specifi (aHR: 1.54; 95% CI: 1.44-1.65) mortality. Living in higher Hispanic density neighborhoods was generally associated with increased mortality, although associations differed by patient ethnicity, birthplace, and neighborhood poverty. For Whites, living in high poverty and/or high Hispanic density neighborhoods was consistently associated with increased mortality. For Hispanics, associations were mixed, and U.S.-born Hispanics received some mortality benefit when living in high Hispanic density, low poverty neighborhoods. **Conclusion:** Further research is needed to identify mechanisms underlying differences in cancer mortality by patient ethnicity, birthplace, and neighborhood of residence.

257 **CPRIT Grantee**

The Comparative Effectiveness Research on Cancer in Texas (CERCIT) Website: A Resource for All Texans *S. Toombs Smith, The University of Texas Medical Branch at Galveston; A. DiNuzzo, The University of Texas Medical Branch at Galveston; J. Goodwin, The University of Texas Medical Branch at Galveston*

Introduction: The Comparative Effectiveness Research on Cancer in Texas (CERCIT) Website (http://www.txcercit.org/) provides an ongoing resource for all Texans working on or concerned with cancer in Texas. **Methods:** A collaboration of the University of Texas Medical Branch at Galveston, the University of Texas - M. D. Anderson Cancer Center, Rice University, and the Texas Cancer Registry, CERCIT provides the infrastructure for research on cancer, the training of junior investigators, and the development of dissemination products. The website highlights research projects and supporting cores focused on cancer prevention, treatment and surveillance. **Results:** For researchers, the website describes current research and links to videos of previous workshops. For junior investigators, it provides training resources and a means of showcasing one's projects. For citizens, it links to news of interest and the opinions of top cancer doctors. For legislators, it provides easy access to fact sheets and special reports. Healthcare providers, patients, health educators, media specialists and more can access late-breaking CERCIT news, as well as videos, reports and the 80+ publications resulting from this consortium. **Conclusion:** The CERCIT website provides a model of dissemination for cancer-related research and information that benefit all Texans.

258 **CPRIT Grantee**

Charitable Food Distribution Sites Offer Novel Opportunities For Cancer Prevention Research and Intervention Among Vulnerable, Hard-to-Reach, and Underserved Populations *S. Pruitt, The University of Texas Southwestern Medical Center at Dallas; R. Higashi, The University of Texas Southwestern Medical Center at Dallas; S. Lee, The University of Texas Southwestern Medical Center at Dallas; E . Cuate, The University of Texas Southwestern Medical Center at Dallas; T. Leonard, University of Dallas*

Introduction: People who live in food-insecure households face significan unmet health needs. At the same time, this population may be under-represented in clinical research studies because of the population's limited and intermittent engagement with the health care system. We describe preliminary results of a research partnership between UT Southwestern Medical Center (UTSW) and Crossroads Community Services (CCS), the largest charitable food distributor of the North Texas Food Bank. The goal of the study is to improve understanding of this population's health- and mammography-related needs, knowledge and service utilization. **Methods:** Eight structured focus groups were conducted in English (n=4) and Spanish (n=4) at CCS. Discussions focused on 13 open-ended questions designed to solicit group communication about members' health status, healthcare access, mammography awareness and utilization, and attitudes toward participation in future health research. **Results:** Participants included 42 CCS clients, about 90% of whom were Hispanic or African-American women. Key findings include: (1) Participants reported multiple co-morbid conditions among themselves and household members, yet utilization of health services was cost-dependent and often limited to emergency triage. (2) Many participants did not know what a mammogram was and utilization was closely linked to having health insurance, which many did not. (3) Despite reporting numerous daily life challenges, the majority were interested in participating in future research-related focus groups as a means of communicating their health needs and obtaining information and emotional support from peers. **Conclusion:** Recruitment from charitable food distribution sites will target a high-need, underserved population that is typically excluded from clinic-based studies. The community-academic partnership between CCS and UTSW has created a robust foundation for cancer prevention research that has already produced important insights about the population's needs and willingness to participate in research. Ongoing research is focused on implementing longitudinal health assessments of CCS clients. These

data will be used to guide future interventions to increase awareness and utilization of cancer prevention services, e.g. mammography, in a population facing multiple barriers to care.

259 **CPRIT Grantee**

Parental Recall of Daughter's HPV Vaccination Status

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Introduction: HPV vaccination is recommended for children and young adults aged 11-26. Parental reporting of vaccination status among children 11-18 is an important element for ensuring that children receive the required doses. We evaluated the accuracy of parental recall of daughters' HPV vaccination status among parents who reported their daughter had not received any doses of the vaccine. We then examined correlates of correct classification. M**ethods:** To assess eligibility for an HPV educational intervention, interviewers asked parents of Hispanic girls, ages 11-17 yrs, about their daughter's HPV vaccination history. Parents who reported that their daughters had no HPV vaccination were eligible for the study. We reviewed clinic medical records to validate parental reports. We calculated the proportion of parents who accurately reported their daughters' vaccination status and used hierarchical logistic regression modelling to determine socio-demographic characteristics associated with accurate vaccination reporting (AVR). **Results:** Among daughters whose parents reported they had not received any doses of the HPV vaccine, medical record review indicated that 28% had been vaccinated prior to enrollment in the study thus making the AVR rate only 72%. AVR was less likely among parents of girls 10-11 yrs relative to 12-14 (OR=0.34, 95% CI=0.19-0.60, p=0.0002), parents of girls with insurance coverage relative to no coverage (OR=0.57, 95% CI=0.36-0.90, p=0.02), and among parents who reported speaking "only English" at home (OR=0.24 (0.070-0.86, p=0.03). **Conclusion:** Misclassificatio of vaccine status may be due to patient factors, or provider tendency to vaccinate older, English speaking, insured girls, with less discussion as compared to Spanish-speaking Hispanic parents. More research is needed to determine potential sources of recall accuracy. Parental recall should also be examined in studies that determine baseline vaccination rates to enable comparisons across groups.

260

Innovative Approaches to Delivery of Patient-Centered Cancer Care: Results of a LIVESTRONG Symposium

R. Schear, LIVESTRONG Foundation; E. Beckjord, University of Pittsburgh Medical Center; D. McGoldrick, LIVESTRONG Foundation; S. Nutt, LIVESTRONG Foundation; R. Rechis, LIVESTRONG Foundation

Introduction: The field of patient-centered care has a 50 year history, but data on cancer-specific patient-centered care research and implementation are somewhat limited. The 2013 Institute of Medicine report, "Delivering High-Quality Cancer Care: Charting a New Course for a System in Crisis," recommends patient-centered cancer care (PCCC) as a way to address challenges facing the cancer care system, but to date, no institution has successfully implemented a comprehensive PCCC model. We have an unprecedented opportunity to determine how to deliver PCCC in a way that results in patients feeling informed, respected and cared for. **Methods:** In June 2014, the LIVESTRONG Foundation convened a symposium with 83 stakeholders to discuss current trends in cancer care; explore solutions to challenges of delivering PCCC; and through a simulation activity, rapidly construct models of PCCC. Symposium participants were equipped with 23 elements of PCCC created by LIVESTRONG based on a thorough literature review. Additionally, presentations, surveys, real-time qualitative data analysis, and human-centered design activities were used to gather input directly from participants about approaches to delivering PCCC. **Results:** Symposium results suggest that the most essential elements of delivering PCCC are accessible, timely, clear, and effective communication between all parties of the patient's care team; providers who identify and communicate realistic goals to patients and caregivers; incorporation of best practices and new evidence; and emotional and psychosocial support for patients and caregivers. Proposed strategies to deliver PCCC included use of patient navigators, health information technology to enable a "learning health care system," and patient-provider communication; incorporation of PCCC into medical education; and psychosocial support. **Conclusion:** Two models of PCCC were proposed by participants, both of which paralleled the structure of the patient-centered medical home. The LIVESTRONG Foundation intends to utilize the proposed strategies to implement the essential elements of PCCC through the LIVESTRONG Cancer Institutes at Dell Medical School at the University of Texas at Austin, focusing on high-priority elements like continually assessing patients' needs, preferences, and values so they serve as the foundation of care decisions; and coordinating and integrating care across multiple disciplines within and outside of oncology. Through the Institutes, we intend to put the patient at the center of the cancer care experience, and to work collectively to make Austin a first of its kind innovative hub for cancer care and a model in how to truly deliver patient-centered cancer care.

261 **CPRIT Grantee**
Poster Session B

Robust statistical causal structures in Multi-omics *A. Yazdani, The University of Texas Medical School at Houston; E. Boerwinkle, The university of Texas, School of public health, Houston; A. Samiei, Hasso-Plattner-Institute; A. Yazdani, The University of Texas Medical School at Houston*

Introduction: The metabolome is a collection of small molecules resulting from a multitude of cellular and physiologic processes and provides opportunities for improved disease prediction and improved understanding of disease mechanisms and outcomes. At an abstract level, the components of most complex systems, such as metabolomics, can be reduced to a series of variables (nodes) that are connected to each other by links so that each link represents an association between two components. Assigning a direction, which represents causal relationship, shows the flow of information between metabolites. Identification of causal relationships among metabolites provides a deeper understanding of metabolomics architecture and facilitates prediction and mechanistic understanding. M**ethods:** The most pragmatic tool for identification and visualization of causal relationships in large-scale data are Directed Acyclic Graphs and a key strategy to achieve stable causal structure in the setting of a Bayesian network is data integration. These causal networks are ideally suited for the analysis of multi-omic and heterogeneous data types, such as DNA sequence, metabolomics and risk factor data collected in a sample of deeply phenotyped individuals. Recently, it has been recognized that genetic information can be used to establish causal relationships among phenotypes organized into networks. We noticed that partial information from genome results in unstable causal structures. Since we do not have a complete knowledge about genome, the extracted information across genome is required to achieve robust structures. This approach improves the performance of Bayesian Network algorithms, where only partial genome information, one gene or even one pathway, is applied. **Results:** For this presentation, we used 691,940 amino acid substitution genotypes to infer the statistical causal network among 122 reliable serum metabolomics measurements determined by a combination of gas and liquid chromatography and mass spectrometry. We then define layers of causal modules and identify metabolites that are influential on the entire network. In addition, we explain the relationship between modules themselves and between modules and individual metabolites. Finally, we document the utility of the causal metabolomics structure with an application predicting onset of congestive heart failure, an important common chronic disease. C**onclusion:** Using genome information as complete as possible results in robust identification of causal structures among phenotypes, such as metabolomics and helps prediction of intervention targets for future experiements. Award: The method of this work was recognized as “exceptionally creative and skillful research” at 2015 Atlantic Causal Inference Conference, at University of Pennsylvania and won The Thomas R. Ten Have Award.

262 **CPRIT Grantee**
Poster Session A

In Vivo Quantification of Skin Inflammation in Zdhhc13 Deficient Mice *C. Perez, The University of Texas M.D. Anderson Cancer Center; D. Kusewitt, The University of Texas M.D. Anderson Cancer Center; F.Benavides, The University of Texas M.D. Anderson Cancer Center*

Introduction: Cutaneous inflammation is characterized by vasodilation, edema, and dermal leukocyte infiltration. In mice, its severity can be quantified by assessing skin redness and thickness in the living animal and by quantifying inflammatory cell influx into the dermis in histologic sections. In vivo techniques are somewhat subjective and histology-based assays require sacrifice of the mice. Here we report the use of a new in vivo imaging technique for assessing cutaneous inflammation in mice that is is rapid, non-subjective, and highly quantitative; sacrifice is not required and inflammation can be measured repeatedly in the same mice. This technique uses two fluorescent imaging agents, one activatable by cathepsins (ProSenseTM 750 FAST) and the other by neutrophil elastase (ProSenseTM 680 FAST), followed by in vivo optical imaging and spectral unmixing. By timing the injection of the two agents, the activity of monocyte/macrophage cathepsins and of neutrophil elastase can be measured simultaneously. We used this method to assess the inflammatory response in the skin of a new, spontaneous recessive mouse mutation in the *Zdhhc13* gene we named luca (symbol: *luc*). This gene is a member of the palmitoyltransferase (PAT) family of genes that catalyzes posttranslational modifications in proteins that also may be involved in the *NF-kappa-B* signaling pathway. Luca is a nonsense base substitution that results in a premature stop codon that generates a truncated form of the ZDHHC13 protein representing a potential loss-of-function allele. The affected homozygous mice show multifocal alopecia and erythema associated with inflammatory cell infiltration. M**ethods:** Homozygous Zdhhc13luc/Zdhhc13luc and WT littermate controls were exposed to either a single dose of UVB or 12-O-tetradecanoyl-phorbol-13- acetate (TPA) and two activatable-fluorescent agents, ProSense 750 and Neutrophil Elastase 680 (PerkinElmer, Waltham, MA) were injected intravenously. Whole-mouse imaging was performed using an IVIS Spectrum system and the acquired images were analyzed using LivingImage 4.4 (Caliper Life Sciences). Values for total radiant efficienc ([p/s] / [µW/cm2]) were collected for statistical comparison. **Results:** A greatly enhanced fluorescence in mutant skin relative to WT in both experiments was observed, suggesting increased neutrophil elastase activity in the ZDHHC13-deficient skin. However, mutant skin showed no differences in macrophage infiltration by IHC and in vivo imaging ith the pan-cathepsin activatable probe. Minimal background was observed in the skin of untreated mice or treated mice not injected with the imaging agents. C**onclusion:** This technique is fast, accurate and offers a simple alternative to other methods of evaluating cutaneous inflammati n.

263 **CPRIT Grantee Poster Session B**

An Orthotopic Lung Tumor Model for Image-guided Microirradiation in Rats *Z. Zhang, The University of Texas Southwestern Medical Center at Dallas; D. Saha, The University of Texas Southwestern Medical Center at Dallas; M. Wodzak, The University of Texas Southwestern Medical Center at Dallas; O. Belzile, The University of Texas Southwestern Medical Center at Dallas; H. Zhou, The University of Texas Southwestern Medical Center at Dallas; S. Stojadinovic, The University of Texas Southwestern Medical Center at Dallas; R. Mason, The University of Texas Southwestern Medical Center at Dallas; R . Brekken, The University of Texas Southwestern Medical Center at Dallas; R. Chopra, The University of Texas Southwestern Medical Center at Dallas; M. Story, The University of Texas Southwestern Medical Center at Dallas; R. Timmerman, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Studies have confirmed that a new lung cancer treatment called stereotactic body radiation therapy (SBRT) can cure certain patients with early stage tumors situated peripherally in their lungs, using a non-invasive, outpatient procedure. While nearly curative for peripheral lung tumors, SBRT also may present life-threatening toxicity for patients with centrally located lung tumors. Moreover, large, hypoxic tumors are at a further risk of radiotherapy failure with SBRT. The efficacy of SBRT application can be improved through the addition of agents designed to sensitize the tumor and/or protect normal tissue. To investigate and validate the response to SBRT, alone or in combination with radiomodulating compounds, it is necessary to develop a tumor model for image-guided high-dose irradiation of rodent tumors and normal tissues in a manner that closely mimics delivery of SBRT in humans. Therefore, the purpose of this study was to develop a rat orthotopic lung tumor model with a solitary intrapulmonary nodule to study the effects of high-dose radiation. **Methods:** Adult female athymic nude rats were anesthetized, intubated and then placed on a ventilator (Inspira, Harvard Apparatus, Holliston, MA) prior to surgery. A small incision (1.0 cm) was made between ribs 5 and 6 on the right side. The right lung was captured using a forceps, clamped with a carotid clamp and H460-luc/A549-luc NSCLC cells (one million cells and matrigel in 20 μl) were then injected into the right lung. The tumor growth was monitored by in vivo bioluminescent imaging (BLI) weekly. Other imaging modalities, including microCT, CBCT, and MRI, were also performed. At the end of study, the rats were sacrificed and lungs were harvested to confirm tumor location and size. **Results:** We achieved 100% survival from this surgical orthotopic implantation procedure. We also achieved >90% success in generating solitary lung tumors in the rats, whereas percutaneous orthotopic injection of tumor cells/chunks in our previous model met only 50 to 60% success in producing solitary tumors. **Conclusion:** This study presents a successful solitary lung tumor model in rodents which provides investigators a useful model to apply conformal radiation treatment, using a sophisticated treatment planning system and significantly preventing normal tissue damage

264 **CPRIT Grantee Poster Session A**

Modeling EMT Genetic Regulatory Circuits in Metastatic Cancer *M. Lu, Rice University; M. Jolly, Rice University; B. Huang, Rice University; S. Hanash, The University of Texas M.D. Anderson Cancer Center; H. Levine, Rice University; E. Ben-Jacob, Rice University; J .Onuchic, Rice University*

Introduction: Epithelial and mesenchymal transitions (EMTs) play crucial roles in embryonic development and tissue repair. During EMT, epithelial cells lose polarity and cell-cell adhesion, and acquire mesenchymal features such as spindle-shaped morphology, cell motility and invasiveness. Aberrantly regulated EMTs are also a hallmark of cancer metastasis, which is responsible for more than 90% of cancer deaths. Yet, understanding the regulation of EMT during cancer metastasis remains a major challenge in cancer biology. It is now established that cells use genetic regulatory circuits to make functional decisions of whether or not to undergo EMT. Our main goal here is to investigate the operational principle of the core EMT circuit in decision making by using computational modeling approach. **Methods:** We constructed the core EMT regulatory circuitry that is consistent to the literature. The regulatory unit consists of two highly interconnected modules - the miR-34/SNAIL and the miR-200/ZEB mutual-inhibition feedback circuits. Since the core EMT circuit contains both transcription factors and miRNAs, we developed a theoretical framework for modeling microRNA-based circuit. The basic new advance of our model is the inclusion of multiple binding sites of miRNAs to an mRNA, and the mechanisms of miRNA-based translational silencing. Thereafter, we applied the modeling method to investigate the functions of the two isolated modules and subsequently of the combined unit when the two modules are integrated into the full regulatory circuit. We further investigated the heterogeneity of EMT in a cell population by modeling cells undergo EMT in a multicellular environment. **Results:** We show that the miR-34/SNAIL module functions as a noise-buffering signal integrator, and the miR-200/ZEB module functions as a three-way switch. This not only allows for the epithelial and mesenchymal phenotypes, but also for a hybrid phenotype with mixed epithelial and mesenchymal characteristics. We observed from our modeling results that the protein level of SNAIL is higher than that of ZEB in the hybrid phenotype due to the different regulations of miRNAs to these genes, which is consistent with recent experiment evidences. We also showed that the inhibition of miR34 by ZEB contributes to the irreversibility of TGB-beta induced EMT. **Conclusion:** By using our modeling approach, we identified the role of the core regulatory circuit in decision making of EMT. Our model explains recent data on the observation of unusual intermediates with specialized cell behavior, such as collective migration, and phenotypic heterogeneity of the EMT observed in various cancer cell lines.

265 **CPRIT Grantee**
Poster Session B

Computational Chemistry Elucidation Of Water Radiolysis And DNA Damage Reactions In Proton Cancer Therapy *J. Morales, Texas Tech University; A. Privett, Texas Tech University; R. Merrit, Texas Tech University; E. Teixeira, Texas Tech University; K. Uppulury, Texas Tech University*

Introduction: Despite established clinical use, a deep understanding of proton cancer therapy (PCT) from its underlying physicochemical and biochemical processes remains elusive. This situation prevents a rational design of PCT that can maximize its therapeutic power, minimize its side effects, and guide the construction of proton accelerators. The poor characterization of PCT processes stems from the fact that state-of-the-art experimental and clinical techniques cannot reveal all the microscopic details of PCT, especially without putting human subjects at risk. Inspired by the 2013 Chemistry Nobel Laureates, we are performing computer simulations of PCT reactions to elucidate all their microscopic details; these simulations are virtual tests harming no patients. **Methods:** Our computation method is the simplest-level electron nuclear dynamics (SLEND). SLEND employs classical mechanics for the nuclei and a single-determinant wavefunction for the electrons. SLEND uniquely describes the numerous processes (scattering, fragmentations electron transfers) that occur simultaneously during PCT. Our SLEND code PACE utilizes various state-of-the-art techniques in computer science: Python, FORTRAN and C++ languages, and intra- and internode parallelization. These techniques allow us to tackle large PCT biomolecules. **Results:** PCT healing power lies in its capacity to inflict nearly irreparable DNA damage in cancerous cells. Therefore, we simulated three types of PCT reactions involving DNA damage: (1) Proton collisions with water clusters $(H_2O)_{1-6}$ at keV energies as prototypes of water radiolysis reactions --the initial PCT reactions in cellular water that generate the radicals, ions, and electrons that damage DNA; simulations provided proton-to-cluster 1-electron-transfer and stopping integral cross sections (ICSs) in very good agreement with experimental results and predicted the formation of OH, O and H radicals involved in DNA damage. (2) Proton collisions with DNA bases at keV energies as prototypes of DNA damage by primary/secondary protons; simulations provided proton-to-base 1-electron-transfer ICSs in reasonable agreement with other theoretical and experimental results and predicted various fragmentations of the DNA bases. And (3) the single strand break (SSB) of a cytosine nucleotide by one electron as a prototype of DNA SSB by secondary electrons; simulations of one added electron to the nucleotide's antibonding molecular orbital on the O-C phosphoester bond lead to SSBs with and without solvation of the PO_4 group. This SSB mechanism is a simpler alternative to the more obvious SSB via electron capture at the DNA base. **Conclusion:** END simulations of prototypical PCT reactions satisfactorily reproduce the essential features of bulk PCT reactions. Further characterization of these PCT reactions is in progress.

266 **CPRIT Grantee**
Poster Session A

A Machine Learning Approach for Predicting Extreme Outcomes in Ovarian Cancer *B. Misganaw, The University of Texas at Dallas; M .Vidyasagar, The University of Texas at Dallas*

Introduction: Response to front line therapy of ovarian cancer patients is not uniform. Initially 70% to 80% of patients appear to respond. For instance, based on the TCGA ovarian clinical data, about 10% of patients have progression-free survival (PFS) of seven months or less. At the opposite end of the spectrum, about 10% of patients enjoy PFS of three years or more. Among the rest, most ultimately relapse and die of disease within three years. We hypothesize that if there is a set of genetic biomarkers and a predictive model that is indicative of patient response, their influence is likely to be more pronounced between the two extreme ends of patient response. **Methods:** The TCGA Agilent gene expression dataset consisting of 565 samples is chosen as the training data set. The bottom one-third, patients with PFS ≤ 283 days, were classified as non-responders (NR), while the top one-third, patients with PFS ≥ 574 days, were classified as super-responders (SR). The most informative genes are filtered with two criteria: (i) t-test statistics, and (ii) fold change between the two classes. Then a recently proposed algorithm, known as "lone star," is applied to the training data, to further reduce the feature size and build a predictive classifie . This resulted in a 25 gene signature that can discriminate between the two extreme classes. **Results:** The signature is tested on the TCGA Affymetrix dataset which consists of the same tumor samples as training dataset but measured on a different platform and the Tothill dataset which is a completely independent dataset measured on an Affymetrix platform. The evaluation is done in two ways. (i) The AUROC for the training, TCGA Affymetrix and the Tothill datasets are 0.8325, 0.7165, 0.6027 respectively. (ii) The discriminant function is computed for all patients in the sample pool. The patients are then grouped into two classes, namely: those whose discriminant value is positive, and those with negative discriminant values. This two groups have statistically significant separation between the the two Kaplan-Meier curves. **Conclusion:** Biomarkers consisting of panels of 25 genes extracted in a data-driven manner are shown to discriminate between extreme clinical outcomes. The signature is validated on a completely independent dataset is able to classify patients into groups with statistically significant survival advantage of one group over the othe .

267 **CPRIT Grantee**
Poster Session B

Histone Mutant Lifespan Screen Reveals That the H3k36me3 Promotes Longevity by Suppressing Intragenic Cryptic Transcription *W. Dang, Baylor College of Medicine*

Introduction: Longevity is regulated by both genetic and epigenetic mechanisms. Epigenetic effects are mediated by reversible DNA methylation, histone post-translational modifications, as well as changes in nucleosome and chromatin conformation. These reversible alterations have been shown to involve in nearly all nuclear and cellular functions; their misregulation has been linked to aging and attributed to many age-related diseases. **Methods:** Here, we report the results of an unbiased lifespan screen in the budding yeast S. cerevisiae using a systematic histone mutant library to identify novel epigenetic mechanisms that regulate aging. **Results:** Through this screen, we identified that methylation of H3 lysine 36 (H3K36) promoted longevity. Eliminating this modification by either mutating H3K36 or deleting the methyltransferase Set2 shortened lifespan; whereas loss of the demethylase Rph1 extended lifespan. The lifespan extension by RPH1 deletion was dependent upon the presence of H3K36, supporting that the longevity effect resulted from histone methylation. The genome-wide levels of H3K36 trimethylation (H3K36me3) decreased as cells age, commensurate with a genome-wide increase in intragenic cryptic transcription in old cells. This is consistent with the function of H3K36me3 in suppressing such cryptic transcription mediated through deacetylation of histones in gene body by recruiting histone deacetylase complex RPD3S. Disruption of the complex components that are required for binding to H3K36me3 also shortened lifespan, supporting this model. Finally, the increased levels genome-wide cryptic transcription were suppressed by deletion of RPH1, suggesting that its longevity effect is mediated by improved genome-wide control of cryptic transcription. These results represent the first evidence that intragenic cryptic transcription is a cause of cellular aging and epigenetic mechanisms can be exploited to antagonize the transcription infidelity associated with aging and to promote longevity. Further, increase in intragenic cryptic transcription was also detected in aged C. elegans and knocking down the H3K36me3 demethylase jmjd-2 extended worm lifespan in a germline-dependent manner. In mammalian cells, we also detected increased intragenic cryptic transcription in replicatively aged mesenchymal stem cells. **Conclusion:** Therefore, elevated intragenic cryptic transcription may be a conserved aging phenomenon and the

H3K36me3-mediated suppression of cryptic transcription could be an evolutionarily conserved mechanism to antagonize aging and age-associated epigenetic changes.

268 **CPRIT Grantee**
Poster Session A

Improving Retrieval of PubMed Articles Using the TopicalMeSH Representation *Z. Yu, The University of Texas Health Science Center at Houston; E. Bernstam, The University of Texas Health Science Center at Houston; T. Cohen, The University of Texas Health Science Center at Houston; B. Wallace, The University of Texas System; T. Johnson, The University of Texas Health Science Center at Houston*

Introduction: Medical Subject Headings (MeSH) was developed by the US National Library of Medicine (NLM) to better manage and search large volumes of articles in MEDLINE. But MeSH has several limitations, including: the lack of concept coverage for newly developing areas; human inconsistency in assigning codes; and the time required to manually index an exponentially growing corpus. In contrast, probabilistic topic modeling approaches can automatically index and summarize corpora. An important practical question is whether automatically generated topics have any advantage over MeSH indexing. To address this question, we introduced the TopicalMeSH representation for biomedical literature by leveraging the correspondence between latent topics (uncovered via topic modeling) and MeSH. We evaluated TopicalMeSH as a representation for document retrieval and classification on a corpus comprising 15 drug reviews. **Methods:** Latent Dirichlet Allocation1 (LDA) is a generative model that considers each document to be a mixture of latent topics, and defines these topics as distributions over words. We propose a method, which combines both LDA and MeSH by computing the correspondence between MeSH terms and topics. We represented each MeSH term as a distribution of the words contained in documents that were tagged with that MeSH term to compute its correspondence similarity with topics from LDA. Then we developed our TopicalMeSH representation by combining this correspondence with the topics' proportions in documents from LDA. We used two tasks to evaluate the utility of the TopicalMeSH representation and the original MeSH representation. The first was a document-retrieval task. We mapped the user's information needs (for a systematic review) to candidate relevant MeSH terms. We used these MeSH terms, both directly and via TopicalMeSH, to rank documents and then calculated the Average Precision (AP). The second evaluation was a document-classification task, in which we applied Support Vector Machines (SVMs) to classify documents with respect to their relevance using both TopicalMeSH and MeSH representations. F1-score (harmonic mean of recall and precision) was calculated to evaluate this task. For both tasks, we used 15 systematic drug review corpora reported by Cohen et al2 **Results:** For the document-retrieval task, TopicalMeSH achieved a higher AP score in 11 of 15 corpora. The average AP improvement from MeSH to TopicalMeSH was about 5%. For the document-classification task, TopicalMeSH achieved a higher F1–score in 14 of 15 corpora compared to MeSH **Conclusion:** The TopicalMeSH representation improves performance on information retrieval and document classification tasks involving PubMed ab tracts.

269 **CPRIT Grantee**
Poster Session B

A Multi-Layered, Hyaluronic Acid-Based Hydrogel System for Automated 3D High Throughput Drug Screening of Cancer-Stromal Cell Co-Cultures *B. Engel, Rice University; P. Constantinou, Rice University; L. Sablatura, Rice University; N. Doty, ESI Bio; D. Carson, Rice University; M. Farach-Carson, Rice University; D. Harrington, Rice University; T. Zarembinski, BioTime*

Introduction: Pre-clinical drug screens, that involve culturing well-annotated cancer cell lines on two-dimensional (2D) tissue culture plastic, poorly recapitulate in vivo tumor characteristics and yield candidate drugs which tend to fail clinical trials. Use of advanced three dimensional (3D) culture platforms for pre-clinical drug screening could increase clinical trial success of candidate drugs. **Methods:** We created a multi-layer, hyaluronic acid (HA)-based hydrogel system that incorporates three layers: an acellular cushion layer; an encapsulated cancer cell layer for growth in three dimensions (3D); and a collagen-containing layer that supports the growth of stromal cells on top of the hydrogel (2.5D). This platform was incorporated into 384-well plates using standard high-throughput robotic delivery from the Gulf Coast Consortium Chemical Genomics High Throughput Core facility (supported by CPRIT grant RP110532-P2). High throughput drug screens using clinically relevant compounds were evaluated with live/dead/nuclei stain using automated imaging systems and automated imaging analysis. **Results:** This formulation provides a highly reproducible system for spheroid culture of prostate (C4-2B) or endometrial (Ishikawa) cancer cell lines in mono- or co-culture with matched stromal cells (HS27a or ESS-1, respectively). Both culture systems provided high cancer cell viability over one week of culture. Compared to 2D and 3D alginate cultures, the 3D HA culture system more accurately predicted efficacy of clinically relevant compounds. **Conclusion:** Cells cultured in our 3D multi-layer HA-based system responded to cytotoxic drugs distinctly from cells grown in 2D and 3D-aliginate, and better predicted the efficacy of chemotherapeutics in clinical trials. Wider adoption of 3D automated screening has the potential to speed drug discovery and increase success of drugs in clinical trials.

270 **CPRIT Grantee**
Poster Session A

Mechanobiology and cell-matrix cues that induce EMT in a lung adenocarcinoma model *R. Han, Rice University; D. Puperi, Rice University; C. Wanna, Rice University; J. Onwenu, Rice University; D. Gibbons, The University of Texas M.D. Anderson Cancer Center; J. Grande-Allen, Rice University*

Introduction: A mouse model of human lung adenocarcinoma driven by mutations in K-ras and p-53 genes was adapted to investigate the interplay between the mechanical environment and cellular epithelial-to-mesenchymal transition (EMT) potential. Tumor cells were grown in collagen gels under different tensile loading conditions with or without TGF-β1, in order to elucidate the resulting mechanobiological behavior and cell-matrix interactions. M**ethods:** Metastatic (344SQ) and non-metastatic (393P) murine lung tumor cells were encapsulated in rat-tail type-I collagen gels, and studied in three systems: 1) under zero tension (free contraction) in 48-well plate wells for 7 days; 2) under static tension (anchored) for 7 days; 3) under dynamic tension (5% cyclic stretch, 0.5Hz) for 3 or 6 days. TGF-β1 was added to stimulate EMT. Collagen gels were imaged using still photography, light microscopy, SEM, and TEM to assess contraction and cell/matrix distribution. The ratio of collagen to non-collagen protein was assessed via semi-quantitative staining and the elastic modulus was determined via mechanical tensile testing. **Results:** Under the no-tension (freely contracting) condition, cells significantly contracted collagen gel within the first 3 days of culture. After 7 days, metastatic cells contracted the gels more than did non-metastatic cells (22.3%, 13.4% and 1.3% more in 0.5, 1.0, and 2.0mg/ml collagen respectively), despite similar collagen/non-collagen protein ratios. Under static tension (anchored), gels containing metastatic cells were 15.3% more contracted and 3.4 folds stiffer than for non-metastatic cells (for 2mg/ml collagen). After 6 days of dynamic (cyclic) tension, the gels containing metastatic cells were 75% stiffer than those with non-metastatic cells. When gels grown under tension were examined microscopically, the non-metastatic cells appeared rounded and clustered together, whereas the metastatic cells were elongated, arranged in rows, and closely aligned with collagen fibers. In all mechanical conditions, the addition of TGF-β1 induced accelerated contraction, an elevated collagen/non-collagen protein ratio, and more collagen fiber alignment. Even smallest concentrations (0.1ng/ml) of TGF-β1 caused gels containing the non-metastatic cells to snap under dynamic tension. C**onclusion:** Under static or dynamic tension, the metastatic cells closely engaged with collagen fibers and contracted the overall collagen gel more than did the non-metastatic cells, resulting in stiffer structures. The TGF-β1 induced EMT enhanced these cell-matrix interactions and generated additional contractile forces, which were borne better by the even dispersion of metastatic cells within the collagen compared to the clustered nature of non-metastatic cells. Further study is required to understand the interaction of mechanical, cytokine, and ECM signaling in lung cancer.

271 **CPRIT Grantee**
Poster Session B

Direct evidence of acinar to ductal metaplasia in human pancreatic tissue *J. Liu, The University of Texas Health Science Center at San Antonio; N. Akanuma, The University of Texas Health Science Center at San Antonio; P. Wang, The University of Texas Health Science Center at San Antonio*

Introduction: Pancreatitis can induce acinar-to-ductal metaplasia (ADM) in both human and mice. Animal studies have indicated that ADM is a key event for KRAS-driven pancreatic ductal adenocarcinoma (PDAC) tumorigenesis. However, ADM-induced changes in acinar cells are still not clear. This is especially true for human acinar cells due to lack of a proper system for research. **Methods:** Flow cytometry-based strategy was developed for lineage tracing of cells in human primary exocrine pancreatic tissues. The primary human tissues were also treated with cytokines and growth factors to identify ADM inducer. Acinar cells were further sorted by flow cytometry to directly test their lineage identity and plasticity. **Results:** Human pancreatic acinar and ductal cells are characterized by the surface staining pattern of UEA-1hghCLA-CD133- and UEA-1lowCLA+CD133+, respectively. During ADM, acinar cells gained expression of ductal marker CD133 but not CLA, a novel ductal marker we identified. ADM also allowed acinar cells to obtain transient proliferation capacity. We further identified TGF-β1 as a potent factor to induce ADM and allow acinar cells to gain ADM-associated alterations. Both Smad and MAPK pathways are involved in TGF-β1-induced ADM in human acinar cells. **Conclusion:** Human acinar cells have the plasticity, which allows them to be converted to ductal-like cells and TGF-β1 is a potent inducer to promote this conversion. Ductal-like cells from acinar not only down-regulate acinar marker genes and up-regulate some ductal marker genes but they also gain transient proliferation capacity. Our results suggest a possible mechanism of how ADM contribute to pancreatic cancer development.

272 **CPRIT Grantee**
Poster Session A

High-throughput Generation and Characterization of Cellular Aggregates by CO2 Laser Ablation of Poly(dimethylsiloxane) *J. Albritton, Rice University; J. Roybal, MD Anderson Cancer Center; S .Paulsen, Rice University; J. Flores-Zaher, Rice University; M. Farach-Carson, Rice University; D. Gibbons, The University of Texas M.D. Anderson Cancer Center; J. Miller, Rice University*

Introduction: Aggregates of cells or multicellular aggregates (MCAs) have been used for decades in the fields of cancer, regenerative medicine, and embryology; however, uniform MCAs are challenging to manufacture in large quantities. Forced aggregation into microwells offers a promising solution, but commercial sources are expensive, and photolithographic methods for generating new well molds are costly, time-consuming, and require significant technical expertise. **Methods:** We demonstrate a cost-effective CO2 laser ablation system for fabricating microwells in poly(dimethylsiloxane) (PDMS). We achieved this by modifying a relatively inexpensive CO2 laser cutter with an open-source 3D printing microcontroller workflo , a z-axis stage, and a vacuum to prevent ablation debris accumulation. Our system produces microwells in the forms of circular inserts for standard multiwell tissue culture plates that can be seeded with cells in a single pipette step. **Results:** Using our system, we generated more than 100,000 MCAs with low diameter polydispersity (62.0 ± 10.8 μm diameter) when seeding at 25 cells/microwell cell density. We show human bone marrow derived mesenchymal stem cell (hBM-MSC), 344SQ murine metastatic adenocarcinoma cells, and C4-2 prostate cancer cells can aggregate in our system. Moreover, 344SQ aggregates maintain hTGF-β sensitive invasive phenotype when plated on Matrigel® in a 2.5D assay, similar to previous results. Additionally, hBM-MSC aggregates show sprouting after 2 days in 3D fibrin gels. **Conclusion:** Open-source hardware and low cost hardware decrease the barriers for other laboratories to adopt new technologies, therefore we expect this technique to find broad utility in the generation and cultivation of primary cell aggregates, cancer aggregates and embryoid bodies.

273 **CPRIT Grantee Poster Session B**

Uncovering Rules Governing Functional Replacement Between Humans and Yeast *J. Laurent, The University of Texas at Austin; A .Kachroo, The University of Texas at Austin; C. Yellman, The University of Texas at Austin; A. Meyer, The University of Texas at Austin; C. Wilke, The University of Texas at Austin; E. Marcotte, The University of Texas at Austin*

Introduction: Owing to its ease of handling and rapid growth, the baker's yeast Saccharomyces cerevisiae is a popular model organism for studying many aspects of eukaryotic biology. Due to the functional conservation still present between many human and yeast proteins, yeast has served an important role in the study of human cancers, especially in regards to control of the eukaryotic cell cycle and elucidation of DNA repair pathways. Inspired by these studies, our lab has been systematically testing which essential yeast proteins are replaceable by their human counterparts, screening for functionality by the human genes' ability to rescue growth in the absence of the yeast proteins. **Methods:** In order to assay rescue of essential yeast genes, we have taken advantage of three separate mechanisms of removing yeast gene function, either by down-regulation with a repressible promoter, removal of the functional yeast protein with temperature-sensitive mutants, or by segregating away the functional allele following sporulation of a heterozygous diploid knockout strain. Human genes were expressed either by constitutive or inducible expression. To determine what factors determine replaceability, we assembled a set of quantitative features of the genes or ortholog pairs, including calculated properties of the genes' sequences and other properties such as protein interactions, mRNA and protein abundances, transcription and translation rates, and mRNA splicing features. **Results:** To date we have tested nearly 600 pairs of orthologs between the two species, and have observed a ~50% rate of replaceability. We have further quantified how well each of over 100 quantitative features predicts replaceability. Strikingly, sequence similarity only weakly predicts replaceability. Instead, replaceability depends most strongly on gene modules: genes in the same process tended to be similarly replaceable (e.g. proteasome, sterol biosynthesis) or not (e.g. DNA replication, TriC chaperonin). **Conclusion:** Our data demonstrate that a substantial portion of conserved yeast and human genes perform much the same roles in both organisms even after >1 billion years of evolution—such that the protein-coding DNA of a human gene can actually substitute for that of the yeast. The pathway-specific pattern of individual replacements suggests that group-wise replacement of genes should be feasible, raising the possibility of humanizing entire cellular processes in yeast. Many of the replaceable genes have important roles in human disease, including cancer. Such 'humanized' strains would simplify drug discovery against human proteins, enable studies of the consequences of human genetic polymorphisms, and empower functional studies of entire human cellular processes in a simplified organism

274 **CPRIT Grantee Poster Session A**

Metabolic MRI: Modeling and Constrained Reconstruction of Hyperpolarized Pyruvate *J. Bankson, The University of Texas M.D. Anderson Cancer Center; C. Walker, The University of Texas M.D. Anderson Cancer Center; M. Merritt, The University of Texas Southwestern Medical Center at Dallas; C. Malloy, The University of Texas Southwestern Medical Center at Dallas; D. Sherry, The University of Texas Southwestern Medical Center at Dallas; S. Lai, The University of Texas M.D. Anderson Cancer Center; J. Hazle, The University of Texas M.D. Anderson Cancer Center*

Introduction: Dissolution dynamic nuclear polarization provides more than 10,000-fold increase in signal from key metabolic agents such as [1-^{13}C]-pyruvate. Hyperpolarized (HP) pyruvate is the most widely studied HP agent to date because of its favorable kinetics, relatively long T_1, and the central role of pyruvate in metabolism. HP pyruvate of particular interest in oncology because metabolism is often altered in cancer and affected by therapy. Imaging of HP agents such as pyruvate is challenging due to the transient and non-renewable signal enhancement that is depleted with each excitation pulse. New imaging methods are needed in order to ensure optimzal measurements under these conditions. We describe a new kinetic model and constrained reconstruction algorithm to reduce the data sampling burden and minimize new information that must be encoded and measured from observations of HP pyruvate and its metabolites. M**ethods:** The kinetic model and constrained reconstruction algorithm were implemented in Matlab. The model was initially tested using data acquired from animals bearing anaplastic thyroid cancer (ATC), glioblastoma, and triple-negative breast cancer dynamic pulse-acquire spectroscopy (TR 2s, 10°-15° excitation). For imaging, a radial multi-band frequency encoded spectroscopic imaging sequence (TR/TE 750ms/165ms, 20° excitation, 3cm FOV) was used to acquire data from anesthetized animals with ATC tumors following administration of 80mM HP pyruvate (200uL). Constrained reconstruction enforced consistency between prior information and observations that were spatially and temporally under-sampled. **Results:** The pharmacokinetic model consisting of two spatial pools (intravascular/extravascular) and two chemical pools (pyruvate/lactate) was found to provide the best compromise between physiological accuracy and computational complexity. This model gives a closed-form solution for signal evolution, providing information about correlations in time that can be exploited to dramatically reduce the amount of raw data that must be measured in order to reconstruct dynamic spectroscopic imaging data. Additional information can be derived from traditional MRI methods to further simplify the model and eliminate unknowns that must be determined from ^{13}C measurements. C**onclusion:** The model-based constrained reconstruction algorithm improved our ability to visualize dynamic evolution of HP pyruvate and lactate from undersampled imaging data. This framework can be integrated with alternative models and/or imaging strategies to allow optimized distribution of spatial and temporal sampling for HP MRI, and will provide important guidance for clinical translation of this exciting new metabolic imaging modality.

275 **CPRIT Grantee Poster Session B**

Novel Murine Model of Dose-Escalated Fractionated Radiotherapy to the Upper Abdomen *C. Taniguchi, The University of Texas M.D. Anderson Cancer Center; T. Fujimoto, The University of Texas M.D. Anderson Cancer Center; J. Molkentine, The University of Texas M.D. Anderson Cancer Center*

Introduction: Definitive chemoradiation to the head of the pancreas is challenging since pancreatic cancer requires radiation doses in excess of 60Gy to achieve local control, but the nearby duodenum can only tolerate a maximum of 50Gy, which limits the dose which can be safely administered to only 50Gy. Unfortunately, there are no FDA-approved treatments to reduce radiation damage to the GI tract that would allow us to achieve this increased radiation dose. We previously published that the PHD proteins are potent regulators of radiation sensitivity in the intestine and the inhibition of the intestinal PHD proteins by genetic knockout or with a small molecule was sufficient to protect mice from a single lethal dose of abdominal radiation. Whether these findings would translate to clinical relevant fractionated radiation, however, was unknown. There are no methods to study the effects of fractionated radiation in mice, as mice were previously thought to be unable to tolerate multiple radiation treatments. **Methods:** Here we tested the logistics and tolerability of a fractionated radiation treatment with a biologically equivalent dose sufficient to ablate pancreatic cancer. We subjected male C57Bl/6 mice 8 weeks of age to 60Gy in15 fractions (BED_{10}=84 Gy) or 75 Gy in 15 fractions (BED_{10}=112.5Gy), with and without the oral PHD inhibitor FG-4592 for radioprotection. Mice were gavaged with FG-4592 or vehicle control 15 minutes before receiving isoflurane anesthesia, which was used for immobilization during radiation. We used a subxiphoid radiation field 15mm in diameter, which would encompass the entire pancreas, duodenum, as well as a portion of the stomach and the left lobe of the liver. Mice were treated with an XRAD225 system with CBCT capability. The endpoints were survival and weight loss. **Results:** Surprisingly, the 60Gy dose was well tolerated with vehicle control, despite exceeded known tolerance for the duodenum. The mice in the 60Gy group gained weight whether they received PHD inhibitor or saline control. All mice were alive after 30 days. In the 75Gy dose group, however, 80% of mice who received daily PHD inhibitor lived beyond 30 days, while control mice all died within 30 days of ending treatment. **Conclusion:** We have developed a novel model to study fractionated, dose escalated radiation treatment to the pancreas and surrounding normal organ in a mouse model. These data should facilitate the study of fractionated radiation treatments in autochthonous tumors models of pancreaticobiliary tumors, something which was thought to be impossible prior to our study.

276 **CPRIT Grantee Poster Session A**

Doxycycline-Inducible CRISPR-Cas9/Cas9n Mice for Cancer Modeling *Y. Huang, Baylor College of Medicine; Y. Lee, Rice University; S. Chan, University of Minnesota; M. Kyba, University of Minnesota; M. Goodell, Baylor College of Medicine*

Introduction: Cancers are often caused by specific gene mutations. Recent discoveries involving the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated RNA-guided endonuclease Cas9 have made eukaryotic genome editing feasible. Cas9 utilizes single-guide RNA (sgRNA) to target genomic DNA sequences, creating mutations or precise corrections with homology-directed repair. Genome editing not only allows us to correct mutations but also enables us to recreate humanized mutations in murine models, which further facilitates cancer research. However, the Cas9 sequence is difficult to deliver due to its large size, and prolonged expression of the Cas9 protein may lead to off-target mutations. Knocking-in the Cas9 sequence under an inducible promoter mitigates these two factors. Nevertheless, off-target mutations might occur due to non-specific sgRNA even when Cas9 is expressed for a limited time. To overcome this obstacle, Cas9 nickase (Cas9n), which is Cas9 D10A point mutation, with a combination of two SgRNAs can increase specificity up to 1000 times. Our goal is to generate a doxycycline-inducible Cas9/Cas9n murine model that enables the establishment of humanized mutations for cancer modeling. **Methods:** In murine embryonic stem cells (mESCs) containing reverse tetracycline-controlled transactivator in ROSA26 loci, Cas9 and Cas9n sequences were engineered into Hprt loci via inducible cassette exchange. Chimera mice were generated by injection of iCas9 and iCas9n mESCs into blastocyst and then bred to C57BL/6J. Under 10mg/ml doxycycline treatment for 5 consecutive days, hematopoietic progenitor cells were purified and we confirmed their Cas9/Cas9n expression. **Results:** In both mESCs and hematopoietic progenitor cells upon doxycycline treatment, Cas9/Cas9n expression was confirmed through Western Blots and Cas9/Cas9n functionality was also confirmed by surveyor assay under transduction of sgRNA targeting Tet2 and Dnmt3a. **Conclusion:** An inducible Cas9/Cas9n system allows for temporal control of Cas9/Cas9n expression, decreasing off target effects. This system also decreases the complexity of genome editing with Cas9, requiring only the introduction of sgRNA. The inducible Cas9 mouse model described in this abstract enables us to generate humanized mutations in mice and provides a screening platform for cancer therapeutic targets.

277 **CPRIT Grantee Poster Session B**

CLAMP (Clinical Language Annotation, Modeling and Processing) As a Toolkit for Processing Free Text Clinical Notes in Cancer Patients *E. Soysal, The University of Texas Health Science Center at Houston; J. Wang, The University of Texas Health Science Center at Houston; M. Jiang, The University of Texas Health Science Center at Houston; Y. Wu, The University of Texas Health Science Center at Houston; H. Xu, The University of Texas Health Science Center at Houston*

Introduction: Cancer is a chronic disease which requires a multidisciplinary approach and coordination among different specialties. Exponentially increasing data size and information exchange requirements make it crucial to use computational methods to process data. Nevertheless, diversity of these information withholds implementation and use of completely structured models in the cancer domain. So, clinical notes become valuable sources of information during the long term follow up of cancer patients. Developments in natural language processing methodologies promise to process these information with an increasing accuracy. **Methods:** We developed a set of state-of-art natural language processing components working as a high performance framework that is tightly coupled with an integrated development environment to visually develop custom algorithms specific to clinical information requirements. Knowledge resources required by these accompanied components like dictionaries, section header list or medical abbreviation list are provided with the toolkit. CLAMP is also integrated with an annotation tool to annotate entities and relationships. This helps user to adapt machine learning algorithms and ease the customization for specifi data and task. **Results:** CLAMP completely automated natural language processing projects as an all-in-one solution. It helped users with limited technical skills to develop task specific machine learning models, starting from development of target specific corpus by annotation of clinical notes to training of machine learning algorithms using these corpora, in a completely integrated environment. **Conclusion:** CLAMP can successfully be utilized in clinical notes of cancer patients by contributing to manage information in clinical notes. CLAMP promises an easy to use desktop application without sacrificing functionality. It serves a complete set of components to achieve the best possible results for clinical text processing, with the best proven approaches using a mixture dictionary based, rule based and machine learning methodologies.

278 **CPRIT Grantee Poster Session A**

Dynamic Regulation of Pol I During Differentiation in a Multicellular Organism *V. Palacios, The University of Texas Southwestern Medical Center at Dallas; M. Buszczak, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Enhanced ribosome biogenesis and protein synthesis help drive tumor formation and growth. rDNA gene transcription represents the first step and limiting step in ribosome biogenesis and tumor cells display increased levels of rRNA synthesis. Furthermore, defects in ribosome biogenesis cause a group of human diseases, collectively known as ribosomopathies, which pre-dispose patients to cancer. Much of what we know about rRNA transcription and ribosome biogenesis has been obtained from studies using yeast and mammalian cell lines. However, emerging evidence shows that ribosome biogenesis and protein production vary greatly among different cell types within multicellular organisms. The mechanisms that regulate RNA polymerase I (Pol I) activity and ribosome biogenesis in vivo remain poorly understood. Pol I exclusively transcribes the 18S, 5.8S and 28S rDNA genes which, together with the Pol III dependent 5S rRNA, form the catalytic domain of the ribosome. Identifying new rRNA transcriptional regulators may lead to novel therapies for the treatment of cancer. Up until recently, few models existed for studying Pol I regulation in an in vivo setting. We recently discovered a Drosophila Pol I regulatory complex (SL1 complex) composed of at least three proteins, Under-developed (Udd), Taf1B and Taf1C-like. We further showed that Pol I activity is dynamically regulated within the Drosophila female germline stem cell (GSC) lineage and that differences in ribosome biogenesis between stem cells and their differentiating daughters have important functional consequences for the fate and function of these cells. **Methods:** To assist in our efforts to further characterize the regulation of Pol I activity, I have generated knockout alleles for three different members of the SL1 complex. I am now generating a series of transcriptional and translational reporters that will help us characterize the mechanisms that govern the expression of SL1 components. Additionally, mass-spec analysis shows that SL1 members physically interact with the importin-beta protein Ranbp9. We are in the process of generating Ranbp9 null mutations using CRISPRcas9 to test the extent to which this factor influences the trafficking of the SL1 complex in and out of the nucleus. **Results: Conclusion:** Data obtained through these studies will help us determine the mechanisms that regulate Pol I activity in vivo. Such information will foster the development of new approaches for combating Pol I-dependent tumor growth.

279 **CPRIT Grantee Poster Session B**

A Publically Available Database for Exploring the Modulators of Multiple Transcription Factors *X. Li, The University of Texas Medical Branch at Galveston; M. Zhu, The University of Texas Medical Branch at Galveston; B. Fongang, The University of Texas Medical Branch at Galveston; A. Brasier, The University of Texas Medical Branch at Galveston; A. Kudlicki, The University of Texas Medical Branch at Galveston*

Introduction: A great amount of expression data based on microarray and RNA-Seq have been increasingly deposited in public databases in the past decades. These data sets provide important information in reverse engineering of the transcriptional and post-transcriptional regulation of gene expression underlying various stimulation, biological or clinical conditions, including cancer progression, trauma and development. However, transcription factor (TF) activity is often regulated by modulators, which enhance, attenuate or invert the activity of the transcription factors in inhibiting or activating gene expressions at the posttranscriptional level. To facilitate the experimental biologist for hypothesis generation and validation, we constructed a modulatory network database in this study. **Methods:** To identify the modulators that affect the TF activity in a target gene unique way, we predicted all the modulator-TF-TG triplets by integrating TF binding data from ChIP-Seq or ChIP-chip experiments, gene expression data and protein-protein interaction, based on a probabilistic model with an interactive term. Based on all the predicted triplets, we construct a database for exploring the modulators of multiple transcription factors (transMOD, http://www.transmod.net/). **Results:** transMOD provides multiple filters and statistical analysis with different options and unique applications for molecular biology experts in different research areas: general or context-specific modulatory network, modulatory network constrained by protein-protein interaction of TFs, kinase-substrate interaction, or non-constrained network. The datasets in the database can be freely downloaded for user oriented research interest as well. **Conclusion:** Our modulatory network database provides experimental biologists important testable modulator-transcription factor-target gene triplet hypothesis in diverse cellular processes for validation.

280 **CPRIT Grantee Poster Session A**

Establishment, Validation, and Drug Resistance Patterns of Cell Lines and Patient-Derived Xenografts Established from Childhood Cancer Post-Mortem Samples *M. Song, Texas Tech University Health Sciences Center; D. Woodburn, Texas Tech University Health Sciences Center; H. Davidson, Texas Tech University Health Sciences Center; H. Hall, Texas Tech University Health Sciences Center; K. White, Texas Tech University Health Sciences Center; K. McCoy, Texas Tech University Health Sciences Center; J. Nance, Texas Tech University Health Sciences Center; R. Blaydes, Texas Tech University Health Sciences Center; A. Hindle, Texas Tech University Health Sciences Center; B. Koneru, Texas Tech University Health Sciences Center; M .Makena, Texas Tech University Health Sciences Center; M. Hogarty, Children's Hospital of Philadelphia (CHOP); J. Maris, Children's Hospital of Philadelphia (CHOP); Y. Mosse, Children's Hospital of Philadelphia (CHOP); A. Marachelian, Children's Hospital Los Angeles (CHLA); A. Erdreich-Epstein, Children's Hospital Los Angeles (CHLA); L. Mascarenhas, Children's Hospital Los Angeles (CHLA); R. Seeger, Children's Hospital Los Angeles; S. Shusterman, Dana-Farber Cancer Institute; S. Roberts, Memorial Sloan Kettering Cancer Center; M. Al-Rahawan, St. Jude Children's Research Hospital Midwest Affiliat Clinic; N. Winick, The University of Texas Southwestern Medical Center at Dallas; M. Irwin, The Hospital for Sick Children; M. Kang, Texas Tech University Health Sciences Center; C. Reynolds, Texas Tech University Health Sciences Center*

Introduction: Cancer cell lines (CLs) and patient-derived xenografts (PDXs) are laboratory models essential for biological and preclinical therapeutic studies. Establishing CLs and PDXs from samples obtained post-mortem (PM) can potentially provide models of disease that has failed all current therapies. M**ethods:** Blood, bone marrow, and/or tumor samples for neuroblastoma (NB), Burkitt lymphoma (BL), glioblastoma multiforme (GBM), Ewing sarcoma (ES), embryonal rhabdomyosarcoma (eRMS), and Wilm's tumor (WT) were collected post-mortem, with written consent of family, from participating Children's Oncology Group (COG) institutions. Processed samples were placed in culture and/or directly xenografted in NOD scid gamma (NSG) mice. All established CLs and PDXs were matched to patient samples via short tandem repeat (STR) analysis, verified to be EBV free via PCR, verified to be mycoplasma free via MycoAlert enzymatic assay, and shown to express telomerase

(TERT) by RT-qPCR. In vitro drug sensitivity was determined by DIMSCAN cytotoxicity dose-response assay. **Results:** Of 54 samples received, 61% were blood (volume: 50-200mL), 11% bone marrow, and 28% tumor. To date, 32 PM continuous CLs (25 NB, 4 BL, 1 GBM, 1 ES, & 1 eRMS) and 13 PM PDXs (10 NB, 1 BL, 1 ES, & 1 WT) have been established and validated through this multi-institutional endeavor; 12 of 13 PM PDXs have matching direct-to-culture CLs. For NBs, 25 of 40 placed in culture (63%) generated a CL and 10 of 16 injected in mice (63%) generated a PDX. Drug resistance was defined as a cytotoxic/inhibitory concentration for 90% of cells (IC90) > clinically achievable drug plasma concentration. For the 25 NB CLs, the percentage manifesting resistance in vitro was 96% (4-hydroperoxy-cyclophosphamide, active metabolite of cyclophosphamide), 88% (melphalan), 72% (carboplatin), 16% (doxorubicin), 36% (etoposide), 44% (SN-38, active metabolite of irinotecan), 28% (topotecan), 16% (vincristine), and 8% (fenretinide). **Conclusion:** The majority of the PM samples obtained were heparinized blood samples, which are readily obtained and had a 70% success rate at establishing a CL or PDX. The 63% success rate of establishing NB PM CLs and PDXs was higher than those observed for 1838 NB pre-treatment (CLs: 6.6%, PDXs: 9.7%) and 246 NB progressive disease (CLs: 17.1%, PDXs: 2.9%) samples. Establishing CLs and PDXs post-mortem is feasible and provides unique laboratory models of chemotherapy-refractory cancers. The CLs and PDXs described here are available via the COG Cell Line and Xenograft Repository (www.COGcell.org).

281 **Poster Session B**

An Integrated Systems Biology Approach to Modeling Cancer Metastasis Circuits *M. Lu, Rice University*

Introduction: Understanding cell fate decisions of epithelial-to-mesenchymal transitions (EMT) during cancer metastasis remains a major research challenge in cancer biology. Previously, we constructed the core EMT regulatory circuit, composed of the miR-34/SNAIL and the miR-200/ZEB mutual-inhibitory feedback loops. We showed that, in addition to the epithelial (E) and the mesenchymal (M) phenotypes, the core EMT circuit allows an additional hybrid phenotype with mixed E and M characteristics. This prediction has been supported by multiple experiments. As a next step, we investigated the behavior of other cellular functions, such as cell motility and metabolism, during EMT. A better understanding of these connections might help to identify new drug targets for improved therapeutic strategies of metastatic cancer **Methods:** We first constructed the core regulatory circuits for both cell motility and metabolism. The cell motility circuit is composed of two GTPases Rac1 and RhoA, and the metabolic regulatory circuit is composed of HIF-1, AMPK and ROS. We further connected these circuits to the EMT circuit. We performed computational modeling to study the coupling among the EMT, cell motility and metabolism. **Results:** We show that the cell motility circuit allows for four stable states that correspond to the amoeboid (A), the M, the A/M, and the E/M phenotypes, respectively. Besides, we predict that the expression of the two miRNAs drive cancer cells away from the metastatic phenotypes by inhibiting both Rac1 and RhoA. Our modeling approach allows mapping of the miRNA levels to the transitions among various cell motility phenotypes. These predictions show good agreement to experimental observations. We also show that cancer cells have three stable metabolic states – the Warburg state (W: high HIF-1, low AMPK), the oxidative state (O: low HIF-1, high AMPK), and the hybrid state (W/O: high HIF-1, high AMPK). Yet, normal cells lack the hybrid phenotype because of their lower mitochondrial ROS production and higher HIF-1 degradation. We propose that the hybrid metabolism contributes to cancer metabolic plasticity, thus allowing cancer cells to adapt to changes in microenvironment and to promote cell proliferation and partial EMT. In addition, our analysis shows that therapies targeting glycolysis and oxidative phosphorylation are distinct in reducing cancer metabolic plasticity, which may explain their different efficacy in cancer treatments. **Conclusion:** By using a modeling approach, we elucidated the interplay among the EMT, cell motility and metabolism. Our model explains the plasticity of cancer cell behavior and suggests improved therapeutic strategies by targeting the hybrid cell phenotype.

282 **Poster Session A**

A High-Throughput Mechanofluidic Screening Platform for Investigating Circulating Tumor Cell Adhesion during Metastasis *A. Spencer, The University of Texas at Austin; C. Spurell, The University of Texas at Austin; S. Nandi, The University of Texas at Austin; M .Crexiell, The University of Texas at Austin; A. Baker, The University of Texas at Austin*

Introduction: A fundamental limitation in the development of new therapies to prevent metastatic cancer is a lack of in vitro systems that can accurately recapitulate the steps of cancer metastasis. Currently, most assays for examining the steps of metastasis fail to incorporate biophysical forces experienced by tumor cells due to blood flo , or are low throughput and thereby not amenable to drug screens or high throughput experimentation. **Methods:** We have developed a novel high throughput mesofluidic platform for assaying cell adhesion under flow in a 96-well format. This device functions like a cone and plate viscometer in each well by inducing shear stress on cells cultured in a standard 96-well plate. We validated the fluid flow and alignment of the device and studied the adhesion of multiple cancer cell lines (MDA-MB-231 and MCF-7 breast cancer cell lines) to extracellular matrix (ECM) molecules, endothelial cells and immobilized platelets. Assays were carried out under flow (0.5 dynes/cm^2 shear stress) and static conditions. **Results:** Our studies show that adhesion assays performed under flow yield markedly different results from static assays. Treatment of breast cancer cells with a small library of integrin inhibitors demonstrated that these compounds had minimal effect on cancer cell adhesion to endothelial cells or immobilized platelets under static conditions, whereas under laminar shear and oscillatory shear conditions many compounds significantly reduced adhesion of cancer cells. A static adhesion assay of breast cancer cells to various types of ECM showed greater adhesion of the less aggressive MCF-7 cells compared to the more aggressive MDA-MB-231 cells. Meanwhile, flow incorporating assays showed increased adhesion of the more aggressive MDA-MB-231 breast cancer cells. Finally, we performed a high throughput screen using a kinase inhibitor library of 80 compounds and found the shear based assay yielded notably different results from a similar screen under static conditions for breast cancer cell adhesion to endothelial cells, immune cell adhesion to endothelial cells and breast cancer cell adhesion to platelets. This shear experiment yielded seven “hits”, many of which match targets of drugs in clinical trials. **Conclusion:** In conclusion, our studies show that adhesion assays performed under

flow yield markedly different results from static adhesion assays, and better identify aggressive cancer cells lines and known pathways for circulating cancer cell adhesion. Thus, this high-throughput screening platform may enable development of novel compounds to inhibit cancer metastasis and facilitate the study of systems level behavior of cancer-endothelium adhesion.

283 **Poster Session B**

Baylor College of Medicine Knock-out Mouse Phenotyping (KOMP2) Program - Opportunities for Researchers *J. Seavitt, Baylor College of Medicine; D. Lanza, Baylor College of Medicine; J. Heaney, Baylor College of Medicine; A. Beaudet, Baylor College of Medicine*

Introduction: Baylor College of Medicine houses NIH-funded Knockout Mouse Phenotyping (KOMP2) production and phenotyping pipelines, generating and evaluating mouse model lines from the KOMP ES cell repository as well as bespoke CRISPR alleles. The project's purpose is to contribute to broad, standardized phenotyping of a genome-wide collection of mouse knockouts. ES cells, model mouse lines, and phenotyping results are publicly available to researchers. M**ethods:** The Baylor KOMP2 program produces conditional-ready and reporter null allele lines in a high-throughput production pipeline. Cohorts are phenotyped in standardized and quality-controlled adult and embryonic platforms to generate robust, high quality datasets. **Results:** The Baylor KOMP2 BaSH Consortium has completed development of its planned production and phenotyping pipelines, as well as its initial production goals of 885 mutant mouse lines. Mouse lines are available either from the Baylor KOMP2 program directly or from the KOMP2 repository. Phenotyping data is transferred to the International Mouse Phenotyping Consortium's Data Collection Center for public curation at mousephenotype.org. **Conclusion:** The Baylor KOMP2 program can provide further resources for Texas investigators. We have extensive production capacity and seek investigators' suggestions for specific genes to target. These lines will be generated by CRISPR and if possible it will be our intention to generate conditional alleles, with loxP sites flanking a critical region. Null alleles will be generated in all cases and cohorts entered into the Baylor KOMP2 phenotyping pipelines. Any alleles generated will be immediately available for distribution. Our strong preference is for genes for which there are no extant mutant alleles and whose generation will support investigators' research interests. The Baylor pipelines are also capable of supporting proposals for secondary examination of lines by investigators, either broadly or upon triage by initial phenotyping results. Finally, we are able to consult regarding KOMP repository ES cell holdings, regardless of their local assignment, for investigators considering obtaining such lines.

284 **Poster Session A**

Computational Study of Allostery of Protein VIVID, a PAS/LOV Domain in Circadian Clock System *P. Tao, Southern Methodist Univesity; H. Zhou, Southern Methodist University; B. Zoltowski, Southern Methodist University*

Introduction: PAS domains mediate cell signaling pathways that control cancer cell growth and differentiation. Allostery is the main mechanism for signal transductions through protein complexes in all PAS mediated signaling pathways. Developing allosteric drugs targeting key proteins in signaling pathways are becoming important revenues in cancer drug discovery. However, the understanding of protein allosteric mechanisms is the major bottleneck in this effort. To make breakthrough, fully understanding allostery that is highly correlated with protein dynamics is inevitable. Recently, we developed a novel simulation method, named as rigid residue scanning (RRS), to systematically identify key residues for allostery. In this study, we employed RRS method to explore the allosteric mechanism of VIVID protein from Light-Oxygen-Voltage (LOV) domains in circadian clock system. **Methods:** In RRS method, an efficien rigid body dynamics integrator we developed previously was applied to carry out MD simulations to systematically probe the effect of removing individual residue internal degrees of freedom on the whole protein dynamics. Several indices are generated based on the cross-correlation matrices from RRS MD simulations: average residue correlation (ARC) index, the residue correlation similarity (RCS) index, and the unbound and bound difference (UBD) index. **Results:** Total of 34 nanosecond (ns) RRS molecular dynamics (MD) simulations were carried out for both the active Light state and inactive Dark state of VIVID with each of residue being held rigid during the simulations. The ARC, RCS and UBD indices were generated to evaluate the impact of internal degree of freedom of each residue on the overall protein dynamics. Several key residues were identified for the allostery of VIVID, including Ser63, Ala88, Val118, Arg124, Asn133, and Val168. The Light state of VIVID protein displayed more correlations, both positive and negative, within the structures than the Dark state. In addition, the principle component analyses (PCA) combining with quasi-harmonic approximation were carried out on all the MD trajectories to reveal the changes of dynamics in different parts of protein when each residue being held rigid. **Conclusion:** The novel RRS method has be demonstrated to be particularly useful to reveal protein allosteric mechanism, especially for the evaluation of individual residue's impact on overall protein dynamics. VIVID protein carries out its function in the Light state through stronger positive and negative correlations within the structure. The PCA combining with quasi-harmonic approximation provided a new way to precisely monitor the impact on the dynamics of different part of the protein from local constraints.

285 **Poster Session B**

Modulation of the antitumoral immune response in a murine HPV-positive oropharyngeal squamous cell carcinoma model of Cisplatin-based fractionated chemoradiotherapy *R. Krupar, Baylor College of Medicine; P. Jayaraman, Baylor College of Medicine; H. Liu, Baylor College of Medicine; A. Sikora, Baylor College of Medicine*

Introduction: Besides direct tumor cell killing, chemoradiotherapy is known to have antitumoral capacities by inducing an antitumoral immune response. But radiation and chemotherapy have also systemic and local immune suppressive effects. Understanding the underlying pathomechanisms of therapy-induced antitumoral immune response and immunosuppression is crucial to improve chemoradiotherapy regimens as the current standard-of-care in order to exploit their immune-modulatory effects in the most beneficial way. We have established a syngeneic murine HPV-positive oropharyngeal squamous cell carcinoma model of fractionated chemoradiotherapy in order to evaluate changes of immune cell subtype composition as well as activation status in the tumor immune microenvironment by fractionated chemoradiotherapy. **Methods:** C57BL/6 syngeneic MTEC tumor bearing mice were treated with weekly low dose Cisplatin and a total dose of targeted 30 Gy X-ray radiation administered as daily single doses of 3 Gy in 10 fractions (conventional) or weekly single doses of 15 Gy in 2 fractions (hypofractionated). Tumor immune responses were analyzed by flow cytometry for lymphocyte subtype composition and activation status (CD8, CD4, Treg, perforin, PD-1). **Results:** Chemoradiation with 2 fractions, but not with 10 fractions, significantly inhibited tumor growth as compared to untreated mice. Tumor microenvironment studies revealed a radiation-induced increase of CD8 and CD4 cells. Tregs in contrast were significantly decreased by radiation in 2 fractions. While Cisplatin alone caused a significant inhi ition of CD8 activation status (perforin expression), 10 fractions of radiation brought it back to baseline and 2 fractions lead to a significant increase in CD8 activity as compared to 10 fractions. Furthermore PD-1 expression on CD8 cells was significantly upregulated by radiation in 10 fractions, but restored to baseline levels by radiation in 2 fractions. **Conclusion:** In our murine model of fractionated chemoradiation, different treatment regimens exert inhibitory and activating effects on the local antitumoral immune response. While hypofractionated chemoradiation strongly promotes antitumoral immune response, conventional chemoradiation also demonstrates inhibitory effects on antitumoral immune response. Therefore the ultimate impact of chemoradiation on tumor immune microenvironment may strongly depend on particular features of a given treatment regimen, including dose, duration, and schedule of radiation. By evaluating local and systemic immune responses of different chemoradiation regimen this preclinical study will guide towards an optimization of the immune modulatory capacities of chemoradiation in future clinical trials and point out relevant immune changes of favorable therapy responses.

286 **Poster Session A**

A rapid and efficient chick-based strategy for cancer xenografts *R. Pathak, Baylor College of Medicine; R. Krupar, Baylor College of Medicine; K. Kanthasamy, Baylor College of Medicine; J. Patel, Baylor College of Medicine; A. Sikora, Baylor College of Medicine*

Introduction: Patient-derived xenograft (PDX) mouse models widely used in cancer research have contributed immensely to our understanding of cancer biology. However, despite being the current standard for PDX studies, there are a number of factors that limit the use of these models. Maintaining these PDX mouse models is laborious, time consuming and expensive. Additionally, xenografts in nude mice have displayed variable viability post implantation with engraftments rates ranging from 25-75% depending on the tumor type. This combined with long experiment turn around time (months to years) limits the reproducibility and degree to which the PDX mouse can be scaled. The CAM that surrounds and nourishes the developing chick embryo is immunodeficient and highly vascularized, properties that we have exploited to create a natural in vivo model capable of supporting tumor growth, angiogenesis, and even metastasis. We demonstrate a fast, cost-effective, and reproducible avian xenograft model that exploits the chorioallantoic membrane (CAM) for cancer xenografts. M**ethods:** Tumor specimens (100-200-mg) are incubated in minimal essential medium (MEM) supplemented with antibiotics for 60-90 minutes. Tumor fragments (intact pieces or tumor mush) are mixed in a suspension of PBS and Matrigel©, and subsequently explanted onto the vascularized CAM of 6-day chick embryos, followed by incubation at 37°C with 60-70% humidity. At day 17, chicks are euthanized via hypothermia (incubation on ice for 1 hour). Tumor explants and the surrounding CAM are assessed for viability, both grossly and microscopically. **Results:** Three-dimensional, vascularized tumors were successfully grown using tumor specimens from breast cancers (mouse PDX derived), skin cancer, oral squamous carcinoma and adenexal carcinomas derived from patient resections. The take rates for the tumor xenografts were between 60-75% for different tumor types, and showed high survival rates (>90%) for all xenografts. The tumors cells grown on CAM histologically resemble the original tumors, with actively proliferating regions within the xenografts. **Conclusion:** Our results successfully demonstrate the efficiency and reproducibility of the chick-based model across multiple tumor types. Furthermore, the model offers a unique advantage of providing easy access to the CAM and the tumor graft/plaque for imaging and other downstream applications. We anticipate that our model will serve as a valuable tool for maintaining cancer tissue bio-banks.

287 **Poster Session B**

Novel Approach to Assess the Functional and Clinical Impact of Genetic Variations *T. Hsu, Baylor College of Medicine; P. Katsonis, Baylor College of Medicine; A. Koire, Baylor College of Medicine; S .Wilson, Baylor College of Medicine; O. Lichtarge, Baylor College of Medicine*

Introduction: Whole genome sequencing uncovers a plethora of genetic alterations that are often with unknown functional and clinical impact. A major challenge is to estimate the consequences of these alterations. **Methods:** As a complement to traditional computational tools that follow machine learning, statistical and biophysical modeling approaches and trained with numerous available data, we sought to assess the impact of novel mutations from a phylogenomic perspective, namely by relating specific genotype variations to specific speciation events. Here we defined the overall change in fitness due to a mutation as a novel biological quantity called the Evolutionary Action (EA) of the mutation and that factors in the size of the mutation plus the site at which it occurs. **Results:** In our retrospective data analysis, this approach correlated with the loss of protein function, separated the disease-associated mutations from the benign and matched the morbidity of monogenic disorders. This approach was also tested by independent judges at the two most recent international CAGI contests and consistently achieved top ranking. In practice, this approach allowed us to stratify patients into subgroups by the impact of mutations in TP53, such that tumors harboring greater deleterious impact TP53 mutations were more invasive and patients with such mutations associate with the poorest clinical outcomes. We also evaluate the functional impact of somatic mutations from the TCGA and identify novel cancer genes by detecting the functional impact bias on cancer mutations during tumorigenesis. **Conclusion:** Together, these results show “Evolutionary Action” (EA) is a novel approach to assess the functional and clinical impact of genetic variations, identify novel therapeutic targets, and may eventually play a role in the stratification of cancer patients for precise treatment selection.

288 **Poster Session A**

Parameter Estimation of a Reaction-Diffusion Model of Low Grade Gliomas *A. Gholami, The University of Texas at Austin; G. Biros, The University of Texas at Austin*

Introduction: One of the key challenges in treating gliomas is their aggressive infiltration into the healthy tissue, well beyond the visible bulk of the tumor in standard clinical imaging modalities. Thus it is hard to decide on how much tissue to resect in surgery or radiate in radiotherapy. Considering a large margin in radiotherapy may destroy healthy tissue, while small margins may result in faster recurrence of the tumor **Methods:** We use a nonlinear reaction-diffusion model for tumor growth. The anisotropic diffusion as well as the extend of tumor infiltration are the unknown parameters that we seek to approximate. We use a constrained optimization method to solve for these unknowns. This results in a system of nonlinear partial differential equations (PDEs). In our formulation, we estimate the parameters using partially observed, noisy tumor concentration data at two different time instances, along with white matter fiber directions derived from diffusion tensor imaging (DTI). The optimization problem is solved with a Gauss-Newton reduced space algorithm. In our experiments the data consists of two or more noisy, partially observed, segmented images of the tumor. The partial observation corresponds to the visible bulk of the tumor observed in the images. **Results:** We present the formulation and outline the numerical algorithms for solving the resulting equations. We test the method using synthetic dataset and compute the reconstruction error for different noise levels and detection thresholds for monofocal as well as multifocal test cases. The quality of the reconstruction is measured by computing Jaccard Index at different time points. Furthermore, we present performance of our novel preconditioners used to solve the optimization problem. **Conclusion:** The key quantities of interest are (i) the full extent of tumor invasion, and (ii) the rate of anisotropic diffusion. We used a nonlinear reaction-diffusion model for glioma growth, and solved the optimization problem with a reduced space Hessian method. State of the art numerical techniques were presented to speed up the time to solution. The design criteria for these techniques were low computational cost and robustness. The preliminary results show that if the partial tumor concentration is given at two or more consecutive time points, it is possible to use an inverse problem method to approximate the parameters of interest for a synthetic dataset. More tests need to be performed using more complex tumor models along with in vivo/vitro datasets.

289 **Poster Session B**

Towards an Integrated System for Image Analysis in Brain Tumor Imaging based on Inversion Methods *A. Mang, The University of Texas at Austin; G. Biros, The University of Texas at Austin*

Introduction: Despite multimodal treatment patients diagnosed with high-grade manifestations of primary brain tumors continue to face poor prognosis. Medical imaging has a central role in brain tumor diagnosis, treatment planning, and monitoring. Clinical decisions based on imaging information are complicated by numerous factors. The highly volatile nature of gliomas and their ability to infiltrate healthy tissue well beyond the tumor bulk visible in imaging studies make a complete removal of the malignancy nearly impossible. Postsurgical radiation results in imaging artifacts that complicate the differentiation of true progression and response from pseudoprogression and/or pseudoresponse. Our overarching goal is the design of an integrated system that combines information from multiple sources, including multimodal imaging, computational models, and medical imaging analysis software in an effort to aid clinical decision making in patients diagnosed with brain tumors. **Methods:** We showcase two pillars of our system: An inversion method for calibrating brain tumor computer simulations to patient individual observations in clinical imaging data. This method operates on segmentations of the visible tumor bulk. Another integral part are inversion methods that operate on the images themselves: We showcase algorithms for deformable image registration. These methods can be used to integrate multimodal imaging data of patients into a common frame of reference for further study and analysis. Our formulation allows us to include prior knowledge about an expected deformation map, for instance incompressibility of tissue. **Results:** We showcase results for a multimodal imaging study (T2-weighted, T1-weighted with and without gadolinium contrast enhancement) of 12 patients. We demonstrate that we can reliably fit tumor growth simulations to imaging data. We validate the simulations by comparison to segmentations generated by expert observers. We also showcase results of our framework for deformable image registration. We demonstrate that we can register medical images with high fidelit . **Conclusion:** We provide enabling technology to aid clinical decision making and management of brain tumor patients. Our framework provides insight and visualizations of how tumors grow, infiltrate, and progress over time. Once clinically validated, our simulations can be used to generate patient individual predictions of tumor growth. This can help us to refine treatment plans or generate virtual scenarios for clinical outcome. Our framework for deformable image registration provides high accuracy, high fidelit , and is efficient. Both methodologies are an integral part of our overall framework for medical image analysis in brain tumor imaging to provide a patient individual decision pathway for tumor treatment.

290 **CPRIT Grantee**

Closing the Loop between Computational and Experimental Models in 3D Printing Vascularized Tissues *S. Paulsen, Rice University; B. Grigoryan, Rice University; J. Miller, Rice University*

Introduction: Though computational approaches have been shown to be effective in modeling flow rates, diffusion, and other key tissue parameters, few research groups are able to experimentally verify the accuracy or significance of their computational models either in vitro or in vivo. In order for computational models to directly aid in developing networks of perfusable vessels for 3D printed tissues, researchers must first close the loop between computational and experimental tissue models. We seek to verify computational models of living tissue to explore how the structure of vascular networks affects the biochemical environment and physiology of cells within a tissue. **Methods:** After 3D printing poly(ethylene glycol) gels containing internal branching micro-channel networks with four discrete channels, we flowed fluorescently labeled beads through the channel networks using inlet flow rates of 10 and 100 μL/min. We then tracked the flow of fluorescent beads at 40 frames per second to calculate flow rates and flow profiles within individual channels of our gels. Additionally, we have assessed transport into the bulk of the gel by tracking the diffusion of FITC-dextran using fluorescence microscopy. Finally, to develop computational models for flow and diffusion through the same micro-channel networks, we scanned the gels using micro-computed tomography to reconstruct a 3D model of the channel networks that we imported into computational fluid dynamics software. **Results:** We assessed flow and diffusion through 5 printed gels containing the same internal vascular structure. Initial results show that flow profile through the gels are parabolic in nature. However, average flow rates varied noticeably between channels, with channels 1 and 4 having the highest flow velocities. Computational models of these gels followed the same patterns of flo . Furthermore, after normalizing the inflow rate for computational models to match that measured in experimental models, predicted flow rates corresponded strongly with the experimentally measured flow rates. These results show the capability of computational modeling to demonstrate non-obvious flow patterns in 3D printed tissues along with the necessity for in vitro verification. **Conclusion:** For future work we will develop computational models for flow and diffusion through these same gels, we used micro-computed tomography to reconstruct 3D models of our printed gels then began initial tests to predict flow rates and patterns through individual channels. By developing reliable computational

models for tissue engineered systems, specifically focusing on vascular transport, we can rapidly assess vascular geometries to generate the desired tissue phenotype for studying disease progression in vitro.

291 **CPRIT Grantee**

Phenotypic Transitions Induced by Extracellular Vesicle-mediated Cell-cell Communication *M. Lu, Rice University; M. Capello, The University of Texas M.D. Anderson Cancer Center; H. Levine, Rice University; S. Hanash, The University of Texas M.D. Anderson Cancer Center; E. Ben-Jacob, Rice University; J. Onuchic, Rice University*

Introduction: Recently, an important new class of cell-cell communication has been found to mediate the horizontal exchange of information though special extracellular vesicles (EVs), such as exosomes and microvesicles, especially in tumor and immune systems. The EVs can carry a broad range of biomolecules, and transport them to specific targeted cells. Notably, the EV-mediated cell-cell communication can cause functional and phenotypic switches in the recipient cells. However, understanding the advantage of EV-mediated cell-cell communication over the other communication mechanisms remains a challenge in cell biology. Here, we address this question by modeling and comparing EV-mediated communication with chemical-mediated communication in reduced models. **Methods:** We explored the contents of exosomes by using bioinformatics analysis on proteomics data of both cells and exosomes in multiple pancreatic cancer cell lines. At the same time, we built reduced models to investigate the role of different types cell-cell communications in noise-induced phenotypic transitions. Here we utilized both the analytical and simulation analyses on the stochastic process of gene regulations in the reduced systems. **Results:** From the above-mentioned bioinformatics analysis, we identified a high enrichment of correlated molecule pairs across multiple cell lines in the exosomes but not in the cells. The observation suggested a special mechanism of EV-based sorting and packaging. Accordingly, we hypothesized that EVs are capable of sending multiple signaling/regulatory molecules of certain ratio altogether.

By utilizing the information, we constructed a model of a cell receiving two signal molecules that are sent either independently in the case of chemical-mediated communication, or simultaneously in the case of EV-mediated communication. We show that EV-mediated communication causes correlated noise of the two signaling molecules, and plays crucial role in the noise-induced phenotypic transitions. Moreover, from the simulation of two-way EV-mediated communication of two cell types, we show that cells could achieve more synchronized decision making by using EVs. Our results are supported by some recent experimental observations. C**onclusion:** We used bioinformatics analysis on the proteomics data, and devised reduced models to study the role of EV-mediated cell-cell communication in inducing phenotypic transitions. We suggest that, due to the special sorting and packaging mechanism, EVs can send multiple signaling/regulatory molecules of correlated noise. By doing so, EVs enable recipient cells to rewire the gene regulatory network, therefore achieving unique and more robust cell phenotypic transitions, compared to the other communication mechanisms. Our modeling approach provides insights into the special advantages of EV-mediated cell-cell communication.

292 **CPRIT Grantee**

Modeling Cellular Plasticity during Cancer Metastasis *B. Huang, Rice University; M. Jolly, Rice University; M. Lu, Rice University; I. Tsarfaty, Tel Aviv University; E. Ben-Jacob, Rice University; J. Onuchic, Rice University*

Introduction: Metastasis causes 90% of deaths of cancer patients. Metastasis of carcinomas occurs when some epithelial (E) cells in the primary tumor lose cell-cell adhesions and gain the ability of movement through a process known as epithelial-to-mesenchymal transition (EMT). The metastatic cancer cells could move either collectively in the partial EMT phenotype (E/M) or individually in the mesenchymal (M), the amoeboid (A) or the hybrid amoeboid/mesenchymal (A/M) phenotypes. Cells of these phenotypes could undergo transitions among each other, such as mesenchymal-to-amoeboid transition (MAT), to adapt to changes in their microenvironment. The rich cellular plasticity during metastasis benefit cancer cells for their survival, causing a major clinical challenge for cancer treatments. Although mechanisms such as EMT and MAT have been intensely studied for each, a comprehensive understanding remains elusive. **Methods:** To provide a more comprehensive picture of cellular plasticity during metastasis, we utilized computational systems biology approach to model the dynamic behavior of the gene regulatory circuit for cell motility. Previously, we constructed the core regulatory circuit for both EMT (miR-200/ZEB/miR-34/Snail) and MAT (Rac1/RhoA). These two circuits are connected since miR-200 and miR-34 inhibit the expressions of Rac1 and RhoA. Here we extended our previous work to study the coupling between the EMT and the MAT circuits. We considered the two miRNAs as external signals to the core MAT circuit, and modeled the response of the circuit dynamics upon the changes in the signals. **Results:** Our modeling shows that the core MAT circuit allows for four stable states that correspond to M, A/M, M and E/M phenotypes respectively. Besides, we show that the expression of the two miRNAs drive cancer cells away from the metastatic phenotypes by inhibiting both Rac1 and RhoA. Our modeling approach allows mapping of the miRNA levels to the transitions among various cell motility phenotypes, which agrees well with experimental observations. **Conclusion:** We developed a tractable theoretical framework towards a comprehensive understanding of cellular plasticity during metastasis by modeling gene regulatory circuits. We explained how the experimentally observed phenotypes can be mapped to the stable steady states in our circuit model, and provided a tentative mechanism for the observed phenotypic transitions among different migration phenotypes such as collective-to-amoeboid transitions (CAT).

293 **CPRIT Grantee**

Identification of variants from ultra-deep Ion Torrent-based next-generation DNA sequencing *J. Fowler, The University of Texas M.D. Anderson Cancer Center; A. Deshpande, The University of Texas M.D. Anderson Cancer Center; W. Lang, The University of Texas M.D. Anderson Cancer Center; H. Kadara, The University of Texas M.D. Anderson Cancer Center; P. Scheet, The University of Texas M.D. Anderson Cancer Center*

Introduction: Numerous variant calling algorithms have been developed for next-generation sequencing (NGS) platforms. Some, such as the genome analysis toolkit (GATK) workflow for Illumina-based experiments, have benefited from broad use and feedback, undergoing successive iterations, and have become the default for a specific technology. **Methods:** To support our NGS experiments using the Ion Torrent sequencing platform, we sought to compare the performance of several available somatic single nucleotide variant (SNV) mutation detectors when applied to ultra-deep (1000X) sequencing of PCR amplicons covering 409 genes targeted in the Comprehensive Cancer Panel (CCP) (Ion Torrent, Life Technologies). We analyzed NGS data from a total of 30 tumors and core needle biopsies (CNBs) derived from 4 patients, as well as one paired normal sample per patient (3 blood samples, one normal lung tissue), and compared the results of tumor-normal analysis using MuTect, Varscan (VS), Ion Torrent's proprietary Ion Reporter somatic detection workflow (IR), and a tumor-normal variant subtraction that we called "Poor Man's somatic detector" (PM). **Results:** We filtered all results by method to remove variants in the normal sample for any of the four patients, as well as variants found in either the 1000 Genomes Project or the Exome Variant Server. Because VS on its default settings produced an order-of-magnitude more calls than the other algorithms, we further filtered its calls to exclude variants that it annotated with a p-value greater than 1e-9 (VS-e9). Over the 409 genes of the CCP, the total number of called variants called from MuTect, IR, PM, and VS-e9 were 9, 607, 1424, and 1200 SNVs, respectively. Of those, 8, 319, 464, and 604 (respectively) were exonic. One exonic variant was found in common by all four methods. Twenty-nine (29) exonic variants were called by IR, PM, and VS-e9. Overall, among IR, PM, and VS, the sets of variants called from VS-e9 and PM were closest in terms of size (number of called variants). However, overall agreement was strongest between the IR and PM methods, which is perhaps not surprising, since they both derive from algorithms provided by Life Technologies (with PM requiring some manual intervention). Over 70% of calls from IR were corroborated by another caller, far more than for any other method. **Conclusion:** While investigations of variant calls from multiple CNBs will lend insights into repeatability of each method, our preliminary results indicate IR is a robust, yet sensitive, caller for high-depth NGS data from the Ion Torrent platform.

294 **CPRIT Grantee**

CATTLE: An Integrated Data Portal For Cancer Treatment Research *E. Soysal, The University of Texas Health Science Center at Houston; H. Lee, The University of Texas Health Science Center at Houston; J. Sun, The University of Texas Health Science Center at Houston; L. Huang, The University of Texas Health Science Center at Houston; Y. Zhang, The University of Texas Health Science Center at Houston; X. Chen, The University of Texas Health Science Center at Houston; J. Zheng, The University of Texas Health Science Center at Houston; T. Cohen, The University of Texas Health Science Center at Houston; J. Chang, The University of Texas Health Science Center at Houston; H. Xu, The University of Texas Health Science Center at Houston*

Introduction: Within the past few decades, cancer treatment research has been greatly accelerated with advances in drug discovery methodologies, producing thousands of drugs that have been approved or are under investigation in clinical trials. While further investigation on these drugs should primarily rely on existing knowledge, acquiring a comprehensive view on a cancer drug can often be a time-consuming task, due to the fact that the relevant information is scattered across different data sources. In order to allow researchers to rapidly grasp the full-spectrum of information available for a drug, a one-stop data resource for cancer drugs is imperative. **Methods:** We developed CATTLE (CAncer Treatment Treasury with Linked Evidence), an integrated resource for cancer treatment, by applying state-of-the-art natural language processing and data integration techniques. A significant amount of manual curation is followed, in order to produce highly accurate data. **Results:** CATTLE provides: 1) an extensive list of cancer drugs including those that are in clinical trial stages as well as FDA-approved, 2) comprehensive information on cancer drugs encompassing pharmacology and pharmacogenomics - indication, target, side effects, pharmacovigilance, gene variations, drug sensitive/resistant genes, and drug induced gene expressions, and 3) evidence from prior research on the drugs covering the whole development cycle of a drug – bioassays, literature, clinical trials, patents and adverse event reports. Users can query the database with drug names to obtain concise summaries of broad knowledge in an effortless way. CATTLE also provides links to external databases and evidence so that the users can quickly access and investigate prior research results. **Conclusion:** To our knowledge, CATTLE is the firs integrated data source with an extensive list of cancer drugs linking to a comprehensive set of evidence. We expect CATTLE to be a valuable resource for cancer treatment research. CATTLE is available through http://drugkb.org.

295 **Oral Presentation**
CPRIT Grantee
Poster Session A

Enzyme-Mediated Checkpoint Inhibition Drives Immune Control of Cancer *M. Donkor, The University of Texas System; K. Triplett, The University of Texas System; N. Marshall, The University of Texas System; J. Blazeck, The University of Texas System; W. Lu, The University of Texas System; T. Triplett, University Health System; M. Yemeny, The University of Texas System; A. Qerqez, The University of Texas System; L. Ehrlich, The University of Texas System; E. Stone, The University of Texas at Austin; G. Georgiou, The University of Texas at Austin*

Introduction: The tryptophan-oxidation product kynurenine is a ligand of aryl hydrocarbon receptor and is known to be highly immunosuppressive . Kynurenine is synthesized by 3 enzymes: IFNg-inducible indoleamine-2,3-dioxygenase 1 (IDO1), IDO2, and tryptophan-2,3-dioxygenase TDO. IDO and TDO are overexpressed in many tumors and independently constitute negative prognostic indicator in cancer patients. Small-molecule inhibitors of IDO and TDO are currently in clinical development. However, pharmacological blockade of kynurenine synthesis results only in marginal anti-cancer activity as monotherapies and combined TDO and IDO inhibition is unlikely to be clinically relevant as a result of significant toxicities. We hypothesized that enzyme-mediated degradation of secreted kynurenine will promote immune activation and anti-tumor activity. M**ethods:** We tested the catalytic activities of different kynureninases (KYNases) and compared their efficacy in C57BL/B6 or Balb/C mice against subcutaneously established aggressive B16 melanoma or 4T1 mammary tumors respectively in a side-by-side comparison with standard-of-care checkpoint blocking anti-CTLA-4 and anti-PD-1 antibodies or the IDO-1 inhibitor NLG919. **Results:** KYNase significantly retarded the growth of B16 melanoma and extended survival in a manner indistinguishable from that of anti-PD1 or anti-CTLA-4 antibody and outperformed the most clinically advanced IDO1 inhibitor NLG919. The efficacy of KYNase was significantly diminished in NOD-scid Il2Rg$^{-/-}$ or Rag1$^{-/-}$ mice or mice in which CD8+ but not CD4+ T cells were depleted using specific antibodies, underscoring a requirement for intact adaptive immunity, specifically CD8+ T cells, for the antitumor function of KYNase. Immune profiling revealed that KYNase significantly enhanced the proliferation, tumor infiltration and antitumor effector functions of CD8+ and CD4+ T cells compared to control deactivated KYNase. Subsequently, KYNase synergized with anti-PD-1 or anti-CTLA-4 antibody to inhibit the growth of B16-OVA tumors and metastatic poorly immunogenic 4T1 mammary adenocarcinoma. KYNase combined with anti-PD-1 stimulated the rejection of B16-OVA tumors with 60% of mice still alive and tumor-free at day 300 compared to 20% in the anti-PD-1 arm alone while all deactivated KYNase treated mice had died by 30 days. These observations underscore kynurenine as a negative regulator of host immunosurveillance and the combination of enzyme-mediated kynurenine depletion and antibody-mediated checkpoint inhibition overcomes tumor resistance. This novel approach to checkpoint inhibition enables therapeutic awakening of T cell immunity for cancer eradication. C**onclusion:** These observations underscore kynurenine as a negative regulator of host immunosurveillance and the combination of enzyme-mediated kynurenine depletion and antibody-mediated checkpoint inhibition overcomes tumor resistance.

296 **Oral Presentation**
CPRIT Grantee
Poster Session A

Improving Therapy for Hepatocellular Carcinoma by Combining a HDAC Inhibitor with an Alkylating Agent *J. Zavadil, The University of Texas Health Science Center at San Antonio; H. White, The University of Texas Health Science Center at San Antonio; C. Walter, The University of Texas Health Science Center at San Antonio*

Introduction: Within the US, approximately 80% of hepatocellular carcinoma (HCC) is diagnosed in late stages when chemotherapy is the only treatment option. Chemotherapy at late stages provides a median survival of only ~10 months, demonstrating the need for improved therapeutic options. We proposed combining temozolomide (TMZ), a monofunctional DNA alkylating agent, with suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, to treat HCC. We hypothesized that these agents would work together by two potential mechanisms: (1) SAHA alters the expression of genes, especially in DNA repair pathways, to impair the cells' ability to respond to DNA damage induced by TMZ, thus lowering the cells' capacity for repair and (2) SAHA increases the amount of DNA that is only loosely bound to histones, allowing TMZ to have greater access to DNA and to induce higher levels of damage. Together, these mechanisms would lower the capacity for DNA repair and increase the amount of DNA damage incurred, thus increasing the number of cells experiencing damage that exceeds repair capacity and results in cell death. **Methods:** We show that this drug combination synergistically reduces cell viability in two human HCC cell lines across multiple doses and treatment conditions. To test the efficacy in vivo, we have used the C3HeB/FeJ mouse strain, which has a ~50% prevalence of spontaneous HCC in males once they reach 10-12 months old. Surgery and ultrasound were used to identify tumor bearing mice and measure tumor volumes prior to initiation of therapy. **Results:** There is variability in % change in tumor burden within each of the treatment groups, with some mice within each cohort (except sham mice) having complete regression of tumors while others had large increases in tumor burden. The TMZ cohort displayed significant decline in tumor burden compared to sham treated mice, and responded better than SAHA alone or SAHA+TMZ. Surprisingly, the solvent dimethyl sulfoxide (DMSO) by itself significantly reduced percentage change in tumor burden compared to sham treated mice. **Conclusion:** Future experiments will test the mechanisms of synergy in vitro and identify gene expression patterns shared among regressing tumors versus those with increasing tumor burden.

297 **Oral Presentation**
CPRIT Grantee
Poster Session A

Gulf Coast Consortium for Chemical Genomics: An Alliance of Core Facilities for Cancer Therapeutics *P. Davies, Texas A&M University System Health Science Center; M. Mancini, Baylor College of Medicine; C. Stephan, Texas A&M University System Health Science Center; G. Bartholomeusz, The University of Texas M.D. Anderson Cancer Center; K. Dalby, The University of Texas at Austin; S. Wong, The Methodist Hospital Research Institute; D. Carson, Rice University; Z. An, The University of Texas Health Science Center at Houston; S. Gilbertson, University of Houston*

Introduction: The John S. Dunn Gulf Coast Consortium for Chemical Genomics, organized by a team of scientists, promotes academic drug discovery research in Texas. The goal has been, and continues to be, to develop the infrastructure necessary to support the translation of basic research discoveries into therapeutic applications. **Methods:** The GCC for Chemical Genomics (GCC-CG) is a component of the Gulf Coast Consortia, a multi-institutional organization that promotes collaboration through the development of inter-institutional agreements that lower the barriers to collaborative research. The GCC-CG has organized a network of collaborating research cores that provide specialized resources to support drug discovery research. This network (1) facilitates the movement of projects between different institutional cores, (2) provides a centralized project application review and tracking system, and (3) promotes exchange of scientific information. With support from multiple CPRIT awards, the program is evolving to include seven core facilities focused on cancer therapeutics: **a. Target Discovery and Validation Core Facility*** (UT M.D. Anderson Cancer Center): uses siRNA and CRISPR technologies to support cancer-related target discovery research. **b. Targeted Therapeutics Drug Discovery Program*** (Univ of Texas – Austin): uses chemistry-based strategies to discover novel, targeted cancer therapeutics. **c. Combinatorial Drug Discovery Program*** (Texas A&M HSC): specialized expertise in drug repurposing and combinatorial drug discovery for cancer therapeutics. **d. Therapeutic Monoclonal Antibody Lead Optimization and Development Core*** (UTHSC-Houston): specialized expertise in the development of engineered monoclonal antibodies as cancer therapeutics. **e. Advanced Imaging Program** (Baylor COM and Texas A&M HSC): provides advanced imaging platforms and informatics for cancer-related drug

discovery research. **f. Bioinformatics Core Program*** (The Methodist Hospital Research Institute): specialized expertise in single agent and combination drug repositioning for cancer therapy.. **g. Synthetic Chemistry Program** (Univ of Houston): a new program to provide synthetic chemistry support for cancer related drug discovery research *CPRIT-funded. **Results:** This integrated approach to support academic drug discovery research is engaging a large number of investigators and research programs in developing novel therapies for many different types of cancer. **Conclusion:** By establishing an organized alliance of research core facilities focused on cancer-related drug discovery research, the GCC-CG is leveraging the specialized expertise and facilities of multiple institutions, avoiding duplication of critical resources and insuring the most efficient use of established capabilities. The GCC-CG is providing the framework to accelerate the movement of basic discoveries into clinical application in a way that best serves the interests of the citizens of Texas.

298 **CPRIT Grantee**
Poster Session B

FoxO transcription factors promote AKT Ser473 phosphorylation and renal tumor growth in response to pharmacologic inhibition of the PI3K-AKT pathway *B. Gan, The University of Texas M.D. Anderson Cancer Center*

Introduction: The PI3K-AKT pathway is hyperactivated in many human cancers, and several drugs to inhibit this pathway, including the PI3K/mTOR dual inhibitor NVP-BEZ235, are currently being tested in various pre-clinical and clinical trials. It has been shown that pharmacological inhibition of the PI3K-AKT pathway results in feedback activation of other oncogenic signaling pathways, which likely will limit the clinical utilization of these inhibitors in cancer treatment. However, the underlying mechanisms of such feedback regulation remain incompletely understood. M**ethods:** The PI3K-AKT pathway is a validated therapeutic target in renal cell carcinoma (RCC). In this study, using various PI3K/AKT inhibitors and renal cancer models, we studied the roles of FoxO transcription factors in PI3K/AKT inhibition mediated feedback reactivation of AKT and renal tumor growth. **Results:** We show that FoxO transcription factors serve to promote AKT phosphorylation at Ser473 in response to the treatment of NVP-BEZ235, a PI3K/mTOR dual inhibitor, in renal cancer cells. Inactivation of FoxO attenuated NVP-BEZ235-induced AKT Ser473 phosphorylation, and rendered renal cancer cells more susceptible to NVP-BEZ235-mediated cell growth suppression in vitro and tumor shrinkage in vivo. Mechanistically, we showed that FoxOs upregulated the expression of Rictor, an essential component of mammalian target of rapamycin complex 2 (mTORC2), in response to NVP-BEZ235 treatment, and revealed that Rictor is a key downstream target of FoxOs in NVP-BEZ235-mediated feedback regulation. We also show that FoxOs similarly modulate the feedback response on AKT Ser473 phosphorylation and renal tumor growth by the treatment of other PI3K or AKT inhibitors, such as MK-2206. **Conclusion:** Our study suggests a model that activation of FoxO mediates PI3K or AKT inhibition–directed reactivation of AKT by upregulating Rictor expression and promoting AKT Ser473 phosphorylation, which eventually limits the impact of the PI3K or AKT inhibitor in RCC treatment. Together, our study reveals a novel mechanism of PI3K-AKT inhibition-mediated feedback regulation. Importantly, a recent RCC clinical trial with MK-2206 revealed a dichotomous response: a subset of patients had dramatic tumor reduction, while another subset of RCC patients showed no decrease in tumor growth rate, and had a very short progression free survival. We will discuss our work to determine the expression/activation status of the FoxO-Rictor-AKT signaling axis in renal cancer patients receiving AKT inhibitor treatment, which may identify FoxO as a novel biomarker to stratify RCC patients for PI3K or AKT inhibitor treat.

299 **CPRIT Grantee**
Poster Session A

Pharmacologic Inhibition of the ΔNp63/DGCR8 Axis as a Novel Therapeutic Strategy for p53 Deficient and Mutant Tumors *M. Napoli, The University of Texas M.D. Anderson Cancer Center; A. Venkatanarayan, The University of Texas M.D. Anderson Cancer Center; P. Raulji, The University of Texas M.D. Anderson Cancer Center; W. Norton, The University of Texas M.D. Anderson Cancer Center; L. Mangala, The University of Texas M.D. Anderson Cancer Center; A. Sood, The University of Texas M.D. Anderson Cancer Center; C. Rodriguez-Aguayo, The University of Texas M.D. Anderson Cancer Center; G. Lopez-Berestein, The University of Texas M.D. Anderson Cancer Center; K. Tsai, The University of Texas M.D. Anderson Cancer Center; H. Abbass, The University of Texas M.D. Anderson Cancer Center; C. Coarfa, Baylor College of Medicine; P. Gunaratne, University of Houston; E. Flores, The University of Texas M.D. Anderson Cancer Center*

Introduction: The p63 gene is a member of the p53 family, whose transcription is driven by two alternative promoters, allowing the expression of two distinct isoforms: TAp63 and ΔNp63. In order to determine their roles in tumorigenesis, we generated isoform-specifi knockout mouse models. TAp63 was found to be a pivotal tumor and metastasis suppressor, which acts by regulating the miRNA biogenesis pathway in a Dicer dependent way. ΔNp63 was to crucially regulate the differentiation of epithelial tissues, such as the epidermis. DNp63 is indeed able to directly induce the expression of DGCR8, a co-factor essential in modulating miRNA biogenesis, sustaining in this manner a proper epidermal differentiation. Accordingly, ΔNp63 knockout mice die shortly after birth because of developmental defects. In addition to characterize its role in skin development, our group has recently demonstrated that ΔNp63 may act as an oncogene by counteracting TAp63 tumor suppressive activities in vivo: specific deletion of ΔNp63 in $p53^{-/-}$ thymic lymphomas caused TAp63 reactivation and consequent tumor shrinkage. **Methods: Results:** Based on all these findings, we hypothesized that ΔNp63 might support tumor formation not only by inhibiting TAp63, but also by affecting miRNA maturation in a DGCR8 dependent manner. In line with this hypothesis, we deem that the inhibition of the ΔNp63/DGCR8 axis might be crucial to curb ΔNp63 tumor promoting activities. To this end, we screened a drug library of more than 850 FDA approved compounds looking for inhibitors of the ΔNp63/DGCR8 axis. We found

several small molecules that were able to reduce ΔNp63 protein stability and, as a result, to decrease the expression levels of its target gene DGCR8 and that of a specific set of miRNAs, whose biosynthesis is DGCR8-dependent. On the contrary, the levels of DGCR8-independent miRNAs were not affected by the drug treatments. In agreement with our hypothesis, curbing the ΔNp63/DGCR8 axis by either these drugs or inhibitors against the identified miRNAs, reduced cell viability of different cancer cell types of both murine and human origin, without affecting that of normal cells. In vivo analyses of these compounds as well as of these miRNA inhibitors have showed their efficacy in counteracting tumor formation and progression in mice. **Conclusion:** In summary, we believe that the in vitro and in vivo characterization of the ΔNp63/DGCR8 axis will provide a novel and effective strategy to target tumors relying on ΔNp63 for their expansion, especially the most therapeutically challenging ones devoid of a functional tumor suppressor p53.

300 **CPRIT Grantee**
Poster Session B

Synthetic Protein Degradation Agents to Clear Oncogenic Proteins From Cells *K. Bowen, The University of Texas at Austin; G. Kago, The University of Texas at Austin; S. Wilmington, The University of Texas at Austin; M. Dao, Harvard Medical School; A. Matouschek, The University of Texas at Austin*

Introduction: A hallmark of cancer is the presence of proteins that inappropriately signal cells to grow and to divide. In many types of cancer, the cells become dependent on these proteins in order to survive. Molecularly-targeted cancer therapeutics that inhibit these overactive proteins with small molecules revolutionized cancer treatment. Similarly, biologics, mainly based on antibodies targeted against cell surface receptors, have had an immense impact on cancer therapies. At the same time, a vast number of cancers lack molecularly targeted therapies and growing resistance against existing treatments underscore the need for new targeting strategies. We are developing a method to purge oncogenic proteins from cells by subverting the cells' own protein removal system, the Ubiquitin Proteasome System (UPS). **Methods:** In order to clear oncogenic proteins from the cell, we have designed protein "adaptors" which shuttle the oncogenic protein of interest (called the "substrate") directly to the proteasome for destruction. The adaptors consist of two parts connected by a linker. The first part binds to the proteasome, while the second domain, based on an antibody derivative, recognizes the substrate. Together, these adaptors bind to the oncogenic target and then bring the target to the proteasome for degradation. The adaptors themselves have been constructed to escape degradation, and thus act catalytically. In order to characterize our system, we used both in-cell and cell-free assays. We attached green fluorescent protein (GFP) to our substrate and monitored decrease in GFP fluorescence over time when incubated with adaptor and yeast proteasome. We also created stable HEK293 cells expressing our model protein and transfected the adaptor constructs, measuring fluorescence decrease with flow cytometry. **Results:** We designed and expressed a set of adaptors and characterized them in vitro by targeting a model protein consisting of GFP attached to a fragment of BCR-Abl. We found that two of the adaptors were effective in vitro, and we chose the best adaptor to target the overexpressed test protein in HEK293 cells. This adaptor depleted 80% of the target protein. **Conclusion:** These results show that the adaptors are highly effective in specifically degrading the target. Future work will focus on examining depletion of endogenous Abl and BCR-Abl in cancer cell lines, as well as targeting other oncogenic proteins. In principle, our method is applicable to any soluble protein in the cytosol or nucleus of cells, making it versatile and potentially applicable to many types of cancer.

301 **CPRIT Grantee**
Poster Session A

Development of a Precision Oncology Decision Support Core to facilitate the implementation of genomically-informed therapy *B. Litzenburger, The University of Texas M.D. Anderson Cancer Center; J. Zeng, The University of Texas M.D. Anderson Cancer Center; V. Holla, The University of Texas M.D. Anderson Cancer Center; A. Bailey, The University of Texas M.D. Anderson Cancer Center; A. Johnson, The University of Texas M.D. Anderson Cancer Center; N. Sanchez, The University of Texas M.D. Anderson Cancer Center; Y. Khotskaya, The University of Texas M.D. Anderson Cancer Center; K. Shaw, The University of Texas M.D. Anderson Cancer Center; E. Bernstam, The University of Texas Health Science Center at Houston; G. Mills, The University of Texas M.D. Anderson Cancer Center; J. Mendelsohn, The University of Texas M.D. Anderson Cancer Center; M. Routbort, The University of Texas M.D. Anderson Cancer Center; R. Team, The University of Texas M.D. Anderson Cancer Center; F. Meric-Bernstam, The University of Texas M.D. Anderson Cancer Center*

Introduction: Next generation sequencing is performed on cancers to identify alterations that can be matched to targeted therapies. With over 4,500 unique CLIA-validated alterations identified at The University of Texas MD Anderson Cancer Center alone, it is impossible for any individual to maintain a comprehensive understanding of targetable alterations. Therefore, there is an urgent need to support oncologists with a decision support system that provides literature- and experimentally-supported annotation of individual variants, and genomically relevant trials. The Precision Oncology Decision Support (PODS) Team at MD Anderson helps oncologists to select targeted therapies based on patients' molecular profiles. The PODS-Team provides local point-of-care support to determine actionability of variants in patients being considered for standard of care (FDA approved) therapies as well as investigational drugs via clinical trials. The PODS-Core aims to extend this support to all oncologists in Texas. **Methods:** Alterations, therapeutic agents, and clinical trials were manually annotated using a combination of literature and publically available databases. The functional significance of each variant was noted with appropriate references and linked to targeted agents and clinical trials. Annotations are aggregated in a dynamic fashion for the development of a comprehensive database that facilitates rapid retrieval of molecular annotations, relevant therapeutic implications, and

proactive alerts on a per-patient basis. **Results:** 12,930 unique alterations have been reviewed by the PODS-Team. 1,347 drugs have been curated, with more than 790 agents linked to their direct or indirect molecular targets. 525 genotype-selected trials and 2,060 genotype-relevant clinical trials were curated. Physicians can search the curated information via a Web-interface. Trial matching is supported through a queryable clinical trial portal and proactive electronic alerts of matched clinical trials. A web-based request form is utilized by physicians to request annotations of their patients' molecular profiles, resulting in the annotation of 597 individual patient reports in the past year. A clinical decision follow-up questionnaire is used to capture how individual reports were acted upon clinically. Information on 652 functionally or therapeutically significan alterations across 26 genes, level of evidence associated with each drug in genotype and tumor type-context, and genotype-matched and -relevant clinical trials is available via https://pct.mdanderson.org/#/. **Conclusion:** The development of the PODS-Core will enhance awareness of targeted therapies matched to each patient's molecular profile and increase accrual to genotype-selected trials. The mission of the PODS-Core is to help clinicians deliver on the promise of genomically-informed therapy using the "right drug to the right patient at the right time."

302 **CPRIT Grantee**
Poster Session B

Retargeting Lentiviruses for Cancer Therapy Using an Engineered Split Intein *A. Chamoun-Emanuelli, Texas A&M University System Health Science Center; G. Wright, Texas A&M University; R. Smith III, Texas A&M University; R. Münch, Paul-Ehrlich-Institut,; C. Buchholz, Paul-Ehrlich-Institut; Z. Chen, Texas A&M University System Health Science Center*

Introduction: Gene therapy represents a promising therapeutic paradigm for addressing many disorders, but the absence of a vector that can be robustly and reproducibly functionalized with cell-homing functionality to mediate the delivery of genetic cargo specifically to target cells following systemic administration has stood as a major impediment. **Methods:** A high-affinity protein-protein pair comprising a splicing-deficient naturally split intein was used as molecular Velcro to append a HER2/neu-binding protein (DARPin) onto the surface of a binding-deficient, fusion-competent lentivirus. **Results:** HER2/neu-specific lentiviruses created using this *in vitro* pseudotyping approach were able to deliver their genetic reporter cargo specifically to cells expressing the target receptor at high levels in a co-culture. **Conclusion:** We envision that the described technology could provide a powerful and broadly applicable platform for the incorporation of cell-targeting functionality onto viral vectors.

303 **CPRIT Grantee**
Poster Session B

Update from the the first Cancer Clinical Research Core Facility established in El Paso, TX *Z. Nahleh, Texas Tech University Health Science Center at El Paso; C. Ochoa, Texas Tech University Health Science Center at El Paso; L. Sanchez, Texas Tech University Health Science Center at El Paso; D. Liss, Texas Tech University Health Science Center at El Paso; R. Heydarian, Texas Tech University Health Science Center at El Paso; S. Gaur, Texas Tech University Health Science Center at El Paso*

Introduction: El Paso, TX is the fourth most populous city in Texas (population of more than 700,000, >85% Hispanics/Latinos). Hispanic cancer patients present at more advanced stages at diagnosis[1] and are underrepresented in cancer research[2-3]. There is a great need to improve access of cancer patients in El Paso, TX to quality cancer research. Me**thods:** The Cancer Clinical Research Core (CCRC) Facility was established in 2012 on the campus of the Texas Tech University, the only health sciences center on the US/Mexico border. The three main goals were to develop: 1)a research infrastructure with appropriately trained personnel to activate local and national cancer research clinical trials and projects; 2)a community outreach program; and 3)a searchable cancer database representative of cancer in Hispanics. R**esults:** Policies and procedures were developed. 8 clinical research and data coordinators were recruited over the years and received formal training in cancer protocols. An independent affiliate membership with SWOG (formerly Southwest Oncology Group) allowed the activation of 6 SWOG/ NCI sponsored cooperative group trials so far. Three seed grants were obtained and 7 investigator- initiated clinical trials were conducted addressing issues relevant to Hispanic cancer patients [4-11]. National and loco- regional research collaboration with University of Texas- El Paso, Texas Tech - Lubbock, and NCI produced 3 additional research protocols. Also, 9 industry-sponsored trials were activated for advanced and metastatic breast and pancreatic cancers. A total of 437 patients were enrolled in various prospective clinical trials. Two annual symposia were established for health care professionals, cancer survivors and the public. Two bilingual booklets were produced [12-13] in addition to quarterly newsletters. An informative website[14] was created and several local and media events (In English and Spanish) were organized[14]. Data on cancer clinical research trials was organized and stored in a Research Electronic Data Capture system (Redcap). A breast cancer database is being built. C**onclusion:** A desperately needed infrastructure for cancer research was established in El Paso, TX, thanks to a CPRIT Core Grant. Over 20 IRB approved cancer clinical trials were activated to date and enrolled over 400 cancer patients. The CCRC is improving access of cancer patients in El Paso, TX to quality cancer clinical trials, and will continue its active cancer research program as well as its community outreach and educational activities to promote cancer research, improve cancer outcome and decrease cancer disparity.

304 **CPRIT Grantee**
Poster Session B

Cellular Mechanisms of Chemotherapy-Induced Peripheral Neuropathy *J. Stockand, The University of Texas Health Science Center at San Antonio; B. Eaton, The University of Texas Health Science Center at San Antonio; K. Hargreaves, The University of Texas Health Science Center at San Antonio*

Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) and associated pain represent a clinically relevant limitation to patient treatment with anti-cancer drugs. Currently, there is no effective treatment for CIPN and the cellular mechanisms underpinning CIPN are obscure. Enhanced excitability of peripheral sensory neurons is thought to contribute to the generation and progression of CIPN. The current studies test the hypothesis that CIPN-causing chemotherapy drugs enhance neuronal activity eventuating neuroexcitotoxicity and neuropathy by directly affecting membrane excitability. **Methods:** We tested our hypothesis by combining a molecular genetics approach in the Drosophila model organism with electrophysiological assessment of neuron and ion channel function in peripheral sensory neurons, and behavioral assays testing fly sensitivity to pain. In complement, candidates identified in Drosophila studies were targeted in a rodent pain model. **Results:** Vinca alkaloids and taxane anti-cancer drugs rapidly and reversibly depolarize Drosophila peripheral sensory neurons by activating ruthenium red sensitive inward cation currents. Ruthenium red inhibits TRP channels. This depolarization of sensory neurons exceeds threshold leading to hyperexcitability. In behavioral studies, vinca alkaloids and taxanes caused pain hyperalgesia, increasing nocifensive responses to what is typically a sub-threshold mechanical stimulus. Such nocifensive responses are dependent on the activity of the peripheral sensory neurons excited by these anti-tumor agents. This effect of vinca alkaloids and taxanes on nociception is abolished in TRPA1 mutants. Similarly, vinca alkaloids fail to activate inward cation currents and depolarize peripheral sensory neurons in TRPA1 mutants. In the rat, vinca alkaloids cause a similar pain response noted by the rapidly development of hyperalgesia when injected into the footpad. **Conclusion:** Anti-tumor drugs in the vinca and taxane classes excite peripheral sensory neurons by direct action on these cells. This hyperexcitability underlies the pain and hyperalgesia caused by these drugs, and may represent the early stages of an excitotoxicity that ultimately leads to neuropathy. The ion channel TRPA1 plays a key role in conveying vinca alkaloid responses in sensory neurons and appears at this early stage of discovery to represent a reasonable target to counter the pain and neuronal death caused by these anti-cancer drugs.

305 **CPRIT Grantee**
Poster Session A

Targeting The Tumor Vasculature With TEM8-Specific T Cells *L. Williams, Baylor College of Medicine; M. Karla, Baylor College of Medicine; S. Kakarla, Baylor College of Medicine; T. Phung, Baylor College of Medicine; D. Rowley, Baylor College of Medicine; S. Gottschalk, Baylor College of Medicine*

Introduction: T-cell immunotherapy with genetically modified T cells expressing chimeric antigen receptors (CARs) has shown promise in preclinical models as well as early clinical studies. However, patients with solid tumors often do not respond as well as patients with hematological malignancies. This lack of efficacy for solid tumors is most likely due to several factors including a) emergence of immune escape mutants, and b) inability of tumor-specific T cells to recognize and destroy the vascular bed of solid tumors, which is critical for their malignant growth. The aim of this project is to generate CARs specific for tumor endothelial marker (TEM)8, and evaluate their anti-vasculature and anti-tumor activity in preclinical tumor models. **Methods:** We generated a retroviral encoding a TEM8-specific CAR consisting of the TEM8-specific single chain variable fragment AF344, a hinge/transmembrane domain, and a CD28.41BB.z endodomain. CD3/CD28-actiavted T cells were transduced with RD114-pseudotyped retroviral particles to generate TEM8-specific T cells and CAR expression was confirmed by FACS analysis. To evaluate the functionality of TEM8-specific T cells we used TEM8-negative cell lines (U373, A549, LM7, 293T) and 293T cells that were genetically modifie to either express human TEM8 (293T.hTEM8) or murine TEM8 (293T. mTEM8). **Results:** TEM8-specific T cells recognized target cells in an antigen-dependent fashion as judged by their ability to secrete pro-inflammatory cytokines (IFN-g and IL-2) in coculture assays, and kill TEM8-positive target cells. Importantly, TEM8-specific T cells readily recognized mTEM8-positive target cells, which will allow us to evaluate the safety and efficacy of TEM8-specific T cells in xenograft and immune competent murine tumor models. **Conclusion:** We have constructed a TEM8-specific CAR and have shown that T cells expressing this CAR recognize and kill hTEM8- or mTEM8-positive target cells. Animal studies are in progress to determine their safety and efficac . Targeting the tumor vasculature with TEM8-specific T cells may improve current T-cell immunotherapies for solid tumors.

306 **CPRIT Grantee**
Poster Session B

Development of Discoipyrrole Analogs for the Treatment of Non Small Cell Lung Cancer *J. MacMillan, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Discoidin domain receptor 2 (DDR2) is a widely expressed cell surface receptor that binds to collagen and activates a downstream signaling cascade, resulting in regulation of the extracellular matrix. DDR2 promotes cell migration, proliferation and survival when activated by ligand binding and phosphorylation. In squamous cell carcinomas (SCC), somatic mutations were present in ~4% of a sample set of 290 lung SCC tumors, and the DDR2 mutations were shown to be oncogenic. We have discovered a natural product, discoipyrrole A, that exhibits potent and selective toxicity to DDR2 mutant cancer cell lines. Since the discovery of discoipyrrole A, we have taken advantage of a 1-pot total synthesis of this natural product to make analogs with improved potency and PK properties. **Methods:** We have taken advantage of the unusual biosynthetic orgins of the discoipyrrole family of natural products to develop a rapid 1-pot total synthesis from three relatively simple starting materials. This has allowed us to make significant structural changes to the molecule to improve potency, soluble and PK properties. All of the analogs have been evaluated against a panel of SCC cell lines. We have also developed a series of photoaffinity probes based on the discoipyrrole scaffold to identify the therapeutic target. The most advanced analogs have been evaluted in xenograft models using the DDR2 mutant cell line HCC366. **Results:** Discoipyrrole A was discovered to have an IC_{50} of 120 nM against the DDR2 mutant SCC cell line HCC366 and >20 μM against the DDR2 WT cell line A549. Due to poor PK properties, it was not possible to carry out in vivo evaluation of the natural product. We have utilized an efficient synthetic route to make >100 analogs, with the best analogs exhibiting IC_{50} values < 100 nM and have improved PK properties. **Conclusion:** We have been able to take advantage of our discovery of the discoipyrrole family of antural products that showed potent cytotoxicity to cell lines with discoidin domain receptor 2 mutations. Based on our understanding of the biosynthesis of these natural products, we were able to design an efficient three step synthesis of discoipyrrole analogs with improved potency and optimized PK properties. We have been able to take two of these analogs into a *in vivo* zenograft model using the non-small cell lung cancer cell line HCC366.

307 **CPRIT Grantee**
Poster Session A

Increasing the Anti-tumor Efficacy of Immunotherapy in Melanoma by using Topoisomerase I Inhibitors. *J. McKenzie, The University of Texas M.D. Anderson Cancer Center; R. Mbofung, The University of Texas M.D. Anderson Cancer Center; S. Malu, The University of Texas M.D. Anderson Cancer Center; P. Hwu, The University of Texas M.D. Anderson Cancer Center*

Introduction: Melanoma is a highly aggressive form of skin cancer, whose rates of morbidity and mortality are increasing. The development of immunotherapeutic agents like anti-PDL1 and anti-CTLA4 antibodies has resulted in fundamental advances in the treatment of melanoma. However, long lasting responses are only observed in a subset of immunotherapy-treated patients. This shortfall highlights the need for a better understanding of the molecular mechanisms that govern tumor response to immunotherapy. **Methods:** To address this need, autologous patient-derived tumor cell lines and tumor infiltrating lymphocytes (TILs) were utilized in an in vitro activated caspase 3-based high-throughput screen, to identify compounds that increase the sensitivity of melanoma cells to T-cell mediated cytotoxicity. The screen consisted of an 850 compound library. One group of compounds that was most able to enhance T-cell killing of melanoma cells was topoisomerase I (Top1) inhibitors including: topotecan, and irinotecan. **Results:** Topoisomerases are a family of DNA enzymes, involved in unwinding DNA and relieving torsional strain during replication and transcription. Our results indicate that treatment of melanoma tumor cells with a Top1 inhibitor prior to exposure to autologous T cells, produced a synergistic increase in tumor cell death, as measured by intracellular staining of activated caspase 3, and computed using CalcuSyn. We have also recapitulated this finding in an in vivo model, where a better anti-tumor effect was observed in tumor- bearing mice treated with an antibody against the co-inhibitory molecule Programmed Death Ligand 1 (PD-L1) in combination with MM398, a nanoparticle liposomal formulation of irinotecan, than in cohorts treated with either antibody or drug alone. These findings suggest synergism between Top1 inhibitors and immune-based therapies in the treatment of melanoma. Genomic and proteomic changes elicited by inhibition of Top1 are now being investigated to identify the molecular factors that mediate the effect of Top1 inhibitors on T cell-mediated killing of melanoma. Our goal is to identify molecular changes mediated by Top1 inhibition in melanoma tumor cells, and/or the tumor microenvironment, that relieves immunosuppression and potentiates the activity of cytotoxic T cell-based immunotherapy. **Conclusion:** Understanding how Top1 inhibitors enhance melanoma killing by immunotherapy will allow for the development of predictive biomarkers, and also augment immune-based therapeutic strategies to ensure durable responses in a larger population of melanoma patients. By using melanoma as a model disease system, we can gain valuable insights into the dynamics of cancer immune response that may be applied to other cancers where effective treatment strategies are also lacking.

308 **CPRIT Grantee**
Poster Session B

The NO-releasing Aspirin Inactivates and Degrades Human MGMT More Efficiently Than O6-Benzylguanine and Greatly Sensitizes Cancer Cells to Alkylating agents *K. Srivenugopal, Texas Tech University Health Science Center at Amarillo; A. Paranjpe, Texas Tech University Health Science Center at Amarillo*

Introduction: The DNA repair protein MGMT (O6-methylguanine-DNA methyltransferase) is a major determinant of resistance to temozolomide and chloroethylnotrosoureas used in glioma therapy. Currently, clinical trials with O6-benzylguanine (BG) to inhibit MGMT are ongoing; however, this is beset with severe alkylation damage to bone marrow and the need for hematopoietic rescue using BG-resistant MGMT gene therapy. Here, we exploited the highly reactive nature of the active-site cysteine (Cys145) of MGMT, which accepts the alkyl groups in a self-inactivating reaction for novel drug design. Cys145 has a pKa of 4.8 and is susceptible for glutathionylation and nitrosylation, both of which inactivate MGMT. This study characterized the effect of nitro-aspirin or NCX-4016, which is a potent nitrosylator of reactive cysteines and tyrosines. NCX-4016, unlike aspirin has no GI adverse effects and is degraded by esterases to release NO in a sustained manner. Me**thods: Results:** In several MGMT-proficient human cancer cell lines (HT29, T47D, and HCT116, UW228, H460), nitroaspirin (NA) at pharmacologically achievable concentrations (5-10 μM) caused 90% inhibition of MGMT activity within 1 h of exposure. Interestingly, the MGMT protein disappeared very rapidly with similar kinetics after NA treatment; approx. 80-90% of MGMT was degraded after 10 μM NA treatment for 2 h. These data are highly comparable and/or superior to those reported for BG. Further, we showed that Cys145 and the neighboring Tyr114, a residue critical for DNA repair, were both modified by nitroaspirin. The involvement of the ub-proteasome pathway in the degradation of nitrosylated MGMT both in vitro and in cells was demonstrated. In cultured cells, MGMT suppression by BG was more prolonged than nitroaspirin, however, the 24-36 h curtailment by NA was adequate for increased production of cytotoxic lesions. Pre-exposure of tumor cells to NA followed by BCNU resulted in (i) a greater Induction and persistence of DNA interstrand crosslinks, and (ii) a huge and prolonged G2/M cell cycle arrest. Experiments in mice have shown that a single injection (100 mg/kg) of NCX-4016 causes 50-60% inhibition of MGMT activity in mice brain and liver. NO-aspirin greatly potentiated the antitumor efficacies of temozolomide and BCNU in xenografts developed in nude mice. C**onclusion:** Because NO-aspirin, is non-toxic, can be administered at high levels, yields a chemopreventive by-product, unlikely to elicit tumor resistance, is lipophilic enough to cross the blood-brain barrier, and its pleiotropic actions are actually beneficial for chemotherapy, we believe NA's ability to potently inhibit MGMT holds great promise for efficient brain tumor therap .

309 **CPRIT Grantee Poster Session A**

Clinical, Molecular and Immune Effects of Combined BRAF and MEK Inhibitor Therapy in Melanoma Patients with Resistance to BRAF Inhibitors *G. Chen, The University of Texas M.D. Anderson Cancer Center; J. McQuade, The University of Texas M.D. Anderson Cancer Center; D. Panka, Beth Israel Deaconess Medical Center; A. Reuben, The University of Texas M.D. Anderson Cancer Center; R. Bassett, The University of Texas M.D. Anderson Cancer Center; C. Hudgens, The University of Texas M.D. Anderson Cancer Center; K. Wani, The University of Texas M.D. Anderson Cancer Center; A. Amin-Mansour, The Broad Institute of Harvard & MIT; X. Mu, The Broad Institute of Harvard & MIT; A. Joon, The University of Texas M.D. Anderson Cancer Center; Z. Cooper, The University of Texas M.D. Anderson Cancer Center; A. Lazar, The University of Texas M.D. Anderson Cancer Center; M. Tetzlaff, The University of Texas M.D. Anderson Cancer Center; L. Simpson, The University of Texas M.D. Anderson Cancer Center; R. Mouton, The University of Texas M.D. Anderson Cancer Center; I. Glitza, The University of Texas M.D. Anderson Cancer Center; S. Patel, The University of Texas M.D. Anderson Cancer Center; W. Hwu, The University of Texas M.D. Anderson Cancer Center; R. Amaria, The University of Texas M.D. Anderson Cancer Center; A. Diab, The University of Texas M.D. Anderson Cancer Center; P. Hwu, The University of Texas M.D. Anderson Cancer Center; J. Wargo, The University of Texas M.D. Anderson Cancer Center; R. Sullivan, Massachusetts General Hospital; K. Kim, California Pacific Medical Center/Research Institute; M. Davies, The University of Texas M.D. Anderson Cancer Center*

Introduction: A subpopulation of patients who progress on single-agent BRAF inhibitor (BRAFi) achieve clinical benefit from the combination of the BRAFi dabrafenib (D) and the MEK1/2 inhibitor trametinib (T). Biospecimens collected from patients enrolled on a phase II trial of D+T in BRAFi-refractory metastatic melanoma patients were analyzed to determine predictors of response and mechanisms of resistance. **Methods:** Twenty-three patients with BRAFi-refractory *BRAF*^{V600} mutant metastatic melanoma were treated with dabrafenib (150 mg BID) plus trametinib (2 mg QD). Responses were evaluated by RECIST1.1 criteria every 8 weeks. Circulating BRAF^{V600} was measured from peripheral blood mononuclear cells (PBMC). DNA and RNA were isolated from pre- and on-treatment tumor biopsies for deep sequencing of 200 cancer-related genes, whole exome sequencing (WES), RT-PCR for BRAF splicing, and RNAseq. Phosphorylated MAPK (pMAPK) and CD8-positive immune infiltrates were evaluated by IHC. **Results:** Among evaluable patients, the confirmed response rate was 10%, disease control rate (DCR) was 45%, and median progression-free survival (PFS) was 13 weeks. Clinical benefit was predicted by duration of prior BRAFi >6 months (DCR 73% vs. 11% for <6 months, p=0.02; median PFS 16 vs. 8 weeks, p=0.01) and decrease in circulating BRAF^{V600} at day 8 of cycle 1 (DCR 75% vs. 18% for no decrease, p=0.015). BRAFi resistance mechanisms known to re-activate MEK (NRAS, MAP2K1 mutations; BRAF amplification and splice variants) were detected in 5 of 15 patients with evaluable pre-treatment tumor biopsies, but their presence did not predict DCR (p=0.36) or PFS (p=0.73). Direct assessment of pre-treatment MAPK pathway activity by IHC and RNAseq also failed to predict disease control (p=1), and analysis of matched on-treatment biopsies revealed that D+T failed to inhibit MAPK activity in 5 of 8 evaluable patients. Immune analysis demonstrated that almost all patients (15/17) had minimal immune infiltration after progression on BRAFi, and D+T induced increased immune infiltration in only 1 of 9 patients. **Conclusion:** Prior duration of BRAFi monotherapy and early decrease in circulating BRAF^{V600} levels predict benefit from D+T therapy in BRAFi-refractory melanoma patients, but MAPK pathway reactivation does not. In contrast to BRAFi-naïve patients, D+T therapy generally failed to achieve MAPK pathway inhibition or to induce immune infiltratio in BRAFi-refractory melanomas. The findings have clinical implications for the sequencing of immune and targeted agents in melanoma patients with *BRAF*^{V600} mutations, and they provide insights into potential causes and markers of therapeutic resistance that should be evaluated in future trials in this patient population.

310 **CPRIT Grantee Poster Session B**

Collateral Lethality: One Step Closer to the Clinic *F. Muller, The University of Texas M.D. Anderson Cancer Center; E. Lin, The University of Texas M.D. Anderson Cancer Center; N. Satani, The University of Texas M.D. Anderson Cancer Center; N. Hammoudi, The University of Texas M.D. Anderson Cancer Center; P. Leonard, The University of Texas M.D. Anderson Cancer Center; J. Marszalek, The University of Texas M.D. Anderson Cancer Center; Y. Sung, The University of Texas M.D. Anderson Cancer Center; W. Bornmann, Bayou Therapeutics; D. Maxwell, The University of Texas M.D. Anderson Cancer Center; B. Czako, The University of Texas M.D. Anderson Cancer Center; M. Difrancesco, The University of Texas M.D. Anderson Cancer Center; P. Zhenghong, Private sector; A. Wang, The University of Texas M.D. Anderson Cancer Center; R. DePinho, The University of Texas M.D. Anderson Cancer Center*

Introduction: Large scale genomic characterization efforts such as TCGA have painted an unprecedentedly detailed picture of the molecular anatomy of human cancer. However, few of the numerous genomic alterations identified to date are therapeutically actionable. While most efforts have focused on identifying and devising molecular targeted therapies against driver genetic events, most genomic alterations are passengers rather than drivers. We have recently shown that passenger or collateral deleted genes can be used as a basis for differential vulnerability and selective killing of cancer cells ("Collateral Lethality"). As proof of principal, we demonstrated that passenger deletion of the glycolytic gene *ENO1*, dramatically sensitizes glioma cells to inhibition of its redundant paralogue, ENO2. However, the tool compound that we employed for these *in vitro* studies, Phosphonoacetohydroxamate (PhAH), has very poor pharmacological properties and was found to be ineffective *in vivo*. **Methods:** We performed structure-activity relationship (SAR) studies to increase inhibitor specificity towards ENO2. In addition, we generated pro-drug ester derivatives to increase cell permeability. We then evaluated novel Enolase inhibitors for selective killing of *ENO1*-deleted glioma cells in culture and anti-neoplastic activity *in vivo* using an orthotopic intracranial xenografted model where tumor growth and response to therapy are monitored by MRI. **Results:** Molecular modeling and SAR studies culminated in a 6-membered cyclized derivative of PhAH, termed HEX, which exhibited between 4-10 fold greater specificit towards ENO2 versus ENO1. We generated a pivoxyl ester derivative of HEX, termed POMHEX, which showed selective killing of *ENO1*-deleted glioma cells in culture at <35nM (versus μM for PhAH or the parent compound, HEX). Non-deleted glioma cells, as well as normal human astrocytes were at least 50-times less sensitive to POMHEX. Using focused metabolomics, we demonstrate a strong correlation between toxicity of POMHEX and accumulation of metabolites upstream, and depletion of metabolites downstream of the Enolase reaction across *ENO1*-deleted and intact glioma lines. Finally, we demonstrate that POMHEX is capable of eradicating xenografted intracranial *ENO1*-deleted gliomas, with mice remaining recurrence-free even after treatment discontinuation. **Conclusion:** Taken together, these results indicate that POMHEX is a potent Enolase inhibitior *in vivo* and a promising starting point for the generation of a clinical candidate. In more general terms, these results constitute *in vivo* proof-of-principal for the concept of using passenger deletions as targetable vulnerabilities for the treament of cancer. Given the large number of passenger deleted genes in human cancer, collateral lethality may be a widely applicable therapeutic strategy.

311 **CPRIT Grantee**
Poster Session A

A Moving-Blocker-Based Strategy for Simultaneous Megavoltage and Kilovoltage Scatter Correction in CBCT Acquired during Volumetric Modulated Arc Therapy *J. Wang, The University of Texas Southwestern Medical Center at Dallas; H. Lee, The University of Texas Southwestern Medical Center at Dallas; L. Ouyang, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Cone-beam computed tomography (CBCT) acquired during beam delivery provides anatomical information of patients during the treatment. However, scatter signal from the treatment beam will degrade the image quality of CBCT. The goal of this work is to evaluate a moving-blocker-based approach in estimating and correcting megavoltage (MV) and kilovoltage (kV) scatter contamination in kV CBCT acquired during volumetric modulated arc therapy (VMAT). **Methods:** During the concurrent CBCT/VMAT acquisition, a physical attenuator (i.e., "blocker") consisting of equally spaced lead strips was mounted and moved constantly between the CBCT source and patient. Both MV and kV scatter signals were estimated from the blocked region of the imaging panel, and interpolated into the unblocked region. A scatter corrected CBCT was then reconstructed from the unblocked projections after scatter subtraction using an iterative image reconstruction algorithm based on constraint optimization. Experimental studies were performed on a Catphan® phantom and an anthropomorphic pelvis phantom to demonstrate the feasibility of using a moving blocker for MV-kV scatter correction. **Results:** Scatter induced cupping artifacts were substantially reduced in the moving blocker corrected CBCT images. Quantitatively, the root mean square error of Hounsfield unites (HU) in seven density inserts of the Catphan phantom was reduced from 395 to 40. **Conclusion:** The proposed moving blocker strategy greatly improves the image quality of CBCT acquired with concurrent VMAT by reducing the MV-kV scatter induced HU inaccuracy and cupping artifacts.

312 **CPRIT Grantee**
Poster Session B

Discovery and Development of Potent Anti-Cancer Compounds via Diverted Total Synthesis *W. Montgomery, The University of Texas at Austin; B. Granger, The University of Texas at Austin; B. Parkinson, University of Illinois at Urbana-Champaign; P. Hergenrother, University of Illinois at Urbana-Champaign; S. Martin, The University of Texas at Austin*

Introduction: During the course of the total synthesis of (±)-actinophylic acid, the natural product and several advanced intermediates were tested in vitro for the their ability to induce cell death in several cancer cell lines. Subsequent diversion of the intermediates led to the discovery of compounds that are cytotoxic at low micromolar concentrations and show no over toxicity. **Methods:** Using synthetic methods optimzed in the synthesis of (±)-actinophylic acid, over 40 anticancer derivatives have been synthesized with the intention of establishing a structure-activity-relationship. These newly synthesized compounds were screen *in vitro* for their ability to elicit cell death in various cancer cell lines. Additionally, derivatives containing photo-affinity crosslinkers are being synthesized to determine the unknown biological target via biotin pull-down studies. **Results:** Through structure-activity-analysis, we have begun to determine what structural characteristics of these compounds are necessary for cancer cell toxicity as well as hemolyitc activity. Further investigations into the mode-of-action have uncovered that the mechanism of cell death is likely due to endoplasmic reticulum stress. The lead compound has shown little to no *in vivo* toxicity and has been shown to be efficacou in a challenging mouse model of metastatic breast cancer. **Conclusion:** Novel anti-cancer compounds have been discovered via the diversion of synthetic intermediates encountered in the total synthesis of (±)-actinophyllic acid. We intend to continue the pre-clinical evaluation of this scaffold with the intention of translating the lead compound to the clinic.

313 **CPRIT Grantee**
Poster Session A

Developing Novel Covalent Ras Effectors *C. Schardon, The University of Texas at Austin; A. Solmonson, The University of Texas at Austin; M. Cammarata, The University of Texas at Austin; H. Kenefick, The University of Texas at Austin; J. Brodbelt, The University of Texas at Austin; E. Mills, The University of Texas at Austin; W. Fast, The University of Texas at Austin*

Introduction: Ras proteins control cancer-promoting pathways. Ras mutations, present in 33% of human cancers, can result in constitutive activation of these pathways. Mutations of the KRas isoform in particular are associated with the highest percentage of all human cancers (21.6%) and result in poor response to standard therapies. KRas is a validated anticancer target but its characteristics have led some to suspect it is "undruggable" in a practical sense. However, recent work applying covalent KRas inhibitors has offered proof-of-principle for this strategy. We are developing a novel class of covalent protein modifiers that can selectively target protein nucleophiles in a context-dependent manner and demonstrate here that a fragment-sized compound can be used to successfully label KRas. **Methods:** Stability and non-enzymatic reactivity of the covalent modifiers were analyzed by UV-Vis and HPLC. In vitro characterization of KRas labeling by fragment-sized covalent modifier was carried out using site directed mutagenesis, click chemistry, and Western blot protocols. In cell effects of the covalent modifiers using pancreatic cancer cell lines MiaPaca2 and BxPC3 were assessed using cell viability assays, Western blotting, and immunoprecipitation. **Results:** The fragment-sized covalent modifiers are shown to not react readily with small molecule thiols in solution. However, KRas is modified at C51 and C118, with a slight preference for the C118 site. The C51A/C118A double mutant and cysteine-less construct (C51S, C80L, C118S) are not modified, showing selectivity for two Cys sites. Modification at the C51 site is unusual, so a triple mutant (C118A, E3A, E49A) was used to show the importance of neighboring residues in catalyzing covalent bond formation at this site. Labeling was shown to be reversible at long time periods, which may ameliorate concerns about an idiopathic immune response to the label. Treatment of pancreatic cancer cell lines MiaPaca2 and BxPC3 with the fragment-sized covalent modifiers showed a statistically significant albeit modest, decrease in cell proliferation. Notably, Ras modificatio is observed in cell culture. **Conclusion:** Covalent KRas inhibitors offer a promising approach to this difficult target. We have developed novel covalent modifiers that achieve selectivity through reliance on catalysis by neighboring protein residues for bond formation. We demonstrate selective targeting of two sites on KRas by a fragment-sized modifier that also inhibits proliferation of pancreatic cell lines and results in targeted modification of KRas in cell culture. These experiments support future derivatization of these scaffolds to increase efficacy toward modulating KRas activity.

314 **CPRIT Grantee**
Poster Session B

Mechanisms of de Novo and Acquired Resistance to Therapeutic Treatment of Bone-Metastatic Prostate Cancer *G. Gallick, The University of Texas M.D. Anderson Cancer Center; S. Lin, The University of Texas M.D. Anderson Cancer Center; N. Parikh, The University of Texas M.D. Anderson Cancer Center; J. Song, The University of Texas M.D. Anderson Cancer Center; Y. Lee, The University of Texas M.D. Anderson Cancer Center; A. Varkaris, The University of Texas M.D. Anderson Cancer Center*

Introduction: While several new agents have been FDA approved for treatment of prostate cancer bone metastasis, their effectiveness is limited both by primary resistance and the development of acquired resistance. Understanding resistance mechanisms will lead to new therapeutic strategies to prolong lives of men with bone metastasis. M**ethods:** Patients in a phase 2 trial and patient-derived xenografts (PDX) were treated with cabozantinib, a VEGFR-2, MET inhibitor. To examine potential mediators of primary resistance, conditioned medium from the bone-forming PDX, MDA PCa118b, which induced bone formation in immunocompromised mice was prepared and subjected to mass spectrometry analyses. To examine acquired resistance, PDX were treated with cabozantinib for twelve weeks until logarithmic growth recurred, and subjected to whole genome sequencing and growth factor receptor profiling **Results:** A total of 121 secretory proteins were identified by mass spectrometry, of which 39 were integrin ligands. Several of these were confirmed to be of mouse origin, indicating that they were derived from mouse osteoblasts. To examine the effects of integrin ligands on cell survival, selected osteocrines identified by mass spectrometry known to bind cognate integrins expressed in C4-2B4 cells (with low integrin activation) were examined. Addition of SPARC, SPP1 or lumican activated focal adhesion kinase (a surrogate marker of integrin activation) and increased the survival of C4-2B4 cells. Next, we tested whether the FAK inhibitor, PF-562271, reduced therapy resistance in combination with cabozantinib. Mice bearing MDA PCa118b tumors were treated with PF-562271 alone, cabozantinib alone, or cabozantinib in combination with PF-562271. Treatment with PF-562271 alone inhibited tumor growth, demonstrating that the integrin pathway is a target for therapeutic intervention. Treatments with cabozantinib or cabozantinib plus PF-562271 resulted in complete tumor growth inhibition on day 14. However, upon cessation of treatments, tumors regrew rapidly. Treatment with cabozantinib plus PF-562271 delayed tumor regrowth, indicating that treatment with PF-562271 led to a reduction in resistant tumor cells. To assess acquired resistance, whole genome sequencing and cognate receptor kinases were examined after resistance developed. Only FGFR-1 was upregulated (both transcriptionally and by increased protein levels). Knockdown of Met in established PCa cell lines also led to increased FGFR-1 expression. **Conclusion:** Ostoecrines secreted by tumor/bone interactions can lead to therapy resistance to PCa bone metastases, in part, through activation of integrins; whereas acquired resistance to Met inhibition can occur through de-repression of FGFR-1 expression.

315 **CPRIT Grantee**
Poster Session A

Cyclopropylamine Containing Inhibitors of Lysine Specific Demethylase 1 Are Potential Therapeutics for MLL-Rearranged Leukemia *F. Wu, Baylor College of Medicine; Y. Yao, Baylor College of Medicine; Z. Feng, Baylor College of Medicine; C. Zhou, Baylor College of Medicine; L. Wei, Baylor College of Medicine; Y. Song, Baylor College of Medicine*

Introduction: Mixed lineage leukemia (MLL) gene translocations are found in ~75% infant and 10% adult acute leukemia, showing a poor prognosis. Lysine specific demethylase 1 (LSD1) has recently been implicated to be a drug target for this subtype of leukemia. However, detailed studies using LSD1 inhibitors have not been performed targeting MLL-rearranged leukemia. **Methods:** Compounds were synthesized and tested for their biochemical activity against LSD1. Potent and selective LSD1 inhibitors were examined for their activities against MLL-rearranged leukemia as well as other cancer cells. **Results:** Potent LSD1 inhibitors with biochemical IC50 values of 9.8-77 nM were found to exhibit high activity against proliferation of MLL-rearranged leukemia cells with EC50 of 10-280 nM, while these compounds are generally non-cytotoxic to several other tumor cells. Mechanistically, the compounds increased histone-H3 lysine-4 (H3K4) methylation, downregulated expression of several leukemia-relevant genes, induced apoptosis and differentiation, and inhibited self-renewal of leukemia stem-like cells. **Conclusion:** One compound showed significant in vivo activity in a systemic mouse model of MLL-rearranged leukemia without overt toxicities. LSD1 inhibition worked synergistically with inhibition of DOT1L, an H3K79 methyltransferase, against MLL-rearranged leukemia.

316 **CPRIT Grantee**
Poster Session B

Inhibition of Cancer-Associated Mutant Isocitrate Dehydrogenases by 2-thiohydantoin compounds *Y. Yao, Baylor College of Medicine; Z. Feng, Baylor College of Medicine; F. Wu, Baylor College of Medicine; L. Wei, Baylor College of Medicine; Y. Song, Baylor College of Medicine*

Introduction: Somatic mutations of isocitrate dehydrogenase 1 (IDH1) at R132 are frequently found in certain cancers such as glioma. With losing the activity of wild-type IDH1, the R132H and R132C mutant proteins can reduce α-ketoglutaric acid (α-KG) to D-2-hydroxyglutaric acid (D2HG). The resulting high concentration of D2HG inhibits many α-KG-dependent dioxygenases, including histone demethylases, to cause broad histone hypermethylation. These aberrant epigenetic changes are responsible for initiation of these cancers. Mutant IDH1 is therefore a drug target for intervention. **Methods:** We synthesized a novel series of 2-thiohydantoin and related compounds. Structure activity relationship investigation, crystal structures, kinetic studies and isothermal titration calorimetry(ITC) studies were performed to unveil their inhibition mode aginst IDH1 mutants. **Results:** Several compounds are found to be selective inhibitors of mutant IDH1 with Ki as low as 420 nM. X-ray crystal structures of IDH1(R132H) in complex with two inhibitors are determined. These, together with kinetic and ITC studies, show the inhibitor-protein interactions. In addition, these compounds can decrease the cellular concentration of D2HG, reduce the levels of histone methylation, and suppress proliferation of stem-like cancer cells in BT142 glioma with IDH1 R132H mutation. **Conclusion:** These compounds can be used as novel chemical probes for studies of mutant IDH1 in cancer and represent a new scaffold for drug discovery targeting IDH1 mutated cancers.

317 **CPRIT Grantee**
Poster Session A

Exosomes as Delivery Vehicles For Cancer Therapeutics *A. Haltom, The University of Texas M.D. Anderson Cancer Center; R. Kalluri, The University of Texas M.D. Anderson Cancer Center*

Introduction: Aberrations in gene expression, caused by mutations or epigenetic alterations, are the main cause of cancer development. The overexpression of oncogenes, such as c-myc and kras, and loss of tumor suppressors, especially p53 and PTEN, predispose many patients to cancer and are found in many cancer types. Attempts to correct these genetic aberrations in cancer cells are an area of intense study. Many drugs currently being tested, such as siRNA and antibody therapy, can have short half lives in the bloodstream and can have difficulty penetrating the cell membrane. However, a promising method of drug delivery is encapsulation by exosomes, which are vesicles 80-150 nm in diameter. Exosomes are a cell's natural transport system, and have been shown to carry mRNA and miRNA from one cell to another. As such, it has been hypothesized that exosomes may be effective drug carriers in a cancer setting. We plan to introduce drugs to exosomes to determine their efficacy in inducing tumor regression. We hypothesize that drug delivery by exosomes is more effective than other delivery methods. To test this hypothesis, we will carry out the following aims:1) Determine whether exosome-mediated siRNA delivery to cancer leads to tumor regression. 2) Introduce a functional copy of tumor suppressors that are lost in cancer and determine whether this can induce tumor regression.
M**ethods:** Aim 1. We will use c-myc siRNA to treat c-myc-overexpressing glioblastoma multiforme (GBM). We will introduce exosomes containing siRNA into a GBM cell line overexpressing c-myc and determine the resulting level of apoptosis, proliferation, and cell viability. Next, we will inject exosomes containing c-myc siRNA into orthotopic and genetic mouse models of GBM to determine if this treatment can induce tumor regression.
Aim 2. We plan to use BRCA1$^{-/-}$ breast cancer cells to introduce a functional copy of BRCA1 as a circular plasmid into exosomes. Cells will first be treated in vitro to determine efficacy of treatment as described in aim 1. Next, we will determine whether the treatment is effective in orthotopic and genetic mouse models.
Results: Results have not yet been obtained. **Conclusion:** Conclusions have not yet been made.

318 **CPRIT Grantee**
Poster Session B

The Dosimetric Impact Of Optimizer-Defined Treatment Field Orientation For Liver Stereotactic Ablative Radiotherapy (SABR) *D. Stanley, The University of Texas Health Science Center at San Antonio; N. Papanikolaou, The University of Texas Health Science Center at San Antonio; A. Gutierrez, The University of Texas Health Science Center at San Antonio*

Introduction: Due to the technological advances in imaging and highly conformal radiation delivery techniques, stereotactic ablative radiotherapy (SABR) has become a desirable treatment option due to its ability to achieve a comparable overall tumor control to that of a surgical resection. Stereotactic ablative radiotherapy uses a course of hypo-fractionated doses of external beam radiation to treat small well defined lesions, often in the liver or lung, while minimizing exposure to surrounding heathy tissue. We dosimetrically compared treatment plans produced by the user-defined non-coplanar beam arrangements to a novel beam selection process with optimizer-defined non-coplanar beam arrangements for liver SABR patients. For each of the plans we examined whether significant gains could be achieved with PTV homogeneity and conformity while concurrently reducing dose to critical OARs. Me**thods:** This study involved ten (n=10) selected patients previously treated to the liver by means of a SABR with user-defined beam arrangements. The patients were selected such that they possessed varying tumor locations and sizes. Within the Pinnacle3 treatment planning system platform(TPS) v.9.6(PhilipsMedical,FitchburgWI), the optimizer-define beam arrangements were generated by applying a novel script in which the 33 non-coplanar beams were superimposed onto the isocenter for each patient. Following the importing of the 33 beam ensembles, each patient was analyzed regarding clearance issues due to the different location of each tumor site. All patients were optimized with the same planning objectives and normalized such that 98% of the PTV received 100% of the prescription dose(45Gy/3) while minimizing the dose to the spinal cord, esophagus,heart, right kidney,duodenum,total lung, and the normal liver. Metrics used for comparison were: homogeneity index(HI),Conformity Number(CN),D2%,Dmean,R50,V80%,V50%, and V30. **Results:** For the PTV,there were no statistically significan differences in D98%,D2% and HIvalues, but the CN/R50 showed an improvement of 5.4%(p=0.04) and 5.0%(p=0.04) respectively for the for the optimized defined non-coplanar beam arrangement plans.

Concerning the OARs, a 6.7%(p<0.01) improvement in theV50% for the liver was demonstrated using the optimizer defined non-coplanar beam arrangement while the D2%,Dmean,V80%,V30% for the liver showed no statistical significance. All other evaluated OARS showed no statistical difference between either plans. C**onclusion:** A retrospective, treatment planning study involving ten patients previously treated to the liver with a SABR technique was analyzed for a correlation plan quality and origin of beam definition. Based on the evaluated results, Optimizer-defined non-coplanar beam arrangements can be considered to be a more effective method of treatment planning for the clinical treatment SABR patients than user-defined non-coplanar beam arrangement

319 **CPRIT Grantee**
Poster Session A

Novel ERK Inhibitors Identified by High-Throughput Screening *R. Sammons, The University of Texas at Austin; T. Kaoud, The University of Texas at Austin; A. Devkota, The University of Texas at Austin; E. Cho, The University of Texas at Austin; K. Dalby, The University of Texas at Austin*

Introduction: Protein docking sites on mitogen-activated protein kinases (MAPKs) facilitate complex formations and help generate signal specificit . Targeting these sites in cancer offers a way to potentially overcome many issues associated with ATP-competitive MAPK inhibitor design. These issues include millimolar cellular ATP concentrations and highly conserved active sites among MAPKs. Extracellular signal-regulated kinases 1/2 (ERK1/2) are MAPKs that are up-regulated in numerous cancers to drive cell proliferation and survival. ERKs possess two protein docking sites that are distinct from the active site: the D-recruitment site (DRS) and F-recruitment site (FRS). In this study, we have developed a high-throughput fluorescence anisotropy screening to identify small molecule inhibitors that target the DRS of ERK by interacting with the Cys-159 residue. **Methods:** DRS inhibitors were detected in a high-throughput assay by fluorescence anisotropy signal. Over 22,000 molecules were screened for the ability to competitively displace a fluorescent peptide from the DRS of inactive ERK2. The top 100 compounds from this screening were validated with a secondary radioactive kinase assay. The nine highest ranked inhibitors were characterized by dose-response assays against wild-type and C159S ERK2, a DRS mutant. Inhibition of ERK2 phosphorylation of an FRS substrate and JNK2 phosphorylation of c-Jun was evaluated to determine compound specificit . The compounds were also assessed in EGF-stimulated HEK293T cells for their ability to prevent ERK1/2, JNK1/2, and p38α phosphorylation using Western blot. Cell phenotype assays and *in vitro* assays with ERK mutants were used to further characterize the top inhibitor. **Results:** Four of the nine identified compounds inhibited the DRS of ERK2 with *in vitro* IC_{50} and K_i values under 5 μM, and did not show significant inhibition of the FRS or C159S ERK2. Two inhibitors reduced ERK activation to basal levels in MAPK pathway-stimulated HEK293T cells at 5 μM. One of the two compounds inhibited clonogenic growth of HEK293T cells at 0.5 μM. In specificity tests it demonstrated ability to also target JNK2 and p38α. This inhibitor was shown to target the DRS of ERK via the Cys-159 residue, as well as Cys-164 in the active site. Both of these interactions contribute

to ERK inhibition. **Conclusion:** The fluorescence anisotropy screening developed here can identify potent inhibitors that target the DRS of ERK. These inhibitors can be used to probe ERK signaling events that are mediated by the DRS and can be further optimized to yield highly selective ERK DRS inhibitors with potential therapeutic value.

320 **CPRIT Grantee**
Poster Session B

The SCORE System for Online Adaptive Radiation Therapy

A. Le, The University of Texas Southwestern Medical Center at Dallas; Z. Tian, The University of Texas Southwestern Medical Center at Dallas; M. Folkerts, The University of Texas Southwestern Medical Center at Dallas; M. Chen, The University of Texas Southwestern Medical Center at Dallas; J. Chang, The University of Texas Southwestern Medical Center at Dallas; W. Lu, The University of Texas Southwestern Medical Center at Dallas; S. Jiang, The University of Texas Southwestern Medical Center at Dallas

Introduction: In current radiotherapy, treatment plans are generated based on patient anatomy during simulation then delivered fractionally. However, patient anatomy may vary significantly from fraction to fraction. Therefore, the novel online adaptive radiation therapy (ART) paradigm has been initiated to allow real-time treatment adaptations based on up-to-date patient anatomy and is promising to maximize compensation for those variations. We have developed a software platform called Supercomputing Online Re-planning Environment (SCORE) to research various aspects of online ART. **Methods:** The SCORE system consists of a user-friendly graphical user interface (GUI), a set of GPU-based real-time re-planning tools, and an interface with commercial treatment planning systems (TPS). The ART planning process includes usage of original treatment plan and CBCT images, auto-contouring utilized rigid and deformation image registration (DIR) to transfer the contours from the planning CT to CBCT, plan re-optimization and final dose calculations. The three computationally intensive steps, DIR-based auto contouring, re-optimization and dose calculation, are implemented in GPU-based platform to enable rapid ART planning. The final plan can be output in DICOM-RT format to the commercial TPS. **Results:** The usage of the SCORE system was tested on an NVIDIA Tesla GPU card for prostate cancer cases. For a typical 9-field prostate IMRT case, the deformable registration can be done in 7 seconds, the dose calculation takes less than 1 second for FSPB method and less than 40 seconds for MC method, and the plan re-optimization takes less than 3 seconds using a DAO method. Considering users' interactions, a new plan can be developed in the time scale of a few minutes. **Conclusion:** We have presented our development of a SCORE system for radiotherapy online re-planning. The functionalities of SCORE are illustrated through an example case of prostate cancer re-planning, in which a new plan can be finished in a few minutes including loading data and exporting the new plans. Our CPRIT grant aims to translate this research software to clinical useful system, after which a virtual clinical trial on head and neck cancer patients will be conducted. In addition, built-in quality assurance tools and mobile interactive planning application will be developed to secure patient safety, treatment plan quality and workflow efficienc . The success of this project is promising to realize online ART in practice for head and neck cancer and broaden the scope of ART for all cancer sites.

321 **CPRIT Grantee**
Poster Session A

Unbiased selection of a lung cancer specific peptide-peptoid hybrid and validation of lipid-phosphatidylserine (PS) as the target

T. Desai, University of Houston; G. Udugamasooriya, University of Houston

Introduction: The heterogeneity of protein expression and cross-talk/compensation between signaling cascades present significant hurdles for the development of single targeted therapeutic agents that provide durable efficacy and are broadly effective. To overcome some of these challenges, we hypothesized sensing cellular differences and initiated a unique unbiased selection approach to target any cancer specifi biomolecule such as protein, lipid, carbohydrate etc., present only on the cancer cell surface that are not found or less abundant on normal cells. We exploited a unique on-bead two-color (OBTC) cell screen using a lung cancer cell line (HCC4017) and normal bronchial epithelial cells (HBEC30KT) derived from the same patient. This screening strategy is unbiased in terms of the nature of target selection allowing equal chance to recognize a protein, lipid or a carbohydrate distinct to cancer and finally identified the target of our compound as highly cancer specifi lipid-phosphatidylserine (PS). M**ethods:** A ~400,000 peptid-peptoid library was synthesized and was screened using OBTC combinatorial cell assay. Magnetic bead, ELISA like and FACS binding assays and cell viability assays were carried out to validate binding and cytotoxicity of the identified compound – PPS1on lung cancer cells. PS, as the target was confirmed using lipid ELISA, membrane lipid array and liposome binding assays. In vivo activity was assessed with mice models of lung cancer. **Results:** Our unbiased selection approach successfully identifie PPS1, a peptide-peptoid hybrid. Our results show that PPS1 selectively bound and the dimeric version PPS1D1 was cytotoxic to multiple different cancer cell types such as lung, breast and prostate. We found that PPS1 bound to anionic phospholipids with high specificity to PS, but do not recognize lipids such as PC and SM found on normal cells validated through ELISA-like assays, membrane lipid arrays and liposome based FACS binding assays. Further, PPS1D1 showed potent cytotoxicity towards multiple cancer cells in vitro and displayed single agent activity in H460 xenografts. In addition, PPS1D1 potently enhanced the anti-tumor activity of docetaxel. C**onclusion:** There is an increasing need for anti-cancer agents that are effective against broad types of cancers due to the less effectiveness of protein targeted standard therapies.

Phospholipid asymmetry and elevated PS levels is observed in the tumor microenvironments of most cancers analyzed to date. We propose that PPS1D1 may have efficacy in multiple tumor types and also has the potential to safely increase the efficacy of standard cancer th rapy.

322 **CPRIT Grantee Poster Session B**

Development of an Effective Cancer Vaccine Platform Using an Attenuated Salmonella Typhi *X. Xu, Baylor College of Medicine; S. Kurbanov, Baylor College of Medicine; J. Galen, University of Maryland School of Medicine; L. Metelitsa, Baylor College of Medicine*

Introduction: Inadequate antigen delivery is one of the major limitations of modern cancer vaccine vectors. To overcome this challenge, we exploited Salmonella Pathogenicity Island 2 (SPI2) and its type III secretion system (T3SS) to deliver a tumor-associated antigen (TAA) of choice into the cytosol of antigen-presenting cells (APC) in situ. The goal of this study was to explore and exploit the potential of SPI2-encoded T3SS of a clinically validated S. typhi strain CVD908 for construction of effective cancer vaccines. **Methods:** We engineered CVD908 to express functionally inactive mutants of human oncoproteins survivin (SVN) and MYCN. To adapt CVD908 to stably express recombinant antigens without antibiotic-dependent selection, we used a recently reported plasmid stabilization system that encodes the single-stranded binding protein (SSB), an essential protein in DNA metabolism, which was deleted from the bacterial chromosome. The resultant constructs maintained bacterial vector stability while expressing human TAAs. **Results:** We found that CVD908Δssb vector effectively infected human dendritic cells (DCs) in vitro and translocated recombinant SVN or MYCN into their cytosol. DCs infected with CVD908Δssb/MYCN or CVD908Δssb/SVN induced in vitro generation of antigen-specific CD8 T cells from human PBMC. CVD908Δssb remained stable and immunogenic in mice. The therapeutic vaccination with CVD908Δssb/MYCN or CVD908Δssb/SVN vaccines produced potent antitumor activity in a murine model of neuroblastoma. **Conclusion:** Oral antigen delivery via SPI2-encoded T3SS of Salmonella typhi may serve as a foundation of an effective cancer vaccine platform.

323 **CPRIT Grantee Poster Session A**

The CPRIT Therapeutic Monoclonal Antibody Lead Optimization and Development Core Facility *Z. An, The University of Texas Health Science Center at Houston*

Introduction: Anti-cancer antibodies represent one of the most significan advances in cancer therapy. During the last 25 years, therapeutic antibodies have become a major drug modality for cancer with more than 40 therapies in clinical use and hundreds more in development. This emerging trend is largely due to their desirable safety profile, high target specificit , and efficac . Despite the success, there is an urgent need for novel antibody cancer targets for all cancer types, especially those of unmet medical needs. Almost all academic researchers engaged in cancer drug target discovery employ antibodies as reagents in both in vitro and in vivo studies. Some of the reagent antibodies exhibit significan efficacy in animal disease models and offer potential for drug development. However, most of the promising antibodies are never advanced further as cancer therapies due to the lack of access to key technologies in therapeutic antibody discovery and development. **Methods:** The CPRIT Therapeutic Monoclonal Antibody Lead Optimization and Development Core Facility is a state-of-the-art platform for cancer therapeutic antibody lead optimization and development, which provide state-wide support and service to advance lead antibodies from academic laboratories to the stage of preclinical development. Therapeutic antibody lead optimization is a complex and technologically demanding process. **Results:** The core facility offers four major functional modules: 1) Lead identification 2) Lead optimization; 3) Lead construction; and 4) Antibody production. The CPRIT antibody core facility is available to independently funded researchers engaged in antibody drug discovery in Texas-based institutions. **Conclusion:** By optimizing lead antibody candidates with "drug-like" properties, researchers will gain competitivity for attracting alternative funding(s) to continue development of the optimized antibodies for cancer therapies.

324 **CPRIT Grantee Poster Session B**

Investigating the therapeutic use of Estrogen Receptor β agonists in breast cancer *C. Samayoa, The University of Texas Health Science Center at San Antonio; N. Krishnegowda, The University of Texas Health Science Center at San Antonio; R. Vadlamudi, The University of Texas Health Science Center at San Antonio; R. Tekmal, The University of Texas Health Science Center at San Antonio*

Introduction: In breast cancer, estrogen receptor status reflects the biology of the tumor and is used to determine the likelihood of responding to endocrine therapy. 70% of all breast cancers express Estrogen Receptor α, which is indicative of estrogen dependence for growth. Current therapies aim to lower estrogen levels, or inhibit estrogen receptor signaling to ultimately prevent recurrence. However, only two-thirds of ERα-positive respond to endocrine therapy. The Estrogen Receptors are ligand-activated transcription factors with similar structures that slightly differ in their DNA binding sites and ligand binding domains. Estrogen Receptor α has been shown to induce the proliferation of mammary gland cells, whereas Estrogen Receptor β exhibits tumor suppressive properties. The objective of this study was to investigate the utility of selective ERβ agonists as a treatment for breast cancer. **Methods:** Using different breast cancer models, we investigated the effects of ERβ agonist treatment on growth, migration, apoptosis, cell cycle distribution and gene expression. To determine if our finding translate in-vivo, we employed xenograft and syngeneic mouse models. **Results:** In this study, we demonstrate that treatment with ERβ agonist results in inhibition of cell growth and migration. Additionally, treatment also impacts cell cycle distribution and affects key proteins involved in cell cycle regulation. We also demonstrate an increase in ERβ mRNA and protein levels upon treatment. This shift in the ERβ to ERα ratio, represents a viable targeting strategy in the treatment of breast cancer. Our in-vivo studies demonstrated reduced tumor volumes in mice treated with ERβ agonists in combination with conventional therapy, confirming the growth inhibitory activity of ERβ agonists. **Conclusion:** Selective activation of ERβ, through the use of ERβ agonists has enabled us to exploit its tumor suppressive function and investigate the utility as a treatment for breast cancer. This study suggest that activation of ERβ signaling is a valuable strategy to inhibit breast cancer growth and progression.

325 **CPRIT Grantee Poster Session A**

Peptide Vaccines Enhance Checkpoint Blockade Anti-melanoma Therapeutic Efficac *Y. Hailemichael, The University of Texas M.D. Anderson Cancer Center; W. Overwijk, The University of Texas M.D. Anderson Cancer Center*

Introduction: Current clinical data shows about 80% of patients with melanoma get little or no benefit from ipilimumab therapy alone. A promising avenue to further increase their efficacy is combination with T cell-inducing vaccination. Surprisingly, addition of gp100 peptide vaccination did not increase but actually decreased clinical efficacy to anti-CTLA-4 in melanoma patients[1]. As a result, it is currently unclear how to combine anti-CTLA-4 with vaccination. And by extension, prospective studies on combining anti-PD-1/ anti-PD-L1 with gp100/IFA also are lacking. We recently reported[2] that vaccination with gp100 peptide in IFA creates a persisting antigen depot that primes antigen-specific CD8+ T cells, followed by their undesirable sequestration at the vaccination site, and eventually their exhaustion and apoptosis, resulting in negligible anti-tumor activity. M**ethods:** To understand the mechanism by which vaccination failed to synergize with checkpoint blockade therapy, we studied the widely used standard treatment model of anti-CTLA-4 therapy of established subcutaneous B16 melanoma[3] and added concurrent vaccination with gp100/IFA together with adoptively transferred transgenic TcR pmel-1 T cells that specifically recognize the gp100 melanoma. For a more comprehensive assessment of anti-CTLA-4 and vaccine activated T cells (CD8+ Teffs), we included CD44, CD11a and CD8 to better quantify their number and survey their localization at the tumor and vaccination site. **Results:** Here, we show that the inflamed, chemokine-rich vaccination site also potently sequestered anti-CTLA-4 activated effector T cells with unrelated antigen-specificit . A fraction of these T cells specificall recognized epitopes from the known melanoma antigens TRP-2, p15E and gp100. In contrast, anti-CTLA-4 monotherapy significantly increased the absolute number of TRP-2-specific or CD44hiCD11ahiCD8lo effector T cells at the tumor site at the time of tumor suppression. A non-persistent vaccine formulation, Vesicular Stomatitis Virus encoding gp100 (VSV. gp100), synergized with anti-CTLA-4 to give a better anti-tumor activity. Interestingly, our results show that anti-CTLA-4 could further synergize with anti-PD-L1 if we were to build up on our non-persistent vaccine platform. CD8+ Teffs ubiquitously expressed (C-X-C motif) receptor 3 (CXCR3) and Leukocyte Function Associated Antigen-1 (LFA-1) in blood. Antibody blockade of CXCR3 and LFA-1 in vivo correlated with the detection of fewer CD8+ Teffs in the tumor and correspondingly reduced tumor suppression, indicating that CXCR3 and LFA-1 may mediate CD8+ Teffs tumor retention and anti-tumor activity. C**onclusion:** In conclusion, a non-persistent vaccine formulation can reverse the undesirable effect of the persistent vaccine formulation and synergizes with anti-CTLA-4 and/or anti-PD-L1 therapies, resulting in significantly improved anti-tumor activity.

326 **CPRIT Grantee Poster Session B**

Improve CBCT Image Quality Through Deformable Image Registration for Thoracic Adaptive Radiotherapy *J. Yang, The University of Texas M.D. Anderson Cancer Center; Y. Zhang, The University of Texas M.D. Anderson Cancer Center; L. Zhang, The University of Texas M.D. Anderson Cancer Center; L. Dong, The University of Texas M.D. Anderson Cancer Center; P. Balter, The University of Texas M.D. Anderson Cancer Center; L. Court, The University of Texas M.D. Anderson Cancer Center*

Introduction: In-room CBCT lacks explicit representation of patient respiratory motion and has poor image quality and inaccurate CT numbers for adaptive treatment planning. We developed an approach to digitally synthesize high-quality daily 4D-CBCT images from the planning 4DCT using deformable image registration. We also addressed the issue of motion discontinuities along different types of tissue in deformable registration and improved the accuracy of CT number mapping inside the lung. **Methods:** A patient-specific respiratory motion model, which was constructed from the planning 4DCT images, together with the CT number defined in the planning CT, was spatially mapped onto the daily CBCT using deformable image registration. (1) Our registration algorithm used the prior knowledge defined in the image context as an additional constraint to regularize deformation, which favored the motions within the same tissue, but penalized the motions across different tissues. Five lung patients, each with 300 landmark pairs, were used to validate this algorithm. (2) Based on the assumption that the mass of lung remains the same during respiration, we created a mass preserving model to correct the CT number mapping inside the lung. The deep-inspiration-breath-hold CT image and the end-of-exhalation phase image in 4DCT of three lung patients were used to validate the CT number correction. (3) We validated the entire process by comparing the synthesized 4D-CBCT against the weekly 4DCT acquired in the same day using two protocol lung patients with both daily in-room CBCT and weekly 4DCT scans. **Results:** (1) For five lung patients, the mean and standard deviation of the landmark displacements were 1.3±0.8mm using our approach, compared with that of 2.3±2.9mm using demons algorithm. (2) Without density correction, the mean and standard deviation of the absolute CT number difference inside the lung were 59±48, 69±49, and 71±42 for the three cases. After density correction, these numbers reduced to 18±35, 20±35, and 14±29, respectively. (3) The Dice values were calculated to assess lung and tumor volume overlaps between synthesized 4D-CBCT and weekly 4DCT. For the lung, the values were 92% and 97.1% respectively. For the tumor, they were 90.2% and 82.5%, respectively. **Conclusion:** We proposed a novel computational framework to generate improved daily 4D-CBCT images through deformable image registration by transferring prior respiratory motion and CT numbers from the planning 4DCT. The synthesized daily 4D-CBCT images have much better image quality than the conventional CBCT thus potentially improving the on-line adaptive treatment planning and dose verification

327 **CPRIT Grantee Poster Session A**

Temozolomide + Irinotecan Followed by Fenretinide/LXS + Ketoconazole + Vincristine Is Active in Post-progressive Disease Neuroblastoma Xenografts *L. Lopez-Barcons, Texas Tech University Health Sciences Center; B. Maurer, Texas Tech University Health Sciences Center; C. Reynolds, Texas Tech University Health Sciences Center*

Introduction: Despite modern aggressive therapy, many children with high-risk neuroblastoma (NB) develop progressive, therapy-resistant disease. Chemotherapy of recurrent neuroblastoma commonly employs cyclophosphamide (cyclo) + topotecan (topo), or temozolomide (TMZ) + irinotecan (irino). Fenretinide (4-HPR) achieved multiple complete responses in a phase I clinical trial in recurrent neuroblastoma when formulated as 4-HPR/LXS oral powder; 4-HPR plasma levels (clinically) and associated anti-tumor activity (pre-clinically) are increased by the P450 inhibitor, ketoconazole (keto). Vincristine (VCR) is an anti-microtubule agent. Using neuroblastoma xenografts we compared TMZ+irino to cyclo+topo, defined the activity of 4-HPR+keto+VCR, and determined if 4-HPR+keto+VCR was active against minimal residual disease (MRD) remaining after optimal re-induction chemotherapy. **Methods:** Multidrug-resistant, human neuroblastoma cell lines (CHLA-119, CHLA-79, CHLA-90m, CHLA-171, FU-NB-2006, CHLA-136, CHLA-136Luc) and patient-derived xenografts (PDX) (COG-N-415x, Felix-PDX, COG-N-452x, COG-N-471x), all from patients with progressive disease, were xenografted into nu/nu mice. Drugs: TMZ (25 mg/kg, p.o.), irino (7.5 mg/kg, i.v.), cyclo (22 mg/kg, i.p.) and topo (0.4 mg/kg, i.p.), were given q.d. x 5 = 1 cycle, every 21 days; 4-HPR/LXS (180 mg 4-HPR/kg, p.o.), keto (38 mg/kg, p.o.) were all given q.d. x 5/week; VCR (0.125 mg/kg, i.v.) was given twice weekly, alternating weeks. **Results:** Comparing TMZ+irino vs. cyclo+topo in 5 cell line and 2 PDX xenografts (subcutaneous) and one disseminated xenograft showed that event-free survival (EFS) of mice at 200 days treated with TMZ+irino was significantl better than cyclo+topo, 51.5% vs 33.3% ($P<0.03$, all models combined); EFS of controls = 1.5%. 4-HPR+keto+VCR showed significantly higher ($P<0.001$) EFS at 100 days in 3 cell line xenografts and 3 PDX xenografts than single agents: 4-HPR+keto+VCR = 72%, 4-HPR = 25%, VCR = 7%, controls = 1%. 4-HPR+keto+VCR given after one cycle of TMZ+irino significantly enhanced mouse event-free survival compared to no further therapy in subcutaneous xenograft models with suboptimal TMZ+irino activity: FU-NB-2006, 153 ± 70 days vs 59 ± 9 days ($P=0.0002$); Felix-PDX, 81 ± 29 days vs. 57 ± 7 days ($P=0.01$). All combination regimens were well-tolerated. **Conclusion:** TMZ+irino was a more effective re-induction regimen than cyclo+topo for recurrent/refractory neuroblastoma subcutaneous and disseminated disease xenograft models. The combination of 4-HPR+keto+VCR was more active than single agents and was active against MRD remaining after TMZ+irino. These preclinical data support clinical trials of novel combination therapies for recurrent neuroblastoma. A phase I study of 4-HPR/LXS oral powder + keto + VCR (SPOC 2013-001) is ongoing in the South Plains Oncology Consortium (www.SPONC.org).

328 **CPRIT Grantee Poster Session B**

Estrogen Receptor β Agonists Induce Differentiation and Apoptosis of Glioma Stem Cells *G. Sareddy, The University of Texas Health Science Center at San Antonio; L. Garcia, The University of Texas Health Science Center at San Antonio; A. Brenner, The University of Texas Health Science Center at San Antonio; R. Vadlamudi, The University of Texas Health Science Center at San Antonio*

Introduction: Glioblastoma (GBM) are the most common primary brain tumors with poor prognosis. A gender bias exists in the development of glioblastoma with incidence of developing GBM is greater in males compared to females. Glioma Stem Cells (GSCs) are implicated in the tumor initiation and therapy resistance in GBM and their elimination is critical for the development of efficient therapeutic strategies. Estrogens play a crucial role during brain development, differentiation and in neuroprotection. Estrogen effects are mediated though its cognate receptors ERα and ERβ and ERβ functions as a tissue-specific tumor suppressor. Emerging evidence suggest the tumor suppressive role of estrogen on brain tumors. However, estrogen as potential therapy for GBM has limited therapeutic application due to safety concerns. Therefore, alternative agents that mimic estrogen effects will have clinical utility in treating GBM. Recent studies identified a plant derived Liquiritigenin and a synthetic LY500307 as selective ERβ agonists with an excellent preclinical profile. The objective of this study is to determine the effect of ERβ agonists on apoptosis and differentiation and to determine their mechanism(s) of action on GSCs. **Methods:** GSCs (CD133+ve) were isolated from established and patient derived GBM cells using FACS. Therapeutic effect of ERβ agonists on GSCs were analyzed by cell proliferation, neurosphere formation and self-renewal assays. Effect of ERβ agonists on stem-ness and differentiation of GSCs were analyzed by western blotting and q-RT-PCR. Global transcriptome changes following ERβ agonists treatment in GSCs were determined by RNA-sequencing analysis. Therapeutic potential of ERβ agonists on GSCs mediated tumor growth and survival was determined using orthotopic models. **Results:** GSCs preferably expressed ERβ with undetectable levels of ERα. ERβ agonists significantly reduced the proliferation of GSCs with no activity on normal astrocytes suggesting its tumor specific effects. ERβ agonists significantly inhibited the neurosphere formation and self-renewal of GSCs. Western blot analysis and qRT-PCR analysis revealed that ERβ agonists reduced expression of stemness markers such as Nestin and Sox-2 and increased expression of differentiation markers GFAP and tuj-1. RNA sequencing analysis revealed induction of pathways related to apoptosis, cell cycle, stemness and differentiation by ERβ agonists. Further, ERβ agonists treatment significantly reduced the GSCs driven tumor growth in in vivo orthotopic model. ERβ agonists reduced the expression of stemness genes and induced the apoptosis in tumors. **Conclusion:** Together, our results demonstrated that ERβ agonists induce differentiation and apoptosis of glioma stem cells and function as novel therapeutic agents for treatment of GBM.

329 **CPRIT Grantee Poster Session A**

Improvements in the QA of Clinical Trials in Radiation Therapy

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Introduction: We address two issues not adequately addressed in current QA of radiotherapy clinical trials: (1) Current dosimetry phantoms used for clinical trial credentialing do not directly assess IGRT processes. (2) Current trial QA does not examine the accuracy of normal tissue contours in patient treatment plans. Tools were created to address these and tested at centers enrolling patients on a CPRIT-funded phase III study of accelerated hypofractionated IGRT in patients with stage II-III NSCLC **Methods:** (1) An IGRT phantom was built out of a low-density body with two inserts. Insert A is used for the CT simulation. Insert B is used for the actual treatment. Relative positions are unknown to the user. The user simulates the phantom (with insert A) as they would a patient, including marking the phantom. A treatment plan is created and sent to the treatment unit. The phantom (with insert B) is then positioned using local IGRT practice. The phantom was tested at 7 centers, selected to include a wide variety of imaging equipment. (2) Atlases for deformable registration and normal tissue segmentation were created for the esophagus, trachea/large bronchus, and brachial plexus. These atlases were used to independently delineate 20 patients **Results:** (1) Approaches tested to mark (and transfer) simulation isocenter included lasers, fiducials and reflective markers. IGRT approaches included kV imaging (Varian Trilogy, Brainlab ExacTrac), kV CT (CT-on-rails), kV CBCT (Varian Trilogy, Varian Truebeam, Elekta Agility) and MV CT (Tomotherapy). Users were able to successfully use this phantom for all combinations of equipment and processes. IGRT-based shifts agreed with the truth within 0.8mm, 0.8mm and 1.9mm in the LR, AP, and SI directions, respectively. (2) On visual inspection, the atlas contour of the esophagus was only acceptable for 65% of cases. For two cases (one trachea/large bronchus case and one brachial plexus case), the auto-segmentation highlighted the fact that not all of the structures had been delineated in the original plan. The average change in maximum dose to the esophagus, trachea, and brachial plexus were 0.2±4.4Gy (range: - 13.6: 4.7Gy), 0.2±1.1Gy (-3.9:0.8Gy), and 3.6±10.7Gy (-1.2:34.1Gy), respectively. **Conclusion:** We have developed an IGRT phantom that can be used for credentialing of clinical trials with an action level of 1mm in AP and LR directions, and 2mm in the SI direction, consistent with TG142. Automatic contouring can help identify gross errors in normal tissue contouring, but is not sufficientl accurate to identify smaller errors.

330 **CPRIT Grantee Poster Session B**

Engineered aglycosylated IgG variants with enhanced anti-tumor activity via CDC without ADCC

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Introduction: We engineered a series of variants of the IgG Fc domain that can mediate the potent killing of cancer cells. These Fc engineered variants are likely to be of great utility for the generation of more potent antibody therapeutics. One of the key mechanisms by which antibodies kill target cells is via the recruitment and activation of proteins of the complement system. The complex between the Fc domains on antibodies bound on pathogenic cells and C1q activates a cascade of biochemical reactions that results in cell killing, a process known as Complement Dependent Cytotoxicity (CDC). The potency of CDC depends on the ability of antibodies to bind to C1q. This interaction is normally completely abolished when the single carbohydrate chain appended to the Fc polypeptide has been removed, i.e. in antibodies that are aglycosylated. **Methods:** Fc enginerred variants were isolated by screening very large combinatorial libraries of Fc mutant proteins by a variation of the methology described earlier (Jung et al. 2010). The binding kinetics between engineered IgGs and C1q were measured by SPR on a BiaCore 3000 instrument. To monitor the ability of the antibodies to elicit CDC, cell lysis was evaluated using the calcein release assay. **Results:** Unlike the glycosylated IgG, aglycosylated IgG antibodies do not bind FcγRs or C1q. We generated aglycosylated IgG variants that bind to C1q with exquisite affinit , higher than that of glycosylated IgG. The A801 IgG variant also showed 213-fold enhanced affinity toward C1q. The purified A801 IgG variant, which Fab is from Rituximab, were incubated with human serum and lymphoid leukemia cells (Raji), which expressed CD20. The Rituximab A801 variant induced cell-killing much more efficiently than clinical grade Rituximab and showed enhanced EC50 values. Also, the three IgG variants did not have any binding characteristics to any FcγRs. In other words, the Rituximab A801 variant elicit only CDC and no Antibody Dependent Cell Cytotoxicity (ADCC), a property that is of great interest both for certain therapeutic applications and also as a tool for determining the relative contribution of CDC and ADCC on antibody therapeutic function. **Conclusion:** We have employed protein engineering to generate aglycosylated IgG variants that not only binds to C1q but with a much higher affinity than that of their glycosylated counterparts. Having an Fc domain that can only induce cancer cell killing by CDC without binding to FcγRs may drastically enhance the therapeutic potency of certain antibody drugs.

331 **CPRIT Grantee Poster Session A**

Reciprocal Inhibition of NKT Cells and Tumor-Associated Macrophages in the Neuroblastoma Microenvironment *A. Courtney, Baylor College of Medicine; D. Liu, Baylor College of Medicine; A. Heczey, Baylor College of Medicine; E. Marinova, Baylor College of Medicine; G. Tian, Baylor College of Medicine; X. Gao, Baylor College of Medicine; L. Guo, Baylor College of Medicine; G. Dotti, Baylor College of Medicine; L. Metelitsa, Baylor College of Medicine*

Introduction: Immunotherapy with Natural Killer T cells engineered to express a GD2-specific chimeric antigen receptor (CAR.GD2 NKTs) demonstrated potent anti-tumor activity in a metastatic model of neuroblastoma in humanized (hu)-NSG mice. However, tumors eventually progressed without the loss of GD2 expression suggesting that tumor escape is due to inhibition of effector function of CAR.GD2 NKTs. Since NKTs co-localize and specifically interact with M2-like tumor-associated macrophages (TAMs), we examined whether TAMs inhibit effector functions of NKT and CAR.GD2 NKTs. **Methods:** To examine how NKTs interact with macrophage subsets, we generated M2 (CD163high) and M1 (CD163low) in vitro and set up co-cultures to evaluate phenotypic changes to both macrophages and NKTs. To determine the effect of NKTs on TAMs in vivo, hu-NSG mice were generated using human cord blood derived CD34+ cells followed by injection of luciferase-transduced human neuroblastoma cells and adoptive transfer of NKTs or CAR. GD2 NKTs. Tumor growth and response to therapy was monitored by bioluminescence imaging. We also performed multi-parameter flo cytometry analysis of tumor-infiltrating NKTs, CAR.GD2 NKTs, and TAMs. **Results:** In vitro experiments revealed a reciprocal inhibition of NKTs and M2-polarized macrophages. We found that NKTs selectively kill M2-polarized macrophages and/or induce their differentiation toward M1-like cells. However, both M2- and M1-macrophages suppressed NKT-cell proliferation in response to TCR or CAR stimulation. This was associated with rapid up-regulation of PD-L1 and PD-L2 on both M2 and M1 macrophages upon addition of supernatant from activated NKTs. The analysis of tumor tissues in hu-NSG mice revealed strong up-regulation of PD-L1 expression exclusively on TAMs and not on neuroblastoma cells after treatment with CAR.GD2 NKTs. Neither neuroblastoma cells nor TAMs expressed PD1 ligands in untreated mice or in primary NB tissues from patients with stage 4 disease. Importantly, a combination of CAR. GD2 NKT cell immunotherapy with anti-PD1 blocking mAb produced significant delay in tumor growth. Furthermore, depletion of TAMs prior to CAR.GD2 NKT cell transfer resulted in durable tumor regression. **Conclusion:** The results provide a rationale for a combined use of CAR-redirected NKTs with PD1-blocking or TAM-depleting therapeutics for immunotherapy of neuroblastoma and possibly other solid tumors.

332 **CPRIT Grantee Poster Session B**

Developing A Self-learning Patient Safety Event Reporting System *H. Kang, The University of Texas Health Science Center at Houston; Y. Gong, The University of Texas Health Science Center at Houston*

Introduction: Each year in the United States, 650,000 cancer outpatients receiving chemotherapy are at high-risk of developing infections. The infections may lead to hospitalization, disruptions in chemotherapy schedules, or even death. Furthermore, many of the patient safety events are very repetitive and could be prevented. To learn from the recurring events, an event reporting system is regarded as an effective way to analyze accumulated events and similarities at a collective level. The ideal reporting system would generate actionable knowledge based upon patient safety event repository, and even suggest common solutions for similar events under investigation. Unfortunately, current reporting systems still remain in the primary stage transitioning from paper forms, lack a logic-based organizational knowledge structure for comparison and analysis, and suffer from poor usability—all of which impede the development of the systems towards the ideal condition. Therefore, we propose to design a self-learning patient safety event reporting system based on structural knowledge that can dynamically measure similarities of patient safety events and thus promote quality, safety and learning effect. **Methods:** Three prevailing algorithms of semantic similarity (Vector Space, Term Overlap, and Information Content) were implemented to measure the similarities of the 326 patient safety events annotated by the AHRQ event taxonomy. The performance of each algorithm was then evaluated by three experts based on a 4-point Likert scale. The consistency between the scales of the algorithms and experts was measured and compared with the scales randomly assigned. The similarity algorithms and scores, as a self-learning and self-updating module, were then integrated into the system. **Results:** The similarity scores of the three algorithms reflect a significantly higher consistency with the experts' review than those randomly assigned. For example, results of the Vector Space algorithm are the most consistent with those of the three experts, with agreements of 80%, 90% and 90% respectively and all p-values less than 0.01. Incorporating the algorithms into our reporting system enables the system to learn and update based upon patient safety event similarity. **Conclusion:** By integrating semantic similarity algorithms into a patient safety event reporting system, the system can learn from previous events and provide hints or common solutions for current events. With this innovative idea, reporting systems will have more useful functions and potentially trigger a revolution for data management and analysis in the field of patient safet .

333 **CPRIT Grantee**
Poster Session A

Inactivation of BTLA inhibitory motifs in TIL exhibits positive signals that mediate anti-tumor control *K. Ritthipichai, The University of Texas Health Science Center at Houston; C. Haymaker, The University of Texas M.D. Anderson Cancer Center; R. Nurieva, The University of Texas M.D. Anderson Cancer Center; P. Hwu, The University of Texas M.D. Anderson Cancer Center; C. Bernatchez, The University of Texas M.D. Anderson Cancer Center*

Introduction: Clinical trials of adoptive T-cell therapy (ACT) using autologous ex vivo expanded tumor-infiltrating lymphocytes (TIL) have demonstrated a potential immunotherapy with 50 percent clinical response in stage IV metastatic melanoma. When we investigated a number of biomarkers in the infused TIL association with clinical response, a subset of CD8+BTLA+ was the strongest predictive biomarker of response to TIL therapy. BTLA, B-and-T lymphocyte attenuator, is known as an inhibitory molecule in different immune cells. Its cytoplasmic region contains three motifs: (Grb2, ITIM, and ITSM). ITIM and ITSM are inhibitory motifs that suppress T cell function upon ligation of BTLA with its ligand, HVEM (Herpes virus entry mediator). Although the functional role of the Grb2 motif of BTLA remains unclear, it is suggested that Grb2 may exhibit positive signal through PI3K. **Methods:** To dissect the signaling pathway of BTLA's motifs, we generated retroviral vector containing BTLA and its mutants by substitution tyrosine (Y) for phenylalanine (F) in either the Grb2 motif (∆Grb2) or ITIM and ITSM (∆ ITIM and ITSM). BTLA and its mutants were overexpressed in either CD8+BTLA-human TIL or BTLA$^{-/-}$ mouse T cells to uncover BTLA downstream signaling pathway and in vivo anti-tumor effect following TIL transfer. **Results:** AKT and MAPK pathway was significantly suppressed in Grb2 mutant upon HVEM ligation. Greater proliferation was pronounced in ITIM and ITSM mutant mouse T cells when stimulated with DC pulsed cognate peptide, while less proliferation was observed when Grb2 inactivated. NOD scid gamma (NSG) mice were engrafted human derived melanoma and TIL overexpressing BTLA mutants for the investigation of anti-tumor effect. ITIM and ITSM mutant TIL exhibited better tumor burden control and present at higher frequency in peripheral blood following adoptive transfer. **Conclusion:** Our study revealed that Grb2 motif provided positive signal that favors anti-tumor response. Therefore, the strategy to inactivate ITIM and ITSM may enhance persistence following infusion and resulting in mediating tumor control.

334 **CPRIT Grantee**
Poster Session B

Combinations of Kinase siRNAs Enhanced Sensitivity of Ovarian Cancer Cells to Paclitaxel *H. Yang, The University of Texas M.D. Anderson Cancer Center; X. Wang, The University of Texas M.D. Anderson Cancer Center; A. Ahmed, University of Oxford; L. Iles, The University of Texas M.D. Anderson Cancer Center; G. Bartholomeusz, The University of Texas M.D. Anderson Cancer Center; L. Zhen, The University of Texas M.D. Anderson Cancer Center; R. Bast, The University of Texas M.D. Anderson Cancer Center*

Introduction: Primary drug resistance imposes a major obstacle in ovarian cancer treatment. All patients with newly diagnosed ovarian cancer are treated with a combination of paclitaxel and carboplatin. While 70% of ovarian cancers respond to carboplatin, less than 50% respond to paclitaxel. Inhibition of kinases that modulate primary resistance to paclitaxel, could enhance response to therapy. M**ethods:** A subscreen of combinations of 14 target kinase siRNAs was performed in six ovarian cancer cell lines in present or absent of paclitaxel after a completed kinome siRNA library screen. Sulforhodamine B (SRB) colorimetric assay and microtubule fractionation assay were used to measure cell viability and microtubule stability respectively. **Results:** Among 45 hits from high throughput screening with a kinome siRNA library, 14 target proteins (AATK, ACRBP, BMP2K, CHUK, EDN2, IKBKB, ILK, RAPGEF3, RAPGEF4, RFP, SIK2, STK24, STK39 and TBK1) regulated sensitivity to paclitaxel and were differentially expressed or overexpressed in a fraction of ovarian cancers. siRNAs against each of these kinases were tested for the ability to enhance sensitivity to paclitaxel in each of 12 ovarian cancer cell lines that reflected the heterogeneity observed in ovarian cancers including mutations of TP53, BRCA1/2, KRAS, BRAF, PI3K and PTEN. Knockdown of 10 individual genes enhanced paclitaxel sensitivity by at least two fold in different cell lines. A subscreen using combinations of these 14 kinase siRNAs in the six most responsive cell lines were carried out to select pairs of kinase siRNAs which could further potentiate pactlitaxel sensitivity. IKBKB and STK39 kinase siRNAs had the greatest activity. We discovered that knockdown of IKBKB and STK39 stabilized microtubules judged by a microtubule fractionation assay and levels of acetylated and detyrosinated tubulin. C**onclusion:** Double knockdown of microtubule stabilizing kinases IKBKB and STK39 resulted in greater enhancement in microtubule stability, leading to additive sensitization of paclitaxel. Small molecule inhibitors are available for IKBKB. As inhibition drugs become available for STK39, these observations can be translated to the clinic.

335 **CPRIT Grantee**
Poster Session A

Spinal Nerve Tolerance to Single-Session Stereotactic Ablative Radiotherapy *P. Medin, The University of Texas Southwestern Medical Center at Dallas; R. Foster, The University of Texas Southwestern Medical Center at Dallas; S. Vernino, The University of Texas Southwestern Medical Center at Dallas; J. Meyer, The University of Texas Southwestern Medical Center at Dallas; A. van der Kogel, University of Wisconsin; J. Sayre, UCLA; Y. Yamada, Memorial Sloan Kettering Cancer Center; R. Timmerman, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Stereotactic ablative radiotherapy (SAbR) is a rapidly expanding treatment modality utilized for an increasing number of cancers. The radiosensitivity of healthy normal tissues, including neural structures, is poorly understood in the setting of SAbR. In recent years, numerous cases of peripheral neuropathy following SAbR have been published and we have observed 11 cases in our own practice that are not yet reported. A better understanding of tolerated dose limits is critical to avoid overestimation, thus prescribing radiation doses that will lead to catastrophic normal-tissue injury, or underestimation resulting in lower prescription doses that are less likely to ablate tumors. A study is underway to define the dose-related incidence of neuropathy resulting from single-session SAbR to the spinal nerve using a porcine model. The influenc of irradiated length on neuropathy will also be evaluated. **Methods:** To date, 10 Yucatan minipigs (out of a planned 50) have been entered in this study. Each animal has received CT and MRI scans for treatment planning followed by single-session SAbR using an image-guided, 6MV linear accelerator. A 1.5cm length of the left-sided C6, C7 and C8 spinal nerves was targeted. Animals were evenly distributed into 5 dose groups receiving 16, 18, 20, 22 or 24Gy. The neurologic status of all animals is being followed by electrodiagnostic exam (-1, 2, and 10 weeks following SAbR) and daily observation of gait. Currently, all animals have been followed between 10-13 weeks since irradiation. Animals will continue to be evaluated with electrodiagnostic exams and gait observation until neurologic deficits occur or the 52-week maximum followup period is reached. Histopathologic examination will be performed on both the irradiated spinal nerves and the corresponding unirradiated contra-lateral nerves. **Results:** To date, motor neurologic deficits have been observed in 3 animals that were in the 20, 22, and 24 Gy dose groups. Affected animals presented with a limp in their left front limb. Deficits were firs

observed 11-12 weeks following irradiation. C**onclusion:** The neurologic deficits observed to date have occurred at the predicted dose levels and latency periods suggesting that our hypothesis is true; the dose-response curves for the spinal nerves and spinal cord are the same. It is still early in the course of this study and additional data is necessary to make fir conclusions. An additional 40 animals will be enrolled in this study over the next 2.5 years so significance will be achieved

336 **CPRIT Grantee**
Poster Session B

Enhanced Hormonal therapy Response by Targeting TRβ in Breast Cancer *G. Gu, Baylor College of Medicine; L. Gelsomino, Baylor College of Medicine; T. Babarinde, Baylor College of Medicine; S. Fuqua, Baylor College of Medicine*

Introduction: Thyroid hormone receptor beta (TRβ) is a nuclear transcription factor that mediates the pleiotropic activities of thyroid hormones, and influences basal oxygen consumption, cardiac contractility, and lipid metabolism. We previously reported that TRβ could regulate chemotherapy response in triple negative breast cancers (TNBC). Treatment of TNBC is a major clinical problem due to the lack of useful therapeutic targets. Several breast cancer groups have tried to restore ER expression in ER-negative cells. It has been reported that thyroid hormones can increase estrogen-mediated transcription in cancer cells. We thus explored the role of TRβ in ERa regulation in both ER+ and ER- cells. **Methods:** TaqMan qRT-PCR. pGIPZ lentiviral infection. Inducible gene expression. MTT and soft agar assays. Western blot. Site-directed mutagenesis. **Results:** TRβ specific agonists GC-1 and KB-141 restored ERα expression in the ER-negative cell line HCC-2185. In the ER positive cell line MCF-7, these agonists were also able to enhance ER protein expression, which could be blocked by TRβ antagonist treatment. Cycloheximide treatment further demonstrated that KB-141 treatment was able to stabilize ERa protein by enhancing its half-life. In HCC2185 cells, treatment of KB-141 significantly increased phosphorylation of ERa including S104/106, S118, S167 and S305phosphorylaiton sites and these were reversed by TRβ antagonist. ERa mutations for each phosphorylation site have been generated and tested in western blot to determine which phosphorylation site initiated the post-translational modification (PTM). When S305 was mutated, KB-141 was not able to enhance the phosphorylation of S118 or S167 indicating that S305 was the "initiating site" of the PTM cascade. The molecular mechanisms underlying TRβ agonist enhancement of pS305 is under investigation. In HCC2185 cells, ERa levels were induced by GC-1 or KB-141 which was sufficient to sensitize to Tamoxifen and Fulvestrant, indicating that modulation of TRβ may also extend hormonal therapy to this traditionally hormonally-insensitive group of tumors. In ER+/Tamoxifen-resistant cell line TR1, a TRβ antagonist was able to reverse the acquired Tamoxifen-resistant phenotype through enhanced P53 expression and PARP cleavage, indicating that TRβ antagonists might be used to modulate ERa and enhance Tamoxifen response. **Conclusion:** Clinical targeting of TRβ in TNBC is a novel strategy to restore hormone response via functional re-activation of ERα. Targeting of the TRβ might extend hormonal therapies to ER-negative patients, and potentially enhance Tamoxifen therapy in ER-positive patients. The molecular mechanisms underlying TRβ regulation may be mediated by S305 of the estrogen receptor.

337 **CPRIT Grantee**
Poster Session A

Inhibition and Suppression of Human DNA Polymerase θ (POLQ) to Influence Radiosensitivity of Breast Cancer *R. Wood, The University of Texas M.D. Anderson Cancer Center; E. Cho, The University of Texas at Austin; M. Yousefzadeh, The University of Texas M.D. Anderson Cancer Center; K. Boulware, The University of Texas M.D. Anderson Cancer Center; A. Devkota, The University of Texas at Austin; M. Lowery, The University of Texas M.D. Anderson Cancer Center; J. Tomida, The University of Texas M.D. Anderson Cancer Center; C. Aldaz, The University of Texas M.D. Anderson Cancer Center; S. Doublié, University of Vermont; K. Dalby, The University of Texas at Austin; K. Takata, The University of Texas M.D. Anderson Cancer Center*

Introduction: While ionizing radiation (IR) is used in treatment for breast cancer, its effectiveness could be improved greatly. We are investigating the suppression of DNA polymerase θ (POLQ) as a means to sensitize breast cancer cells to IR or topoisomerase inhibitors. Higher levels of POLQ gene expression are associated with poorer outcome in breast cancer. POLQ-defective cells lack one pathway of DNA double-strand break repair. We aimed to test whether reduction of POLQ activity in breast cancer cells increases sensitivity to IR, and to identify agonists of POLQ activity by high-throughput compound screening. **Methods:** A selection of genetically characterized breast cancer cell lines is being analyzed for radiosensitization following silencing of POLQ by lentiviral delivery of shRNA. Endogenous expression of POLQ is quantified before and after introduction of shRNA into cells. Radiosensitivity is measured by colony formation ability and growth suppression. **Results:** We first focused on the Basal A cell line MDA-MB-436 and Basal B cell line HCC1806. Quantitative PCR was used to measure POLQ expression and the efficiency of suppression. Five different Sigma Mission shRNAs were tested and the three highest suppressing RNAs were selected for analysis. Stable knockdown and relevant control cell lines were generated after lentiviral transduction of shRNA constructs and puromycin selection. POLQ transcript levels were reduced to ~60% of normal in HCC1806 cells harboring stable shRNAs against POLQ. This yielded detectable IR sensitivity of HCC1806. No shRNAs were found that completely suppressed POLQ expression. A real-time fluorescence assay was established for high throughput small molecule inhibitor screening. An active recombinant fragment of POLQ was used to catalyze DNA synthesis and strand displacement

with a reporter–quencher substrate. The assay was optimized for DNA strand lengths, buffers, time of incubation, and other factors including well format. POLQ was screened in parallel with RB69 gp43 DNA polymerase as a specificity control. Over 1,700 small molecules were screened, comprising commercially available nucleotide analogs, an FDA approved drug library, and kinase inhibitors with known activities. A few compounds demonstrated moderate reduction of polymerization activities of POLQ. Two compounds were selected as lead candidates and are being evaluated further by testing radiosensitization of cells. POLQ has unique abilities to extend poorly paired DNA that might be exploited in second generation screens with other DNA substrates and additional compounds. **Conclusion:** Suppression of POLQ increases radiosensitivity of some breast cancer cell lines.

338 **CPRIT Grantee**
Poster Session B

The Chemokine CXCL12 and its Receptors CXCR4 and CXCR7 in Antiangiogenic Resistance of Glioblastoma *A. Gruslova, The University of Texas Health Science Center at San Antonio; D. Cavazos, The University of Texas Health Science Center at San Antonio; M. Garcia, The University of Texas Health Science Center at San Antonio; A. Brenner, The University of Texas Health Science Center at San Antonio*

Introduction: Angiogenesis, the formation of new vessels, is a key property in tumor development and progression. There is increasing evidence that the vascularization comes not only from the sprouting of pre-existing vessels, but also the formation of new blood vessels from recruiting a heterogeneous population of bone-marrow-derived cells (BMDCs) in response to oncogenic signals. One critical factor that mobilizes BMDCs into the blood stream and retention in the tumor is chemokine CCXL12 and its two receptors: CXCR4 and CXCR7. **Methods:** To validate the role of each receptor, we combined two novel methods, cranial window imaging and fluorescent chimeric mice. This allows us to capture the chronological interactions among tumor cells (RFP), circulating BMDCs (GFP) and the cerebral vessels (FITC dextran) during tumor growth. In vivo optical imaging were performed weekly (before, during and after treatment) on a Nikon Eclipse FN-1 microscope. **Results:** While VEGFR inhibition slows tumor growth, inhibition of CXCR4 or CXCR7 was not additive to this effect. In fact, inhibition of CXCR4 resulted in significan increase in tumor size. Inhibition of VEGFR by sunitinib leads not only to a significant decrease in the number of small vessels as previously published, but also results in a likely compensatory response of increase in size of the large vessels and vasculomegaly. Addition of CXCR7 inhibition to sunitinib prevented vasculomegaly, but did not impact the loss of smaller vessels. CXCR4 inhibition had no effect on the vasculature. Further, CXCR7 inhibition decreases the number of bone marrow derived cells recruited to the vasculature, while CXCR4 inhibition decrease the number of cells recruited outside the vessels and in the tumor itself. These tumoral BMDCs are IBA1 positive and suggest a role of CXCR4 in recruitment of microglia. **Conclusion:** Our results suggests that the compensatory response of vasculomegaly to trimming of small vessels by sunitinib may be mediated by CXCL12 in a CXCR7 dependent process. CXCR7's inhibitory effect on the number of BMDCs cell recruited into the vessels, suggests that the impact on vasculomegaly may be due to impact on recruitment of endothelial progenitor cells into vessels or their survival following recruitment. VEGFR inhibition not only impacts recruitment of progenitor cells to the vessels in a CXCR7 dependent fashion, but also outside the vessels in a CXCR4 dependent fashion. We propose that CXCL12 receptors, CXCR4 and CXCR7, have a unique roles in response to loss of VEGF signaling and subsequent hypoxia.

339 **CPRIT Grantee**
Poster Session A

The Systematic Design and Biophysical Evaluation of Cationic Sulfonamide Aminolipids for siRNA Delivery *J. Miller, The University of Texas Southwestern Medical Center at Dallas; V. Tieu, University of California, Berkeley; D. Siegwart, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The use of RNA interference (RNAi) as a therapeutic is an exciting and rapidly developing field because it offers a promising alternative to small molecule drugs for the treatment of cancer. Small interfering RNA (siRNA) can be designed against any mRNA target, and upon loading into the RNA-induced silencing complex (RISC) can enable the sequence-specific recognition and degradation of the target oncogene. Because siRNA has a high molecular weight (~13 kDa) and is highly hydrophilic and anionic, it is unable to passively diffuse across cell membranes and into the cell. To date, many successful carriers have been designed using amphiphilic lipid-like compounds containing amine-rich cores, but the challenge of efficient endosomal release remains a major bottleneck in the field of RNAi therapeutics. Cationic sulfonamide amino lipids (CSALs) are a novel class of modular synthetic lipids that possess chemical and structural properties that make them promising candidates for siRNA anticancer therapies. Sulfonamide functionalities, though prevalent in many pharmaceutical drugs, have not been explored in lipid carriers for siRNA delivery. Due to a positively-charged quaternary ammonium, CSALs are excellent candidates for enhanced siRNA binding and endosomal membrane interaction and disruption upon cell uptake, which enables siRNA release. **Methods:** CSALs were readily prepared from commercially available reagents via a zwitterionic aminolipid intermediate bearing a sulfobetaine. The characteristic sulfonamide was introduced by sequential reaction of this intermediate with thionyl chloride and a primary amine to append the headgroup. A systematic, modular design enabled the evaluation of the effect of structural modification on the biophysical properties of CSAL nanoparticles in terms of size, surface charge, siRNA encapsulation, and delivery efficacy *in vitro* in human cancer cell lines. **Results:** Systematic SAR of CSALs was established by modifying the linker length between the sulfonamide and the amine headgroup, sterics at tertiary amine headgroup and quaternary ammonium, the number of hydrophobic tails, and a sidearm alcohol functionality on the periphery of the amine core in terms of both sterics and electronics. Lead materials showed excellent uptake into cells,

efficient siRNA encapsulation (97%) and *in vitro* target gene silencing in human cancer cells with up to 90% knockdown and minimal material-derived cytotoxicity. **Conclusion:** Systematic rational design of a library of CSALs, a novel class of synthetic lipids, has elucidated SAR in properties governing effective siRNA delivery. This led to the discovery of lead CSAL materials that enable efficacious siRN delivery to cancer cells.

340 **CPRIT Grantee**
Poster Session B

A Trojan Horse For Light-Triggered Delivery of Toxic Iron to Cancer Cells *D. Kurtz, The University of Texas Health Science Center at San Antonio; E. Boice, The University of Texas at San Antonio; D. Cioloboc, The University of Texas at San Antonio; C. Kennedy, The University of Texas at San Antonio*

Introduction: Traditional photodynamic cancer therapy relies on generation of singlet oxygen. However, therapeutically effective singlet oxygen requires well-oxygenated tissues, whereas many tumor environments tend to be hypoxic. We have developed a potential alternative photodynamic therapy that generates the highly toxic hydroxyl radical in a tumor-targeted fashion. Hydroxyl radical can be generated by the Fenton reaction of ferrous iron with hydrogen peroxide under hypoxic conditions. **Methods:** We created a "Trojan horse" by modification of a spherical 24-subunit iron storage protein which has the unique property of binding heme groups in its protein shell. We substituted the hemes with porphyrin photosensitizers and loaded the inner cavity with ~2000 irons as a ferric oxyhydroxide polymer. We also fused a tumor-targeting peptide (TTP) to the 24 subunits. **Results:** Irradiation of the iron-loaded porphyrin-TTP-protein with visible light triggered release of ferrous iron, and in the presence of hydrogen peroxide, hydroxyl radical. The iron-loaded protein was capable of light-dependent killing of melanoma cells. **Conclusion:** Our results constitute a proof of principle for a "Trojan horse" strategy for photosensitized delivery of toxic levels of Fenton-reactive iron to tumors. This approach is potentially applicable to many types of cancers.

341 **CPRIT Grantee**
Poster Session A

Degradable Disulfide Crosslinked Nanogels for siRNA Therapeutic Delivery Application *S. Elkassih, The University of Texas Southwestern Medical Center at Dallas; D. Siegwart, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The synthesis and construction of degradable disulfid crosslinked nanogels for siRNA therapeutic delivery application is described. Nanogels are networked structures of polymer chains crosslinked to each other, where polydisulfides specifically degrade in response to a change in redox potential through thiol-disulfide exchange reactions. In tissue, the extracellular space is oxidizing and the intracellular space is reducing due to the presence of reducing molecules such as glutathione found in millimolar concentrations. Therefore, disulfid crosslinked nanogels degrade in physiological settings (i.e. in cells) with reduced cytotoxicity, analogous to how disulfide cross-linked proteins are broken down. **Methods:** Deprotonation using a base followed by the addition of an oxidant allowed dithiol monomer and thiol crosslinker anions to undergo single-electron-transfer reactions to form disulfide bonds via a radical type mechanism. Nanogels formed using a nanoprecipitation method, utilizing a nonionic surfactant for increased particle stability. **Results:** Both synthesis of bulk gels and nanogels is accomplished using described method. Control over the crosslinking density and size of nanogels is possible by varying the feed ratio or changing the monomer/crosslinker. Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance spectroscopy (H1-NMR) confir that disulfide crosslinking occurs. A procedure using Ellman's Reagent quantified the nanogel crosslinking density and reveals that some free thiols persist. The free thiols can be used to attach siRNA directly via a "click-type" reaction. CellTiter-Glo Luminescent Cell Viability Assay from Promega using HeLa cells demonstrates that nanogels have low toxicity. Nanogels can degrade in the presence of dithiothreitol according to preliminary experiment. Degradation, dye entrapment, and kinetic studies are currently on going. Further cell-based in vitro studies and in vivo studies will be carried out. **Conclusion:** Colloidally stable disulfid crosslinked nanogels were successfully synthesized using an optimized nanoprecipitation method. Nanogels hold great promise as carriers for siRNA therapeutic application.

342 **CPRIT Grantee**
Poster Session B

Creating Novel Translation Inhibitors to Target Pro-Survival Proteins in Chronic Lymphocytic Leukemia (CLL) *M. Zhu, Baylor University; R. Chen, The University of Texas M.D. Anderson Cancer Center; Y. Chen, The University of Texas M.D. Anderson Cancer Center; W. Skillern, The University of Texas at Austin; O. Robles, Texas A&M University; W. Wierda, The University of Texas M.D. Anderson Cancer Center; K. Hull, Baylor University; D. Romo, Baylor University; W. Plunkett, The University of Texas M.D. Anderson Cancer Center*

Introduction: The overall goal of this project is to identify a small molecule inhibitor of translation initiation that is suitable for clinical development for the treatment of chronic lymphocytic leukemia (CLL) and overcome drug resistance. We targeted the biological rewiring that is characteristic of CLL, namely the overexpression of short-lived pro-survival proteins that protect the leukemic cells from apoptosis. We hypothesize that transient inhibition of translation will cause a lethal decrease in those short-lived, pro-survival proteins in CLL cells to initiate apoptosis. Pateamine A (PatA) was isolated from the marine sponge *Mycale sp.* by bioassay-guided fractionation based on its cytotoxic activity against P388 murine leukemia cells (IC_{50}=0.27 nmol/L). Des-methyl, des-amino Pateamine A (DMDAPatA) is a simplified analog that is easier to synthesize with potent anti-proliferative action against >30 human cancer cell lines. PatA and DMDAPatA inhibit cap-dependent translation initiation by binding to eukaryotic initiation factor 4A (eIF4A). Preliminary data suggests that DMDAPatA is highly bound to human plasma proteins and may lack sufficient *in vivo* potency required for development as a therapeutic agent. To address this, we designed a family of PatA-based inhibitors with the goal of improving the physical properties and cytotoxic potency against CLL. **Methods:** Apoptosis was measured by annexin V and propidium iodide double staining followed by flow cytometry. Plasma protein binding was determined by equilibrium dialysis. The combination effect of DMDAPatA and ABT-199 was evaluated with the median-effect method. **Results:** The PatA analog DMDAPatA induced apoptosis in primary CLL cells through the intrinsic pathway regardless of patient prognosis characters and reduced the short-lived anti-apoptotic protein Mcl-1, but not Bcl-2. DMDAPatA and ABT-199 target two parallel arms of apoptosis regulation and induce cell death synergistically, indicating that they could be used effectively in combination to treat CLL. As expected, DMDAPatA was >99% bound in human plasma. A series of new PatA derivatives

were synthesized and several of them exhibited greater cytotoxic potency toward CLL cells and lower human plasma protein binding. **Conclusion:** These studies demonstrate that inhibition of protein translation through perturbation of eIF4A by PatA analogs is a valid therapeutic strategy for CLL either alone or in mechanism-based combinations. Several candidates were identified for further drug development. Planned preclinical studies with these leads will enable a full evaluation of their therapeutic potential in CLL.

343 **CPRIT Grantee**
Poster Session A

Tankyrase Inhibitor Attenuates Wnt/beta-catenin Signaling Pathway and Promotes Telomere Shortening in Human Cells *H. Chen, The University of Texas Southwestern Medical Center at Dallas; O. Kulak, The University of Texas Southwestern Medical Center at Dallas; B. Holohan, The University of Texas Southwestern Medical Center at Dallas; X. Wu, The University of Texas Southwestern Medical Center at Dallas; H. he, The University of Texas Southwestern Medical Center at Dallas; D. Borek, The University of Texas Southwestern Medical Center at Dallas; Z. Otwinowski, The University of Texas Southwestern Medical Center at Dallas; K. Yamaguchi, University of Tokyo; L. Garofalo, The University of Texas Southwestern Medical Center at Dallas; Z. Ma, The University of Texas Southwestern Medical Center at Dallas; W. Wright, The University of Texas Southwestern Medical Center at Dallas; C. Chen, The University of Texas Southwestern Medical Center at Dallas; J. Shay, The University of Texas Southwestern Medical Center at Dallas; X. Zhang, The University of Texas Southwestern Medical Center at Dallas; L. Lum, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Tankyrase proteins (Tnks1 and Tnks2) belong to the superfamily of poly (ADP-ribose) polymerase (PARPs) that catalyze the addition of poly (ADP-ribose) onto protein substrates. In Wnt/beta-catenin signaling, Tnks enzymes indirectly control the abundance of the transcriptional co-activator b-catenin by regulating the turn-over rate of Axin proteins, cytoplasmic scaffolding molecules that assemble components of a b-catenin destruction complex. The ability of Tnks inhibitors to promote beta-catenin destruction in cells devoid of the colorectal cancer tumor suppressor Adenomatous polyposis coli (APC) has fueled efforts to develop such chemicals as anti-cancer therapeutic agents. **Methods:** In this study, we investigated the cellular activity of novel Tnks inhibitors by bringing together structural, biochemical, and cell biological observations. **Results:** Previously we identified about 60 chemicals with the potential for disrupting cellular responses to Wnt ligands from a large chemical library screen. Using a biochemical counter-screen to identify additional Tnks inhibitors in this chemical collection, we identified two novel chemical scaffolds that exhibited Tnks inhibitory activity (IWR-3 and IWR-8). The binding mode for IWR-3 and IWR-8 was revealed by solving the crystal structure of each compound with a Tnks enzyme. Our structural analysis suggested that IWR-3 exploits both the nicotinamide- and adenosine-binding pockets to engage with Tnks2, while IWR-8 only interacts with the adenosine-binding pocket. Thus Tnks inhibitors can be segregated according to the active site pocket(s) they engage. We then examined the effect of Tnks inhibitors on telomere length maintenance by first scoring their ability to promote telomere dysfunction-induced foci (TIFs). From this approach, we found that all of the Tnks inhibitors induced a rapid DNA damage response at telomere ends, which correlated with their ability to promote shortening of telomere upon long term treatment in cultured cells. **Conclusion:** Our data suggests that the clinical development path for Tnks inhibitors as anticancer agents should include not only an understanding of their actions in Wnt/beta-catenin signaling but also their effects on telomere length maintenance in normal and cancerous cells.

344 **CPRIT Grantee**
Poster Session B

Metabolic modulation by combinatorial intervention with natural compounds for prostate cancer prevention and treatment *B. Wang, The University of Texas at Austin; A. Saha, The University of Texas at Austin; X. Lu, The University of Texas at Austin; J. DiGiovanni, The University of Texas at Austin; S. Tiziani, The University of Texas at Austin*

Introduction: Prostate cancer is one of the three major cancer types in men and is ranked as the second in death rate and first in new cases for men cancers according to the US cancer statistics 2015. Natural compounds have recently drawn large attention for cancer prevention and treatment mainly for their low toxicity to normal tissue. **Methods:** In this study, a natural compound library of 132 compounds was screened on cultured mouse prostate tumor cells from HiMyc mice (HMVP2 cells) and ATP and reactive oxygen species (ROS) bioluminescence measurements were performed on treated and untreated cells. Based on ATP and ROS results, three hits, ursolic acid (UA), curcumin (CURC) and resveratrol (RES) were selected for a more in depth metabolomic and lipidomic analyses. High-resolution liquid chromatography mass spectrometry (LC-MS) and magnetic resonance spectroscopy (MRS) were applied for large scale untargeted metabolic and lipidomic profiling of intra and extracellular prostate cancer extracts after treatment with the three drugs and their combinations at different time points. **Results:** Following 12 hours of treatment, 115 metabolites in KEGG database and 12 classes of lipids (664 features) were included in the study. Principal component analysis (PCA) showed that the combination of UA and CURC exerts the most profound metabolic perturbation on HMVP2 cells compared to the individual treatment or the combination of other selected hits. Further data mining showed that the CURC+UA had at least an additive effect by altering metabolic pathways associated with alanine, aspartate, proline and glutamate metabolism. Moreover, key intermediates in glycerophospholipid and ceramides metabolism were highly perturbed in CURC +UA indicating a relevant response of lipid mechanism to treatment with these combined agents. In in vivo study, we established an allograft mouse model of prostate cancer by injecting the HMVP-2 spheroids into FVB/N mice. This model gives rise to palpable tumors within 10-14 days post injection. Dietary administration of CURC+ UA and UA+RES showed significant inhibition of tumor growth compared to the individual compound when used alone, and CURC+ UA combination yields the

most effective combination. **Conclusion:** In summary, amongst the 132 screened compounds CURC, UA and RES exerted the most prominent metabolic effects on cells, the combined CUR and UA treatment showed at least an additive effect on cell metabolism and the CURC+UA treatment significantly affected key metabolic pathways active in mitochondria, most likely via lipid metabolisms.

345 **CPRIT Grantee**
Poster Session A

Discovery of Tumor-Specific Irreversible Inhibitors of Steroyl CoA Desaturase *P. Theodoropoulos, The University of Texas Southwestern Medical Center at Dallas; S. Gonzales, The University of Texas Southwestern Medical Center at Dallas; S. Winterton, The University of Texas Southwestern Medical Center at Dallas; C. Rodriguez-Navas, The University of Texas Southwestern Medical Center at Dallas; J. McKnight, The University of Texas Southwestern Medical Center at Dallas; L. Morlock, The University of Texas Southwestern Medical Center at Dallas; A. Owen, The University of Texas Southwestern Medical Center at Dallas; Y. Duan, The University of Texas Southwestern Medical Center at Dallas; J. Moreno, The University of Texas Southwestern Medical Center at Dallas; J. Hanson, The University of Texas Southwestern Medical Center at Dallas; B. Cross, The University of Texas Southwestern Medical Center at Dallas; A. Lemoff, The University of Texas Southwestern Medical Center at Dallas; H. Mirzaei, The University of Texas Southwestern Medical Center at Dallas; B. Posner, The University of Texas Southwestern Medical Center at Dallas; N. Williams, The University of Texas Southwestern Medical Center at Dallas; J. Ready, The University of Texas Southwestern Medical Center at Dallas; D. Nijhawan, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Growth of individual non-small-cell lung cancers (NSCLC) is driven by a unique set of mutations known as an oncogenotype, and it is clear from existing data that this profile influences their response to therapy. We hypothesized that compounds that selectively targeted an individual oncogenotype would also show a higher degree of selectively for tumor versus normal tissues and that biomarkers would exist to identify sensitive tumors. **Methods:** We evaluated toxicity of the UT Southwestern Medical Center (UTSW) High Throughput Screening (HTS) compound library toward twelve NSCLC lines and one immortalized but non-cancerous human bronchial epithelial cell line (HBEC) looking for compounds that were reproducibly toxic to at least one tumor line, were non-toxic to HBEC, and showed indication of selectivity against other lines. Compounds selected for further evaluation were also subjected to metabolic stability analysis in the presence of sensitive and resistant cell lines using LC-MS/MS to determine whether differential metabolism or intracellular accumulation could account for differences in sensitivity. Probe compounds generated from selected hits allowed identification of targets using mass spectrometry. **Results:** We identifie two scaffolds, aminobenzothiazoles and oxalic acid diamides, which were selectively toxic to the same four of 12 NSCLC lines but not to HBEC. Surprisingly, the compounds showed metabolic instability upon incubation with sensitive but not resistant cell lines. Further analysis revealed that sensitivity to these inhibitors was predicted by expression of CYP4F11, which metabolized the chemicals into irreversible stearoyl CoA desaturase (SCD) inhibitors. In xenograft models with mice bearing CYP4F11hi sensitive H2122 tumors or CYP4F11lo resistant H1155 tumors, only the sensitive H2122 tumors showed growth inhibition in response to therapy with a bioavailable aminobenzothiazole, SW203668. Although the metabolite was identified at low levels in plasma of mice bearing both types of tumors, intratumoral levels were four-fold higher in the CYP4F11hi sensitive H2122 tumors versus the CYP4F11lo resistant H1155 tumors. SCD has been recognized as a promising biological target in cancer and metabolic disease. However, SCD is essential to sebocytes and accordingly existing SCD inhibitors cause skin toxicity. Mouse sebocytes were unable to activate the aminobenzothiazoles or oxalic acid diamides into SCD inhibitors and in vivo were preserved using dosing schedules of the bioavailable aminobenzothiazole that were efficaciou in the xenograft model. **Conclusion:** The results suggest a means to pharmacologically target SCD in cancer, taking advantage of high CYP expression in a subset of NSCLC.

346 **CPRIT Grantee**
Poster Session B

Insights into the Molecular Mechanism of Investigational New Drug Phosphaplatin (PT-112) *S. Tripathi, University of Houston; L. Belkacemi, University of Houston; M. Cheung, University of Houston; R. Bose, na*

Introduction: Platinum (Pt) based chemotherapeutic drugs (cisplatin, carboplatin and oxaliplatin) are currently used in the treatment of 50% or more cancer patients worldwide. In vitro and in vivo assays demonstrated that phosphaplatin compounds, developed by Rathindra Bose's group, have superior drug activity with lesser toxicity and resistance than the commonly used Pt drugs. One of the phosphaplatin compounds; PT-112 is currently undergoing clinical trial (ClinicalTrials.gov identifier NCT02266745). In this study, we explored the mechanism of action of PT-112 by correlating the drug activity with the gene transcript level for the US National Cancer Institute 60 (NCI-60) human cancer cell lines and compared that with the other three Pt drugs. **Methods:** The drug activity data were obtained from the Developmental Therapeutics Program at NCI/NIH based on the GI50 (the concentration that causes 50% growth inhibition) values of the cancer cell lines. For each drug the GI50 data was converted to z-scores and used as an input to compare with the gene transcript and activity levels of other drugs using CellMiner (NCI-60 analysis tools). Genes that showed significant correlations with the drug activity based on statistical significance (p-value < 0.05) were considered for gene network and pathway analysis. **Results:** We found that out of nearly 150 Food and Drug Administration approved drugs; PT-112 exhibited the highest correlation with oxaliplatin activity (Pearson correlation coefficient (PCC) is 0.72). On the other hand, the PCC of PT-112 with cisplatin and carboplatin activity was only 0.34 and 0.24, respectively. We identified a total of 2012 genes for which the transcript level significantly correlates with the activity of PT-112. For 35% of these genes the transcript level significantly correlates with oxaliplatin activity as well. On the other hand, transcript level of only 1.5% and 2% of the 2012 genes of PT-112 significantly correlates with the activity of cisplatin and carboplatin, respectively. Clustering analysis of the 1290 genes that correlate with PT-112 activity only (but not to the activity of other three Pt drugs) revealed that some genes associated with cell proliferation were down regulated while others linked to apoptosis were up regulated. **Conclusion:** Our results demonstrate that the non-DNA binding Pt drug PT-112 has some unique mode of actions to activate/deactivate genes

related to cell proliferation and apoptosis pathways when compared to the other commonly used Pt drugs, making PT-112 an attractive alternative to the current available chemotherapeutic drugs.

347 **CPRIT Grantee**
Poster Session A

Tumor therapy with PS-Targeting Antibodies in Mice *R. Sharma, The University of Texas Southwestern Medical Center at Dallas; R. Velmurugan, Texas A&M University System Health Science Center; L. Li, The University of Texas Southwestern Medical Center at Dallas; R. Mason, The University of Texas Southwestern Medical Center at Dallas; A. Schroit, The University of Texas Southwestern Medical Center at Dallas; E. Ward, Texas A&M University System Health Science Center*

Introduction: In normal non-tumorigenic cells, the transbilayer distribution of phosphatidylserine (PS) is highly regulated and is preferentially segregated to the internal plasma membrane surface. In many tumor cells, apoptotic cells and in tumor vasculature endothelial cells, PS redistributes to the outer membrane leaflet where it is amongst the most specific markers of the tumor vasculature. Several preclinical studies have demonstrated the therapeutic efficacy of PS-directed vascular targeting agents especially when combined with chemotherapeutic agents (Ran & He; Clin Cancer Res. 2005; 11:1551-1562). Bavituximab, a first generation PS-targeting agent, is currently in phase III clinical trials. In an effort to develop highly effective PS targeting agents we generated and characterized two new variants of the PS-targeting (B2-glycoprotein specific) antibody, wild type 1N11 (1N11-WT): a tetravalent variant of 1N11 (1N11-T) with four binding sites per molecule and 1N11-N297Q that has substantially reduced binding affinities for human FcγRs (hFcgRs). Therapeutic efficacy of these antibodies was assessed in SCID mice bearing orthotopic breast carcinomas. **Methods:** Therapeutic antibodies were expressed in CHO and HEK 293T cells, purified on protein G-Sepharose and analyzed on HPLC. The antibodies were characterized for PS/B2-glycoprotein binding by flow cytometry and ELISA using PS-expressing cells and PS, respectively. The binding activity of 1N11-N297Q for hFcγRs was assessed using surface plasmon resonance (BIAcore). For the orthotopic breast tumor models, groups of 8-10 female SCID mice were injected with MDA-MB-231 human mammary carcinoma cells into the mammary fat pad. Therapy was initiated when the tumors reached an average diameter of 0.5 to 0.7 cm. The mice were then treated with 100 μg molar equivalents of different antibodies three times/week and Docetaxol (10 mg/kg) once/week for 3 weeks. The mice were continuously monitored for tumor size and body weight. **Results:** 1N11-WT, 1N11-T and 1N11-N297Q were predominantly monomeric following purification and exhibited similar (1N11-N297Q) or increased (1N11-T) binding to PS on cells (flow cytometry) and using ELISA to that observed for 1N11-WT. 1N11-N297Q has substantially reduced binding affinitie for both high and low affinity hFcgRs. Combination treatment with the therapeutic Abs and docetaxel retarded tumor growth by >50%. Although 1N11-T exhibited better binding to PS in vitro, therapeutic efficacy was similar to that observed with the parent bivalent antibody, 1N11-WT. Unexpectedly, 1N11-N297Q was therapeutically equivalent to 1N11-WT. **Conclusion:** These data suggest that antibodies with substantially reduced binding affinity for FcgRs retain therapeutic efficac . Additional studies will investigate the activity of additional 1N11 variants with altered FcgRs binding affinities

348 **CPRIT Grantee**
Poster Session B

Pilot Quality Assurance Study of Stereotactic HYpofractionateD RadioAblative (HYDRA) Treatment of Advanced Laryngeal Cancer *W. Mao, The University of Texas Southwestern Medical Center at Dallas; W. Lu, The University of Texas Southwestern Medical Center at Dallas; X. Gu, The University of Texas Southwestern Medical Center at Dallas; Y. Yan, The University of Texas Southwestern Medical Center at Dallas; S. Jiang, The University of Texas Southwestern Medical Center at Dallas; B. Sumer, The University of Texas Southwestern Medical Center at Dallas; D. Schwartz, The University of Texas Southwestern Medical Center at Dallas*

Introduction: We have initiated a multi-institutional phase I trial of 5-fraction stereotactic body radiotherapy (SBRT) for Stage III-IVa laryngeal cancer with CPRIT support. To confirm treatment quality and deliverability, we compared daily delivered treatment doses to intended doses in study patients treated with SBRT. We also tested the feasibility of using an in house software platform (SCORE) for adaptive replanning to correct dose deviations during daily treatment. **Methods:** We evaluated seven cases; three patients were enrolled on this clinical trial and four patients were enrolled onto a concurrent phase I SBRT trial for early-stage glottic larynx cancer. Daily cone-beam CT (CBCT) or diagnostic CT images were acquired prior to every fraction of treatment for all study patients. Baseline and daily SBRT treatment plans were generated per dictates of the HYDRA trial protocol. Evaluation and adaptive replanning procedures were carried out on SCORE and a commercially available treatment planning system (Eclipse, Varian Medical Systems). Reference simulation CT images were deformably registered to daily images. Planning contours and reference SBRT plan were copied to deformed daily CTs. Delivered dose distributions were obtained by re-calculating doses on the deformed CT images, added together, and propagated back to the reference CT images for comparison to the original SBRT plan. Dosimetric differences were evaluated via dose-volume-histograms (DVHs). **Results:** In all seven cases, delivered daily and total doses were completely evaluated in <10 minutes. Prescribed D95% coverage of GTV and CTV target volumes was preserved in all cases; however, PTV D95% coverage was less than intended in half of cases (total cohort mean: 93.5%, range: 84%-96.6%). One patient had a single treatment where PTV D95% was only 84%, although intended CTV coverage remained intact. Maximum bystander point dose limitations to arytenoids, carotids,

and spinal cord were preserved in all cases at <3.8 Gy variance. We found that daily treatment plans could be re-optimized with our SCORE planning system to satisfy initial planning dosimetric constrains within 1-2 minutes. **Conclusion:** Although GTV and CTV dose coverage was preserved with 3D image guidance, PTV coverage could vary significantl from intended plans. Use of online adaptive treatment replanning appears necessary to maintain treatment quality. We are currently validating our SCORE software platform to satisfy this role.

349 **CPRIT Grantee**
Poster Session A

Magnetic Enhancement of Vascular Permeability for Targeted Drug Delivery *S. Tong, Rice University; Y. Qiu, Georgia Institute of Technology; L. Zhang, Rice University; G. Bao, Rice University*

Introduction: Current drug delivery to solid tumors relies on overexpressed biomarkers or enhanced vascular permeability due to tumor angiogenesis. However, targeting specificity and intratumoral distribution of therapeutic agents are often compromised by the heterogeneity of tumor microenvironment. Here we demonstrate using magnetic iron oxide nanoparticles (MNPs) to increase vascular permeability in the target tissue, whereas external magnetic field is used to enhance the uptake of MNPs by vascular endothelium in the target tissue and open up endothelial cell-cell junctions by disrupting actin filaments with intracellular magnetic force. In this study, we designed the magnetic field with numerical simulations, and investigated magnetic force induced structural and functional changes of vascular endothelium in endothelialized microfluidic channels and in live animals. M**ethods:** Magnetite nanocrystals were synthesized by thermodecomposition of Iron acetylacetonate. As-synthesized nanocrystals were made water-dispersible by coating the nanocrystals with phospholipid-poly(ethylene glycol). Magnetic field was constructed with N52 grade rare-earth block magnets. Distribution of the magnetic field and its orientation with respect to blood flow were optimized through numerical simulations. In endothelialized microfluidic channels, actin filaments and VE-cadherin were labeled and examined with confocal microscopy before and after addition of the magnetic field. In in vivo study, MNPs were injected i.v. in nude mice and the magnetic field was added to an intact lateral tail vein. The change in vascular permeability was examined with indocyanine green angiography (ICGA). **Results:** In endothelialized microfluidi channels, endothelial cells formed actin filaments along the flow direction of culture medium. We found that endocytosis of MNPs by endothelial cells was enhanced by magnetic force. Subsequently, a magnetic force perpendicular to the flow direction on intracellular MNPs caused re-arrangement of actin filaments and disrupted the distribution of VE-cadherins in cell-cell junctions. Similarly, we demonstrated that external magnetic field could increase the accumulation of MNPs in the lateral tail vein in live mice. After ICG was injected systemically, its accumulation in treated mouse tails increased by 3 fold compared with untreated tails, indicating increased permeability in local vasculature. **Conclusion:**

We demonstrate that vascular permeability in the target tissue can be increased by controlling systemically administrated MNPs with an external magnetic field. It provides an engineering approach to increase targeted delivery of systemically administrated therapeutic agents, which does not rely on specific disease conditions and is not limited by the ability to conjugate/encapsulate therapeutic agents with drug carriers.

350 **CPRIT Grantee**
Poster Session B

Treatment With Neoadjuvant BRAF/MEK Inhibitors Yields High Response Rates in Patients With Resectable Metastatic Melanoma *P. Prieto, The University of Texas M.D. Anderson Cancer Center; H. Jiang, The University of Texas M.D. Anderson Cancer Center; A. Reuben, The University of Texas M.D. Anderson Cancer Center; J. Austin-Breneman; C. Spencer, The University of Texas M.D. Anderson Cancer Center; J. Gershenwald, The University of Texas M.D. Anderson Cancer Center; J. Cormier, The University of Texas M.D. Anderson Cancer Center; J. Lee, The University of Texas M.D. Anderson Cancer Center; R. Royal, The University of Texas M.D. Anderson Cancer Center; A. Lucci, The University of Texas M.D. Anderson Cancer Center; M. Ross, The University of Texas M.D. Anderson Cancer Center; K. Griffin, The University of Texas M.D. Anderson Cancer Center; R. Amaria, The University of Texas M.D. Anderson Cancer Center; Z. Cooper, The University of Texas M.D. Anderson Cancer Center; M. Tetzlaff, The University of Texas M.D. Anderson Cancer Center; J. Wargo, The University of Texas M.D. Anderson Cancer Center*

Introduction: We have made significant advances in the treatment of melanoma through the use of targeted therapy – specifically with the use of BRAF and MEK inhibitors. Combined BRAF+MEK inhibition is FDA-approved in the treatment of stage IV disease, however its use is now being expanded to patients with earlier stage melanoma - including those with resectable stage III disease. Currently the standard of care in these patients is upfront surgery, however the majority of patients treated in this manner will relapse and die of disease. Our hypothesis is that treating patients with high-risk resectable melanoma with neoadjuvant + adjuvant BRAF+MEK inhibitors will result in a lower rate of relapse and improved long-term survival compared to those treated with standard of care upfront surgery. We are now running a clinical trial to test this hypothesis. This trial is currently underway, and critical correlative studies to better understand molecular and immune determinants of response are being performed via CPRIT support (RP150030). **Methods:** As a basis for this clinical trial, we surveyed a group of patients with resectable metastatic melanoma (with a known BRAF mutation) who were treated off-protocol with BRAF+/-MEK inhibitors prior to surgical resection. Tumor measurements were performed pre-treatment and just prior to surgical resection, and biopsies were also acquired. Immune analysis (via 12-marker IHC panel) was performed on pre-treatment, on-treatment and surgical samples

when feasible. **Results:** In the small cohort of patients, all patients demonstrated radiographic responses (with 4 achieving a partial response and 1 achieving a complete response). Pathologic evaluation at the time of surgical resection demonstrated a complete pathologic response in 3 patients (with no viable tumor cells identified), and a partial pathologic response in 2 patients (with < 50% viable tumor cells). IHC staining of immune markers (PD-L1, PD-1, CD4, CD8, CD45RO, Granzyme B and FoxP3) was performed demonstrating up-regulation of immune markers in surgical specimens compared to pre-treatment tumor biopsies, with the highest infiltrate in the patient with a complete pathologic response to therapy. **Conclusion:** Genomic and deep immune profiling studies are underway on this cohort and in patients on our ongoing clinical trial, and will provide insight into differential responses to therapy as well as actionable strategies to overcome therapeutic resistance. The ongoing clinical trial has significant potential to change the standard of care and to improve outcomes for patients with melanoma.

351 **CPRIT Grantee**
Poster Session A

Olfactory Memory Deficits in an Animal Model of Pediatric Radiotherapy *E. Perez, University of Houston; J. Leisure, University of Houston; M. Gaber, Baylor College of Medicine; T. Inoue, Baylor College of Medicine*

Introduction: Radiation is effective in eradicating tumors, however, cranial radiation therapy results in long-term neurocognitive deficits in survivors of pediatric cancer. Patients also report impairment in the ability to taste shortly after radiation treatment, which may result in part from a deficit in olfaction. Moreover, previous reports on olfaction have determined that radiation produces a deficit in olfactory memory in adult mice. Testing olfactory recognition memory in rodents offers an ethologically relevant means by which to probe memory deficits caused by radiotherapy damage. The olfactory bulb is replenished with new neurons from the subventricular zone of the brain and these new neurons are said to take part in olfactory behavior. We predict that whole-brain radiation, with the olfactory bulb shielded, in a young mouse will impair olfactory behavior. M**ethods:** In this study, male C57BL/6J mice were divided into two groups in a design examining the effects of radiation on olfaction discrimination and memory. Animals received a single dose of 5 Gy X-rays to the whole brain at postnatal day 31; olfactory bulbs and the rest of the body were shielded from radiation. The novel odor recognition test developed by M. J. Spinetta (2008) was used to assess olfaction discrimination and memory in an irradiated group of mice versus an age-matched control (non-irradiated) group at approximately 12 weeks post-radiation. The firs part of the test consists of presenting the animal with familiar odors as well as one novel odor (NO1) in multiple trials; 24 hours later, the animal is presented with familiar odors, NO1, and a second novel odor. **Results:** Our results showed that irradiated mice, like controls, could distinguish novel odors from familiar ones. Each group showed significantly higher exploration of NO1 on the first trial versus the second or third trials at both time points, thus, both groups habituated to the novel odor. However, unlike controls, irradiated mice did not spend significantly more time with NO2 on the second day, suggesting that irradiated mice do not remember NO1. **Conclusion:** Our findings suggest that olfactory memory but not discrimination is affected by cranial radiation. Our work presents an interesting model to study radiation-induced memory deficits using olfaction as our sensory measurement. We plan on utilizing this model to test interventions aimed at ameliorating side effects of cranial radiotherapy.

352 **CPRIT Grantee**
Poster Session B

IgGA: A "Cross-Isotype" Engineered Human Fc Antibody Domain that Displays Both IgG-like and IgA-like Effector Functions *J. Lee, The University of Texas at Austin; W. Kelton, ETH; C. Lee, The University of Texas at Austin; N. Mehta, Stanford University; W. Charab, The University of Texas at Austin; D. Glass, Stanford University; T. Kang, The University of Texas at Austin; T. Kojima, Nagoya University; J. Jung, The University of Texas at Austin; G. Georgiou, The University of Texas at Austin*

Introduction: Our current understanding of therapeutic antibodies against tumor is that immune effector cytotoxic mechanisms (antibody-dependent cell cytotoxicity [ADCC], antibody-dependent cell phagocytosis [ADCP], and complement-dependent cytotoxicity [CDC]) mediate the clearance of cancer cells via binding to Fc receptors and complement (C1q). Presently, all clinically approved antibodies for cancer therapy are of the IgG isotype; however, it has been well established that IgA can elicit potent ADCC, by neutrophils and eosinophils, and ADCP, by macrophages, via FcαRI receptor binding. The challenges in developing IgA isotype therapeutic antibodies are associated with the difficulties in expression and quality controls due to its heavy-glycosylation and the lack of FcγR- and complement-mediated effector functions. Developing therapeutics with the combined potency of IgG and IgA could open an entirely new avenue for cancer therapy. **Methods:** "Cross-isotype" antibody, IgGA, that combines the effector functions of IgG and IgA was engineered by constructing and testing different chimeras of IgA and IgG. Its binding kinetics with various Fc receptors were measured on BiaCore 3000. ADCC, ADCP, and CDC assays were performed as described (Kelton, W. et al. 2014). Further engineering of IgGA were achieved through screening of combinatorial libraries of Fc variants through the method previously developed (Jung, S. et al. 2010). **Results:** IgGA antibody displays high affinities to FcαRI while retaining binding to the activating FcγRs, FcγRI and FcγRIIa. Although IgGA does not show affinity for FcγRIIIa, our cell-based assays demonstrate that IgGA potently activates both neutrophils and macrophages to kill Her2(+) tumor cells through the FcαR and FcγR-dependent mechanisms of ADCC and ADCP. Additionally, it displays improved binding to C1q compared to IgG1, which translates to greater CDC activity. In order to overcome the limitations of IgGA resulting from the loss of bindings to FcγRIIIa and to FcRn in a pH-dependent manner, we have further engineered it and regained bindings to FcγRIIIa and FcRn, which is expected to translate into greater potency and longer serum half-life. **Conclusion:** We have developed hybrid antibodies of IgG and IgA that hold a great promise as novel antibody therapeutics for cancer. Our work also demonstrates the concept of cross-isotype antibodies that may be further extended to the creation of other types of cross-isotype antibodies, highlighting the potential of novel antibody engineering platform.

353 **CPRIT Grantee Poster Session A**

Comparative Analysis of MIM and Velocity's Image Deformation Algorithm Using Simulated kV-CBCT Images for Quality Assurance *K. Cline, The University of Texas Health Science Center at San Antonio; G. Narayanasamy, The University of Texas Health Science Center at San Antonio; M. Obeidat, The University of Texas Health Science Center at San Antonio; D. Stanley, The University of Texas Health Science Center at San Antonio; S. Stathakis, The University of Texas Health Science Center at San Antonio; H. Kim, University of California San Francisco; N. Kirby, The University of Texas Health Science Center at San Antonio*

Introduction: Deformable image registration (DIR) is used routinely in the clinic without a formalized quality assurance (QA) process. Using simulated deformations to digitally deform images in a known way and comparing to DIR algorithm predictions is a powerful technique for DIR QA. This technique must also simulate realistic image noise and artifacts, especially between modalities. This study developed an algorithm to create simulated daily kV cone-beam computed-tomography (CBCT) images from CT images for DIR QA between these modalities. **Methods:** A Catphan and physical head-and-neck phantom, with known deformations, were used. CT and kV-CBCT images of the Catphan were utilized to characterize the changes in Hounsfield units, noise, and image cupping that occur between these imaging modalities. The algorithm then imprinted these changes onto a CT image of the deformed head-and-neck phantom, thereby creating a simulated-CBCT image. CT and kV-CBCT images of the undeformed and deformed head-and-neck phantom were also acquired. The Velocity and MIM DIR algorithms were applied between the undeformed CT image and each of the deformed CT, CBCT, and simulated-CBCT images to obtain predicted deformations. The error between the known and predicted deformations was used as a metric to evaluate the quality of the simulated-CBCT image. Ideally, the simulated-CBCT image registration would produce the same accuracy as the deformed CBCT image registration. **Results:** For Velocity, the mean error was 1.4 mm for the CT-CT registration, 1.7 mm for the CT-CBCT registration, and 1.4 mm for the CT-simulated-CBCT registration. These same numbers were 1.5, 4.5, and 5.9 mm, respectively, for MIM. **Conclusion:** All cases produced similar accuracy for Velocity. MIM produced similar values of accuracy for CT-CT registration, but was not as accurate for CT-CBCT registrations. The MIM simulated-CBCT registration followed this same trend, but overestimated MIM DIR errors relative to the CT-CBCT registration. Part of this project was supported by the CPRIT Training Grant RP 140105 to Kristen Cline, MS.

354 **CPRIT Grantee Poster Session B**

Genetic Markers For Chemotherapy-Related Pancreatitis In Childhood ALL *A. Grimes, The University of Texas Health Science Center at San Antonio; H. Bansal, The University of Texas Health Science Center at San Antonio; C. Aguilar, The University of Texas Health Science Center at San Antonio; G. Tomlinson, The University of Texas Health Science Center at San Antonio*

Introduction: Although ninety percent of children with standard risk acute lymphoblastic leukemia (ALL) remain disease-free five years after treatment, quality of life is greatly affected by treatment-related toxicities. Acute pancreatitis is one of the more severe toxicities, limiting further use of a critical chemotherapeutic agent and delaying subsequent therapy. Host factors, including genetic variability, play a significant role in susceptibility to certain toxicities and are thought to contribute to treatment-related pancreatitis. However, gene studies to evaluate risk for pancreatitis in childhood leukemia have not been performed. The development of pancreatitis during leukemia treatment is associated with L-asparaginase (L-Asp), a key medication in achieving and sustaining remission. Pancreatitis is the most common cause of L-Asp intolerance, noted in 5-18% of children during leukemia therapy and can result in prolonged hospitalization, delays in cancer treatment, and numerous complications including secondary diabetes, pancreatic pseudocysts, abscess formation and death. Much speculation exists regarding which underlying genetic factors confer greater risk of developing treatment-related pancreatitis. Children with ALL who develop pancreatitis may harbor mutations in one or more genes, such as those associated with familial, chronic, or idiopathic pancreatitis or those involved in asparagine metabolism which increase vulnerability. Therefore, the overall hypothesis is that underlying gene alterations in some children with ALL lead to acute pancreatitis when exposed to L-Asp therapy. **Methods:** Blood samples from children with ALL with and without pancreatitis during therapy were evaluated for isolation of total DNA. A custom 18-gene DNA-sequencing panel was designed to include known pancreatitis genes and genes involved in asparagine metabolism. We recognize that alterations in genes which have not been previously investigated may be present in the subset of ALL children who develop pancreatitis, increasing individual susceptibility to this adverse treatment effect. Therefore, using DNA isolated from blood samples from leukemic children, we utilized whole exome-sequencing techniques to compare the gene alteration profiles in children who developed pancreatitis versus those who did not. **Results:** An increased number of gene alterations is present the asparaginase metabolism gene AGXT among case patients. These findings, however, are preliminary and results from whole exome sequencing to evaluate flanking regions and validate these findings are underway. **Conclusion:** Identifying mutations correlated with pancreatitis in childhood ALL will provide an innovative screening method to identify those at greatest risk for pancreatitis prior to treatment. Such knowledge would greatly impact supportive care, early interventions, and alter therapeutic protocols according to toxicity risk.

355 **CPRIT Grantee**
Poster Session A

Image-Guided Cancer Therapy using High-Intensity Focused Ultrasound (HIFU) *R. Chopra, The University of Texas Southwestern Medical Center at Dallas*

Introduction: High-Intensity Focused Ultrasound (HIFU) is a form of image-guided therapy capable of noninvasive tissue ablation and drug delivery. Similar to the concentration of light energy to a focal point using a lens, HIFU can concentrate acoustic energy within the body to a region of a few millimeters in dimension. Within this focal region, a range of bio-effects can be generated in tissue. HIFU devices and systems have been developed in oncology for the treatment of prostate, breast, bone, and brain tumors. **Methods:** Through a multi-year CPRIT grant, the HIFU research program in the Department of Radiology at UT Southwestern Medical Center is exploring novel applications of focused ultrasound energy in oncology. Major research foci include the use of HIFU for tissue ablation under real-time MRI guidance, generation of mild-heating for targeted delivery of chemotherapeutics, and opening of the blood brain barrier for non-invasive drug delivery in the brain. Research spans technical development, preclinilcal evaluation, and clinical trials. **Results:** To date, localized delivery of doxorubicin using HIFU and temperature-sensitive liposomes has been demonstrated in a rabbit VX2 tumor model. A stereotaxic system for targeted opening of the blood brain barrier in rodents has been developed. Novel MRI methods for monitoring temperature in bone and evaluating thermal damage from HIFU treatments have been developed. Finally a clinical trial for the treatment of uterine fibroids has been established as a stepping stone to the launch of clinical trials for the treatment of prostate cancer, and pediatric cancers. **Conclusion:** This novel image-guided cancer therapy has the potential to transform many conventional forms of cancer therapy into non-invasive, image-guided outpatient procedures. Further, the unique bio-effects of ultrasound energy in the body creates opportunities for novel approaches to cancer therapy.

356 **CPRIT Grantee**
Poster Session B

In vivo Gene Delivery to Lymphocytes Using Novel Biodegradable Nanoparticles *M. Zhao, Baylor College of Medicine; F. Feng, Baylor College of Medicine; A. Sizovs, Baylor College of Medicine; X. Song, Baylor College of Medicine; J. Wang, Baylor College of Medicine*

Introduction: Immunotherapy has gained increasing attention in cancer therapy recently based on the unprecedented success in clinical trials. As one of the immunotherapies, anti-CD19 chimeric antigen receptor (CAR)-modified T cells demonstrated remarkable effectiveness in refractive acute lymphoblastic leukemia (ALL) in a Phase I trial. Despite the tremendous success of CAR-T cell therapy, the main drawback of this treatment is the highly expensive *ex vivo* procedures to produce genetically modified CAR-T cells. We rationalize that direct *in vivo* production of CAR-T cells can significantly reduce the treatment cost and eliminate the potential side-effects involved in the *ex vivo* procedures. Herein, we report a novel star-shaped polymer (SP) based non-viral gene delivery system that can deliver genes to primary lymphocytes *in vivo* through intravenous administration. M**ethods:** We used plasmids encoding firefl luciferase pCMV-Luc2 and enhanced green fluorescen protein (EGFP) as model systems to test the gene transfection efficienc and specificity of SP formed nanoparticles (NPs) in the immune cells *in vivo*. Histochemsitry was performed to evaluate *in vivo* toxicity. Gene expression in lymphocytes *in vivo* was confirmed by bioluminescence live imaging, by immunohistochemistry (IHC), and by flow cytometry through staining lymphocyte surface markers. **Results:** (1) Systemic intravenous administration of pCMV-Luc2-SP NPs showed a transient but strong luciferase expression in major lymph nodes *in vivo*, which was confirme by whole body luminescence imaging. (2) Systemic intravenous administration of pCMV-GFP-SP NPs led to effective transfection in various primary lymphocytes in lymph nodes. Flow cytometry followed by staining surface markers of different lymphocyte subsets demonstrated that 20% of CD4+ T lymphocytes, 19% of CD8+ T lymphocytes and 15% of total lymphocytes were GFP positive 24 h after administration. The percentage of cells expressing GFP decreased over a 4-day period based on flow cytometry measurements. (3) Antigen presenting cells (APCs) and B lymphocytes were also transfected by SP NPs 24 h after administration with lower percentage than T lymphocytes. Flow cytometry showed that more than 10% of APCs were GFP positive 24 h after administration. Staining of surface marker further confirmed that more than 17% of B lymphocytes were GFP positive. C**onclusion:** In this study, we successfully developed a method to deliver genes into lymphocytes directly *in vivo*. In addition, the SP polymer is biodegradable and induces minimal toxicity. In future studies, we will use SP NPs to directly deliver plasmids encoding CAR to CD8+ T lymphocytes and test its therapeutic efficacy compared to current CAR- cell therapy.

357 **Poster Session A**

Antibody-Mediated Blockade of Phosphatidylserine Combined With Intense Radiation Improves Survival and Tumor Eradication in Rat Models of NSCLC *O. Belzile, The University of Texas Southwestern Medical Center at Dallas; Z. Zhang, The University of Texas Southwestern Medical Center at Dallas; X. Huang, The University of Texas Southwestern Medical Center at Dallas; D. Saha, The University of Texas Southwestern Medical Center at Dallas; R. Brekken, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Stereotactic body radiation therapy (SBRT) uses image guidance to deliver high doses of radiation, in no more than 5 fractions, to tumors. SBRT has shown remarkable benefit in patients with peripheral lung tumors but can cause severe toxicity to central organs when the tumor is proximally located. Bavituximab, a therapeutic monoclonal antibody in Phase III clinical testing in non-small cell lung cancer (NSCLC) patients, targets the immunosuppressive lipid, phosphatidylserine (PS), which becomes exposed on blood vessels and cells in tumors and is increased by SBRT. Antibody-mediated blockade of PS signaling stimulates innate and adaptive immune anti-tumor activity. Using orthotopic rat models, we hypothesized that treatment with the antibody 2aG4 (a murine version of bavituximab) would synergize with SBRT and allow tolerable doses of radiation to be used to treat centrally-located NSCLC without sacrificing efficac . **Methods:** Immunodeficient nude rats, bearing established orthotopic A549-luciferase NSCLC tumors utilizing an improved lung implantation technique, were enrolled in a therapy study after the bioluminescence signal of their tumor was greater than 10 million photons per second (typically 2-3 weeks post implantation of tumor cells). Animals were treated with either 3x12 Gy of radiation alone (n=10), 2aG4 alone (n=8; 4 mg/kg, twice per week), or a combination of radiation and 2aG4 (n=11). Tumor growth was monitored by bioluminescence weekly. **Results:** Rats treated with the combination of radiation and 2aG4 had a 100% survival rate 185 days after implantation, compared with 60%, 62.5%, and 18% for radiation, 2aG4, and untreated, respectively ($p = 0.022$, 0.029, and <0.0001, respectively). Additionally, tumors were completely eradicated in 8/11 (73%) of animals treated with the combination. Immunofluorescence staining analysis confirme that exposure of PS was induced by intense radiation in orthotopic lung tumors. Additionally, we performed endpoint experiments with the same treatment arms to evaluate immune cell infiltration into lung tumor tissue after radiation therapy +/- antibody-mediated PS blockade.

Follow-on toxicity studies were conducted in which 3x12 Gy of radiation was delivered to central organs of tumor-free rats in presence or absence of 2aG4. The presence of 2aG4 did not significantly prevent or exacerbate normal radiation-induced toxicity as measured by lung fibrosis, thickness of esophageal tissues, or bronchus integrity. **Conclusion:** In conclusion, these results provide a pre-clinical rationale for the administration of bavituximab in combination with radiation in patients with centrally-located NSCLC.

358 **Poster Session B**

Radiofrequency Disrupts Drug Resistant Cancer Cells *M. Ware, Baylor College of Medicine; V. Keshishian, Baylor College of Medicine; J. Ho, Baylor College of Medicine; S. Corr, Baylor College of Medicine; B. Godin, The Methodist Hospital Research Institute; S. Curley, Baylor College of Medicine*

Introduction: One of the major clinical challenges with current therapeutic modalities for pancreatic adenocarcinoma (PDAC) is cell insensitivity or the development of resistance to standard therapeutic regimens. Thus, there is an urgent need to develop novel therapeutic approaches for PDAC, especially for tumors that have poor response or develop resistance to standard chemotherapy and radiation. Fibroblastoid phenotype, incorporating tunneling nanotubes (TNTs), is a major characteristic that is developed over prolonged GCB exposure, which, we think, rescues apoptotic cells and conserves the health of the population during drug treatment via the transfer of cellular constituents from cell to cell. Mild hyperthermia, administered via our non-invasive radiofrequency device has been shown to disrupt TNT formation and hence may provide a method of disrupting protective mechanisms, such as TNTs, in cancer cell population. **Methods:** We created super-GCB resistant PDAC cells by exposing PDAC cells to incremental doses of GCB over a one-month period. After a short recovery period cells were dosed with high concentrations of GCB and both cell death and morphological responses were recorded over time. Super-GCB resistant and untreated cells were exposed to non-invasive RF to achieve a thermal dose of 44°C for 2 mins to assess TNT expression and synergistic cell killing. **Results:** Our results indicate that TNTs are involved in maintaining cell population health via drug resistance transfer, possibly via the transfer of mitochondria. Furthermore, we show that TNTs exist abundantly in 3D PDAC spheroids and are a relevant phenomenon in solid tumors. We show that the administration of a single RF treatment to PDAC in-vitro via our non-invasive RF machine causes a significant suppression of these structures for at least 24h and results in greater cell death when combined with GCB. **Conclusion:** Our research highlights the specific phenotypes found in PDAC cell populations and their heterogeneous response to standard of care GCB therapy over multiple time scales. Based on this, we propose that TNTs play a significant role in the transfer and development of drug resistance in PDAC and are therefore a new target, which needs to be explored for the development of novel PDAC treatment regimens. Currently there are no approved treatments that specificall

target TNTs in tumors. We have shown that our non-invasive RF machine can suppress the formation of TNTs for at least 24h, which can pave the way to a novel therapy of PDAC that may enhance therapeutic efficac against this deadly disease and will benefit PDAC patients

359 **Poster Session A**

Non-thermal Radiofrequency Disrupts Normal Pancreatic Adenocarcinoma Phenotype *V. Keshishian, Baylor College of Medicine; M. Ware, Baylor College of Medicine*

Introduction: Poor drug diffusion, cell insensitivity and the development of resistance to standard therapeutic treatments represent major clinical challenges in the treatment of pancreatic adenocarcinoma (PDAC). Consequently, the development of new therapeutic approaches for PDAC is urgently needed. Our group has developed a non-invasive means of exposing tumor tissues to radiofrequency (RF) fields for either thermal or non-thermal therapies. Much of our previous work has focused on RF-induced hyperthermia, with and without the use of nanomaterials as thermal 'activators'. This has been shown to alter solid tumor drug diffusion and enhance PDAC cell susceptibility to chemotherapy (data not shown). Investigations into non-thermal electromagnetic interactions are also warranted and are the basis of the work described herein. **Methods:** PDAC cell populations were exposed to high power (900 W) non-invasive RF for 15 min whilst simultaneously cooling the culture plates using a high-powered fan. The temperature of the PDAC cells remained between 36-37 °C during RF exposure, so it was assumed that any PDAC responses seen are due to non-thermal electromagnetic interactions. We analyzed key biophysical parameters of the PDAC cells that indicate changes in the behavior and/or health of the population through a battery of tests conducted over time. Control PDAC cells were subjected to the same temperature changes by removing them from an incubator. **Results:** Our results indicate that non-thermal RF exposure induces overexpression and reorganization of filamentous actin in the cytoskeleton via the formation of stress fibers . Our group has previously shown that the formation of stress fibers, cytoplasm retraction and cell balling (data not shown) alter 3-D tumor architecture. Hence, non-thermal RF may provide a means of enhancing drug diffusion through solid tumors when used in combination with standard chemotherapy regimens. Non-thermal RF also induced slight but significant cell death 0-24 h after treatment when compared to control PDAC cells. Additionally, non-thermal RF significantly affects the 'renewability' of PDAC stem cells when analyzed in-vitro using the sphere forming assay. This result suggests that non-thermal RF decreases the viability of these important sub-populations, which are thought to be key players in chemo and radiation resistance along with the progression of the disease. **Conclusion:** Our research suggests that non-thermal RF exposure induces several biophysical responses in PDAC cells, which

affect population-wide behavior and health. Further investigation into the effects of non-thermal RF therapy on PDAC cells is warranted and may pave the way to the development of novel PDAC therapies.

360 **Poster Session B**

A Zwitterion Hexavanadate Nanocluster Targeting Redox Homeostasis: Selective Cancer Killing and Overcoming Drug Resistance *J. Song, Emory University; J. Mi, Emory University, Georgia State Univeristy*

Introduction: Personalized therapy is the final effective solution to fight with diseases and it is also the ultimate goal of precision medicine. Nanotechnology provides a novel platform by engineering drug molecules with new physicochemical properties in addressing the challenges of disease treatment. Such therapeutic agents commonly refer to active molecule(s) loaded on engineered nanoparticle vehicles. However, nearly all the vehicles are designed and deemed as inert carriers to bio-systems in order to boost their applications in medicine. Few studies are focused on the therapeutic effects of nanoparticle itself. Here we report that a zwitterion nanocluster polyoxoanion of vanadium (VPOA-6) of uniform size and atomically precise structure, selective kills cancer cells and overcome drug-resistance via targeting cancer redox metabolism. **Methods:** Synthesis: This 1nm sized of VPOA-6 is conveniently synthesized in two-step via hydrothermal method using commercially available vanadium salts and water as the starting materials. VPOA-6 was obtained in an orange crystalline solid. Characterization: We fully characterize the structure and stability of this new nanoscale compound using multiple physicochemical techniques. X-ray single crystal diffraction confirms its 3D zwitterionic structure composing of the vanadium atoms covalently-linked by oxygen atoms. FT-IR, UV-Vis, and NMR collectively demonstrate its hydrolytic stability in aqueous solution (PBS buffer) and cell culture conditions. DLS shows this zwitterion induced the bovin serum albumin aggregation forming ca. 25nm aggregates in physiological conditions. **Results:** We discovered that this vanadium-containing zwitterion nanocluster, which is assembled from the vanadium, a naturally occurring element in the human body, is a potent anti-cancer drug in selectively killing cancer cells by increasing the level of reactive oxygen species (ROS). This hydrolytically stable inorganic compound significantly inhibits the growth of a broad spectrum of cancer cells including the drug resistant types in the micro molar range while exhibiting no cytotoxicity to normal cells. In vivo study demonstrates that VPOA-6 has more favorable chemotherapeutic profiles than cisplatin. VPOA-6 inhibits tumor growth in the KB-3-1 xenograft mouse model while showing no organ toxicity including the nephrotoxicity. Mechanistic studies further reveal that VPOA-6 dramatically decreases the level of reduced glutathione (GSH), leading to a significant increase of ROS and cell apoptosis. Our results demonstrate that VPOA-6 selectively induces cancer cell death through perturbing the natural balance of GSH/GSSG. **Conclusion:** We discovered a new class of inorganic nanotherapeutics that preferentially eradicates cancer cells and overcomes drug-resistance by targeting the redox homeostasis.

361 **Poster Session A**

Levetiracetam Mitigates Fluorouracil-Induced Synaptic and DNA Damage in Neurons *A. Tsvetkov, The University of Texas Health Science Center at Houston; J. Moruno Manchon, The University of Texas Health Science Center at Houston; N. Uzor, The University of Texas Health Science Center at Houston; J. Wefel, The University of Texas M.D. Anderson Cancer Center; Y. Dabaghian, Rice University*

Introduction: Neurotoxicity may occur in cancer patients and survivors during or after chemotherapy. Cognitive deficits associated with neurotoxicity can be subtle or disabling and frequently include disturbances in memory, attention, executive function and processing speed. **Methods:** Establishing cause-and-effect relationships in the fixed animal brain samples can be misleading due to the complexity of processes occurring in the brain during chemotherapy. To overcome these limitations, we use a model of chemotherapy-induced changes in neurons based on cultured cortical neurons derived from embryonic rats and mice. Neurons are cultured in a 96-well or 384-well plate for 2 or more weeks to allow them to mature synaptically prior to treatment with different concentrations of a drug or with a combination of drugs for various times. Cells are then fixed, stained with antibodies against different proteins (e.g., synaptic proteins), and imaged in an automated fashion. Images are then analyzed for protein expression levels and subcellular localization. This represents an unparalleled fluorescence microscopy–statistical modeling system for rapid, robust and sensitive assessment of the effects of chemotherapeutics on neuronal health that is 1000-fold more sensitive than conventional approaches. **Results:** Searching for pathways altered by anti-cancer treatments in cultured primary neurons, we discovered that fluorouracil, a commonly used anti-neoplastic drug, reduced synaptic density and promoted the formation of DNA double-strand breaks (DSB). Pretreatment of neurons with levetiracetam, an FDA-approved anti-epileptic drug, prevented fluorouracil-associated synaptic loss and reduced the formation of DNA DSBs. **Conclusion:** Thus, levetiracetam might be part of a valuable new approach for mitigating synaptic damage and, perhaps, for treating cognitive disturbances in cancer patients and survivors.

362 **Poster Session B**

Design, Synthesis, and Evaluation of Amino Acid Prodrug Conjugates of Amino-Combretastatin, Dihydronaphthalene and Benzosuberene Derivatives as Potential Vascular Disrupting Agents *C. Lin, Baylor University; L. Devkota, Baylor University; T. Strecker, Baylor University; Y. Wang, Baylor University; R. Lopez, The University of Texas Southwestern Medical Center at Dallas; L. Liu, The University of Texas Southwestern Medical Center at Dallas; E. Hamel, NIH; D. Chaplin, OXiGENE; R. Mason, The University of Texas Southwestern Medical Center at Dallas; M. Trawick, Baylor University; K. Pinney, Baylor University*

Introduction: Significant differences exist between tumor-related and normal blood vasculature, which identifies tumor-related vasculature as an intriguing target for anticancer therapy. Vascular disrupting agents (VDAs) cause the vascular structure feeding a solid tumor to collapse, depriving the tumor of the nutrients and oxygen it needs to survive, which ultimately leads to tumor cell necrosis. Structure-activity relationship studies of VDAs that inhibit tubulin polymerization (through an interaction at the colchicine binding site) led to the design and synthesis of amino-based combretastatins (KGP06, KGP08), dihydronaphthalene (KGP05), and benzosuberene (KGP156) derivatives. These compounds demonstrate potent inhibition of tubulin polymerization and pronounced cytotoxicity against human cancer cell lines. In order to increase water solubility and potentially bioavailability, their corresponding water-soluble amino acid prodrug conjugates (AAPCs) were synthesized. **Methods:** Water-soluble AAPCs were synthesized utilizing general peptide coupling reactions followed by salt formation. These compounds were evaluated by an enzyme cleavage assay (in vitro) using leucine aminopeptidase. In addition, the AAPCs were screened for cytotoxicity against selected human cancer cell lines, and separately for their ability to inhibit tubulin polymerization. Bioluminescence imaging (BLI) studies in a SCID mouse model bearing the MDA-MB-231-luc breast tumor were carried out as a preliminary means of assessing the capability of AAPCs to disrupt the vessels feeding tumors, and thus function as VDAs. **Results:** Each of the parent (non-prodrug) amino-bearing anticancer agents demonstrated excellent cytotoxicity against selected human cancer cell lines, and were potent inhibitors of tubulin polymerization. Several of the synthetic AAPCs were cleaved (quantitatively) upon treatment with leucine aminopeptidase to generate their corresponding parent amino-bearing anticancer agents. In general, the extent of enzyme-mediated cleavage (in vitro) mirrored the cytotoxicity (in vitro) of these AAPCs. One of the AAPCs exhibited a pronounced reduction in bioluminescence in a tumor bearing (MDA-MB-231-luc) SCID mouse model two hours post treatment (15 mg/kg) indicating tumor vascular disruption. **Conclusion:** A series of water-soluble amino acid prodrugs (AAPCs) based on amino-bearing anticancer agents (designed from the combretastatin, dihydronaphthalene, and benzosuberene compound classes) were prepared by chemical synthesis. An enzyme-mediated assay (in vitro) identified a subset of these prodrugs that were readily cleaved to generate their parent anticancer agents. A preliminary in vivo BLI study with one of the AAPCs highlighted its vascular damaging capability. Collectively these results confirm the potential importance of these AAPCs for further development as anticancer agents.

363 **Poster Session A**

A Liposomal-Iodinated Contrast Agent For CT-Image Guided Treatment of Liver Tumors *K. Ghaghada, Texas Children's Hospital; K. Dixon, The University of Texas M.D. Anderson Cancer Center; A .McWatters, The University of Texas M.D. Anderson Cancer Center; S. Gupta, The University of Texas M.D. Anderson Cancer Center*

Introduction: CT imaging plays an important role in image-guided catheter-based treatment of liver tumors. One of the major challenges, however, with conventional CT imaging techniques is the inability to visualize target lesions during the interventional procedure. In this work, we investigated the utility of a long circulating liposomal-iodinated contrast agent and CT imaging for prolonged visualization of liver tumors. **Methods:** Studies were performed in a rabbit model of orthotopic VX2 liver tumor. Tumor cells were surgically implanted at upto 3 locations within the liver. CT imaging was initiated once the tumors reached 4-5 mm in size. The liposomal-iodinated contrast agent was intravenously administered as a slow infusion. CT imaging was performed pre-contrast and then at multiple time points (upto 2 weeks) post-administration of the liposomal contrast agent. The effect of contrast agent dose on lesion visualization was also investigated. **Results:** The liposomal contrast agent enabled clear visualization of liver tumors. The lesions appeared as hypo-enhanced in the early-phase imaging (scans acquired within a few hours post-contrast). In the delayed-phase imaging (in CT images after 24 hr), the lesions appeared as hyper-enhanced with a peripheral ring of signal enhancement due to extravasation and accumulation of liposomal-iodine via the leaky tumor vasculature. Tumors as small as 5 mm were clearly visible in the post-contrast scans. The lesions were visible with contrast dose as low as 165 mg I/kg (4-fold lower than a standard clinical dose of iodine contrast agent). Maximum CT signal in the tumors was observed at 48 hr post-contrast; however, lesions remained visible for upto 7 days post-administration of the liposomal contrast agent. **Conclusion:** A single injection of liposomal-iodinated contrast agent facilitated visualization of liver tumors for up to 7 days. The ability to visualize liver tumors for prolonged period could greatly enhance the effectiveness of CT-image guided interventions for treatment of liver tumors.

364 **Poster Session B**

Assessing Potential Differential Efficacy of Vascular Disrupting Agents on Diverse Tumor Lines *L. Liu, The University of Texas Southwestern Medical Center at Dallas; L. Delgado, The University of Texas Southwestern Medical Center at Dallas; K. Pinney, Baylor University; M. Trawick, Baylor University; R. Mason, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Development of neovasculature is critical for tumor growth, survival, and metastasis, and therefore vascular disrupting agents (VDAs) offer a powerful potential therapy [1]. In developing any new drug, non-invasive assays of efficacy provide vital insight into activity with respect to dosing and pharmacodynamics of action. We have previously demonstrated the utility of dynamic bioluminescence imaging (dBLI) to evaluate VDA activity. A novel benzosuberene-based agent (KGP265) has shown efficacy in the MBA-MD-231 human breast mammary fat pad tumor model [2] and we have explored its effects on additional diverse tumor lines. **Methods:** Human prostate cancer PC3, lung cancer A549 and mouse breast tumors 4T1 cells were implanted in male SCID/Balb/c mice. KGP265 dose ranged from 3 to 15 mg/kg and the classic VDA combretastatin (CA4P; 120 mg/kg) was used as positive control. Dynamic BLI was performed at baseline, 4, 24 and 48 hours after injection IP administration of VDA. Fresh D-luciferin was administered at each time point. As a control, additional tumor bearing mice were injected with the same volume of saline. **Results:** Treatment with KGP265 led to significantly decreased and delayed light emission indicative of vascular disruption in different tumor types. BLI signal was reduced by 90% at 4 hours compared to baseline for PC3 and 4T1, A549 was about 70%. Light emission remained significantly depressed up to 48 hours, whereas CA4P showed substantial recovery by 24 hours and further recovery by 48 hours. Meanwhile light emission was highly consistent following administration of saline as control with reproducible kinetics and intensities. Optimal doses response of KGP265 was seen at 7.5 mg/kg, for 4T1 and 10 mg/kg for PC3 and A549. Treatment of 4T1-luc tumor with 7.5mg/kg KGP265 greatly reduced signal output in BLI images. By 4 hours, 90% of signal was reduced and the signal stayed down for the next 48 hours post-injection. 4T1 tumors are known to be highly metastatic and indeed BLI signal indicated distinct metastases. These showed more modest response likely because they were much smaller with less developed vasculature. **Conclusion:** The results indicate that KGP265 causes significant vascular disruption. KGP265 is water soluble, but

maintains substantial vascular disrupting efficac . Compared to CA4P (optimal dose 120 mg/kg), the new VDA (KGP265) achieved similar imaging results at lower doses suggesting enhanced efficac , which we are continuing to explore.

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365 **Poster Session A**

Differential Structural Properties, Physicochemical Behavior, and Biodistribution of Malonodiserinolamide-Derivatized [60] Fullerene in a Murine Orthotopic Breast Cancer Model *N. Lapin, Baylor College of Medicine; L. Vergara, Baylor College of Medicine; Y. Mackeyev, Rice University; M. Atsushi, Hitachi High Technologies America, Inc.; M . Pulikkathara, Baylor College of Medicine; C. de la Cerda, Baylor College of Medicine; L. Wilson, Rice University; S. Curley, Baylor College of Medicine; R. Serda, Baylor College of Medicine*

Introduction: Here we have investigated the differential structure and biodistribution of water-soluble malonodiserinolamide-derivatized [60] fullerene (C_{60}-ser) and its silica nanoparticle conjugate (C_{60}-ser-SiO_2-NP). We first characterized the structure of these agents, then tracked their localization 1) *in vitro*, in 4T1 cells, HeLa cells, and fibroblasts incubated with these agents and 2) *in vivo*, in mice with orthotopically implanted 4T1 breast tumors. Tumor vasculature is characterized by increased permeability of vascular walls (owing to tumor-associated inflammation that causes extravasation of molecules and particles normally confine to the vasculature. **Methods:** Lyophilized C_{60}-ser self-assembled sheets and C_{60}-ser-SiO_2-NP were characterized using cold field emission (CFE)-scanning and scanning transmission electron microscopy (SEM and STEM). In the *in vitro* cellular studies, 4T1 cells, HeLa cells, and fibroblast were incubated with either C_{60}-ser or C_{60}-ser-SiO_2-NP for two hours at 37°C, and their intracellular localization was monitored with electron microscopy and light microscopy using confocal and super-resolution technologies. In the animal model, mice with orthotopically implanted 4T1 breast tumors were anesthetized and secured under an intravital microscope and IV injected with PF-633 fluorophore-labeled C_{60}-ser and C_{60}-ser-SiO_2-NP (accompanied in some cases by vascular contrast dyes) to observe the kinetics and localization of these agents within the tumor. **Results:** Electron microscopic characterization of the nanomaterials revealed hollow shells of C_{60}-ser (lyophilized) and clusters of C_{60}-ser conjugated SiO_2-NP. Imaging at high resolution enabled visualization and size quantification of clustered C_{60}-ser molecules, observed as sheets of discrete grains. Microscopic analysis of 4T1 cells, HeLa cells, and fibroblasts showed nanoparticle capture at the cell surface, cellular internalization of C_{60}-ser-SiO_2-NP (in confocal z-stacks), and nuclear localization of C_{60}-ser. Intravitally, PF-633-labeled C_{60}-ser extravasated rapidly through the walls of the tumor vasculature into the tumor interstitium as evidenced by progressive fluorescence increase across selected vascular and interstitial regions. In contrast, C_{60}-ser conjugated SiO_2-NPs were almost entirely contained within the vasculature over a one hour observation period. **Conclusion:** CFE-SEM and STEM captured images of pure C_{60}-ser sheets, which appeared as hollow spheres, but did not detect a difference between native and C_{60}-conjugated silica. Intravital microscopy was successfully applied to observe and relatively quantify rates of extravasation and intratumoral biodistribution of fluorophore labeled C_{60}-ser in aggregate and NP-conjugated forms, with the C_{60}-ser aggregates rapidly diffusing into the interstitium from tumor-associated vasculature.

366 **Poster Session B**

TGF-β1 primed myeloid derived suppressor cells lose their ability to suppress T cell and decrease tumor growth *P. Jayaraman, Baylor College of Medicine; R. Krupar, Baylor College of Medicine; F. Parikh, Baylor College of Medicine; A. Sikora, Baylor College of Medicine*

Introduction: Myeloid derived suppressor cells (MDSC) consist of myeloid progenitor and immature cells, which are recruited to the tumor site by cancer-related inflammation. Conventional MDSC's suppress T cell proliferation and promote tumor growth/angiogenesis by various mechanisms, and we have previously shown that functional maturation of MDSC depends on iNOS-produced nitric oxide (NO). TGF-b1 is a pleiotropic cytokine abundantly expressed in the tumor microenvironment with diverse effects on myeloid, lymphoid and tumor cells. In many situations, TGF-b1 is highly immunosuppressive. The aim of this study is to determine the effect of TGF-b1 in the generation and function of MDSC, including its effects on T cell proliferation and tumor growth. **Methods:** Ex vivo MDSC generation: Bone marrow progenitor cells were derived from WT C57bl/6 mice and cultured in the presence of MTEC (murine pharyngeal epithelial cells expressing HPV E6, E7, and ras oncogenes) tumor supernatants ± TGF-b1 for 5 days at 370C. Cells were then harvested, processed into single cell suspensions, and stained for MDSC surface markers, NO by DAF-DA, iNOS and other functional markers and analyzed using flow cytometry (FACS). T cell proliferation assay MDSCs were generated ± TGF-b1 with supernatants from MTEC cells and co-cultured with CFSE labeled T cells activated with anti CD3 & anti CD28 antibodies. T cell proliferation was measured by using CFSE dilution, which was analyzed by FACS. Effect of MDSC on tumor growth Control and TGF-b1 conditioned MDSC were co-cultured with murine (MTEC) or human (T-hep3) head and neck cell lines for 72 hrs at the end of which proliferation of the spheroid was assessed using Ki-67 or tumor numbers was determined by FACS **Results:** We found that TGF-b1 primed MDSC's failed to inhibit T cell proliferation compared to control MDSC. Further, TGF-b1 primed MDSC inhibited tumor growth in an ex vivo co-culture system. The spheroids co-cultured with TGF-b1 conditioned MDSC revealed decreased ki-67 expression compared to control. It was also seen that TGF-b1 treated MDSC's down regulate both iNOS and NO expression compared to control MDSC while not altering the expression of other MDSC functional markers like Arginase, PD-1 and PD-L1 **Conclusion:** We can therefore conclude that TGF-b1 reprograms MDSC to a) decrease their ability

to suppress T cells; and b) directly suppress tumor cell growth. These observations have a direct translational implication wherein the inherent pro-tumor nature of MDSCs can be reprogramed with TGF-b1 and directed against the tumor thereby impacting tumor growth.

367 **Poster Session A**

Effect of TGF-b1 on human Myeloid derived suppressor cells (MDSC) *F. Parikh, Baylor College of Medicine; R. Parihar, Baylor College of Medicine; P. Jayaraman, Baylor College of Medicine; R . Krupar, Baylor College of Medicine; A. Sikora, Baylor College of Medicine*

Introduction: Myeloid derived suppressor cells (MDSC) are a heterogeneous population of myeloid progenitor cells and immature myeloid cells. MDSC plays an important role in tumor-mediated immunosuppression. MDSCs mediate T cell suppression through a variety of mechanisms, including arginase-1 (ARG-1)–mediated local arginine depletion, inducible NO synthase (iNOS) and NADPH oxidase (NOX2) production of reactive oxygen and nitrogen species, VEGF expression, and cysteine depletion. TGF-b1 is a pleiotropic cytokine abundantly expressed in the tumor microenvironment with diverse effects on myeloid, lymphoid and tumor cells. The aim of this study is to determine the effect of TGF-b1 in the generation and function of MDSC, including its effects on T cell proliferation. **Methods:** Generation of MDSC: PBMCs were derived from healthy individual and co-cultured with either cytokines (GM-CSF+IL-6) or tumor supernatants (DHEP3 and SCC47) in the presence or absence of TGF-b1 for 6-7 days at 370C. Cells were then harvested and stained for MDSC surface markers (CD33+CD11b+HLADR-); samples were acquired by flow cytometry. CD33+ cells were sorted either using beads or FACS. T cell proliferation assay: MDSCs were generated in the presence or absence of TGF-b1 with cytokines or tumor supernatants and co-cultured with CFSE labeled healthy donor-derived T cells activated with anti CD3 and anti CD28 antibodies. T cell proliferation was measured by using CFSE dilution, which was analyzed by flow cytometry. **Results:** While the percentage of MDSC increases (2 fold) in presence of TGF-b1, TGF-b1 primed MDSC's (99% proliferation) lost the ability to inhibit T cell proliferation unlike control MDSC (59% T cell proliferation) which suppressed T cell proliferation in dose dependent fashion. **Conclusion:** We conclude that, while TGFb-1 is immunosuppressive in many contexts (such as induction of Treg), it acts to decrease the ability of MDSC to suppress T cell proliferation. Thus these findings suggest a potential feedback mechanism to limit TGF-b induced immunosuppression by down regulating the suppressive capacity of MDSC.

368 **Poster Session B**

Gedunin Inhibits Pancreatic Cancer by Altering Sonic Hedgehog Signaling Pathway *R. Subramani, Texas Tech University Health Science Center at El Paso; E. Gonzalez, Texas Tech University Health Science Center at El Paso; A. Arumugam, Texas Tech University Health Science Center at El Paso; S. Nandy, Texas Tech University Health Science Center at El Paso; D. Alabi, Texas Tech University Health Science Center at El Paso; F. Camacho, Texas Tech University Health Science Center at El Paso; J. Medel, Texas Tech University Health Science Center at El Paso; P. Tandon, Texas Tech University Health Science Center at El Paso; R. Lakshmanaswamy, Texas Tech University Health Science Center at Dallas*

Introduction: The lack of efficient treatment options for pancreatic cancer highlights the critical need for the development of new, novel and effective chemotherapeutic agents. The medicinal properties found in plants have been used to treat many different illnesses including cancers. This study focuses on the anticancer effects of gedunin, a natural compound extracted from Azadirachta indica. **Methods:** Anti-proliferative effect of gedunin on pancreatic cancer cells was assessed using MTS assay. We used matrigel invasion assay, scratch assay, and soft agar colony formation assay to measure the anti-metastatic potential of gedunin. Immunoblotting was performed to analyze the expression of key proteins involved in pancreatic cancer growth and metastasis. Gedunin induced apoptosis was measured using flow cytometric analysis with Annexin V and Propidium iodide staining. **Results:** Gedunin treatment is highly effective in inducing death of pancreatic cancer cells. Our data demonstrates that pancreatic cancer cell death occurs via death receptor and mitochondrial mediated apoptosis. Gedunin inhibited proliferation of pancreatic cancer cells through reduction of AKT/mTOR signaling. Our data further indicates that gedunin inhibited metastasis of pancreatic cancer cells by decreasing their invasive, migratory and colony formation capabilities. The reduced levels of expression of key markers of epithelial to mesenchymal transition (N-cadherin, Vimentin, Slug, Snail, Zeb and Notch) along with increased levels of E-cadherin establishes the anti-metastatic effect of gedunin. Moreover, our experiments with recombinant sonic hedgehog analog and Gli inhibitor (Gant-61) demonstrated that gedunin induces its anti-metastatic effect through inhibition of sonic hedgehog signaling. Our data also demonstrated that inhibition of Gli enhances the chemo-sensitivity of pancreatic cancer cells to gedunin. C**onclusion:** Overall, our data suggests that gedunin could serve as a potent anticancer agent against pancreatic cancers.

369 **Poster Session A**

Drug Effects Modeling on Tumor Growth *X. Li, Prairie View A&M University; L. Qian, Prairie View A&M University*

Introduction: Motivated by the frustration of translation of research advances in the molecular and cellular biology of cancer into treatment, this study calls for cross-disciplinary efforts and proposes a methodology of incorporating drug pharmacology information into drug therapeutic response modeling using a computational systems biology approach. **Methods:** A realistic drug pharmacology model is taken into account in the proposed model, including drug pharmacokinetics and pharmacodynamics information linked through a state-space approach. The objectives are two fold. The first one is to involve effective mathematical modeling in the drug development stage to incorporate preclinical and clinical data in order to decrease costs of drug development and increase pipeline productivity, since it is extremely expensive and difficult to get the optimal compromise of dosage and schedule through empirical testing. The second objective is to provide valuable suggestions to adjust individual drug dosing regimens to improve therapeutic effects considering most anticancer agents have wide inter-individual pharmacokinetic variability and a narrow therapeutic index. **Results:** It is demonstrated that there exists an optimal drug dosage and interval administration to reduce the tumor growth. **Conclusion:** It is promising that such study using effective mathematical modeling in the drug development stage to incorporate preclinical and clinical data would advance research in effective and affordable treatment of genetic diseases like cancer.

370 **Poster Session B**

Synthesis and Biochemical Evaluation of Benzoylbenzophenone Thiosemicarbazone Analogues as Potent Inhibitors of Cathepsin L *E. Parker, Baylor University; J. Song, Baylor University; G. Kumar, Baylor University; S. Odutola, Baylor University; G. Chavarria, Baylor University; A. Charlton-Sevcik, Baylor University; T. Strecker, Baylor University; A. Barnes, Baylor University; D. Chaplin, OXiGENE; M .Trawick, Baylor University; K. Pinney, Baylor University*

Introduction: Progression of tumor growth into the latter stages of cancer is characterized by the degree of metastasis. Cathepsin L (CTSL) is a powerful cysteine protease that is upregulated in certain types of cancer and is involved in the degradation of proteins comprising the extracellular matrix. CTSL has emerged as a potential drug target for the development of small-molecule inhibitors that function mechanistically as antimetastatic agents. We have designed and synthesized a series of CTSL inhibitors based on the benzoylbenzophenone molecular scaffold and evaluated them biologically. **Methods:** Structure activity relationship considerations developed from our lead benzophenone thiosemicarbazone CTSL inhibitors, **KGP94** and **KGP119**, along with empirical observations led to the design and synthesis of a privileged series of functionalized benzoylbenzophenone thiosemicarbazone analogues. Each of these molecules incorporated a benzoyl moiety along with functional group motifs associated with our most potent benzophenone thiosemicarbazone inhibitors. The resultant benzoylbenzophenone thiosemicarbazone analogues were evaluated as inhibitors of CTSL, CTSB, and CTSK. Selected active analogues were further evaluated for their ability to inhibit the invasion of MDA-MB-231 breast cancer cells. **Results:** A variety of CTSL inhibitors, functioning in the low nanomolar range, emerged from this series including 3-benzoylbenzophenone thiosemicarbazone (**KGP207**), 1,3-bis(4-fluorobenzoyl)benzene thiosemicarbazone (**KGP244**), and 1,3-bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone (**KGP312**). Selectivity for CTSL compared to CTSB was observed for all active analogues; in addition, **KGP244** demonstrated greater than 100 fold selectivity for CTSL with respect to CTSK. The most potent CTSL inhibitor, **KGP312**, inhibited invasion through Matrigel of MDA-MB-231 breast cancer cells by 70% at 10 μM and displayed low cytotoxicity toward normal primary cells [in this case human umbilical vein endothelial cells (HUVECs)]. **Conclusion:** A small library of benzoylbenzophenone thiosemicarbazone analogues were prepared by chemical synthesis and evaluated for their inhibitory activity against CTSL, CTSB, and CTSK. The high potency against CTSL and ability to inhibit the invasion of tumor cells in vitro coupled with the desirable property of low cytotoxicity towards normal cells, positions the most active members of this series as viable candidates to consider for further pre-clinical development as antimetastatic agents.

371 **Poster Session A**

Quantitative Monitoring and Control of Tumor Vascular Permeability In Vivo using Microbubble Contrast Agents *S. Sirsi, The University of Texas at Dallas*

Introduction: Over the last decade, microbubble contrast agents have frequently been cited as promising vehicles for targeted drug delivery applications. Microbubbles are gas filled spheres between 1-10 μm in diameter that circulate in the blood stream when injected systemically. When insonified under specific ultrasound conditions, microbubbles can alter vascular permeability by a technique called “sonoporation”. By spatially controlling the application of ultrasound energy, drug uptake can be targeted to specific regions, making this technique well suited for tumor-targeted drug delivery. In this study, we develop a novel technique to simultaneously monitor tumor perfusion and apply sonoporation to enhance tumor drug delivery. We expect that this technique will be useful for predicting drug delivery efficacy in tumors using quantitative perfusion imaging techniques. Me**thods:** Matrigel plugs (BD Biosciences, Franklin Lakes, NJ) were used as mock tumors that promote neovascular growth when injected subcutaneously in CD-1 mice. The vasculature was allowed to grow for 10-14 days in the matrigel plug then imaged with contrast-enhanced ultrasound (US) using lipid microbubble contrast agents to quantitatively monitor vascular perfusion. Perfusion was monitored using an Acuson Sequoia 512 ultrasound imaging scanner (Siemens Healthcare, Malvern, PA) with a 15L8 probe and custom Labview Software to analyze US video data (National Instruments, Austin, TX). After perfusion imaging, matrigel plugs were sonoporated using a therapeutic ultrasound machine (SoundCare Plus, Austin, TX) at 0-3 W/cm2 (1 Mhz, 10% duty cycle) for 10 minutes with a high dose of microbubbles (1x109 MB’s) mixed with 5 mg of FITC-dextran (Sigma Aldrich, 3-5 kDa) as a model drug (Figure 1A). Matrigel plugs were imaged again using US to monitor changes in perfusion then excised to evaluate drug uptake by dissolving the plugs (Dispase, BD Bioscience) and quantifying FITC-Dextran using a fluorescence plate reader (Genios Plus, Tecan, San Jose, CA). R**esults:** Matrigel sonoporation demonstrated significantly higher levels of FITC-Dextran uptake with increasing US intensity. No significant differences in contrast volume was detected at any US intensity, however, contrast agent reflow rates were inversely correlated with FITC-Dextran uptake. C**onclusion:** Currently we are able to demonstrate significantly improved drug uptake in mock tumors using ultrasound mediated sonoporation. In this study we expect that quantitative 3D perfusion imaging could be used to predict levels of drug uptake in tumors, which would have significan clinical impact for designing tailored drug treatment regiments for patients.

372 **Poster Session B**

Directed Evolution of Molecular Imaging Agents *S. Millward, The University of Texas M.D. Anderson Cancer Center; S. Fiacco, EvoRx Technologies Inc.; L. Kelderhouse, The University of Texas M.D. Anderson Cancer Center; A. Hardy, EvoRx Technologies Inc.; Y .Peleg, University of Southern California; B. Hu, University of Southern California; A. Ornelas, The University of Texas M.D. Anderson Cancer Center; P. Yang, The University of Texas M.D. Anderson Cancer Center; S. Gammon, The University of Texas M.D. Anderson Cancer Center; S. Howell, University of Southern California; P. Wang, University of Southern California; T. Takahashi, University of Southern California; R. Roberts, University of Southern California*

Introduction: Despite the success of Her2-targeted therapies, there are no clinically approved imaging agents to visualize and quantify Her2 expression in vivo. An ideal molecular imaging agent for Her2 would combine the affinity and selectivity of monoclonal antibodies with the synthetic accessibility, stability, and rapid clearance of small molecules. While peptides can have antibody-like affinities and specificities, their generally poor biostabilities preclude their use in most in vivo settings. We have developed a new directed evolution strategy to select ultra-stable cyclic peptides using an expanded genetic code and validated the most promising candidate as an in vivo Her2 imaging agent. **Methods:** mRNA Display libraries were translated with a 21st amino acid, covalently cyclized, and pre-selected for protease resistance. Libraries were subsequently selected for binding to Her2-overexpressing breast cancer cells in culture and the most promising candidates obtained by next-generation sequencing. The resulting SUPR (Scanning Unnatural Protease Resistant) peptides were converted into optical and PET imaging agents to measure their uptake, selectivity, and biodistribution in mouse models of Her2-positive breast cancer. **Results:** The Cy5-labled SUPR peptide EVO-004 showed antibody-like affinity and selectivity in vitro along with rapid, Her2-selective tumor uptake in vivo. Competition experiments revealed that EVO-004 bound to the affibody epitope on Her2 but did not compete with either Pertuzumab or Trastuzumab. EVO-004 was efficiently labeled with 18F using fluoroethylazide chemistry on a GE Tracerlab platform and purified to >90% radiochemical purity. PET/CT imaging was performed to quantify tumor uptake, biodistribution, and clearance rate. **Conclusion:** The EVO-004 SUPR peptide showed excellent in vivo properties with essentially no medicinal chemistry or stability optimization. EVO-004 can be efficiently radiolabeled with 18F on an automated platform and does not compete with clinical anti-Her2 antibodies making it an attractive candidate for further development as PET radiotracer. We believe that SUPR peptides represent a general approach for rapid discovery and translation of novel radiotracers and radiopharmaceuticals for targeted molecular imaging and cancer therapy.

373 **Poster Session A**

Near-infrared Optical Imaging Modality For Use As An Adjunct To Oncologic Liver Surgery *S. Vasudevan, Texas Children's Hospital; Z. Starosolski, Texas Children's Hospital; Y. Shi, Baylor College of Medicine; R. Patel, Texas Children's Hospital; C. Patel, Texas Children's Hospital; J. Jin, Baylor College of Medicine; A. Annapragada, Texas Children's Hospital; K. Ghaghada, Texas Children's Hospital*

Introduction: Curative surgical resection for hepatocellular malignancies is highly dependent on proper extirpation of the tumor with negative margins. Being a parenchymous organ, the tumor and blood vessels of the liver are hidden by the opaque tissues of the native liver. Novel imaging techniques that could enable intra-operative, real-time imaging of the tumor and major hepatic blood vessels could substantially refine liver surgery, resulting in safer and more accurate liver resection. In this work, we present preliminary pre-clinical results using a prototype 3D near-infrared (NIR) optical imaging system and novel contrast agents for use as an adjunct to liver surgery. **Methods:** The NIR imaging prototype consists of a 780 nm wavelength light source, optimized for use with a clinically-approved agent, indocyanine-green (ICG), and three detectors mounted on a rotatable gantry. 3D reconstructions were performed with structure-to-motion technique using images collected by 63 virtual detectors with an angular rotation of 42°. In vitro vascular phantom studies were performed, using ICG and a liposomal version of ICG (liposomal-ICG) to determine maximum depth of vessel visualization. Tumor imaging studies were performed using a novel orthotopic xenograft for liver cancer. In vivo NIR imaging was performed at 48h post-administration of ICG. **Results:** Studies using the vascular phantom demonstrated visualization of vessels up to 3.5 mm in depth below an opaque surface mimicking hepatic parenchyma. The liposomal-ICG contrast agent, which exemplifies a long-circulating vascular imaging agent, demonstrated ~ 6-fold higher signal intensity compared to the clinically-approved ICG. In vivo studies in mice models of primary liver tumor demonstrated that ICG is selectively retained in the liver tumor, thereby enabling NIR visualization of the tumor tissue. **Conclusion:** The techniques presented in this work demonstrate the feasibility of using a near-infrared imaging system for imaging of hepatic vasculature and tumors. The use of this technique could greatly advance liver surgery and therefore warrant further investigations for clinical translation.

374 **Poster Session B**

A Human Therapeutic Enzyme Specifically Sabotages Tumor Metabolism by an Engineered Cystine/Cysteine Degrading Activity *S. Cramer, The University of Texas at Austin; A. Saha, The University of Texas at Austin; S. Tadi, The University of Texas at Austin; S. Tiziani, The University of Texas at Austin; W. Yan, The University of Texas at Austin; K. Triplett, The University of Texas System; S. Alters, Aeglea Biotherapeutics; D. Johnson, Aeglea Biotherapeutics; Y. Zhang, The University of Texas at Austin; J. DiGiovanni, The University of Texas at Austin; G. Georgiou, The University of Texas at Austin; E. Stone, The University of Texas at Austin*

Introduction: Cancer cells experience higher levels of reactive oxygen species (ROS) stress than non-malignant cells and therefore enhanced anti-oxidant mechanisms are essential for their survival and proliferation. The cellular anti-oxidant axis is critically dependent on the concentration and redox state of the L-cysteine (L-Cys)-containing tripeptide glutathione (GSH). L-Cys is produced in most tissues from L-Methionine (L-Met) via the transsulfuration pathway, comprised of the enzymes cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL). However, under elevated ROS conditions, L-Cys flux via the transsulfuration pathway is insufficient, necessitating supplemental L-Cys import (predominantly in its oxidized form L-cystine (CSSC)) for survival. This heightened requirement of tumor cells for L-Cys/CSSC relative to non-malignant tissues constitutes a critical metabolic vulnerability and a unique therapeutic opportunity. Therefore, we hypothesized that prolonged, systemic depletion of the serum L-Cys/CSSC pool using engineered human enzyme(s) may constitute a potent and completely novel cancer therapeutic with minimal toxicity. We propose that an optimized human L-Cys/CSSC degrading enzyme suitable for clinical evaluation can be engineered by introducing mutations into the human CGL enzyme. **Methods:** CGL can already accept CSSC and L-Cys as substrates, however its kinetics (k_{cat}/K_M <11- and 45-fold lower, respectively, compared to its physiological substrate, L-Cystathionine) are too slow to be relevant for clinical applications. Rational design and scanning saturation mutagenesis were used to generate libraries of CGL variants which were subjected to a novel high throughput screen for L-Cys/CSSC lyase activity in order to find muteins with enhanced activity. **Results:** Indeed in an initial protein engineering campaign exploring this hypothesis we *engineered and pharmacologically optimized a prototype human L-Cys/CSSC degrading enzyme* (Cyst(e)inase) that displayed a 25- and 50-fold improvement in L-Cys and CSSC

degrading activity. We found that **(i)** bi-weekly administration of Cyst(e) inase in mice results in near complete depletion of the serum L-Cys/ CSSC pool; **(ii)** mediates complete inhibition of murine prostate tumors in allograft models and significant growth retardation of human PC3 tumors in xenografts and **(iii)** importantly treatment for over a month was very well tolerated with no weight loss nor gross toxicities. **Conclusion:** Enzyme-mediated depletion of the serum CSSC/L-Cys pool represents a novel, potent and well tolerated approach for the treatment of tumors displaying high levels of ROS.

375 **Poster Session A**

Treatment compliance and outcomes for women with locoregionally advanced cervical cancer treated in a safety-net health system

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Introduction: Platinum-based chemotherapy is commonly used to sensitize locoregionally advanced (bulky stage IB- IVA) cervical cancer to radiotherapy, but not all women are able to complete at least 6 cycles of weekly cisplatin. The purpose of this study is to assess treatment compliance and outcomes among women undergoing definitiv chemoradiation with weekly cisplatin for cervical cancer within a safety-net health system and to quantify the impact of chemotherapy compliance. **Methods:** All women definitively treated for FIGO IB2-IVA cervical cancer between April, 2008 and May, 2014 in our institution were identified Treatment delays were categorized as due to toxicity, comorbid conditions, system issues, or patient-initiated. Disease-free (DFS) and overall survival (OS) of subjects who received <6 vs. ≥6 doses of weekly cisplatin at 40mg/m2 were compared using Kaplan-Meier analyses. **Results:** A total of 119 women (mean age: 48.5±11.8 years) were identified. Most (n=112, 94.1%) completed definitive radiotherapy, requiring 56.5±20.1 days. Sixty-four subjects (57.1%) completed XRT in ≤56 days. Only 44 women (36.4%) received ≥6 cycles of cisplatin. Of 122 delayed cycles, reasons for delay were as follows - grade 2 or higher toxicity (n=70, 57.4%), medical comorbidity (n=12, 9.8%), system issues (n= 9, 7.4%), and patient-initiated (n=14, 11.5%). Multiple issues complicated treatment for 3 doses (2.5%). Reasons for delay were not documented in 14 (11.5%) doses. In patients who received ≥6 cycles, DFS was improved by 17.4 months (61.1±3.7 vs. 43.7±4.3 months, p=0.002) and OS was improved by 8.6 months (68.7±2.3 vs. 60.1±3.7 months, p = 0.011). Two-year progression-free survival for all patients is 65%.. For patients who completed all 6 cycles of chemo, the two year progression-free survival was 83% and for patients who received less than 6 cycles of chemo, it was 53%. A subgroup analysis showed that patents with hepatic disease were less likely to complete all planned chemotherapy (p=0.037) and patients with bipolar disorder were more likely to have worse disease free survival even after controlling for other factors (p=0.008). **Conclusion:** Higher rates of toxicity and psychosocial barriers to chemotherapy compliance adversely impact survival among those who seek care in low resource settings. In our population, administration of all 6 cycles of cisplatin was necessary for greatest local control and survival benefit. Future efforts to improve cervical cancer outcomes should address preventable reasons for treatment delays among the under- or uninsured.

376 **Poster Session B**

In Vivo 3D Interrogation of Tumor Vascular Heterogeneity Using High-Resolution CT Imaging and Nanoparticle Contrast Agent

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Introduction: Abnormal and heterogeneous tumor vasculature is one of the hallmarks of developing solid tumors. The heterogeneity in tumor vasculature gives rise to variability in vascular permeability, one of the consequences being non-uniform intra-tumoral uptake and distribution of therapeutics, especially nanoparticle-based chemotherapeutics. An imaging agent that can assess heterogeneity of tumor vasculature could therefore enable personalized nano-chemotherapy. In this work, we utilized high-resolution CT imaging and a liposomal-iodinated contrast agent to interrogate tumor vasculature heterogeneity and nanoparticle uptake in orthotopic mouse models of high-risk Neuroblastoma (NB). **Methods:** MYCN-amplified human NB cells (NGP) or MYCN non-amplified human NB cells (SH-SY5Y) were surgically injected beneath the renal capsule in female nude mice for generation of orthotopic tumors. A long circulating liposomal nanoparticle stably encapsulating iodinated contrast agent molecules (110 mg I/mL) was administered i.v. at an iodine dose of 2.2 mg/g of body weight. High-resolution CT imaging (35 micron isotropic resolution) was performed at 5 days post-contrast administration to assess the 'leakiness' characteristic of tumor vasculature. Subsequently, a second equivalent dose of nanoprobe was administered and a high-resolution CT angiogram of tumor vasculature acquired. Studies were performed as a function of tumor age and volume. The mice were sacrificed after imaging and the tumors removed for histological evaluation. **Results:** CT imaging demonstrated highly heterogeneous uptake and distribution of nanoprobe within NB tumors, exemplifying the spatio-temporal variability in tumor vascular permeability. Quantificatio of CT signal enabled assessment of liposomal uptake as a function of tumor age and volume. CT angiograms acquired immediately injecting the blood-pool liposomal CT agent enabled exquisite 3D visualization of intra-tumoral vessels of varying dimensions and tortuosity. Registration and super-imposition of the nanoprobe distribution pattern on the vascular map facilitated in identifying low and high permeable regions of the tumor vascular architecture. **Conclusion:** High-resolution CT imaging using the long circulating liposomal-iodinated contrast agent enabled interrogation

of tumor vascular architecture. The use of such techniques could be useful in studying nano-chemotherapeutic and vascular-targeting therapies.

377 **Poster Session A**

Contribution of Carbon and Silica Nanoparticles to Heating and Cell Death in a Non-invasive Radiofrequency Field *S. Suki, Baylor College of Medicine; Y. Mackeyev, Rice University; N. Lara, Rice University; M. Pulikkathara, Baylor College of Medicine; C. Mark, Baylor College of Medicine; N. Kumar, Baylor College of Medicine; M. Cheney, Rice University; J. Flores-Arredondo, Baylor College of Medicine; L .Vergara, Baylor College of Medicine; L. Wilson, Rice University; S .Curley, Baylor College of Medicine; R. Serda, Baylor College of Medicine*

Introduction: Currently, radiofrequency (RF) thermal ablation involving image-guided placement of needle electrodes is used for localized treatment of neoplastic tissue. The use of non-invasive RF energy to induce mild thermal and non thermal effects in cancer tissue is currently under study as an adjuvant to chemo or immuno therapy. This study examined the potential of a variety of nanomaterials to elevate heating rates or enhance biological effects in cancer cells. Nanoparticles in solution with a net surface charge have an electrostatic potential due to the boundary between ions associated with the surface and counter ions in the dispersant. We sought to determine if this potential could alter the heating rate of cells following internalization. **Methods:** The effect of cell type on sensitivity to RF fields was determined in endothelial, fibroblast macrophage and cancer cells using an RF generator and flow cytometry. Human HeLa cervical adenocarcinoma cells were further pretreated with water soluble [60]fullerene (C60-ser) nanoparticles, free or conjugated to the surface of silica (SiO2), followed by exposure to an RF field at 900 watts using 13.56 MHz energy. Heating rates were determined using a FLIR infrared camera. **Results:** Altering the percent serum or adding C60-ser, SiO2 or SiO2-C60-ser nanoparticles failed to alter the heating rate of cell culture media or change cell viability, however, cationic SiO2-C60 nanoparticles were cytotoxic and increased cellular sensitivity to RF exposure. Treatment of cells with heating at 41°C in a water bath did not induce cell death, however, RF exposure that elevated the recorded temperature to 41°C caused a 20% increase in cell death. Thus non-thermal effects of RF may negatively impact cell viability, and we are in the process of confirming this with more direct temperature measurements. **Conclusion:** As previously reported, changes in conductivity alter heating rates in an RF field. Nanoparticle formulations of varying surface potential failed to elevate the heating rate of cell culture media, however, nanoparticle cytotoxicity sensitized cancer cells to cell death by RF treatment.

378 **Poster Session B**

Pre-clinical Evaluation of the Vascular-Disrupting Activity of OXi8006 in Activated Endothelial Cells and its Prodrug OXi8007 in Breast Tumor Xenografts *S. Odutola, Baylor University; T. Strecker, Baylor University; L. Liu, The University of Texas Southwestern Medical Center at Dallas; R. Lopez, The University of Texas Southwestern Medical Center at Dallas; D. Chaplin, OXiGENE; R. Mason, The University of Texas Southwestern Medical Center at Dallas; K. Pinney, Baylor University; M. Trawick, Baylor University*

Introduction: Tubulin-binding small-molecule vascular disrupting agents (VDAs) are being investigated for their ability to elicit anti-tumor activity by causing shutdown in blood flow to solid tumors, resulting in extensive tumor-cell necrosis, while leaving blood flow in normal tissues intact. These compounds function as anticancer agents by causing rapid depolymerization of microtubules in tumor vascular cells leading to their tumor vascular disrupting effects. As part of a collaborative project between the Trawick and Pinney laboratories at Baylor University, an indole-based tubulin-binding compound OXi8006 and its phosphate prodrug OXi8007 were developed and investigated as VDAs. **Methods:** 1) In vitro experiments were done using activated/rapidly proliferating human umbilical vein endothelial cells (HUVECs) to model the tumor vasculature. 2) Immunofluorescence microscopy was used to observe the effects of compound treatment on cytoskeletal elements and cell signaling in HUVECs. 3) The sulforhodamine B assay was used to determine cytotoxicity of compounds against rapidly proliferating HUVECs. 4) Flow cytometry was used to determine the cell cycle effects of these compounds. 5) Bioluminescence imaging was used to investigate antivascular effects of OXi8007 in vivo in a tumor xenograft mouse model. **Results:** OXi8006 and OXi8007 caused depolymerization of microtubules, and resulted in increased phosphorylation of both non-muscle myosin light chain and focal adhesion kinase. This led to an increase in action-myosin contractility and focal adhesion formation in rapidly proliferating/activated human umbilical vein endothelial cells (HUVECs). These effects were markedly diminished by an inhibitor of RhoA kinase, a downstream effector of RhoA. OXi8006 and OXi8007 demonstrated strong cytotoxicity against rapidly proliferating HUVECs, disrupted in vitro tubular networks of HUVECs, and also caused cell cycle blockade at G2/M. Furthermore, OXi8007 had significant dose-dependent antivascular activity assessed by bioluminescence imaging in an MDA-MB-231-luc breast cancer xenograft mouse model. **Conclusion:** Despite the encouraging positive pre-clinical studies and human clinical trial results of members of this class of anticancer agents, no VDA has yet been approved by the FDA. This study was directed towards the expansion of the compound landscape available for therapy. We confirmed the VDA properties and provide information on the biochemical and biological mechanism of action of OXi8006 and its phosphate prodrug OXi8007 as VDAs. We demonstrated conclusively that OXi8007 is able to effectively disrupt the tumor vasculature in a human breast cancer xenograft model in SCID mice at a well-tolerated dose. These results show that OXi8006 and OXi8007 are potent VDAs and function through a mechanism that is mediated via RhoA.

379 **Poster Session A**

MiR-509-3p Inhibits Osteosarcoma Migratory Properties And Enhances Sensitivity To Cisplatin *S. Patil, University of Houston; P. Gunaratne, University of Houston; J. Yustein, Baylor College of Medicine*

Introduction: Osteosarcoma is the most common primary malignancy of bone within the pediatric population, with metastasis being the leading cause of death for patients afflicted with this very deadly disease. Chemotherapeutic approaches have been employed to inhibit metastasis in osteosarcoma, but it often leads to chemo-resistance and relapse. Previous research in our lab found that has-miR-509-3p can significantl inhibit migration, invasion and proliferation of ovarian cancer cells. To test if this microRNA can be used as a metastatic inhibitor of osteosarcoma, we used six osteosarcoma cell lines with different metastatic nature and heterogenetic background of p53 and Rb genes in our study. **Methods:** Wound healing assay was performed on the six osteosarcoma cell lines with different metastatic nature and heterogenetic background of p53 and Rb genes treated with scrambled microRNA, non-treated and miR-509-3p treated cells. In order to test whether miR-509-3p can sensitize osteosarcoma cells to cisplatin wound healing assay was performed on cells treated with scrambled microRNA, non-treated and miR-509-3p treated cells in combination with cisplatin. **Results:** The wound healing assays showed that miR-509-3p can significantly inhibit migration of all the six osteosarcoma cell lines that we studied, compared to non-treated and scramble miRNAs transfected cells. We also found that miR-509-3p can sensitize osteosarcoma to cisplatin, a chemotherapeutic drug widely used to treat the disease. Our results indicate that miR-509-3p and cisplatin combination effectively inhibit the migration of osteosarcoma cell lines regardless of their genetic background. **Conclusion:** Our results indicate that miR-509-3p and cisplatin combination effectively inhibit the migration of osteosarcoma cell lines regardless of their genetic background.

380 **Poster Session B**

Searching for Allosteric Modulators to Restore Fbw7 Loss of Function Caused by Cancer Mutations *J. Liu, The University of North Texas Health Science Center at Fort Worth*

Introduction: Cancer is an allosteric disease. Allostery, the alternation of protein activity at the sites other than active sites, regulates many proteins involved in cancer, including oncogenes and tumor suppressor genes. Allosteric inhibitors have been successfully developed targeting many oncoproteins, such as BRAF and MEK1, demonstrating that targeting allosteric mechanism is an effective cancer drug development approach. E3 ubiquitin ligase Fbw7, a tumor suppressor, degrades oncogenes including Notch-1, cyclin E, c-Myc, c-Jun etc., and is frequently mutated in gastric cancer, colon cancer, breast cancer, lung cancer, and pancreatic cancer. Mounting evidences showed that restoration of Fbw7 function is a very promising therapeutic strategy for cancer treatment. However, no small molecules have ever been developed to enhance Fbw7. Here, we demonstrated our efforts to design allosteric modulators to restore function of Fbw7 mutants. **Methods:** We have developed a method and successfully applied on E3 ubiquitin ligase pVHL to identify allosteric mutants to restore pVHL loss of function of cancer mutations. This research validates the feasibility to allosterically restore function of tumor suppressors. Here, we improved the method on Fbw7. We performed molecular dynamics simulations for two states of Fbw7, the unbound state and when Fbw7 bound to the substrate Cyclin E. We then compared the correlated motions of bound and unbound states during the simulations to identify the residues with the largest change correlated to linker region. Sequence-based co-evolutionary methods and another methods we recently developed, Rigid Residue Scanning (RRS), were used to validate the identified allosteric residues as allosteric sites. Virtual screening was performed to identify compound that may restore the function of mutated Fbw7. **Results:** Fbw7 has the conserved structure with another E3 ubiquitin ligase Cdc4, the yeast homologue of Fbw7. We first validated our methods by successfully identifying the allosteric sites of Cdc4, consistent with the identified allosteric inhibitor binding sites. Because the previous identified Cdc4 inhibitor does not work with Fbw7, we predicted allosteric sites for Fbw7, which are significantly different from those of Cdc4. These identified sites were validated by other methods including RRS and co-evolutionary analysis. The cancer-related mutations were simulated and shown to disrupt Fbw7 binding to Cyclin E, but our designed mutations at the identified allosteric sites can restore the binding. We further identified allosteric modulators targeting these sites. **Conclusion:** Here, we demonstrated that loss of Fbw7 function caused by cancer-related mutations could be restored allosterically by mutation of allosteric residues or binding of allosteric modulators.

382 **CPRIT Grantee**

Rapid Synthesis of a Lipocationic Polyester Library as Potent Formulated siRNA Delivery Nanoparticles In Vitro and In Vivo *D. Siegwart, The University of Texas Southwestern Medical Center at Dallas*

Introduction: The ability to control chemical functionality is an exciting feature of modern polymer science that enables precise design of drug delivery systems. Ring-opening polymerization of functional monomers has emerged as the most versatile method to prepare clinically translatable degradable polyesters. A variety of functional groups have been introduced into lactones; however, the direct polymerization of tertiary amine functionalized cyclic esters has remained elusive. In this presentation, we will report a strategy that enabled us to rapidly synthesize >130 lipocationic polyesters directly from functional monomers without protecting groups. These polymers are highly effective for short interfering RNA (siRNA) delivery at low doses *in vitro* and *in vivo*. **Methods:** In the spectrum of delivery systems, polymers have many advantages including tunable structural composition, degradability, and biocompatibility. Yet, they are currently less effective than lipid-based delivery vehicles. To overcome this challenge, we have incorporated key ionizable amines and hydrophobic alkyl chains into polyesters. We synthesized a library of lipocationic polyesters directly from functional monomers in high yield (>95%), fast time (~2 minutes), and in gram scale. This was accomplished with precise monomer incorporation ratios to enable tunable hydrophobicity and pKa. **Results:** Formulated nanoparticles enabled siRNA mediated silencing *in vitro* and *in vivo* at low doses. Strikingly, the delivery efficacy strongly correlated with chemical structure because cationic and hydrophobic moieties were incorporated at precise ratios. Lead polymers enabled >90% silencing at a dosage of only 2.4 nM (100% silencing at doses greater than 10 nM) *in vitro*. In contrast, RNAiMax was much less effective in silencing luciferase expression head-to-head at the same doses. Notably, nanoparticles could localize to tumors *in vivo* after intravenous (IV) delivery. This was visualized by whole animal and *ex vivo* tumor and organ fluorescence imaging. Nanoparticles were also able to silence gene expression in tumor-bearing mice, quantified by bioluminescence imaging and in tissue lysates normalized against total protein level or total tissue amount. **Conclusion:** Because activity strongly correlated to structure, these synthetic methods provide a versatile way to directly synthesize lipocationic polymers for gene delivery. Moreover, lipocationic polyesters were able to silence *in vivo* expression of targeted

genes in tumors. This new class of lipocationic polyesters is a promising step towards closing the activity gap between lipids and polymers.

383 **CPRIT Grantee**

Multifunctional Superparamagnetic Iron Oxide Nanoparticles for Combined Chemotherapy and Hyperthermia Cancer Treatment *G. Bao, Rice University; C. Quinto, Rice University; S. Tong, Rice University*

Introduction: Conventional cancer chemotherapy treatments are often compromised by systemic toxicity which stems from a lack of tumor specificity when anticancer drugs are delivered. Superparamagnetic iron oxide nanoparticles (SPIOs) have the potential for use as a multimodal cancer therapy agent due to their ability to carry anticancer drugs and generate localized heat when exposed to an alternating magnetic field, resulting in combined chemotherapy and hyperthermia. Although previous studies have utilized SPIOs for hyperthermia and drug delivery separately, only very limited efforts have been made to optimize the SPIO-based approach for a combinatorial hyperthermia and chemotherapy. **Methods:** To explore this potential, we synthesized SPIOs with a core size of ~14 nm and a phospholipid-polyethylene glycol (PEG) coating, and the chemotherapeutic drug Doxorubicin (DOX) was loaded onto the SPIOs by incubating the drug with nanoparticles at a 1:1 mass ratio in deionized water for 24 hours at room temperature. The drug release profile was characterized by injecting 1 mg of SPIOs into a 20 kDa MWCO Slide-A-lyzer dialysis cassette, and an alternating magnetic field (29.36 kA/m 355 kHz) was generated within the coil, causing the SPIOs to produce heat. The combinatorial response to magnetic fluid hyperthermia and DOX delivery with SPIOs was evaluated using HeLa cells. **Results:** DOX was loaded to SPIOs with 30.8% w/w loading capacity when the PEG length is optimized. We found that DOX-loaded SPIOs exhibited a sustained DOX release over 72 hours where the release kinetics could be altered by PEG length. In contrast, the heating efficiency of the SPIOs showed minimal change with PEG length. With a core size of 14 nm, the SPIOs could generate sufficient heat to raise the local temperature to 43°C, enough to trigger apoptosis in cancer cells. Further, we found that DOX loaded SPIOs resulted in cell death comparable to free DOX, and that the combined effect of DOX and SPIO-induced hyperthermia enhanced cancer cell death in vitro. **Conclusion:** This study demonstrates the potential of using phospholipid-PEG coated SPIOs for chemotherapy-hyperthermia combinatorial cancer treatment with increased efficac . This SPIO-based approach offers many potential benefits for effective cancer diagnosis and treatment, including the integration of diagnostic imaging with targeted drug delivery and hyperthermia.

384 **CPRIT Grantee**

Elderly Texas patients' adherence to treatment guidelines for regional colorectal cancer *H. Zhao, The University of Texas M.D. Anderson Cancer Center; M. Ding, The University of Texas Health Science Center at Houston; J. Niu, The University of Texas M.D. Anderson Cancer Center; V. Ho, Rice University; N. Zhang, The University of Texas M.D. Anderson Cancer Center; X. Du, The University of Texas Health Science Center at Houston; S. Giordano, The University of Texas M.D. Anderson Cancer Center*

Introduction: In Texas, colorectal cancer (CRC) is the third leading cancer in both incidence and mortality. We evaluated whether the treatment of CRC patients was adherent to guidelines, factors associated with adherence, and the association with survival in older regional stage colorectal cancer patients using Texas Cancer Registry (TCR) and Medicare data linked data. **Methods:** We identified 5984 regional stage colorectal cancer patients who were 66 years and older diagnosed from 2001 to 2007. We estimated the treatment adherence rates using binomial distribution. We used chi-square test and multiple logistic regression to identify factors such as age, sex, ethnicity, Health Services Regions, income and education level, year of cancer diagnosis, lymph node, tumor size, and comordibity score associated with adherence. We used Kaplan-Meier survival curves and Cox regression to evaluate the association between adherence and survival. **Results:** For colon cancer, about 55.9% of patients had at least 12 lymph nodes removed, 59.4% had chemotherapy. For rectal cancer, about 56.5% received radiation treatment, and 49.8% had chemotherapy. People with younger age, female gender, higher education and lower comorbidity score were more likely to have care that was adherent to the surgery guideline in colon cancer patients. Adherence rates were significantl different by Texas Health Services Regions. We found adherence to treatment guidelines was associated with a 50% lower risk of death, after adjusting for age, gender, ethnicity, percent below poverty level, percent of not graduate with high school education, Texas Health Service Regions, tumor size, tumor lymph nodes positive and comorbidity level. C**onclusion:** These results indicate that many patients with colorectal cancer are not receiving guideline concordant care, and that lack of guideline concordance was associated with poorer survival. Future studies to better understand the reasons for poor guideline adherence and to improve adherence are warranted. This research was supported in part by the Cancer Prevention & Research Institute of Texas grant RP140020, and by the Cancer Prevention Fund established at MD Anderson by the Duncan Family Foundation.

385 **CPRIT Grantee**

SF2312, A Natural Phosphonate Inhibitor of Enolase *F. Muller, The University of Texas M.D. Anderson Cancer Center; E. Lin, The University of Texas M.D. Anderson Cancer Center; D. Maxwell, The University of Texas M.D. Anderson Cancer Center; P. Leonard, The University of Texas M.D. Anderson Cancer Center; P. Zhenghong, Private sector; N . Satani, The University of Texas M.D. Anderson Cancer Center; D. Sun, Private sector; N. Hammoudi, The University of Texas M.D. Anderson Cancer Center; B. Czako, The University of Texas M.D. Anderson Cancer Center; M. Difrancesco, The University of Texas M.D. Anderson Cancer Center; W. Bornmann, Bayou Therapeutics; R .DePinho, The University of Texas M.D. Anderson Cancer Center*

Introduction: Despite being critical for energy generation in most forms of life, few if any natural antibiotics specifically inhibit glycolysis. **Methods:** To develop a specific inhibitor of the glycolytic enzyme Enolase 2 for the treatment of cancers with deletion of Enolase 1, we modeled the tool compound inhibitor, Phosphonoacetohydroxamate (PhAH) into the active site of human ENO2. A ring-stabilized backbone of PhAH was predicted to increase binding affinity by stabilizing the inhibitor in a bound conformation. **Results:** Unexpectedly, structure based searches revealed that our hypothesized back-bone-stabilized PhAH bears strong similarity to SF2312, a phosphonate antibiotic of unknown mode of action produced by the fungus Micromonospora, which is exclusively active under anaerobic conditions. Here, we present multiple lines of evidence, including a novel X-ray structure, that SF2312 is a highly potent, low nM inhibitor of Enolase. That SF2312 is an Enolase inhibitor fully explains why its antibiotic activity is most potent under anaerobic conditions: hypoxic and anaerobic conditions prevent respiratory energy generation shifting bioenergetic reliance on glycolytic fermentation. **Conclusion:** As far as we are aware, SF2312 is the most potent naturally occurring inhibitor of glycolysis ever reported.

386 **CPRIT Grantee**

Integrated strategy reveals the Fe-S cluster-containing NEET proteins mitoNEET and NAF-1 as chemotherapeutic targets in breast cancer *F. Bai, Rice University; F. Morcos, The University of Texas at Dallas; P. Jennings, University of California; R. Mittler, University of North Texas; R. Nechushtai, Hebrew University of Jerusalem; J .Onuchic, Rice University*

Introduction: Cancer is a leading cause of death worldwide, with the identification of novel drug targets and chemotherapeutic agents being a high priority for cancer therapy. The NEET proteins mitoNEET (mNT) and nutrient-deprivation autophagy factor-1 (NAF-1) were recently shown to be required for cancer cell proliferation and tumor growth[1, 2]. NAF-1 is an important partner for Bcl-2 at the endoplasmic reticulum to functionally antagonize Beclin 1-dependent autophagy[3, 4]. An important goal is to determine the structural interaction of the NAF-1–Bcl-2 complex, identify bioactive small molecules to NEET proteins and study their pharmacological potential in cancer treatment by using a combination of experimental and computational techniques. **Methods:** We used an integrated strategy combing direct coupling analysis (DCA), peptide array screening, and deuterium exchange mass spectrometry (DXMS) that helped us determine the NAF-1–Bcl-2 interaction interface[5] which was then identified to be overlapping with a druggable binding site on NEET proteins determined by a computational method for drug binding site identification. By utilizing chemical synthesis, molecular docking, NMR, and bioassay experiments with control (MCF-10A) and malignant (MDA-MB-231 or MCF-7) human epithelial breast cells, we designed a mitocan cluvenone (CLV) derivative, termed MAD-28, and evaluated its molecular binding mechanism to mNT/NAF-1 and anti-cancer effects. **Results:** Docking analysis and NMR suggested that MAD-28 bound to NEET proteins, at the vicinity of their 2FE-2S clusters, is located at the interaction interface of complex NAF-1–Bcl-2. MAD-28 seems to break the coordinative bond between the His ligand and the cluster's Fe of mNT/NAF-1 and facilitated cluster destabilization, in contrast to CLV that formed a hydrogen bond network that stabilized the 2Fe-2S cluster of these proteins[6]. Bioassay results revealed that MAD-28 has a high specificity in the selective killing of cancer cells, without any apparent effects on normal breast cells. MAD-28 was found to target the mitochondria of cancer cells and displayed a surprising similarity in its effects to the effects of mNT/NAF-1 shRNA suppression in cancer cells. The effects of shRNA suppression included a decrease in respiration and mitochondrial membrane potential, as well as an increase in mitochondrial iron content and glycolysis. As a corollary, since NEET proteins are targets of MAD-28, cancer cells with suppressed levels of NAF-1 or mNT were less susceptible to the drug. **Conclusion:** Our results identify the NEET proteins as novel drug targets in the chemotherapeutic treatment of breast cancer and suggest MAD-28 as a first lead to a new class of anticancer drugs that target hem.

387 **CPRIT Grantee**

Antiemesis Prophylaxis Among Breast Cancer (BC) Patients Receiving Anthracycline-Based Chemotherapy *M. Chavez-MacGregor, The University of Texas M.D. Anderson Cancer Center; W .He, The University of Texas M.D. Anderson Cancer Center; H. Zhao, The University of Texas M.D. Anderson Cancer Center; L. Elting, The University of Texas M.D. Anderson Cancer Center; S. Giordano, The University of Texas M.D. Anderson Cancer Center*

Introduction: Chemotherapy-induced emesis has important medical implications and is associated with a decrease in quality of life (QoL). Guidelines recommend that patients treated with highly-emetogenic chemotherapy receive a 5-HT3 antagonist and steroids, and since 2006, also a neurokinin-1 (NK1) antagonist. We evaluate adherence to antiemesis prophylaxis guidelines among BC patients. **Methods:** 5,569 BC patients >65 years, diagnosed between 2005-2009 were identifie in the SEER/Texas Cancer Registry (TCR)-Medicare database. 25,971 BC patients <65 years, diagnosed between 2005-2012 were identifie in the MarketScan database. All patients received AC, FAC or TAC. Antiemetics given within 1 day of the first cycle of chemotherapy were recorded, adherence with NCCN guidelines was determined according to treatment year. Emesis-related ER visits/hospitalizations occurring between the 1st and 2nd cycle of treatment were identified. Descriptive statistics and logistic regression models were used. **Results:** 22.4% and 28.2% of patients in the SEER/TCR-Medicare MarketScan databases received guideline-adherent prophylaxis. There was a dramatic decrease in guideline-adherence in 2006 with a slow increase in the adherence rates according to time. In multivariable analysis, in the SEER/TCR-Medicare cohort, year of treatment was associated with a decrease in the adherence rate. African-American patients (OR 0.62 95% CI 0.41, 0.94) and those with the longest time between surgery and initiation of chemotherapy (OR 0.63; 95%CI 0.46-0.87) were less likely to receive guideline-adherent treatment. In the MarketScan cohort, a significan association was observed for year and time to initiation of chemotherapy. In addition, compared to patients treated with AC, those receiving FAC (OR 0.24; 95% CI .18-.32) and TAC (OR 0.87; 95%CI were less likely to receive guideline-adherent treatment. Among SEER/TCR participants treated with NK1 antagonists, the rate of ER visits/hospitalizations was 3.6% compared to 3.0% in patients that did not receive NK1 (p=0.64). Among MarketScan participants the rates were 2.0% vs 1.9% (p=0.68). In multivariable model the predictors of emesis-related ER visits/

hospitalizations included older age, comorbidities and TAC regimen. **Conclusion:** A large proportion of breast cancer patients treated with AC, FAC or TAC do not receive guideline-adherent antiemesis prophylaxis. Variation in adherence according to year shows slow uptake of changes made in the guidelines, particularly among elderly patients. Lack of use of NK1-antagonists was not associated with an increase in ER visits or hospitalizations; however the databases used do not allow for evaluation of severity of symptoms, QoL or physician interventions not associated with a claim.

388 **CPRIT Grantee**

Potent Functional Inactivation of the DNA Repair Protein by Dithiocarbamate Compounds Increases the Efficacy of Temozolomide in Human Glioblastoma cells *H. Madala, Texas Tech University Health Science Center at Amarillo; S. Venugopal, Texas Tech University Health Science Center at Amarillo; S. Punganuru, Texas Tech University Health Science Center at Amarillo; K. Srivenugopal, Texas Tech University Health Science Center at Amarillo*

Introduction: There is an urgent need for the design and discovery of new and potent inhibitors for the DNA repair protein MGMT (O6-Methylguanine-DNA-methyltransferase) in glioma therapy. MGMT is highly expressed in brain tumors, and plays a primary role in conferring resistance to alkylating agents. The psuedosubstrates for MGMT such as the O6-benzylguanine have not been successful in the clinic due to prolonged inhibition of DNA repair in the bone marrow stem cells. Recently, we showed that the anti-alcoholism drug, disulfiram (DSF) inhibits MGMT activity in the same way as ALDH by conjugating with the active-site cysteine 145 (Carcinogenesis 35, 692, 2014). DSF, a symmetrical molecule, is metabolized and split in half to yield dithiocarbamate residues. Since the dithiocarbamates resulting from DSF decomposition are the ultimate reactive species that inactivate the aldehyde dehydrogenase and other signaling targets, we surmised that dithiocarbamate derivatives by themselves, will be active, inhibit MGMT and exert anticancer activities. M**ethods:** Therefore, we tested the pyrrolidine dithiocarbamate (PDTC); diethyldithiocarbamate (EDTC); dimethyldithiocarbamate (MDTC) and dibenzyldithiocarbamate (BDTC) on MGMT activity, protein levels, and other redox-sensitive proteins such as the NF-κB and GSTP1. **Results:** The cytotoxicity of these dithiocarbamates against the MGMT-proficient SF-188 glioblastoma cell was comparable with that of DSF, with the MDTC being most effective and the benzyl derivative BDTC least potent. Western blot analysis in HT29 and SF-188 cells revealed a concentration-dependent degradation of MGMT by the dithiocarbamates and DSF. All dithiocarbamates except the BDTC were superior to DSF in degrading MGMT. MDTC was the most potent followed by PDTC and EDTC in depleting the MGMT protein from tumor cells. Further, MDTC was also effective in reducing the cellular levels of NF-κB transcription factor. We also established that MDTC binds to the active site cysteine145 in MGMT leading to its inactivation. Currently, we have developed the pegylated PLGA nanoparticles loaded with MDTC or zinc-chelated MDTC to target the glioblastoma and other cancers. The efficacy of these formulations in intracranial glioma models developed in nude mice is being evaluated. C**onclusion:** The lack of cytotoxicity and inability to bind with MGMT by BDTC suggests that the bulky group attached to the dithiocarbamate may hinder interaction with target proteins or interfere with their metabolism. Since MDTC has a good potential to cross the blood brain-barrier, possess reactive thiol groups that can interact with not only MGMT but also numerous signaling proteins, our strategy using this repurposed compound holds promise in glioma treatment.

389 **CPRIT Grantee**

Design of a Rofecoxib-Combretastatin Hybrid Drug That Exerts Potent and Improved Antimicrotubule and Angiogenesis Properties *S. Punganuru, Texas Tech University Health Science Center at Amarillo; H. Madala, Texas Tech University Health Science Center at Amarillo; C. Cobos, Texas Tech University Health Science Center at Amarillo; M. Constantinos, Texas Tech University Health Science Center at Amarillo; K. Srivenugopal, Texas Tech University Health Science Center at Amarillo*

Introduction: Given the molecular complexity of cancers, including the extreme heterogeneity in their genetic makeup, their plasticity, innate or acquired resistance to anticancer drugs, there is an increasing need for the development of single drugs that can effectively and simultaneously target multiple pathological processes to achieve greater antitumor efficac . M**ethods:** Chemical synthesis, In vitro tubulin polymerization assay, Flow cytometry, Cytotoxicity assay, Immunocytochemistry. This study developed a hybrid drug by combining the Cox-2 selective NSAID rofecoxib with a trimethoxy aryl group found in combretastatin A4 (CA4) which is a potent antimicrotubule and anti-angiogenesis agent. Two of methoxy groups of the CA4, were, however, replaced with iodine in the hybrid drug named KSS-19. The structural design of KSS-19 also preserved the CA4 nucleus in the cis configuration and prevented its isomerization to the biologically inactive trans form. **Results:** KSS-19 was highly potent in specifically killing the tumor cells of various human cancer types at 2-50 nM range. These included the colon (HT29, HCT116), breast (MDA-MB-231, SK-BR-3), lung (A549, H1299), brain (SF188, GBM10, GBM6), pancreas (MIA-PaCa-2) and fibrosarcoma (HT1080). Particularly significant was that KSS-19 was 100 time more potent than CA4 against the HT29 colon cancer cells. Various biochemical and immunocytochemical assays revealed that KSS-19 retained the microtubule disrupting effects of compound CA4, including microtubule loss, the formation of aberrant mitotic spindles, and mitotic arrest. It also inhibited the polymerization of purified tubulins in vitro. Furthermore, KSS-19 potently inhibited the formation of tubes in three-dimensional cultures of the HUVEC (Human umbilical Vein Cell) at 250 nM drastically decreasing the tube length and junctions. The drug also markedly downregulated the tumor invasion and migration in designated assays. The Cox-2 dependent properties of KSS-19 and its antitumor impact in xenograft models are being tested. C**onclusion:** In summary, the designs of such single molecule hybrid anticancer drugs hold great promise for treatment of colorectal and other cancers.

390 **CPRIT Grantee**

The Role of Mutagenesis in Inducing Endocrine Resistance in Estrogen Receptor Positive Breast Cancer *S. Haricharan, Baylor College of Medicine; M. Ellis, Baylor College of Medicine*

Introduction: ER+ breast cancer exhibits a relentless relapse pattern that extends several decades after diagnosis, despite endocrine interventions. About 20% of all breast cancer diagnoses, or 40,000 women a year, will be resistant to standard-of-care endocrine depletion therapy, despite the tumor being histologically ER+. Further, these tumors will be late stage, and potentially fatal, by the time they are identified as endocrine resistant. There are few effective therapies for endocrine resistant ER+ tumors and women with these tumors have over-all survival comparable to those with ER- tumors. It is critical, therefore to investigate the underlying cause of resistance and identify such tumors early in order to treat patients effectively. Since resistance is caused by somatic mutation in ESR1 as well as other genes, we hypothesize that one of the fundamental processes driving poor outcome is somatic mutation rate. This rate may be accelerated by the administration of mutagenic chemotherapy, which is often given to patients with ER+ disease, despite a low therapeutic index compared to ER- disease. Indeed the long term follow-up of NSABP B18 in which patients were treated with neoadjuvant doxorubicin and cyclophosphamide demonstrated that women older than 50 (a surrogate for ER+ postmenopausal disease) have worse late relapse than women younger than 50 (largely ER- disease). **Methods:** In vitro enrichment assays, comet assays, and cell viability assays were used to test whether cells previous exposed to mutagenizing chemotherapeutic agents are more viable in the absence of estrogen. **Results:** High somatic mutation load of ER+, but not ER-, breast cancer associates with poor clinical outcomes and specifically with resistance to endocrine therapy. Experimentally inducing mutagenesis in ER+ breast cancer cells by exposing the cells to hypoxic conditions results in increased ability of these cells to grow in the absence of estrogen. Cells exposed to mutagenizing chemotherapeutic agents (5-Fluoruracil, cisplatin, and cyclophosphamide) demonstrate a dose-dependent decrease in viability but continue to accumulate mutations even at very low doses when they no longer have decreased viability. Indeed, ER+ cells exposed to lower doses of these chemotherapeutic agents have increased ability to grow in the absence of estrogen. **Conclusion:** The impact of this investigation will be to demonstrate the potential harm of inappropriate chemotherapy administration and to define DNA repair defects that can be subject to more tailored therapeutic interventions that have less risk and greater therapeutic index. The results of this study may inform treatment regimens for ER+ breast cancer.

391 **CPRIT Grantee**

Genetically Engineered iPS Cell-Derived Mesenchymal Stem Cells Expressing Cytosine Deaminase Exhibit Tumor-Suppressive Properties *M. Ullah, Texas A&M University System Health Science Center; Y. Kuroda, Texas A&M University System Health Science Center; S. Adams, Texas A&M University System Health Science Center; J. Beaver, Texas A&M University System Health Science Center; D. Prockop, Texas A&M University System Health Science Center; T. Bartosh, Texas A&M University System Health Science Center*

Introduction: One promising new oncolytic strategy involves targeted delivery of cytotoxic agents to cancers using mesenchymal stem/stromal cells (MSCs) as vehicles that have a remarkable ability to home to tumors and integrate within the tumor environment. However, applications of MSCs for cancer therapy are hindered by variations in quality among different cell preparations and conditional tumor-promoting effects of the cells. MSCs derived from human induced pluripotent stem cells (iPSC-MSCs) have emerged as a viable replacement to MSCs obtained from adult tissues and could potentially provide unlimited numbers of tumor-tropic cells with uniform biological properties. In the current study, we assessed the capacity to produce banks of iPSC-MSCs genetically programmed to express the suicide gene that codes for cytosine deaminase (CD), an enzyme that converts the non-toxic prodrug 5-fluorocytosine (5-FC) locally into the chemotherapeutic agent 5-fluorouracil (5-FU). We then tested effectiveness of these modifie cells for cancer therapy. M**ethods:** The iPSC-MSCs were transduced with a lentiviral vector containing green fluorescent protein (GFP) and the suicide gene CD fused to uracil phosphoribosyltransferase (UPRT). Transduction efficiency was determined by measuring expression levels of GFP and CD. The transduced cells were extensively expanded and cryopreserved for use in later experiments. Maintenance of MSC surface markers on transduced cells was assessed by flow cytometry. Efficacy of the CD:UPRT-expressing iPSC-MSCs was first evaluated in co-cultures of MSCs with various human cancer cell lines (melanoma, breast carcinoma, ovarian cancer) and then in vivo using a human tumor xenograft model. **Results:** Engineered iPSC-MSCs constitutively expressed high levels of CD and maintained surface features characteristic of tissue-derived MSCs. The iPSC-MSCs showed remarkable ability to kill all cancer cell lines tested in vitro after addition of 5-FC to the cultures. Level of killing was dependent on time, concentration of 5-FC, and numbers of MSCs used in the assay. In immune-deficient mice, injections of iPSC-MSCs expressing CD:UPRT not only limited formation of human tumors following systemic administration of 5-FC, but also caused significan regression of established tumors and reduced development of metastatic disease. Importantly, activation of the prodrug also resulted in elimination of the modified iPSC-MSCs thus providing a safeguard against wayward stem cell progeny. **Conclusion:** Taken together, the results here provide evidence that iPSC-MSCs have immense potential as cellular carriers of therapeutic transgenes. Moreover, our findings bring new insights into the mechanism of the anticancer effects induced by modified iPSC-MSCs

392 **CPRIT Grantee**

Evaluation and Application of Novel MELK Inhibitors and Derivatives to Understand MELK Signaling Interactions in TNBC

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Introduction: The cell cycle is dysregulated in cancer cells, and its protein components represent a wealth of putative drug targets. Current evidence supports a role for maternal embryonic leucine zipper kinase (MELK) in regulation of mitotic entry, promotion of cell proliferation, cancer stem cell maintenance, cancer progression, apoptosis, and tumorigenesis. MELK is overexpressed in glioblastoma, lung, colorectal, and notably in triple-negative breast cancer (TNBC) cells, where overexpression level correlates with poor prognosis. Thus, MELK inhibition offers an attractive novel therapeutic strategy for TNBC, but such potential is hindered by incomplete understanding of its cellular role and additional ramification of its inhibition. M**ethods:** In order to test and develop MELK-targeting inhibitor, we designed an E. coli-based expression and purificatio system for the catalytic domain of MELK (MELK 340) using Ni-NTA and ion-exchange chromatography. Using a thioredoxin/calmodulin binding peptide (CBP) containing vector designed in-house, a suitable protein substrate for activity assays, Bcl-GL, was purified with calmodulin linked affinity resin. Previous work involving high-throughput screening identifie an inhibitor (MELK-1) with an IC50 of 51.5 ± 8.5 nM. Rational derivatives to improve selectivity were designed using information gained through molecular modeling of MELK-1 against published crystal structures of MELK. We developed HCC70 TNBC cell lines with stable shRNA knockdown of MELK to investigate downstream effects of MELK activation using western blot and phosphoproteomic approaches. **Results:** Bcl-GL and MELK 340 were robustly expressed, each with an average target yield of 3.5 mg / L culture. Calmodulin-based purification of Bcl-GL reduced the need for additional columns through removal of a contaminating band observed following ion exchange chromatography, resulting in pure sample. Five MELK-1 derivatives (denoted MELK-2 through MELK-8) showed IC50s ranging from 0.5 nM to incomplete inhibition. Preliminary studies with HCC70 cells have shown that knockdown of MELK results in decreased phosphorylation of JNK and c-JUN following serum starvation and/or stimulation with thapsigargin as compared to scramble shRNA control. **Conclusion:** Addition of CBP to the Bcl-GL construct improves recovery efficiency and purity of the protein purification protocol. The most potent inhibitors, MELK-7 and MELK-8, displayed IC50s of 0.5 nM and 10 nM, respectively. Additionally, co-crystallography screening efforts are currently underway. Western blot analysis of shRNA knockdown of MELK with the JNK pathway serves as proof-of-concept for a model system to evaluate the effect of MELK-1 and derivatives on MELK activity in HCC70 TNBC cells. Ongoing research will include the HCC70 knockdown line to identify novel MELK interacting partners using phosphoproteomics.

393 **CPRIT Grantee**

Counteracting Mitochondrial Oxidative Phosphorylation-mediated Resistance to MAPK Pathway Inhibitors in Melanoma

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Introduction: FDA-approved BRAF and MEK inhibitors achieve clinical responses in the majority of metastatic melanoma patients with a $BRAF^{V600}$ mutation. However, a significant proportion of patients have minimal responses, and virtually all patients will develop acquired resistance. An improved understanding of resistance mechanisms to these agents will lead to more effective therapeutic approaches. **Methods:** We used a combination of whole genome siRNA synthetic lethality screening, transcriptional profiling and Reverse Phase Protein Array (RPPA) proteomics to identify the molecular mediators of resistance to MAPK inhibitors in molecularly characterized human melanoma cell lines. Implicated genes and candidate therapeutic strategies were interrogated functionally using in vitro and in vivo melanoma models. **Results:** Both whole genome siRNA screening and global transcriptional profiling implicated increased oxidative phosphorylation (OxPhos) as a key mediator of resistance to MAPK pathway inhibitors in a pilot set of human melanoma cell lines, including melanomas with and without $BRAF^{V600}$ mutations. Additional testing confirmed that elevated OxPhos characterizes 30-50% of BRAFV600-mutant, MAPKi-resistant cell lines and patients with acquired resistance to BRAF +/- MEK inhibitors. The high OxPhos phenotype was dependent upon the transcriptional co-activator PGC1α, which is regulated by the master melanocytic transcriptional regulator MITF. Unexpectedly, treatment with a dual mTORC1/2 inhibitor markedly downregulated PGC1α expression and OxPhos through a novel mechanism involving the subcellular localization of the transcription factor MITF. Elevated OxPhos predicted sensitivity to combined inhibition of MAPK and mTORC1/2 inhibition, and the combination demonstrated marked synergy in vivo. Further functional testing of the high OxPhos melanomas was performed using IACS-10759, a novel and potent inhibitor of Complex I of the mitochondrial electron transport chain. IACS-10759 induced OxPhos inhibition, in vitro and in vivo growth arrest, and apoptosis induction at low doses in a subset of MAPKi-resistant human melanoma lines with high OxPhos, whereas non-transformed melanocytes, skin fibroblasts and other melanoma cell lines were insensitive. Transcriptional and RPPA analyses revealed a decrease of PGC1α and AMPK-activation associated effects as mediators of sensitivity to IACS-10759, in addition to the inability of the cells to use alternate metabolic pathways for energy generation. **Conclusion:** Our findings demonstrate that a subset of melanomas are characterized by a high OxPhos metabolic phenotype that can cause de novo and acquired resistance to MAPK pathway targeted therapies. Both mTORC1/2 and direct OxPhos inhibition are promising therapeutic strategies for this melanoma subtype. Studies are ongoing to optimize the clinical testing and evaluation of such agents in this disease.

394 **CPRIT Grantee**

Inhibition of the TRPM7 Kinase Domain Inhibits Breast Cancer Cell Migration and Invasion and Tumor Metastasis *T. Kaoud, The University of Texas at Austin; X. Xie, The University of Texas M.D. Anderson Cancer Center; J. Park, The University of Texas M.D. Anderson Cancer Center; C. Tavares, The University of Texas at Austin; S. Mitra, The University of Texas M.D. Anderson Cancer Center; M. Cano, The University of Texas M.D. Anderson Cancer Center; R. Sammons, The University of Texas at Austin; M. Radwan, Faculty of Pharmacy, King Abdulaziz University; C. Bartholomeuz, The University of Texas M.D. Anderson Cancer Center; K. Dalby, The University of Texas at Austin*

Introduction: TRPM7 (transient receptor potential melastatin 7) is a non-selective cation channel fused to protein kinase domain at the C-terminal whose activity is linked to the control of actomyosin contractility. TRPM7 mediates adhesion and migration of breast cancer cells and promotes breast tumor metastasis. The lack of cell-permeable inhibitors of the kinase domain represents a barrier to understand the kinase function. M**ethods:** Herein, we describe the discovery of the first small molecule (KD-1) that targets TRPM7 kinase activity and characterize its mechanism of action in-vitro, in-cells and in-vivo. R**esults:** Mg2+ starvation, which promotes TRPM7 kinase activity, induces phosphorylation of eEF2. Treatment of Mg2+-starved HEK293 cells with KD-1 decreased eEF2 phosphorylation, consistent with TRPM7 kinase activity suppression in-cells. KD-1 decreased the binding of Myosin IIB to TRPM7 in HEK293 and MDA-MB-231 cells. And when MDA-MB-231 cells were treated with increasing doses of KD-1, no change in cell viability was seen. Interestingly, KD-1 inhibited MDA-MB-231 cells migration and invasion that is reportedly regulated by TRPM7 kinase activity. Finally, the bioluminescent signals (to assess metastasis) were significantly lower in KD-1-treated mice (25 and 50 mg/kg/day) than in mice treated with vehicle control ($P \leq 0.05$, 2-sided t-test.). C**onclusion:** The discovered compound represents the first inhibitor that targets the kinase activity of transient receptor potential melastatin 7 (TRPM7). Inhibition of TRPM7 kinase activity may reduce or block breast tumor progression and/or metastasis.

395 **CPRIT Grantee**

Validating Hetero-Multimers as Dual Targeted Therapy *S. Raghunathan, University of Houston; D. Shukla, University of Houston; D. Udugamasooriya, University of Houston*

Introduction: Current single targeted therapies are less effective due to complexity of targeted protein expression and signaling cascade overlaps. Therefore using a single drug to block a single protein/receptor is not sufficient. Combination therapy practice in the clinic as a solution for this problem brings high risks of side effects. We aim to address these failures of single targeted therapy by focusing on developing dual targeted single therapeutics. Our plan is to develop biologically active heterodimeric peptoids that can target two different biological targets synergistically. In our first example, we plan to target Lipid- phosphotidylserine (PS) and vascular endothelium growth factor (VEGF) that are two biomolecules which have been found to be strongly co-expressed on tumor endothelial cells. VEGFR2 is the main player in tumor blood vessel formation (angiogenesis) and PS plays the role of evading immune attack. We have previously identifie and validated two peptoids – PPS1D1 & GU40C4 which target PS and VEGF respectively and have exhibited significant cytotoxic activity as individual homo-dimeric compounds. In this study, we synthesized multiple heterodimers of PPS1D1 and GU40C4 to develop more specific, selective and high affinity drug-leads for dual targeted therapy. **Methods:** First, active homo-dimers of each of PPS1D1 and GU40C4 were synthesized on low-loading capacity Nova Syn TGA beads utilizing standard 2 hour peptide synthesis and microwave-assisted peptoid synthesis with different linkers and azido or alkyne groups loaded on the c-terminus. Then hetero-multimers were developed by bringing these two together with 'click' chemistry. We used Porcine Aortic endothelial cells (PAE/KDR) and H441 lung cancer cells that express PS and VEGFR2 as our model systems. PS expression on PAE/KDR and H441 cells was evaluated using Annexin V-FITC binding assay. Heterodimer peptoid activities were evaluated through 5 day MTS cell proliferation assay wherein the cells were serum starved, but induced proliferation in the presence of VEGF. **Results:** PS expression was observed on the tested cell lines. It was observed that heterodimers generated by linking PPS1D1 to GU40C4 provided much better cytotoxic effect at lower nanomolar level over the individual and as a mixture of both peptoids. **Conclusion:** We have developed dual targeted drug-leads that are more specific and effective than their individual counterparts. Other hetero-multimers targeting different combination of receptors can also be developed to target cancer.

396 **CPRIT Grantee**

Pediatric Cranial Radiation Therapy Affects Mouse Behavior *T. Inoue, Baylor College of Medicine; M. Gaber, Baylor College of Medicine; E. Perez, University of Houston; J. Leisure, University of Houston*

Introduction: Radiation therapy (RT) is currently the most effective treatment in the fight against pediatric brain tumors due to its efficienc in eliminating solid tumors. However, the utilization of RT in pediatric populations results in the destruction of neural stem cells required for normal brain development, as well as, the damage of surrounding healthy tissue and vasculature required for normal functioning. The heavy costs of impacting these tissues within the brain include a lifelong cognitive impairment, along with neuroendocrine deficits of these pediatric cancer survivors. As a result, the primary goal of this study is to develop a pediatric animal model of brain radiation therapy, which can then be followed up with the testing of potential neuroprotectants in a brain tumor/RT-based treatment paradigm. M**ethods:** Four-week old, male C57Bl/6J mice were obtained from Jackson labs and were cranially irradiated with a single-dose of 5Gy at 31 days of age. They were observed on a weekly basis and were tested using open fiel apparatus (OFA), novel object recognition (NOR) and conditioned fear (CF) beginning at one-month post-RT and again at 3-months post-RT. All animals were used in accordance to the guidelines set by the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine. **Results:** RT mice have significantly lower body weight compared to SHAM mice beginning as early as 31 days post-RT. At 1-month post-RT, we see deficits in distance covered in the two minutes of OFA, both of which indicate a decrease in locomotor activity in the RT group compared to the SHAMs. No differences in NOR were observed between the SHAM and RT mice. At 3-months post-RT, differences observed in locomotor activity at the 1-month post-RT time point are no long present. In CF, we see a distinct decrease in freezing behavior in the RT mice compared to SHAM mice indicating a loss of memory recall associated with contextual memory, but we do not observe a similar deficit in the cued memory recall. These results point towards a deficit in hippocampal function paired with what appears to be normal amygdala function. C**onclusion:** Exposure to 5Gy of ionizing radiation caused deficits in both behavior and cognition in our mice. Our next step will be to pair this RT exposure with our putative neuroprotectant and assess whether we can ameliorate the deficits we observed post-RT.

397 **CPRIT Grantee**

Pediatric Cranial Radiation Therapy Alters Brain Phenotype of Rats *M. Gaber, Baylor College of Medicine; T. Inoue, Baylor College of Medicine; O. Sabek, The Methodist Hospital Research Institute*

Introduction: Radiation therapy (RT) currently plays a major role in the effective treatment of pediatric brain tumors. It is often used in conjunction with resectioning of the afflicted area and/or chemotherapy. However, survivors often suffer from lower IQ scores and lifelong cognitive impairment as RT results in the damage of surrounding healthy tissue required for normal development and function. RT dose and changes in white matter have been shown to be highly correlated within patients. As a result, we utilized a specialized MR imaging modality, diffusion tensor imaging (DTI), which measures the integrity of white matter tracts. Our goal is to develop a rat model of cranial RT and apply neuroprotective strategies in order to preserve the neuronal architecture. **Methods:** Four-week old, male Fischer 344 rats were obtained from Harlan Labs and were cranially irradiated with four fractions of 5Gy each day for four consecutive days. Twelve months post-RT, rats were transcardially perfused and whole heads were collected and prepared for DTI. Images were analyzed for whole brain volumes and FA, as well as specific brain region volumes and FA. All animals were used in accordance to the guidelines set by the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine. **Results:** DTI imaging of rat brains 12months post-RT showed that irradiated rats have a significantly smaller brain volume (~11% smaller) compared to age-matched shams. A number of specifi brain regions were assessed to see where these volumetric differences arose. We found that there were significant decreases in brain volumes within the cerebellum, diencephalon, hindbrain, isocortex, midbrain and olfactory structures of RT rats compared to SHAM. Finally, we assessed the FA of the entire brain and found that there were no differences in FA of the entire brain of the RT rats compared to SHAM, however, when we looked at specific regions, we see a slight but significant increase in FA within the substantia nigra of RT rats compared to SHAM. **Conclusion:** Pediatric brain RT results in brain phenotype alterations that involve changes in brain size and FA of specific regions. Our rat model of pediatric brain RT will allow a platform in which to test novel neuroprotective strategies for protecting neuronal tissue in brain tumor patients.

398

Sensitivity of Kinetic Rate Variables in Signaling Pathways to Drug Responses *X. Li, Prairie View A&M University; L. Qian, Prairie View A&M University; S. Bamgbose, Prairie View A & M University*

Introduction: The complexity of biological signaling networks, especially the uncertainties associated with the model parameters, present challenges for understanding the behavior of such networks and hence hamper the translation of the modeling study into drug development process. Sensitivity analysis can help to determine which parameters are the "key drivers" of the model's output. How to tailor the sensitivity study under drug perturbation based on the knowledge of available existing or potential drugs are considered in this study. **Methods:** Local Sensitivity Analysis (LSA) and Global Sensitivity Analysis (GSA) techniques are the main techniques used to investigate the effects of variations in parameters. Since most biochemical reactions networks yield models of a nonlinear nature, LSA method can be of limited use when the analysis aims to assess the relative importance of uncertain factors. On the other hand, GSA investigates the sensitivity over the entire parameter space by simultaneously examining a whole range of parameters values. This technique is more appropriate when models are nonlinear or parameter values have large uncertainties. Examples of GSA techniques include: (i) sampling based methods, such as, Monte Carlo filtering (ii) global screening methods, such as, Morris method, and (iii) variance based methods, such as, Sobol's method, and Fourier amplitude sensitivity test (FAST). In this study, we try to find the sensitivity of kinetic rate variables in signaling pathways to drug responses using computationally efficien methods. Specificall , we test the hypothesis whether the molecular mechanism of the drug Bortezomib affect the nuclear NF-κB (nNF-κB) expression level significantly in the NF-κB pathway. We apply both the classical GSA methods, such as Sobol's method, and our proposed systematic sampling method to obtain the sensitivity of kinetic rate variables in NF-κB pathway. **Results:** Both methods indicate that the drug Bortezomib indeed affect the most sensitive kinetic rate variables in the NF-κB pathway. We obtained theoretical results on how much our proposed method would reduce the computational complexity comparing to the classical GSA methods and the conditions that the classical GSA methods would be equivalent to our proposed method due to uncertainty. **Conclusion:** Sensitivity analysis (SA) can help us improve the predictive capacity of the signaling pathway model and refine parameter estimates by identifying the most influential parameters. However, how to tailor existing SA methods for drug effect analysis needs further study.

400 **CPRIT Grantee Poster Session A**

Flow-proteometric Platform for Detecting Individual Signaling Complexes and Validating Therapeutic Antibody Efficacy in Tumor Tissue *C. Chou, The University of Texas M.D. Anderson Cancer Center; P. Tsou, The University of Texas M.D. Anderson Cancer Center; H. Lee, The University of Texas M.D. Anderson Cancer Center; Y. Wang, The University of Texas M.D. Anderson Cancer Center; J. Kameoka, Texas A&M University; M. Hung, The University of Texas M.D. Anderson Cancer Center*

Introduction: Cellular signaling complexes, which are made up of mostly proteins and nucleic acids, play a major role in signal transduction by carrying and delivering messages that coordinate basic biological functions. These processes are mainly relayed through protein-protein and protein-nucleic acid interactions and it is known that deregulated signaling transduction is related to many diseases, especially in cancer. We have developed a multiplex flow-proteometric platform that can analyze individual signaling complexes directly from tissue, which enables us to accurately acquire the information from in vivo signal transduction. In addition, this technology can also be applied for validating therapeutic antibody efficiency in xenograft model through direct detection of antigen-antibody complexes. **Methods:** To demonstrate that single signaling complexes can be directly detected in lysates from fluorescent-labele tissue, we selected STAT3, p300, and genomic DNA as targets to detect and quantify individual complexes from xenograft tumor tissues. All the detected events were presented in a 3D fluorescence plot, revealing seven different types of events. For in vivo therapeutic antibody efficienc test, fluorescence labeled anti-EGFR antibody (Cetuximab) was i.v. injected into mice which have EGFR-GFP expressed tumor. Fine-needle biopsy were performed to collect the tumor tissue and then subjected to single complex analysis. **Results:** Among all of the detected STAT3 events in the signaling complexes analysis, on average 7.04% interacted with both p300 and genomic DNA in the same complex, 2.99% interacted with p300 only, and 15.23% interacted with DNA only. Looking at the data from the standpoint of p300, on average 5.88% of total p300 protein molecules interacted with STAT3 and genomic DNA, 2.5% with STAT3 only, and 3.06% with DNA only. For testing Cetuximab efficiency in mice xenograft model, about 30% of EGFRs were recognized by Cetuximab in 5 minutes after i.v. injection. The amount of Cetuximab-EGFR complexes has no obvious difference after 30 minutes post i.v. injection. **Conclusion:** Our data showed that not only the STAT3, p300, and DNA in a single complex was identified but also quantified the distribution of lone proteins and complexes, which currently cannot be analyzed by conventional methods. We also demonstrated the analysis of therapeutic antibody efficiency in the in vivo xenograft model. All these applications for single complex analysis will not only benefit to basic cancer signaling research but also provide a new tool for identifying new therapeutic agents for cancer treatment.

401 **CPRIT Grantee Poster Session B**

The Novel Small Molecule BC-2059 Inhibits Wnt-dependent Gene Transcription in Cancer Cells by Disrupting TBL1/β-catenin Complex *R. Soldi, Beta Cat Pharmaceuticals LLC; S. Horrigan, Beta Cat Pharmaceuticals LLC; J. Bearss, Huntsman Cancer Institute; K. Bhalla, The University of Texas M.D. Anderson Cancer Center; J. Northrup, Beta Cat Pharmaceuticals LLC; H. Vankayalapati, Huntsman Cancer Institute; S. Sharma, Huntsman Cancer Institute*

Introduction: Aberrant activation of the Wnt/β-catenin signaling pathway is involved in the development and growth of a large variety of human cancers, making this pathway attractive for development of cancer therapy. Unfortunately, the lack of conventionally druggable targets has hindered the development of inhibitors of this pathway. Direct targeting of the β-catenin protein has been difficult largely due to the lack of selective high affinity binding sites on β-catenin protein or partner proteins that are amenable to small molecule binding. Using a novel cell-based high throughput screening system we have identified high affinity compounds that disrupt β-catenin interaction with TBL1 (Transducin Beta-Like protein 1) to effectively inhibit oncogenic signaling. Discovery of the central role of the TBL1/ β-catenin complex in oncogenic signaling has opened new opportunities for targeting this important cancer pathway. M**ethods:** High throughput cell-based screening, and lead optimization was used to identify and optimize of a series of anthracene-9, 10-dione- dioxime compounds. These small molecules were tested on a battery of cancer cell lines and found to inhibit Wnt pathway signaling and induce degradation of β-catenin protein. Computational and structural analysis followed by protein interaction studies determined that these compounds disrupt the TBL1/β-catenin complex, resulting in inhibition of Wnt-mediated signaling. Elisa-based analysis, immunoprecipitation, ChIP and PLA assays were used to investigate the mechanism of this disruption. The lead compound, BC2059, was found to have efficacy in xenograft models of epithelial and hematological malignancies. **Results:** We have identified a small molecule inhibitor, BC-2059, that promotes the reduction of nuclear β-catenin levels, inducing cell cycle arrest in G1 phase and cell apoptosis. BC-2059 causes the disruption of the TBL1/β-catenin interaction in both cellular and cell free systems. ELISA based competition binding experiments, immunoprecipitation analysis and PLA assays in colorectal cancer cell lines characterized by activated Wnt signaling, demonstrate that BC-2059 directly disrupts the TBL1/β-catenin complex, resulting in the decrease in β-catenin protein levels in a proteasome dependent manner. In addition, TCF reporter assays and TBL1 and β-catenin ChIP assays show that BC-2059 inhibits Wnt-mediated gene expression and disrupts oncogenic signaling. **Conclusion:** We show that BC-2059 disrupts the TBL1/β-catenin interaction by directly binding at the interface of the complex and results in the decrease in β-catenin protein levels, inhibition of Wnt/β-catenin-mediated gene transcription, and inhibition of cancer cell growth in cellular and animal models.

402 **CPRIT Grantee**

IL4 Receptor as a Therapeutic Target in Breast and Colon Cancer

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Introduction: The Th2 cytokine interleukin 4 (IL4) is a well-characterized lymphocyte survival, proliferation and activation factor. On lymphocytes, it binds a heterodimeric receptor consisting of the common gamma chain and interleukin-4 receptor alpha (IL4Ra). A second or type II IL4 receptor is found on non- hematopoietic cells. The type II receptor consists of IL4Ra and IL13Ra1, and is able to bind both IL4 and the related cytokine IL13. Expression of this receptor has been reported in several tumor types. Given the role of IL4 in lymphocyte survival and proliferation, we hypothesized that type II IL4R signaling in tumor cells would regulate similar processes thus contributing to tumor progression. **Methods:** Tissue sections or whole cell lysates were immunostained for IL4Ra and downstream effectors. Cell line IL4Ra expression was modulated using shRNAs. The murine mammary cell lines 4T1 and PyVT-R221a and colon line MC38 were used for in vivo and vitro assays. MDA-MB-231 human breast cancer cells and a panel of human colon adenocarcinoma cell lines were used for in vitro experiments with recombinant human IL4 treatment. Experimental metastases in liver and lung were generated by intra-splenic or intravenous injection, respectively. **Results:** Immunostaining of a human breast cancer tissue microarray revealed 82% of the tumors were positive for IL4Ra expression, with approximately half of those also positive for pStat6, the predominant downstream effector of IL4 signaling. To test whether tumor IL4Ra contributed functionally to breast cancer progression, particularly metastatic disease, we generated IL4Ra-knockdown cell lines. In vivo metastasis assays with knockdown cells showed that outgrowth of both liver and lung lesions was significantl attenuated, a phenotype also seen when unmodified cells were implanted in IL4-null mice. Mechanistically, we found that IL4 resulted in increased Akt, Erk and mTOR activation and blockade of these pathways prevented IL4-mediated colony growth. In further work, we have shown that metabolic pathways are altered by IL4 in tumor cells and these alterations contribute to enhanced proliferation. Similarly to breast cancers, we found IL4Ra expressed in over 60% of human colon adenocarcinomas and in human and murine colon cancer cell lines. Additionally, proliferation and survival of colon tumor cells in vivo is reduced when an IL4 receptor antagonist is administered to mice. C**onclusion:** Together, our data suggest that the type II IL4 receptor is expressed in adenocarcinoma cells and contributes functionally to tumor progression. Blockade of this receptor offers a potential therapeutic strategy, with particular relevance to metastatic disease.

403 **CPRIT Grantee**
Poster Session B

An Adnexal Mass Patient Registry For The Development Of Innovative Ovarian Cancer Diagnostics

D. Munroe, Vermillion, Inc.; G. Poveda, Vermillion, Inc; J. Wolf, Vermillion, Inc.; J. Brown, The University of Texas M.D. Anderson Cancer Center

Introduction: In 2010 an Austin-based company, Vermillion Inc, launched OVA1®, the first proteomics-based multivariate test to gain FDA clearance. Developed in collaboration with Johns Hopkins University, OVA1 is used prior to planned surgery for an ovarian (adnexal) mass, to identify patients at high risk of ovarian cancer. OVA1 features high sensitivity across all ovarian cancer stages and subtypes, and high negative predictive value when the test predicts low risk. As a result, OVA1 identifies patients who may benefit from pre-surgical consultation or referral with a gynecologic oncologist (Gyn Onc), and greater confidence that a low risk mass will prove to be benign. This year a second-generation version of the test was developed, which is currently under review by FDA. Despite these advances, early detection of ovarian cancer remains a serious unmet need. Approximately 22,000 new cases occur each year among a much higher number of gynecologic surgeries. In addition, over 14,000 women die from the disease annually, nearly as many deaths as all other gynecologic cancers combined. Early detection is challenging, since most ovarian cancers present in late stage, with 5-year survival of about 25%. M**ethods:** To address this challenge, re-targeting of Vermillion's multivariate technology has been proposed to aid in early detection and surgical decision-making. Vermillion and the MD Anderson Cancer Center are partnering with CPRIT to establish a Texas-led, multi-institutional, Adnexal Mass Registry. The registry will support the development of the re-tuned algorithm, code-named "OVA-AID." Enrollment will begin at first identification of a suspicious pelvic mass. Serum, imaging and clinical data will be collected at intervals as the patient is monitored, and eventually operated. Specimens, pathology, imaging and post-operative follow-up will enable diagnosis and classification of the malignant and benign conditions that led to surgery. **Results:** Treatments and outcomes will also be captured, so that diagnostic, prognostic and predictive factors can be identified. Several products may be possible, depending on the disease type, progression and therapeutic response. Just as OVA1 related 5 biomarkers to the presence of ovarian cancer, other markers and/or clinical factors may enable differential diagnosis of cancer, endometriosis, polycystic ovaries and other conditions that co-present with ovarian cancer. In addition, the Registry will generate publications to address crucial requirements of modern healthcare: safety, reliability, better outcomes, cost savings and improved healthcare delivery. **Conclusion:** Together, Vermillion and MD Anderson will lay a foundation for precision medicine while improving the care of women with ovarian cancer and other important diseases.

404 **CPRIT Grantee**
Poster Session A

An Integrated System for Targeted Next-Generation Sequencing that Analyzes DNA Mutations, RNA Fusions and Gene Expression in Cancer *R. Blidner, Asuragen, Inc.; R. Zeigler, Asuragen, Inc.; J. Plyler, Asuragen, Inc.; S. Sah, Asuragen, Inc.; L. Chen, Asuragen, Inc.; H. Zhu, Asuragen, Inc.; A. Hadd, Asuragen, Inc.; J. Fujimoto, The University of Texas M.D. Anderson Cancer Center; V. Papadimitrakopoulou, The University of Texas M.D. Anderson Cancer Center; I. Wistuba, The University of Texas M.D. Anderson Cancer Center; B. Haynes, Asuragen, Inc.; G. Latham, Asuragen, Inc.*

Introduction: The interrogation of a broad range of DNA mutations in a single sequencing assay has increasingly shifted diagnostics from single-target tests to highly multiplexed NGS panels. Current panels offer a range of content but do not streamline the analysis of RNA and DNA markers into a unified assay. We present a comprehensive approach for targeted clinical NGS that enables simultaneous quantification of DNA and RNA, a streamlined workflow compatible with low-input total nucleic acid (TNA), and specimen compatibility that includes FFPE, FNA, and liquid biopsies. **Methods:** Sample QC was performed using a novel qPCR assay that quantifies and partitions functional DNA and RNA from TNA isolations. PCR-based target enrichment was conducted using QuantidexTM targeted NGS reagents and sequenced on the MiSeq™ (Illumina) or the PGM™ (Thermo Fisher). Library sequences were analyzed using Quantidex™ Reporter, a bioinformatic analysis suite that directly incorporates pre-analytical QC information to improve the accuracy of variant calling, fusion detection and RNA quantification **Results:** We present two unified RNA/DNA cancer panels: 1) a thyroid cancer panel that covers 56 DNA targets and 90 RNA targets, including 50 gene fusions and 40 mRNA targets; and 2) a lung cancer panel that covers 55 DNA targets and 130 RNA targets including over 100 gene fusions and mRNA expression markers associated with clinical actionability. Both panels interrogate RNA and DNA events from a single TNA sample in one sequencing run. The thyroid panel was evaluated on 123 FFPE thyroid lesions and 65 FNAs and revealed >98% agreement with independent methods. A diagnostic classifier that was migrated from FFPE to FNA increased diagnostic sensitivity from 74%, using DNA markers alone, to 98%, using RNA and DNA markers, while maintaining high specificit . The lung panel was assessed with cell-line and synthetic controls as well as >100 NSCLC FFPE specimens. Analytical concordance between matched FFPE, fresh frozen and liquid biopsies was also performed. Integration of a customized bioinformatics pipeline and variant caller with wet-lab QC enhanced mutation call sensitivity for variants present at less than 10% mutation, improved PPV for low-input specimens, and achieved absolute quantification of RNA. **Conclusion:** The Quantidex™ NGS system features optimized sample QC and enrichment chemistries that unify RNA and DNA targets and enhance quantitative variant calling and expression analyses to enable reliable, accurate and comprehensive molecular characterizations of tumor specimens. These approaches can be utilized with multiple types of cancer biopsies and NGS platforms to advance diagnostic, prognostic and theranostic applications.

405 **CPRIT Grantee**

Characterization of TP53 Mutations by DNA and RNA-Sequencing of Platinum Sensitive Ovarian Cancer FFPE Samples *B. Haynes, Asuragen, Inc.; D. Ilsley, Asuragen, Inc.; M. Fahey, Asuragen, Inc.; G. Latham, Asuragen, Inc.; E. Somers, Morphotek Inc.; D. O'Shannessy, Morphotek Inc.*

Introduction: *TP53* is the most frequently mutated gene across all cancers at approximately 50% and in cancers such as high grade serous ovarian up to 96%. p53 suppresses tumorigenesis through apoptosis, senescence or cell cycle arrest in response to genomic insult. In contrast to other tumor-suppressors, *TP53* possesses a spectrum of mutations including both loss and gain of function. Despite the centrality of *TP53* in cancer, its prognostic significance remains unclear and the development of targeted therapies is ongoing. Asuragen has developed sensitive assays and algorithms to characterize DNA mutations and RNA expression profiles from the most challenging poor quality clinical samples. In order to enable a more complete understanding of the functional consequences of *TP53* mutations, we present an application of these approaches to characterize *TP53* function in FFPE ovarian cancer samples through an integrated analysis of DNA and RNA sequencing. **Methods:** Archival FFPE tumor samples were collected from a cohort of 200 platinum sensitive ovarian cancer patients. DNA from tumor and matched germline (PBL) specimens was profiled by NGS using the Quantidex™ *TP53* Assay (Asuragen, Inc.), and sequencing analysis was performed using Quantidex™ Reporter. *TP53* mutation status was independently assessed by the AmpliSeq™ Cancer Hotspot Panel (Thermo Fisher Scientific). Whole transcriptome RNA-Seq was performed on isolated total RNA material. Gene and isoform expression quantitation as well as SNV and indel calling was performed on the RNA-Seq libraries using a custom bioinformatics pipeline. **Results:** The Quantidex *TP53* Assay design provided more complete coverage of COSMIC mutations, 99% compared to 68% for AmpliSeq. Although the majority of *TP53* positives detected by the Quantidex Assay were concordant with AmpliSeq, some calls were negative by AmpliSeq due to coverage differences. The remaining discordant calls in commonly covered regions were assessed with matched RNA-Seq data. This comparison illuminated specific cases of allele-specific preferential expression of *TP53* gain-of-function mutations. Analyses of RNA-Seq expression profiles also identified gene signatures that associate with different *TP53* mutation classes. **Conclusion:** *TP53* profiling through orthogonal DNA-Seq assays and matched RNA-Seq has enabled the identification of a set of high confidence associations between *TP53* mutations and RNA expression signatures in ovarian cancer. These results point to a path for clinical assays in which DNA mutations and pathway signatures are integrated to help advance cancer drug development, and diagnostic and precision medicine applications.

406 **CPRIT Grantee**

Comparisons of Three Pan-cancer Next-generation Sequencing Products Expose Differences in Specimen Compatibility and Input, Sample QC, Workflo , and Turn-around Time *G. Latham, Asuragen, Inc.; J. Houghton, Asuragen, Inc.; B. Printy, Asuragen, Inc.; J. Plyler, Asuragen, Inc.*

Introduction: Next-generation sequencing (NGS) gene panels are increasingly used to identify clinically-actionable cancer variants and individualize patient management. Two of the most commonly used panels are based on AmpliSeq® and TruSight® chemistries. In this study, we compared and contrasted the analytical performance and time-motion workflows of these two targeted NGS products using 60 FFPE tumor samples. We also compared a recently launched product, the Quantidex™ Pan Cancer Kit. **Methods:** Residual clinical FFPE biopsies (n=60) from 6 different tissues, along with defined mixtures of FFPE DNA, were analyzed. Extracted DNA was quantified using 4 different methods (spectrophotometry, fluorescent dye binding, and two distinct qPCR assays). DNA samples were qualified for enrichment based upon the suppliers' instructions, and processed using the AmpliSeq Cancer Hotspot Panel v2 (ACHP, Thermo Fisher), TruSight Tumor sequencing panel (TT, Illumina) and Quantidex Pan Cancer Kit (QPC, Asuragen). NGS data were generated on an Illumina MiSeq (TT and QPC) and Ion Torrent PGM (ACHP). Bioinformatic analyses were performed using each vendor's pipeline. Time-motion analyses were assessed for each method. **Results:** Median values of 62.8 ng/µl (spectrophotometry), 10.6 ng/µl (fluorescent assay), and 1901 amplifiable DNA copies/µl (Quantidex™ DNA Assay) were obtained across 60 FFPE tumor DNA samples. The median percent of amplifiable templates was 9.6. Using the suppliers' provided DNA QC criteria, 57/60 samples could be analyzed with ACHP and QPC, and 29/60 with TT. The median A260 DNA input for qualifie DNA into targeted enrichment was 14.3 ng for QPC, 58.5 ng for ACHP and 2000 ng for TT. Passing DNA samples were enriched for cancer-associated genes using each kit, and sequenced to a median read depth of >1000x. The sample-level agreement in variant calls across all three kits was >95% across shared gene loci. However, differences in the detection of low-level variants from low-copy DNA inputs were revealed using defined FFPE DNA mixtures. Finally, time-motion analyses demonstrated 50% or more reduction in hands-on time and time-to-result for QPC compared to ACHP and TT. **Conclusion:** This study describes a detailed accounting of sample QC measures, analytical performance, and time-motion workflows for three pan-cancer NGS enrichment kits using 60 FFPE tumor biopsies. The results support high levels of variant call accuracy for all three products using the vendors' protocols, but expose marked differences in DNA input amount, the fraction of samples passing QC, and hands-on and overall turn-around time.

407 **CPRIT Grantee Poster Session A**

Inhibitor of Cohesin-Protease, Separase, as Novel Agents for Breast Cancer Therapy *N. Zhang, Baylor College of Medicine; H. Do, University of Houston; S. Demerzhan, University of Houston; L. Dobrolecki, Baylor College of Medicine; M. Lewis, Baylor College of Medicine; S. Berg, Baylor College of Medicine; S. Gilbertson, University of Houston; D. Pati, Baylor College of Medicine*

Introduction: Separase, a chromosomal cohesion-resolving enzyme during cell division, is an oncogene. It is overexpressed in multiple human tumors, including breast, bone and brain. Separase overexpression is found in >60% of breast cancer (BC) specimens, 50% of triple-negative BC (TNBC) tumors, and >80% of luminal-B BC tumors. Separase overexpression strongly correlates with aneuploidy, a high incidence of relapse and metastasis, as well as with a lower 5-year overall survival rate. In mouse models, Separase overexpression has been shown to induce aneuploidy, genomic instability, mammary and osteogenic tumorigenesis, and intratumoral heterogeneity **Methods:** Small molecule Separase inhibitors were identified using a high throughput screen. The effect of Sepin-1 on cancer cell growth was studied using tissue culture and patient-derived orthotopic xenografts in mice. **Results:** Knockdown of Separase inhibits the growth of Separase overexpressing mammary tumor cells but has no effect on cells with a normal Separase level. Using a high throughput screen, we have identified a small molecule Separase inhibitor 1 (Sepin-1) that inhibits Separase activity in a non-competitive way. In vitro, Sepin-1 inhibits growth of neuroblastoma, leukemia, and breast tumor cells with IC50 ranging from 1-30μM, and thus represents a lead candidate to target tumors that overexpress Separase. In mice, Sepin-1 is well tolerated, with no significant toxicity or side effect up to a dose of 80mg/kg. Studies using patient-derived orthotopic xenografts of TNBC render significant survival advantages for Sepin-1 treated mice. Sepin-1 inhibits the growth of Separase-overexpressing human TNBC xenografts in mice, but had no appreciable effect on TNBC tumors with low-Separase expression, suggesting the specificity and efficacy of this compound in targeting tumors addicted to Separase overexpression. Targeting Separase by Sepin-1 results in high level of apoptosis. C**onclusion:** These results suggest that inhibition of Separase represents a new line of therapy to treat breast and other tumors addicted to Separase overexpression. Blocking overexpressed Separase activity as a strategy to eliminate tumors, or to sensitize resistant cancer cells to chemotherapy, is a new therapeutic approach, and will significantly impact cancer treatment. Furthermore, developing anti-cancer therapeutics that target chromosome instability is a new field of research. Currently, Investigational New Drug (IND)-enabling studies are in progress to bring Sepin-1 to the clinics for phase-I clinical trial.

408 **CPRIT Grantee Poster Session B**

Cystathionine–β–Synthase (CBS) Inhibition For Colon Cancer: Enhancement Of The Therapeutic Efficacy Of Aminooxyacetic Acid (AOAA) Via The Prodrug Approach *M. Hellmich, The University of Texas Medical Branch at Galveston; C. Chao, The University of Texas Medical Branch at Galveston; C. Coletta, The University of Texas Medical Branch at Galveston; J. Zatarain, The University of Texas Medical Branch at Galveston; A. Mrazek, The University of Texas Medical Branch at Galveston; N. Druzhyna, The University of Texas Medical Branch at Galveston; P. Johnson, The University of Texas Medical Branch at Galveston; Y. Ding, The University of Texas Medical Branch at Galveston; H. Chen, The University of Texas Medical Branch at Galveston; K. Yanagi, The University of Texas Medical Branch at Galveston; B. Szczesny, The University of Texas Medical Branch at Galveston; J. Zhou, The University of Texas Medical Branch at Galveston; C. Szabo, The University of Texas Medical Branch at Galveston*

Introduction: We have recently shown that colon cancer cells contain high levels of CBS and demonstrated that its product, hydrogen sulfid (H2S), promotes the growth and proliferation of colorectal tumor cells in vitro and in vivo. We also showed that the CBS inhibitor aminooxyacetic acid (AOAA) suppresses the proliferation of colon cancer cells in vitro and reduces tumor growth in vivo. The potency of AOAA in recombinant CBS (IC50 10 μM) was markedly lower than the potency of the compound as an antiproliferative agent in the colon cancer cell line HCT116 in vitro (IC50 600 μM) or as an inhibitor of cancer growth (xenotransplanted HCT116 cells in nude mice) in vivo (IC50 6 mg/kg/day). We hypothesized that the difference between enzyme potency and cell-based efficacy may be related to a limited cellular uptake of AOAA. Therefore, we designed and synthesized several prodrugs of AOAA and tested them in vitro and in vivo. **Methods:** Chemical synthesis; in vitro and cell-based CBS activity assays; HCT116 cell proliferation assays (XCelligence), mouse xenograft models (HCT116 and PDTX). **Results:** The prodrugs did not inhibit recombinant CBS, but potently inhibited CBS activity in HCT116 cells, consistent with cellular uptake and intracellular cleavage of the compound. The antiproliferative effect of several prodrugs produced exceeded the potency of AOAA (IC50 30-300 μM). One selected prodrug, YD1-71 was further studied in vitro and in vivo. YD1-71 exhibited high antiproliferative potency in vitro (IC50 100 μM); its effects were additive to the chemotherapeutic agents 5-fluorouracil and oxaliplatin. The efficacy of YD1-71 as an inhibitor of HCT116 growth was also tested in vivo, in comparison to AOAA. NOD-scid IL2Rgnull mice were subjected to subcutaneous injections of 106 HCT116 cells. On post-injection day 6, after a visible tumor was established, the mice were randomized into several groups and were treated via subcutaneous vehicle, AOAA (1, 3 or 9 mg/kg/day) or YD1-71 (0.1, 0.5 or 1 mg/kg/day) daily for 5 days a week for 3 weeks. Tumor volume was significantly inhibited by 9 m/kg/day AOAA, but not at the lower doses. YD1-71 was more efficacious; tumor volume was significantly inhibited at 0.5 and 1 mg/kg/day. The systemic toxicity of YD1-71 was lower than that of AOAA **Conclusion:** The prodrug approach, as exemplified by YD1-71, is a viable strategy to increase the potency of AOAA, most likely by increasing its uptake to the cells and tissues, followed by intracellular release of the parent compound.

409 **CPRIT Grantee Poster Session A**

ESR1 Coregulator Binding Site Inhibitors (ECBIs) as Novel Therapeutics to Target Hormone Therapy Resistant Breast Cancer *R. Vadlamudi, The University of Texas Health Science Center at San Antonio; G. Sareddy, The University of Texas Health Science Center at San Antonio; S. Viswanadhapalli, The University of Texas Health Science Center at San Antonio; T. Lee, The University of Texas at Dallas; S. Ma, The University of Texas Southwestern Medical Center at Dallas; W. Lee, The University of Texas Southwestern Medical Center at Dallas; M. Mann, The University of Texas Health Science Center at San Antonio; S. Krishnan, The University of Texas Health Science Center at San Antonio; V. Gonugunta, The University of Texas Southwestern Medical Center at Dallas; D. Strand, The University of Texas Southwestern Medical Center at Dallas; R. Tekmal, The University of Texas Health Science Center at San Antonio; J. Ahn, The University of Texas at Dallas; G. Raj, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Estrogens contribute to the progression of breast cancer via estrogen receptor 1 (ESR1) and current therapies involve either antiestrogens (AE) or aromatase inhibitors (AI). However, most patients develop resistance to these drugs. Critically, therapy-resistant tumors retain ESR1-signaling. Mechanisms of therapy resistance involve the activation of ESR1 in the absence of ligand or mutations in ESR1 that allow interaction between the ESR1 and coregulators leading to sustained ESR1 signaling and proliferation. For patients with therapy-resistant breast cancers, there is a critical unmet need for novel agents to disrupt ESR1 signaling by blocking ESR1 interactions with its coregulators. **Methods:** Using rational design, we synthesized and evaluated a small organic molecule (ESR1 coregulator binding inhibitor, ECBI) that mimics the ESR1 coregulator nuclear receptor box motif. Using in vitro cell proliferation and apoptosis assays, we tested the effect of ECBI on several breast cancer cells and therapy-resistant model cells. Mechanistic studies were conducted using established biochemical assays, reporter gene assays, RTqPCR and RNASeq analysis. ESR1+ve (MCF7 and ZR75) xenografts were used for preclinical evaluation and toxicity. The efficacy of ECBI was tested using an ex vivo cultures of freshly extirpated primary human breast tissues. **Results:** In estrogen induced proliferation assays using several ESR1+ve model cells, ECBI inhibit growth at 300-500 nM. Importantly, ECBI showed little or no activity on ESR1 negative cells. Further, ECBI also reduced the proliferation of several ESR1 positive hormonal therapy resistant cells, directly interacted with MT-ESR1 with high affinity and significantly inhibited MT-ESR1 driven oncogenic activity. Mechanistic studies showed that ECBI interacts with ESR1, efficientl blocks ESR1 interactions with coregulators and reduces the ESR1 reporter gene activity. RNA sequencing analysis revealed that ECBI blocks multiple ESR1 driven pathways, likely representing the ability of a single ECBI compound to block multiple ESR1-coregulator interactions. Treatment of ESR1-positive xenograft tumors with ECBI (10 mg/Kg/ oral) significantly reduced tumor volume. Further, ECBI also significantl reduced the proliferation of coregulator-overexpressed breast cancer cells in xenograft model. Using human primary breast tissue ex vivo cultures, we have provided evidence that ECBI has potential to dramatically reduce proliferation of human breast tumor cells. C**onclusion:** The ECBI is a novel agent that targets ESR1 with a unique mechanism of action. Remarkably, ECBIs block both native and mutant forms of ESR1 and have activity against therapy resistant breast cancer cell proliferation both in vitro and in vivo and against primary human tissues ex vivo.

410 **CPRIT Grantee Poster Session B**

Development of AEB1102, an Engineered Form of Human Arginase 1 for Patients with Solid and Hematologic Malignancies *D. Lowe, Aeglea Biotherapeutics; S. Rowlinson, Aeglea Biotherapeutics; J. Tyler, Aeglea Biotherapeutics; S. Alters, Aeglea Biotherapeutics; A. Lowe, Aeglea Biotherapeutics; C. York, Aeglea Biotherapeutics; M. Okamoto-Kearney, Aeglea Biotherapeutics; D. Johnson, Aeglea Biotherapeutics; M. Newman, Aeglea Biotherapeutics*

Introduction: Normal cells make their own supply of arginine using the enzymes ornithine transcarbamylase, argininosuccinate synthase, and argininosuccinate lyase. In many tumor cells, silencing one or more of these enzymes disables arginine synthesis, making tumor cells dependent on extracellular arginine uptake for survival. This makes tumors potentially vulnerable to arginine depletion by AEB1102, an engineered form of human arginase 1. The goal of AERase, Inc. is to leverage funding from CPRIT (DP140031) to perform all non-clinical and chemistry, manufacturing & controls (CMC) activities and initiate clinical development in solid tumors and hematologic malignancies. **Methods:** A previous CPRIT Grant (RP100890) provided insight into non-clinical animal pharmacology and manufacturing for AEB1102. Using AEB1102 from this prior grant, IND-enabling in vitro and in vivo non-clinical oncology studies were performed at South Texas Accelerated Research Therapeutics (START) and pilot dosing in mice and monkeys was carried out at MPI Research. Bioanalytical assays to determine PK and PD were developed and validated at Intertek. Drug product CMC activities optimized at KBI included producing material to support GLP toxicology studies which were subsequently performed at MPI Research. A Phase 1 clinical trial is anticipated to start enrolling solid tumor patients at START in San Antonio and the University of Colorado in the fourth quarter of 2015. **Results:** A panel of 10 xenograft cell lines were tested with AEB1102 and all were shown to be sensitive to arginine depletion. The A375 melanoma cell line was the most sensitive to arginine depletion. AEB1102 administered once weekly significantly delayed A375 tumor growth in mice and yielded a survival benefit. Bioanalytical assays were used to measure arginine and AEB1102 levels in mouse and monkey plasma. These data was used to establish doses used in the GLP toxicology studies that identified an NOAEL in both species. Completed CMC development activities include: a) optimized expression in bacteria to 8 gm/liter; b) PEGylation procedures that ensure reproducible PEGylation of AEB1102, and c) buffer formulation conditions that maintain product integrity and stability. **Conclusion:** AERase, Inc. has successfully executed on all non-clinical and CMC activities necessary to support the clinical development of AEB1102. The Phase 1 clinical trial in solid tumors will start enrolling patients this quarter with a second Phase 1 study in hematologic malignancies planned for 2016.

411 **CPRIT Grantee Poster Session A**

Identification Of Drug Leads As Inhibitors Of The OLIG2 Pathway: New Opportunities For The Treatment Of Glioblastoma *G. Alton, Curtana Pharmaceuticals, Inc.; G. Beaton, Curtana Pharmaceuticals, Inc.; G. Stein, Curtana Pharmaceuticals, Inc.; N. Nomura, University of California at San Diego; S. Pingle, University of California at San Diego; R. Mukthavaram, University of California at San Diego; S. Kesari, University of California at San Diego*

Introduction: Glioblastoma (GBM) is the most common and deadliest of the malignant primary brain tumors in adults. In the U.S., there are approximately 10,000 new cases per year; world-wide there are approximately 50,000 cases. There remains significant unmet clinical need as the median survival is less than 15 months, 2-year survival is only 30%, and 5-year survival is less than 10%. OLIG2 is a transcription factor that is almost exclusively expressed in the CNS and has been linked to the tumorigenesis of GBM cancer cells and their resistance to radiotherapy. Inhibitors of the OLIG2 pathway are therefore of interest as potential treatments for GBM and other OLIG2-expressing gliomas. **Methods:** Curtana Pharmaceuticals licensed a series of compounds identified from a computational strategy that demonstrated moderate cytotoxicity toward several GBM cancer cell lines and functional effects on OLIG2 pathways. Follow-on medicinal chemistry was conducted with the goal to improve both the potency of cytotoxicity and the ability of the compounds to penetrate the CNS. Leads from this effort were profiled in animal models for both their ability to penetrate the CNS and for their efficacy against GBM tumor models. **Results:** Lead optimization identified several compounds that were cytotoxic at sub-micromolar concentrations to patient-derived GBM cells. Two such compounds, CT-178 and CT-179, were highly cytotoxic to OLIG2-expressing GBM cells with a high therapeutic index for non-OLIG2 expressing cell types. CT-179 demonstrated functional inhibition of the OLIG2 pathway as demonstrated by effects on protein activity downstream of OLIG2. A cell pathway analysis indicated that CT-179 impacts GBM proliferation and may have immunomodulatory features. CT-179 was shown to penetrate the CNS to attain brain concentrations at levels far in excess of its cytotoxicity to GBM cells *in vitro*. Efficacy studies reveal dose dependent reductions of tumor growth which is enhanced with radiotherapy. CT-179 demonstrated efficacy in an intracranial model of GBM. **Conclusion:** Available data suggests that inhibitors of the OLIG2 pathway show promise for the treatment of GBM, for which Curtana Pharmaceuticals has identifie several promising inhibitors of this pathway. Pre-clinical studies indicate that these inhibitors show promise as both cytotoxic agents and as potential radiosensitizers for use in the treatment of GBM.

412 **CPRIT Grantee Poster Session B**

Increasing The Purity, Potency And Specificity Of EBV-Specific T-Cells To Improve The Treatment Of EBV-Positive Lymphoma *S. Sharma, Baylor College of Medicine; S. Perna, Baylor College of Medicine; B. Mehta, Baylor College of Medicine; N. Lapteva, Baylor College of Medicine; R. Rouce, Baylor College of Medicine; M. Ngo, Baylor College of Medicine; C. Ramos, Baylor College of Medicine; V. Torrano, Baylor College of Medicine; A. Gee, Baylor College of Medicine; A. Leen, Baylor College of Medicine; H. Heslop, Baylor College of Medicine; C. Rooney, Baylor College of Medicine*

Introduction: Up to ~40 % of Hodgkin and non-Hodgkin lymphomas carry the Epstein-Barr virus (EBV) genome and express EBV type 2 viral latency proteins (T2LPs) EBNA-1, LMP-1, LMP-2 and BARF-1. EBV specific T-cells (EBVSTs) can be generated from the blood of EBV positive individuals, but are usually dominated by T-cells specific for viral proteins not expressed in type 2 latency lymphomas. M**ethods:** To overcome this problem, we used dendritic cells pulsed with overlapping peptide libraries (pepmixes) spanning T2LPs in the presence of IL-4 and IL-7 to stimulate patient peripheral blood mononuclear cells. Responder T-cells were then expanded using pepmix-pulsed, autologous activated T-cells and HLA-negative K562 cells expressing costimulatory transgenes. **Results:** Although T2LP-specific EBVSTs could be generated from healthy-donors, we were unable to consistently generate EBVSTs with significant T2LP specificity from patients. We hypothesized that patient T-cells were anergic to antigen expressed within tumors, and since IL-15 has been shown to rescue tolerant or anergic T-cells, we replaced IL-4 with IL-15 (in combination with IL-7). This improved antigen specificity up to 10-fold. Further dose optimization showed significant advantages in cytolytic activity, proliferation and antigen specificity with higher dose of IL-15. We achieved higher fold expansion (3 fold mean increase in absolute EBVST numbers) and enhanced specificity for T2LPs (high vs. low IL-15 concentration: EBNA1: 156±218 vs. 13±33, LMP-1: 130±223 vs. 33±68, LMP-2: 518±466 vs. 88±122 and BARF-1: 109±147 vs. 24±40; SFC/105 cells; n=11). High dose IL-15 also increased the frequency of central memory T cells in the final T-cell product (36.81±17 vs. 13.81±22 % (IL-15 Hi vs. IL-4)). EBVSTs manufactured using all three conditions were used to treat 20 patients with multiply relapsed, EBV-positive lymphoma; as adjuvant therapy after stem cell transplantation or chemotherapy in 9 patients and as treatment for disease in 11 patients. All patients in remission at the time of infusion, remain in remission. Of patients with active disease at the time of infusion, there was one stable disease among 4 patients who received IL-4/7-expanded T-cells, one PR and one CR in 3 patients who received low does IL-15/7-expanded T-cells and two CRs and one PR among 4 patients whose T-cells were expanded in high dose IL-15 (one is too early to assess). C**onclusion:** Our results suggest that a high frequency of antigen-specific T-cells in the infused product is critical for clinical activity and strategies to further improve specificity will enhance overall tumor responses.

413 **CPRIT Grantee**
Poster Session A

Combination of texaphyrin and platinum (IV) prodrugs as a potential new anticancer therapy *G. Thiabaud, The University of Texas at Austin; J. Sessler, The University of Texas at Austin*

Introduction: Platinum-based chemotherapy has been used a lot these last 4 decades or so, more precisely the platinum (II) complexes cisplatin, oxaliplatin and carboplatin. These have showed very potent antiproliferative activities but patients receiving these treatments suffer from many side effects due to a very poor therapeutic ratio (toxic dose / effective dose) and also a lack of selectivity for cancer tissues. Indeed it's very difficult to contain the reactivity of these drugs to only their target: the DNA in cancer cells. **Methods:** A very elegant way of harvesting their reactivity is to use their oxidized analogues platinum (IV), which are kinetically less reactive than platinum (II). These are usually called prodrug because they need to be reduced to Pt(II) to be active. This activation by reduction occurs intracellularly where the concentration in reducing agents is higher. It would be interesting to take advantage of the relative inertness of some Pt(IV) complexes and activate them by reduction preferentially in the cancer cells. Ideally, this requires Pt(IV) complexes that are very hardly reduced in physiological conditions and a redox catalyst which accumulates preferentially in cancer cells. The toxicity would be high in cancer tissues and much lower in healthy tissues. **Results:** In one of our previous works we showed that motexafin gadolinium (MGd), a expended porphyrin that belongs to a class of molecules named texaphyrin developed by Prof. Sessler and co-workers, not only accumulates preferentially in cancer tissues but also acts as a redox mediator between biological reducing agents (sodium ascorbate) and oxygen. This leads to the formation of Reactive Oxygen Species (ROS) that are toxic for cells. In this case, the final electron acceptor is oxygen. We recently discovered that in presence of a platinum (IV) complex, MGd catalyzes the reduction of the metal ion, leading to the formation of the active form Pt(II). Since MGd tends to accumulate more in cancer tissue, this activation is selective. **Conclusion:** The reduction of some Pt(IV) anticancer prodrugs can be drastically accelerated by combining these metal complexes with texaphyrins, molecules that accumulate preferentially in cancer tissues. We believe that this general strategy has a real potential since it could be applied to other metal ion-based prodrugs. The synthesis of platinum(IV) complexes, the experiments in vials (monitored by RP-HPLC) and in cellular culture will be presented.

414 **CPRIT Grantee**
Poster Session B

CD62L+ NKT cells have superior in vivo persistence and anti-tumor activity *G. Tian, Baylor College of Medicine; A. Courtney, Baylor College of Medicine; B. Jena, The University of Texas Health Science Center at Houston; G. Dotti, Baylor College of Medicine; L. Cooper, The University of Texas Medical School at Houston; L. Metelitsa, Baylor College of Medicine*

Introduction: Va24-invariant Natural Killer T cells (NKTs) have potent antitumor properties and are being developed for cellular immunotherapy of cancer. Such therapy requires extensive ex vivo expansion of primary NKTs while preserving their longevity and function. However, the mechanism responsible for NKT-cell maintenance remains poorly understood and has been the subject of this investigation. **Methods:** We induced proliferation of primary human NKTs using in vitro culture with autologous PBMC pulsed with a synthetic ligand αGalactosylceramide (αGalCer). The phenotype of the numerically expanded NKTs was compared with that of the freshly isolated cells by multi-color FACS. The functional significanc of the enriched markers was examined after magnetic cell sorting into positive and negative subsets followed by comparative analysis of their in vitro proliferation and in vivo persistence in NSG mice. Finally, we used a xenogenic lymphoma model in NSG mice to compare the therapeutic activity of the adoptively transferred NKT-cell subsets. **Results:** First, we found that antigen-induced in vitro expansion of primary NKTs is associated with the accumulation of a CD62L-positive subset. Following magnetic sorting, only CD62L-positive cells survived and proliferated in response to TCR-stimulation in vitro. After transfer to NSG mice, CD62L-positive NKTs persisted 5 times longer and had higher therapeutic efficacy in a lymphoma model compared with CD62L-negative NKTs. Proliferating CD62L-positive cells downregulated or maintained CD62L expression when they were activated via TCR alone or in combinations with co-stimulatory receptors, respectively. After testing 161 clones of K562 cells, genetically modified to express CD1d and various combinations of co-stimulatory molecules, we selected the B-8-2 clone (HLAnullCD1dmedCD86high4-1BBmedOX40Lmed) which induced the highest rate of NKT-cell expansion with the preservation of CD62L-positive cells. Compared with NKTs expanded with autologous PBMC, those expanded with B-8-2 exhibited a prolonged in vivo persistence and superior antitumor activity. **Conclusion:** Our results reveal a previously unanticipated functional hierarchy in human NKTs that can be exploited for cancer immunotherapy.

415 **CPRIT Grantee**
Poster Session A

Advances in the Clinical Development of DNX-2401 for Recurrent Glioblastoma *F. Tufaro, DNAtrix; I. Alibhai, DNAtrix*

Introduction: Glioblastoma is a devastating, incurable primary brain tumor that is resistant to conventional therapies. Following recurrence, available treatment options are limited and ineffective, with median survival of only 4 to 6 months. DNX-2401 is a highly potent oncolytic adenovirus that has demonstrated the ability to trigger a durable antitumor response in several ongoing clinical trials in the US and Europe. DNX-2401 has been granted Fast Track and Orphan Drug designations by the FDA and its development is supported by a 3-year product development grant from the Cancer Prevention and Research Institute of Texas (CPRIT). **Methods:** DNX-2401 was manufactured at Lonza (Houston) using conventional cell factories followed by chromatography to purify the virus for clinical use. Two phase Ib, open-label studies of DNX-2401 are being conducted in recurrent glioblastoma with temozolomide or interferon gamma, which provide further evaluation of the recommended Phase II dose and expansion of the safety database prior to initiating Phase II clinical studies. **Results:** DNX-2401 has been administered to more than 100 subjects with recurrent glioblastoma. Intratumoral administration of DNX-2401 has been well tolerated with no unexpected virus-related toxicities observed to date. Remarkably, in a Phase I dose escalation study, a single intratumoral administration of DNX-2401 induced durable responses and provided survival benefit of 1 to 4 years in responding patients. Histological analysis of resected tumors demonstrated that DNX-2401 triggered an immune response as indicated by macrophage and CD8 T-cell infiltration of lesions. This viral-induced anti-tumor immunity likely plays a role in the durability of clinical responses. Additional data is being accumulated from ongoing studies. **Conclusion:** DNX-2401 has demonstrated promising clinical activity in several Phase I and Ib studies for recurrent glioblastoma, a devastating disease with a critical unmet medical need. These early results provide the groundwork for the upcoming Phase II, single-arm, open-label study supported in part by CPRIT funding.

417 **CPRIT Grantee**
Poster Session A

Novel Cancer Targets for Adoptive Cell Therapies *S. Walter, Immatics US Inc.; M. Bharathan, Immatics US, Inc.; C. Yee, The University of Texas M.D. Anderson Cancer Center; S. Kutscher, Immatics Biotechnologies GmbH; O. Schoor, Immatics Biotechnologies GmbH; S. Bunk, Immatics Biotechnologies GmbH; A. Alpert, Immatics US Inc.; L. Alten, Immatics Biotechnologies GmbH; L. Stevermann, Immatics Biotechnologies GmbH; J. Fritsche, Immatics Biotechnologies GmbH; D. Maurer, Immatics Biotechnologies GmbH; S. Kuttruff, Immatics Biotechnologies GmbH; C. Reinhardt, Immatics US Inc.; T. Weinschenk, Immatics US Inc.; P. Hwu, The University of Texas M.D. Anderson Cancer Center; H. Singh, Immatics US Inc.*

Introduction: Adoptive Cellular Therapy (ACT) bears the promise of eradicating established tumors with highly specific T cells. However, most current ACT approaches bear several restrictions: first, targets are limited to few validated antigens, especially for solid tumors; second, targeting malignancies using chimeric antigen receptors may only address the ~25% of the human proteome expressed on the cell surface. **Immatics Biotechnologies** is a globally leading clinical-stage company. Since more than 10 years, Immatics is developing the **XPRESIDENT®** platform which enables the discovery of novel, relevant, and highly cancer specifi antigens derived from both intra-cellular and surface proteins, together with specific T-cell receptors (TCRs). **Immatics US Inc.**, based in Houston, TX, was recently launched by Immatics and the MD Anderson Cancer Center to develop next-generation ACTs for the treatment of cancer based on novel targets and TCRs. Immatics US Inc. has been recently granted a CPRIT New Product Development Award totaling up to $19.7m. **Methods:** XPRESIDENT® uses quantitative mass spectrometry, differential transcriptomics, immunology and bioinformatics for the high-throughput identifcation of novel antigens. Tumor-associated peptides (TUMAPs) are eluted and sequenced from human tissues. TCRs are identified by single-cell RACE from HLA-matched or mismatched donor T cells that were primed by precisely controlled artificial antigen presenting cells. Novel targets will be validated in three development tracks: ACT with autologous endogenous T cells (**ACTolog**), ACT with autologous TCR-engineered T cells (**ACTengine**) and ACT with allogeneic TCR-engineered T cells (**ACTallo**). **Results:** According to our knowledge, Immatics' Immunopeptidome database is the largest of its kind, containing hundred thousands of peptide sequences, and the only database containing both

qualitative and quantitative data of cancer and normal samples. A recent in-depth search for novel ACT targets identified a pipeline of more than 20 HLA-A*02 restricted TUMAPs, the majority of them being novel, showing highly specific, cancer-germline-like expression and being applicable to multiple indications, including glioblastoma and pancreatic cancer. Using the first TUMAP COL6A3-002, we have already identified 91 candidate TCRs. In-depth characterization of these TCRs is ongoing and showing several highly specific TCRs. **Conclusion:** To translate the success of ACT into solid cancers, novel relevant and safe targets are urgently needed. We have shown that using XPRESIDENT®, such targets can be readily identified. Clinical trials to demonstrate safety and initial signs of efficacy of these targets using endogenous T cells (ACTolog) or TCR-engineered T cells (ACTengine, ACTallo) are currently being prepared with the aim to begin treating patients in 2016.

418 **CPRIT Grantee**
Poster Session B

Tumor Membrane-based Immunotherapy in Combination With Anti-CTLA-4 Antibody, But Not Anti-PD-L1 Antibody, Confers Protection Against Metastatic Triple Negative Breast Cancer *C. Pack, Metaclipse Therapeutics Corporation; J. Patel, Dept of Pathology, Emory University School of Medicine; E. Bozeman, Emory University School of Medicine; R. Bommireddy, Metaclipse Therapeutics Corporation; S. Reddy, Metaclipse Therapeutics Corporation; P. Selvaraj, Dept of Pathology, Emory University School of Medicine*

Introduction: The 4T1 triple negative breast cancer model resembles many of the hallmarks of advanced triple negative breast cancer in humans, such as high metastatic potential, poor immunogenicity, and resistance to immunotherapy. However, immunotherapies combining immune stimulating approaches along with immune checkpoint inhibitors have generated encouraging results for some indications. In this study we investigate if combining a membrane-based immunotherapy with immune checkpoint blockade in an established orthotopic tumor model could effectively generate protective immunity, reduce metastasis, and prolong survival in the aggressive 4T1 model. Our membrane-based immunotherapy uses a tumor-specific approach in conjunction with the glycosyl phosphatidylinositol (GPI)-anchored immunostimulatory molecules B7-1 and IL-12 to generate effective cellular and humoral immune responses to specific tumors. This personalized, membrane-based strategy is particularly well-suited to indications such as triple negative breast cancer that exhibit a high degree of heterogeneity and lack well-defined tumor antigens. **Methods:** 4T1 tumor tissue was harvested from BALB/c mice and processed to generate tumor membrane vesicles (TMVs). TMVs were then incorporated with GPI-B7-1 and GPI-IL-12 by protein transfer and used for immunization in conjunction with immune checkpoint inhibitors. Survival was assesed using a Kaplan-Meier survival curve and significance determined using a Log-rank test for comparison analysis. Metastasis was assessed by clonogenic assay. Immune response was assessed by IFN-γ ELISPOT assay in the spleen. Antibody response against 4T1 cells was assessed in the serum by flow cytometry. Cell depletion studies were performed with anti-CD4 and anti-CD8 antibodies. Significance for clonogenic and immune response assays was determined using a student's t test. **Results:** TMV-based immunotherapy in combination with anti-CTLA-4 antibody significantly improved survival, reduced pulmonary metastasis, and increased tumor-specific CD8 T cell response as measured by IFN-γ ELISPOT. Interestingly, either treatment alone failed to have a significant impact on survival, metastasis, or immune response. Further, the reduction observed in metastasis appears to be due to anti-tumor CD8 T cell activity. Interestingly, anti-PD-L1 antibody combination with TMV did not confer significant protection. No significan antibody response was observed against 4T1 cells either with or without anti-CTLA-4 antibody. **Conclusion:** These results strongly suggest that a tumor membrane-based immunotherapy in combination with anti-CTLA-4 antibody can effectively generate tumor-specific immunity capable of reducing metastasis, prolonging survival, and enhancing anti-tumor immunity in the highly malignant 4T1 triple negative breast cancer model. Such a combinatorial approach could potentially translate into an effective clinical treatment for metastatic triple negative breast cancer, a significan area of unmet medical need.

419 **CPRIT Grantee**
Poster Session A

Biocompatible, Targeted Drug Delivery System for Ovarian Cancer Therapeutics *A. Lacko, The University of North Texas Health Science Center at Fort Worth; A. Sood, The University of Texas M.D. Anderson Cancer Center*

Introduction: A robust drug delivery vehicle has been developed with substantial advantages over current technologies. Our technology is based on nanoparticles that are assembled from the ingredients of a natural blood component (high density lipoproteins /good cholesterol carrier) and a drug that constitutes their payload. The drug carrying nanoparticles have been found to be stable (non-leaking) for several months and have been especially effective for systemic delivery of chemotherapeutic agents to tumors. The rHDL nanoparticles deliver anti-cancer agents to their destinations via a receptor-mediated mechanism that provides a robust selective targeting vehicle for chemotherapy. The receptor mediated uptake of anti-cancer agents is an important part of the concept for efficient drug delivery because cancer cells have a high expression of the HDL (CLA-1/SR-B1) receptor compared to normal cells, due to the excessive need for cholesterol to facilitate membrane biogenesis for their high rate of proliferation. **Methods:** Studies have been conducted to compare the therapeutic efficacy of the rHDL formulations vs. unincorporated anti-cancer agents. The experimental models included cultured cancer cells and xenografts of human tumors carried in mice. The expression of the SR-B1 receptor was also monitored in cancer cells and tumors. **Results:** Proof of concept studies have so far provided ample support for the model described above. The impact of the delivery of small interfering RNA (siRNA) on tumors has been particularly impressive. These studies resulted in a 90% human ovarian tumor ablation (carried in mice) and marked reduction of angiogenesis and upregulation of apoptosis. The substantially higher accumulation of siRNA in tumor tissue vs. normal tissue suggests that this technology, when applied in a clinical setting, is likely to reduce or eliminate toxic side effects of cancer chemotherapy. **Conclusion:** Drug delivery via the rHDL nanoparticles represents a platform technology that has nearly unlimited upsides. Numerous drugs, including anti-cancer agents, are currently less than fully effective because of their poor water solubility or the induction of drug resistance during therapy. The rHDL nanoparticles can be applied to solve these problems and thus renew or "reposition" the application of a host of therapeutic agents with essentially no foreseeable technological

barrier to product development. The major goal of this project is to promote the rHDL drug delivery technology to application at the bedside, at the earliest opportunity. Specificall , the initiation of Phase I clinical trials is projected as the next phase of development for this technology.

420 **CPRIT Grantee**

Inhibition Of Pancreatic And Colon Cancer Stem Cells By Targeting IL4-Rα With MDNA55 *F. Merchant, Medicenna Therapeutics Inc.; S. Fidai, Medicenna Therapeutics Inc.; B. Patel, Virginia Commonwealth University School of Medicine*

Introduction: Despite recent therapeutic advances, the outcome in patients with metastatic gastrointestinal (GI) cancers remains poor mainly due to treatment failure or recurrence. Recently, cancer stem cells (CSCs) have been implicated in resistance to conventional chemotherapy and subsequent relapse. Hence targeting CSCs is an attractive therapeutic approach for deadly cancers including GI cancers. Recent reports indicate that IL-4 signaling plays an important role in promoting growth of CSCs. Here, we evaluated MDNA55 [IL-4(38-37)-PE38KDEL], a targeted toxin composed of a circular permuted IL-4 fused to a *Pseudomonas aeruginosa* exotoxin A, which has been shown to be highly cytotoxic to IL-4R-positive tumors in preclinical and clinical studies, for its ability to inhibit GI CSC growth. M**ethods:** FOLFOX (combination of oxaliplatin and 5-FU) surviving colon cancer HCT-116 cells and pancreatic cancer ASPC cells were analyzed for cancer stem-like cells by tagging them with CD44 antibodies and subsequent sorting by flow cytometry. Sphere forming ability (a CSC property) of chemo-surviving cells was examined in serum deprived, growth factor enriched media. HCT-116 and ASPC chemo-surviving cells were incubated in stem cell media and treated with vehicle, anti-IL4 neutralizing antibody (anti-IL4 NA) or MDNA55 and number of colonospheres/10,000 cells was analyzed after 5 days. **Results:** Most pancreatic cancer cell lines examined using immunocytochemistry expressed IL-4R, with ASPC cells showing the highest level of expression. Treatment of ASPC and colon CSCs with anti-IL4 NA and MDNA55 resulted in inhibition of sphere formation as compared with vector treated controls. However, MDNA55 showed greater inhibition of sphere formation compared with anti IL4-NA at an equimolar concentration. Moreover, combination of MDNA55 with FOLFOX resulted in a greater inhibition of colonosphere formation than either modality alone. More importantly, MDNA55 inhibited secondary spheroid formation, a measure of CSC self renewal property, especially in combination with FOLFOX. Treatment with MDNA55 and FOLFOX resulted in inhibition of CD24, CXCR4 and ABCG2 which are all putative CSC markers. This effect was greater in cells treated with MDNA55 and FOLFOX than either modality alone. We also observed a modest induction in caspase-3 activity following MDNA55 or FOLFOX treatment. However combination of MDNA55 and FOLFOX resulted in a robust 3-fold induction of caspase-3 activity suggesting that MDNA55 and FOLFOX might inhibit CSCs growth at least in part by induction of apoptosis. C**onclusion:** These data demonstrate that MDNA55 potently inhibits growth of GI CSCs, and could have an important role as an agent for targeting chemotherapy resistant tumors.

421 **CPRIT Grantee**
Poster Session A

Synergy Between miR-34 Mimics and Epithermal Growth Factor Receptor Tyrosine Kinase Inhibitors in EGFR Mutant NSCLC *J. Zhao, Mirna Therapeutics, Inc.; A. Guerrero, Mirna Therapeutics, Inc.; K. Kelnar, Mirna Therapeutics, Inc.; H. Peltier, Mirna Therapeutics, Inc.; A. Bader, Mirna Therapeutics, Inc.*

Introduction: Multiple generations of Epithermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib afatinib and rociletinib etc, were developed for the treatment of patients with Non-small cell lung carcinoma (NSCLC), predominantly those that harbor activating EFGR mutations. Unfortunately, a fraction of patients still do not experience disease response despite harboring EGFR-mutant disease. In addition, dose-limiting toxicity remains challenge for target therapy. Mirna Therapeutics develops mimics of naturally occurring microRNAs that are designed to restore tumor suppressor activity and aid appropriate tumor immune response. The company's lead product candidate, MRX34, a naturally occurring microRNA-34 (miR-34) encapsulated in a liposomal nanoparticle formulationis, is currently the subject of a Phase 1 clinical trial in patients with primary hepatocellular carcinoma, other solid tumors and hematological malignancies. Our previous studies indicated that miR-34 can sensitize lung cancer cells with primary resistance and acquired resistance. Here, we investigated the effects of miR-34 mimics in combination with afatinib and rociletinib in NSCLC cells with primary and acquired erlotinib resistance. M**ethods:** A panel of NSCLC cell lines was used to assess the combinatorial effects of miR-34 and TKIs. These include EGFR-wild type (A549, H460, H1299, H226) and EGFR-mutant (H1975, HCC827 parental and HCC827 erlo-res) cell lines. The combination studies were carried out using single drug ratios (~IC50 ratio of TKI and miR-34) and as well as at multiple ratios above and below. Cells were reverse-transfected with miR-34 and incubated with rociletinib or afatinib one day thereafter for 3 days. Cellular proliferation was determined by AlamarBlue. Synergistic, additive, or antagonistic effects were determined according to Chou-Talalay's algorithm and analyzed by combination index (CI) plots, isobolograms and curve shift analyses. **Results:** The data indicate strong synergistic interaction between rociletinib and miR-34 mimics, as well as afatinib and miR-34 mimics in all EFGR-mutant cells tested. Unlike afatinib, synergy of the miR-34+rociletinib combination was also observed in most EGFR-wild type cells. The synergistic effects were observed across a range

of different dose levels and drug ratios. Maximal synergy was detected at dosages that provide a high level of cancer cell inhibition beyond the one that is induced by the single agents alone and, thus, is of clinical relevance. **Conclusion:** The data complement our previous results with erlotinib and suggest that miR-34 synergizes with multiple EGFR-TKIs including 2nd- (afatinib) and 3rd-generation (rociletinib) inhibitors. The miR-34 combination therapy may prove particularly useful in EGFR-mutated lung cancer patients that progressed on prior EGFR-TKI therapy.

422 **CPRIT Grantee**
Poster Session B

Efficac , specificity and safety of systemic delivery of VISA-Claudin4-BikDD-liposome, a proapoptotic protein Bik-based gene therapy for breast cancers *L. Nie, The University of Texas M.D. Anderson Cancer Center; M. Hung, The University of Texas M.D. Anderson Cancer Center; O. Rahal, The University of Texas M.D. Anderson Cancer Center; Y. Sun, The University of Texas M.D. Anderson Cancer Center*

Introduction: BikDD is a phosphorylation-mimic constitutive mutant of Bik, which induces apoptosis by binding to multiple anti-apoptotic molecules such as Bcl2. We previously demonstrated BikDD exhibits greater binding affinity to anti-apoptotic proteins and induces stronger apoptosis than wild-type Bik. We developed a BikDD expression platform under control of breast cancer cell-specific promoter Claudin4 termed VISA-Claudin4-BikDD, and showed systemic delivering BikDD/liposome complexes to xenograft nude mice has a potent activity against tumor initiating cells (TICs), and combination between tyrosine kinase inhibitors and BikDD therapy yielded synergistic effect on breast tumors. A clinical trial of VISA-Claudin4-BikDD gene therapy has been approved by the NIH RAC Advisory Committee. Prior to moving to clinical trial, however, it is imperative to test whether BikDD-based gene therapy in immunocompetent mice has therapeutic efficacy and tolerable side effects. Additionally, we also explored therapy-resistant mechanism to VISA-Claudin4-BikDD. M**ethods:** Immunocompetent MMTV-HER2/Neu transgenic mice were used for examining BikDD–induced inhibitory effects on tumors, especially on TICs and cytotoxic responses. Tail vein injection was used for systemic delivery of VISA-Clauding4-BikDD/liposomes into the mice. Additionally, the breast cancer cell lines expressing BikDD and additional mutants were used in vitro assays to explore combination therapy. **Results:** Systemic delivery of VISA-Clauding4-BikDD/liposomes into the tumor-bearing MMTV-HER2/Neu transgenic mice significantl reduced mammary primary tumor burdens and slowed down residual tumor growth post cessation of therapy as compared to vector control cohorts. The anti-tumor effects of BikDD in immunocompetent hosts were consistent with decreased TIC population determined by specific TIC markers (CD49fhighCD61high or CD24+Jagged1-) and by tumorsphere formation assay of freshly isolated tumor cells. Importantly, systemic administration of BikDD did not result in significant cytotoxic responses in standard toxicity assays and body weight changes. Taken together, our studies validates that selectively expressing BikDD gene in the primary mammary tumors significantly inhibits tumor growth and decelerates progression at off-therapy stage by eliminating TICs. Moreover, we found that exogenously expressed BikDD protein undergoes proteasome-mediated degradation via both ubiquitin-dependent and -independent pathways. Inhibition of proteasome activity significantly increases the protein stability of BikDD, enhancing the apoptotic effect of BikDD on the breast cancer cells. Hence, high proteasome activity may be a mechanism by which intrinsic and acquired resistance occurs in BikDD gene therapy. C**onclusion:** VISA-Claudin4-BikDD-based gene therapy is an effective therapy with tolerable side effect and can safely move to clinical trial of breast cancer patients. Combining BikDD with proteasome inhibitors may enhance therapeutic efficacy and overcome breast cancer resistance to VISA-Claudin4-BikDD.

423 **CPRIT Grantee**
Poster Session A

TACCT: Advancing Cellular Therapies for Cancer in Texas *A. Gee, Baylor College of Medicine; C. Rooney, Baylor College of Medicine*

Introduction: Texas Assistance for Cancer Cell Therapy (TACCT) is a program established under funding from CPRIT. Its aim is to facilitate transition of promising new cellular therapies for cancer, developed by Texas researchers, into early phase clinical trials. It does so by (1) moving production of the cell product from the research laboratory into clinical scale manufacturing in a clean room environment under current Good Manufacturing Practices; (2) developing release criteria testing to permit distribution of the product for clinical administration; (3) providing regulatory assistance for preparation of the Investigational New Drug (IND) application for submission to the Food and Drug Administration. **Methods:** TACCT has established a website providing information on the program and on-line applications at http://www.texascelltherapy.org. With support from CPRIT TACCT has expanded its capabilities with new equipment and software for project management and quality assurance. Incoming applications are reviewed by an internal review panel followed by a distinguished external advisory board of scientists from cell therapy programs nationwide. The TACCT team then works with successful applicants to provide the requested assistance at a reduced cost. **Results:** The TACCT program is currently manufacturing T cell therapy products with specificity to tumor-associated antigens (PRAME/SSX2/MageA4/NYESO1/Survivin peptides), following development of standard operating procedures for manufacturing and testing and validation of the production methods. The IND was approved and two patients have received autologous primed T cells. The second with metastatic squamous cell carcinoma has shown an excellent response. Patient accrual continues. **Conclusion:** TACCT provides an excellent opportunity for Texas cancer researchers to overcome the common hurdle of getting new therapies from the research laboratory into early phase clinical trials. We have established a service that provides product manufacturing, release testing and regulatory advice and assistance at a partially subsidized cost that is considerably lower than that offered by commercial companies. Within the first year we have been able to help an applicant treat patients on a new T cell trial. A second trial has been submitted for FDA approval. We encourage all investigators working on cellular therapies within the State to make use of this valuable resource.

424 **CPRIT Grantee Poster Session B**

Expanding the Market and Success Rates for Myeloablative Cancer Treatments Using PUL-042, an Innate Immune Stimulant

B. Scott, Pulmotect, Inc.; D. Markesich, Pulmotect, Inc.; J. Schaumberg, Pulmotect, Inc.

Introduction: Pulmotect, Inc. was founded to translate inventions for inducing innate immune resistance in the lung into therapeutics that will provide protection from inhaled pathogens for cancer patients. Our inhaled therapeutic, PUL-042, provides immediate protection against a broad spectrum of respiratory pathogens by activating the innate immune defenses in the respiratory epithelium. Immunosuppressed cancer patients include those undergoing induction therapy or conditioning regimens and immunosuppressive therapy. This increases the susceptibility to infectious complications for these patients. Our central hypothesis is that these patients can respond to PUL-042 and mediate innate protection in the lungs. By stimulating innate immune responses in the epithelial lining of the respiratory tract, patients will be protected from infections, thereby reducing morbidity and mortality related to disease and treatment. **Methods: Results:** Following successful phase I clinical trials in healthy subjects, we next aim to demonstrate activity in cancer patients by measuring the overall incidence and severity of pneumonia. The primary objective is to evaluate the safety and efficacy of PUL-042 for prevention of clinically documented lower respiratory tract infections, who have documented parainfluenza upper respiratory tract infection. Exploratory objectives include: 1) evaluate the effect of PUL-042 on parainfluenz viral titers and neutralizing antibodies 2) evaluate the effect of PUL-042 over time on biomarkers of drug activity and disease severity and 3) determine whether PUL-042 induces neutralizing antibodies against PUL-042 components. Initially, we will assess safety and tolerability of two different dose levels of PUL-042 in up to 20 patients from the target population at MD Anderson Cancer Center. Doses will be based on the maximum tolerated dose determined in Phase I trials in healthy volunteers. We will complete the trial with up to 180 more patients at additional sites. Completion of the trial will require the engagement of multiple centers and multiple departments within the anticipated clinical setting such as Pulmonary, Hematology/Oncology, and Infectious Disease to properly identify, recruit, treat and provide follow-up for individual patients during the conduct of the trial. **Conclusion:** The successful achievement of these objectives will demonstrate proof-of-concept in man and further validate the technology for market use. The company is well positioned to advance existing collaborations, partnerships and discussions with leading large pharma and biotechnology organizations, accelerating the path to the market to address the serious unmet needs faced by patients with cancer.

425 **CPRIT Grantee Poster Session A**

Depletion of Kynurenine Using an Engineered Therapeutic Enzyme Potently Inhibits Cancer Immune Suppression

J. Blazeck, The University of Texas System; E. Stone, The University of Texas at Austin; M. Donkor, The University of Texas System; K. Triplett, The University of Texas System; N. Marshall, The University of Texas System; T. Triplett, University Health System; W. Lu, The University of Texas System; L. Ehrlich, The University of Texas System; G. Georgiou, The University of Texas at Austin

Introduction: The kynurenine pathway is a key mechanism of immune suppression in cancer, a function exerted primarily via Kynurenine (KYN), an AHR ligand that potently impairs adaptive immune responses. Inhibition of Kynurenine synthesis, mediated by IDO1, TDO, and possibly IDO2, is of great interest for cancer immune checkpoint inhibition, with at least three IDO1 inhibitors currently in clinical development. However: (1) IDO1 inhibition has marginal anti-cancer effects as a monotherapy; (2) IDO1 inhibitors block only one of the two major Kynurenine synthesis pathways (the other being through TDO). We postulated that administration of a Kynureninase enzyme (Kynase) that degrades Kynurenine into non-toxic and immunologically inactive metabolites (alanine and anthranilic acid) would potently relieve cancer immune suppression. **Methods:** An extensive protein engineering campaign, conducted to develop a Kynureninase suitable for therapeutic administration, resulted in the production of a highly active Pf-Kynase enzyme that was the PEGylated to enable long circulation persistence. Pf-Kynase was evaluated in the B16-OVA melanoma model in wild type C57BL/6J mice as a monotherapy and in combination with an α-PD-1 checkpoint inhibitor. Quantitation of tumor size/regression, histology, and flow cytometric analyses assessing immune cell populations in the spleen, tumor, and tumor-draining lymph nodes were used to demonstrate therapeutic efficac . **Results:** Administration of pegylated Pf-Kynase in B16-OVA melanoma allografts in C57BL/6J mice reduced serum Kynurenine level, resulted in significan tumor growth retardation (treatment initiated @ tumor size 25-40 mm2), and extended survival in a manner indistinguishable from that observed with immune checkpoint inhibitor antibodies α-PD-1 (clone RMP1-14) or α-CTLA-4 (clone 9D9). Consistent with the hypothesis that depletion of Kynurenine relieves immune inhibition, we observed a marked increase in CD8+ tumor-infiltrating lymphocytes expressing Granzyme B and IFNγ, enhanced proliferation of CD4+ and CD8+ T cells in the tumor-draining lymph nodes, as well as decreases in tumor neovascularization. Importantly, combination therapy of Pf-Kynase and α-PD-1 resulted in complete tumor eradication in 60% of mice (n=10 per group) for >100 days, with all surviviors completely rejecting tumors following re-challenge. For comparison, α-PD-1 treatment alone resulted in only 20% long-term survival. **Conclusion:** We demonstrated that Pf-Kynase displays a significant anti-tumor efficacy as a monotherapy and excellent synergy with an α-PD-1 checkpoint inhibitor. The observed increase in proliferation and tumor infiltration of cytotoxic CD8+ T cells demonstrates that Pf-Kynase alleviates immune suppression that normally occurs in the tumor microenvironment due to Kynurenine accumulation. Thus, Pf-Kynase represents a “first in class” immune checkpoint inhibition enzyme.

426 **CPRIT Grantee**
Poster Session B

Development of AVID100, a Highly Active Antibody-Drug Conjugate for the Treatment of Solid Tumours *M. O'Connor, Armada Pharmaceuticals; J. Koropatnick, UWO; M. Jaramillo, NRC; I. Tikhomirov, Armada Pharmaceuticals*

Introduction: AVID100 is a novel epidermal growth factor receptor (EGFR)-targeting antibody-drug conjugate (ADC). EGFR is an important oncogene overexpressed by many types of solid tumors, including lung, breast, head and neck, and others. Unlike currently marketed anti-EGFR therapeutics that depend on EGFR blockade for anti-cancer activity, AVID100 has an additional mechanism of action of direct cytotoxicity via conjugated payload. This is expected to result in significantly enhanced anti-cancer activity of AVID100 compared to currently marketed anti-EGFR agents. We conducted in vitro and in vivo studies to evaluate the anticancer activity of AVID100. **Methods:** Effects of AVID100 were tested against EGFR+ cancer cell lines, including those resistant to marketed anti-EGFR agents. Activity of AVID100 against non-transformed EGFR+ keratinocytes was also evaluated. Pharmacology studies investigating the anti-cancer activity of AVID100 were performed in mice bearing human tumor xenograft models, including breast and head and neck cancers. **Results:** AVID100 demonstrated potent and broad activity against multiple cell lines with IC50 values in the pM to nM range. The ADC was also active against cell lines resistant to marketed to anti-EGFR therapeutics. In vivo, AVID100 demonstrated significant anti-cancer activity in multiple cancer models including complete remissions in breast and head and neck cancer xenografts. Importantly, AVID100 was demonstrated to be minimally toxic against normal EGFR+ keratinocytes. Skin toxicity is a class effect of anti-EGFR therapeutics and this result suggests AVID100 skin toxicity will be comparable to other agents in the class, despite significantly higher potency of AVID100 on tumors. Tolerability of AVID100 was subsequently confirmed in non-human primate studies. **Conclusion:** AVID100 is a promising anti-cancer therapeutic for the treatment of EGFR+ positive tumors, including tumor types resistant to currently marketed anti-EGFR agents. AVID100 is currently undergoing IND-enabling development with clinical trials planned for 2016.

427 **CPRIT Grantee**
Poster Session A

Targeting STAT3 with an Oral Small-Molecule to Reverse Chemotherapy and Radiation Resistance *D. Tweardy, The University of Texas M.D. Anderson Cancer Center; U. Bharadwaj, The University of Texas M.D. Anderson Cancer Center; S. Lai, The University of Texas M.D. Anderson Cancer Center; L. Dobrolecki, Baylor College of Medicine; T. Eckols, The University of Texas M.D. Anderson Cancer Center; S. Hilsenbeck, Baylor College of Medicine; M. Lewis, Baylor College of Medicine*

Introduction: STAT3 has been validated as a target to treat many cancers, including triple-negative breast cancer (TNBC) and head and neck squamous cell carcinoma (HNSCC), especially to eliminate cancer stem cells, which are linked to chemotherapy and radiation resistance in these and other tumor systems. Yet, a STAT3 inhibitor has not entered the clinic. **Methods:** To identify a small-molecule STAT3 inhibitor, we used virtual ligand screening (VLS), surface plasmon resonance-based pY-peptide binding, microscale thermophoresis, cytokine-stimulated pY-STAT3, GFP-STAT3 nuclear translocation, and breast cancer cell line growth inhibition assays. We also did hit-to-lead studies consisting of 2-D and 3-D similarity screening, structure activity relationship-based medicinal chemistry, *in vitro* ADMET, maximum tolerated dose determinations in mice, PK studies in mice, rats, and dogs, and xenograft growth-inhibition assays using TNBC patient derived xenograft (PDX) and radiation-resistant (RR) HNSCC tumor cell lines. **Results:** C188 was identified in the VLS scree and was used in our hit-to-lead program to identify C188-9. C188-9 binds STAT3 with high affinity (4.7 ± 0.4 nM) and potently inhibits STAT3 binding to its pY-peptide ligand and cytokine-stimulated pY-STAT3. C188-9 (50 mg/kg/d) was examined alone and in combination with docetaxel (DT) for anti-tumor effect in two pY-STAT3-positive, DT-resistant TNBC PDX models—BCM-4195 and BCM-4272—and one pSTAT3-positive, DT-sensitive model—BCM-3107. Using RECIST criteria, C188-9 converted BCM-4195 from DT-resistance to DT-sensitive. While not reversing DT resistance in BCM-4272, addition of C188-9 to DT prevented tumor growth vs. DT alone ($p<0.05$). Importantly, C188-9 reduced pY-STAT3 levels in PDX tumors harvested 24 hours after the last treatment ($p<0.05$). As expected, complete remission was observed in 5 of 6 (83%) of mice bearing BCM-3107 tumors treated with either DT alone or with DT plus C188-9; however, while tumors relapsed in 75% of mice treated with DT alone, tumors relapsed in only 20% of mice treated with DT plus C188-9.

C188-9 also reduced constitutive pY-STAT3 levels in the RR-HNSCC cell line, UM-SCC-17B ($p<0.05$) and inhibited its anchorage dependent and independent growth ($p<0.05$ for both). In addition, treatment of nude mice bearing UM-SCC-17B xenografts with C188-9 prevented tumor xenograft growth ($p<0.05$) and modulated many STAT3-regulated genes involved in oncogenesis and radioresistance, as well as radioresistance genes regulated by STAT1. C188-9 demonstrated excellent oral bioavailability, was well tolerated in mice, rats, and dogs, and was concentrated 3-fold in tumors. **Conclusion:** Thus, C188-9 shows promise in improving responses in patients with pY-STAT3-positive TNBC, including those with chemoresistant disease, and in patients with pY-STAT3- and/or pY-STAT1-positive RR HNSCC.

429 **Poster Session A**

Pre-Clinical Development of miRNA-Based Cancer Therapy *C. Daige, Mirna Therapeutics, Inc.; J. Wiggins, Mirna Therapeutics, Inc.; L. Priddy, Mirna Therapeutics, Inc.; T. Muenzer, Mirna Therapeutics, Inc.; K. Vadnagara, Mirna Therapeutics, Inc.; D. Enzler, Mirna Therapeutics, Inc.; D. Brown, Mirna Therapeutics, Inc.*

Introduction: A liposome-formulated mimic of the tumor suppressor miRNA, miR-34, is currently being evaluated in cancer patients as part of a Phase I clinical trial. M**ethods:** Multiple mouse models of liver cancer have been used to characterize the pharmacokinetics and pharmacodynamics of the therapeutic candidate. **Results:** Intravenous injections of the miR-34-based drug candidate in mice with mature liver tumors reduces the expression of multiple oncogenes which results in substantial to complete tumor regression. The drug candidate has also proven to be effective in liver cancer models when used in conjunction with sorafenib, a drug that has been approved for treating patients with non-resectable liver tumors. C**onclusion:** Data from the mouse model studies are being used to supplement the clinical program for the miR-34 drug candidate.

430 **Poster Session B**

Engineered Human L-Methioninase For Therapeutic Purposes

W. Lu, The University of Texas System; W. Yan, The University of Texas at Austin; O. Paley, The University of Texas at Austin; K. Triplett, The University of Texas System; A. Ellington, The University of Texas at Austin; Y. Zhang, The University of Texas at Austin; E. Stone, The University of Texas at Austin; G. Georgiou, The University of Texas at Austin

Introduction: In cancer biology research, it has been found that cancer cells exhibit different metabolism compared to normal cells and it has been shown that some types of cancer cells, such as glioblastomas, medulloblastomas and neuroblastomas are much more sensitive than normal cells to methionine starvation. Past studies have shown that methionine-dependent tumor cells are not able to survive if the serum methionine is decreased to ≤ 5μM. Systemic depletion of serum methionine can be achieved by *Pseudomonas putida* methionine gamma-lyase (pMGL) but it has proven to be rapidly inactivated *in vitro* and be highly immunogenic in primate models. In order to apply systemic methionine depletion to human cancer therapy, we engineered human cystathionine gamma-lyase to accept methionine as a substrate and have isolated several human Methioninase (hMETase) variants with high activity. **Methods:** We engineered and tested several variants of human Methioninase (hMETase) for catalytic activities, thermostabilities, serum stabilities and pharmacodynamics. Furthermore, we tested the efficacy of the most active variant (hMETase V8.4) on NSG mice bearing A375 human melanoma xenografts. **Results:** Several active hMETase variants isolated from a phylogenetic analysis library and the best variant, hMETase V8.4 showed a 10-fold improved K_M< (12.2 vs. 1.8mM) and 10-fold better k_{cat}/K_M values (0.59 to 5.3 1/s.1/mM) in degrading methionine compared to our previous version of variant, hMETase V3.1. Furthermore, hMETase V8.4 showed greater stability in thermal melting analyses (melting temperature: 63.2 vs. 70.2°C) and also in serum stability (half-life: 75 vs. >100 hours) compared to hMETase V3.1. In pharmacodynamic analyses, hMETase V8.4 efficiently lowered serum methionine concentration from 75 μM to ~15 μM in 48 hours without the requirement of a methionine restricted diet (one dose: 50 mg/ kg). In addition, we tested the efficacy of hMETase V8.4 on NSG mice bearing A375 human melanoma xenografts and it significantly improved the median survival from 35 – 43 days compared to the control group. **Conclusion:** The hMETase V8.4 efficiently lowered serum methionine concentration in pharmacodynamic analyses. Also, it significantly improved the survival time of NSG mice bearing A375 human melanoma xenografts. The hMETase V8.4 is a promising therapeutic enzyme candidate for systemic methionine depletion in cancer therapy.

431 **Poster Session A**

Development of SaliPhe, a Radiosensitizing V-ATPase Inhibitor

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Introduction: About 60% of cancer patients will receive ionizing radiation therapy (IRT). While very effective for many cancers, others like nonsmall cell lung cancers (NSCLC) have a poor long term prognosis. One approach for more effective treatments is chemoradiation therapy (CRT) where IRT is combined with a chemotherapeutic agent that enhances the cytotoxicity and effectiveness of IRT. One target for a new chemoradiation therapeutic is the vacuolar H+-ATPase (V-ATPase) protein complex that pumps acid (i.e. H+) across cell membranes of eukaryotes. It has been implicated in development of resistance to IRT and many chemotherapeutics. Saliphenylhalamide (SaliPhe) is a potent inhibitor of V-ATPase and lead candidate. It is active against many cancer targets including mTORC1, Wnt, Bcl-xL, and Notch signaling. Results stem from a Phase I SBIR contract from the NCI to show the feasibility of using SaliPhe in CRT. **Methods:** SaliPhe for use in CRT was evaluated by seven methods. First, its clonogenic dose enhance ratio (DER) was determined for seven human NSCLC lines and three human bronchial epithelial cell (HBEC) lines. Second, cell cycle analysis studies were performed for three NSCLC lined treated with SaliPhe, IRT, and SaliPhe+IRT. Third, the effects of SaliPhe on DNA double stranded breaks were determined. Fourth, the effect of SaliPhe on autophagy was analyzed. Fifth, the effects of SaliPhe and/or IRT on various aspects of cellular metabolism and extracellular acidification were studied using a SeaHorse Bioscience xf24 instrument. Sixth, differential gene expression was determined across four NSCLC lines when treated and untreated with SaliPhe. Finally, a tumor growth delay study (TGD) was performed with SaliPhe delivered by continuous infusion. **Results:** The DERs ranged from 1.11 to a high of 2.22 for A549 and 1.1 to 1.67 for the HBEC pane. Cell cycle analysis showed SaliPhe increased %G2 for A549 and H1299. DSB analysis at 24h showed 20% DSBs for A549, <10% DSBs for H1299, and complete repair for HCC827. Autophagy was unchanged for SaliPhe+IRT over SaliPhe alone. SaliPhe had small to large effects on various aspects of metabolism that was cell line dependent. Gene expression analysis showed 120 genes segregated by SaliPhe exposure. SaliPhe, IRT, and CRT were all similarly effective in a TGD study. **Conclusion:** SaliPhe shows considerable potential as a CRT agent for some NSCLCs as well as a chemotherapeutic. It is a very attractive candidate for further development as a CRT and chemotherapeutic.

432 **Poster Session B**

Nanotechnology-enabled, Targeted Treatment and Imaging of Cancer *J. Harris, NanoHybrids Inc; R. Deschner, NanoHybrids Inc; J. Cook, NanoHybrids Inc; N. Viswanathan, NanoHybrids Inc; S. Emelianov, The University of Texas at Austin; L. Pagliaro, Siva Therapeutics Inc; K. Homan, NanoHybrids Inc*

Introduction: Approximately 1 in 7 men will be diagnosed with prostate cancer in their lifetime, with an estimated 220,800 new cases per year in the US. Though prostate cancer is a slow-progressing disease and very treatable by surgery and radiation, every year tens of thousands of men elect not to be treated due to the high risks of impotence and incontinence inherent with traditional therapies. Precise, targeted, focal treatment of the tumor site via photothermal therapy (PTT) offers an attractive alternative, limiting damage to the prostate and surrounding nerves. Partnering to fight prostate cancer, NanoHybrids Inc and Siva Therapeutics are co-developing a prostate-sparing cancer treatment platform that selectively kills prostate cancer using nanoparticle-mediated PTT and monitors therapeutic doses to ensure preservation of healthy tissues. **Methods:** NanoHybrids is developing a molecularly-targeted nanotechnology agent, termed ProGold, which is optimized for the focal treatment of prostate cancer via PTT. The ProGold platform is a modifie plasmonic gold nanorod that is excited by non-invasive near infrared light to produce efficient and stable heating (hyperthermia). In conjunction, Siva Therapeutics is developing methods of using nanotechnology-enabled PTT to create hyperthermia selectively in tumors, providing molecularly targeted and highly-localized ablation of cancer cells. **Results:** The ProGold platform's small size (dimensions ranging 5-60 nm) and specialized biocompatible coating has enabled accumulation in tumors after systemic injection. ProGold-enabled PTT has also been performed in murine models of cancer. Furthermore, the PTT therapy process has been visualized in vivo using photoacoustic (PA) imaging. Using PA imaging, the presence of ProGold at the tumor site was confirmed and the PTT-induced hyperthermia was dynamically monitored to ensure effective and localized thermal dosage at the tumor, leaving healthy tissue viable and intact. Significant reduction in tumor volumes and doubling of survival were found in the treated mice. **Conclusion:** The breakthrough technology being developed by NanoHybrids and Siva Therapeutics will reduce prostate cancer mortality and increase patient quality of life by lowering the associated risks of impotence and incontinence that come with typical surgery, radiation, and cryoablation treatments. Tens of thousands of prostate cancer patients every year elect for no treatment; our aim is to sway those patients towards early focal treatment options using this platform of targeted PTT combined with ProGold for the safe, selective, localized eradication of prostate cancer.

433 **Poster Session A**

Providing Isotopes for Targeted Cancer Therapy *M. Raizen, The University of Texas at Austin; K. Dorius, Pointsman Foundation*

Introduction: Atomic isotopes are important tools in diagnostic imaging and cancer therapy, yet they are among the rarest and most expensive commodities on earth. The separation of isotopes dates back to the invention of the Calutron in the 1930's. Today, most isotopes are still separated with this old and inefficient technology, now only operating in Russia and subject to uncertain supply due to the age of the machines as well as political concerns. M**ethods:** A new and efficient method for isotope separation was recently demonstrated, Magnetically Activated and Guided Isotope Separation ("MAGIS"). This method relies on laser optical pumping of atoms, preparing the desired isotope in a particular internal state that is repelled by an external magnetic field. Atoms are then separated in a slightly curved guide lined with permanent rare-earth magnets. There is no direct line-of-sight between the atomic source and the collector, so that only the desired isotopes can reach the collector by reflecting off the strong magnetic field. MAGIS is described in "Demonstration of Magnetically Activated and Guided Isotope Separation" published at Nature Physics online on June 29, 2014. After publication and award of a patent (8,672,138), the method has been licensed to the Pointsman Foundation, a not-for-profit 501(c)(3) organization headquartered in Austin, TX. **Results:** The Pointsman Foundation and MAGIS technology will enable expanded research, clinical trials, and promising new therapies for cancer. For example, targeted radio-therapies using short-range radio-isotopes have proven to be effective against a range of metastatic and blood-borne cancers. Initially, MAGIS will be employed for producing highly enriched Yb-176 for use in making Lu-177, a short-range beta-emitter being tested in targeting prostate, endocrine, and liver cancers. MAGIS will later be used to efficientl isolate and purify a shorter-range alpha-emitter, Ac-225, opening the door to targeted alpha-therapies shown to be highly-effective for metastatic bone, brain, lung, breast, ovarian, prostate, pancreatic and blood-borne cancers. The foundation will also provide isotopes for existing and new diagnostic imaging modalities (PET & SPECT scans), and brachytherapy. C**onclusion:** The Pointsman Foundation will partner with top medical centers for joint grant proposals and research for a range of medical applications. MAGIS can separate 130+ isotopes of 30+ elements. The foundation is currently pursuing grants and philanthropic support to establish a beta-therapy isotope production line. In the longer term, the foundation will be mostly self-sustaining from isotope royalties, enabling expansion of efforts and development of additional isotope production lines and medical isotope applications.

434 **Poster Session B**

Tumor Suppressors Mediate Anti-Tumor Immune Responses: Pre-Clinical and Clinical Results *R. Sobol, MultiVir Inc; K. Menander, MultiVir Inc; S. Chada, MultiVir Inc*

Introduction: Multiple studies have demonstrated the induction of anti-tumor immune responses by the tumor suppressors p53 and IL24. **Methods:** Replication incompetent adenoviral vectors expressing p53 (Ad-p53) or IL24 (Ad-IL24) were constructed and evaluated in pre-clinical and Phase 1/2 clinical studies with assessments of innate and adaptive anti-tumor immune responses. **Results:** Cellular senescence induced by p53 treatment activated innate anti-tumor immune responses mediated by natural killer (NK) cells, neutrophils and macrophages. These properties provided the rationale for combining Ad-p53 treatment with 5FU chemotherapy which is known to selectively eliminate myeloid derived suppressor cells. In a Phase 2 trial of 13 patients with liver metastases of colorectal cancer, combined intra-arterial Ad-p53 and 5FU (as FUDR) demonstrated a statistically significant increase in survival compared to patients treated with 5FU/FUDR alone (18 vs 10 months; p = 0.0030). IL24 is a tumor suppressor known to increase adaptive T cell mediated anti-tumor immunity. In Phase 1/2 trials in melanoma patients, Ad-IL24 resulted in clinical responses in 100% of treated lesions in all 7 patients at the highest dose level. These responses were associated with tumor infiltration of lymphocytes and increased circulating tumor specific CD8+ T cells. p53 has been shown to upregulate tumor expression of NK cell ligands NKG2D-L and DNAM1-L which facilitate NK mediated tumor cell killing. IL-24 was shown to upregulate interferon-gamma expression and to inhibit Wnt/beta-catenin signaling which are associated respectively with anti-PD-1 responsiveness and reversal of anti-PD-1 resistance. **Conclusion:** Ad-p53 and Ad-IL24 therapies induce innate and adaptive anti-tumor immune responses supporting future evaluation of these tumor suppressor treatments in combination with innate and adaptive immune checkpoint inhibitors.

435 **CPRIT Grantee**

Pre-IND Development of Gadolinium Texaphyrin Platinum Conjugates *J. Sessler, The University of Texas at Austin*

Introduction: The FDA approved platinum agent cisplatin is one of the most important antitumor agents in the clinic. It has demonstrated significant activity in several cancer types, but is plagued by intrinsic and acquired resistance that translates into low 5-year survival rates. This resistance is multifactorial and includes both biochemical and pharmacologic mechanisms (i.e., reduced drug uptake, reduced DNA lesions). Typically, resistance is found to be 2-3 fold higher in wild-type p53 cancers relative to mutant p53 cancers. This constitutes an unsolved problem. As formulated, current platinum drugs do not permit concurrent imaging. The ability to "see" these agents could improve mechanistic understanding and may improve treatment protocols. Achieving this object thus represents a second unmet challenge. M**ethods:** Texaphyrins are "expanded porphyrins", a proprietary class of macrocycles that have been explored as experimental therapeutic agents. The gadolinium complex show strong tumor localizing properties as evidenced by MRI analyses of patients enrolled in clinical studies. To address the problems associated with platimnum resistance, we are working to develop texaphyrin-platinum conjugates designed to overcome both the uptake/retention and DNA repair mechanisms of Pt resistance. These systems also permit concurrent MRI imaging. Synthesis and initial cell-based testing is being carried out in the PI's laboratory by Drs. Gregory Thiabaud and Tridib Sarma. More advanced in vitro studies are being carried out in conjunction with Dr. Zahid Siddik of the MD Anderson Cancer Center. In vivo analyses are being carried out under the direction of Drs. Alan B. Watts and Greg D. Lyness (Univ. of Texas at Austin Drug Dynamics Institute) and Rick A. Finch (Department of Veterinary Sciences, MD Anderson Center). **Results:** We have found that by conjugating specific platinum(II) agents to a texaphyrin core, we are able to overcome platinum resistance in a wild-type p53 cell line typically used as an ovarian cancer model. New work involving Pt(IV) also show promise. With both systems, both targeting and controlled release has been demonstrated in vitro. Current work under the aegis of this grant and a previous CPRIT research grant has been focused on demonstrating efficacy in vivo, confirming the expected reduced platinum-based toxicity, and commencing IND-enabling studies in animals. Scale up of synthesis for lead compounds is also ongoing. **Conclusion:** Texaphyrin-Pt conjugates show promise and further developmental work will, with luck, allow this promise to be translated into advanced stage leads suitable for testing as MRI-detectable therapeutics in patients.

436 **CPRIT Grantee**
Poster Session B

IL-4 Receptor Overexpression In Recurrent Glioblastoma (rGB) And Metastatic CNS Tumors Supports Clinical Development Of MDNA55 *F. Merchant, Medicenna Therapeutics Inc.; S. Fidai, Medicenna Therapeutics Inc.; N. Merchant, Medicenna Therapeutics Inc.; P. Tsang, Centre for Translational and Applied Genomics, University of British Columbia; S. Yip, University of British Columbia*

Introduction: MDNA55 [IL-4(38-37)-PE38KDEL] is a targeted toxin in clinical development for treatment of rGB administered as an intratumoral infusion using convection enhanced delivery. It consists of an engineered, circularly permuted interleukin-4 (IL-4) fused to a *Pseudomonas aeruginosa* exotoxin A (PE). MDNA55 binds selectively and with high affinity to IL-4 receptor (IL-4R) overexpressed on tumor cells. To support a planned Phase 2 study of MDNA55 in patients with rGB at first recurrence, an IL-4R based *in vitro* companion diagnostic (CDx) is under development to select GB patients most likely to respond to MDNA55 treatment. **Methods:** As a first step toward development of an IL-4R based CDx, Medicenna optimized an immunohistochemistry (IHC) based assay using formalin fixed paraffin-embedded tissue sections of human brain tumor biopsies obtained from the Brain Tumor Bank (London, Ontario). The IHC assay was developed using a commercially available anti-human IL-4Ra antibody and the Ventana Discovery XT automated slide staining system along with an Ultramap DAB chromogenic detection kit. Tissue sections were graded by a neuro-oncology pathologist for IL-4Ra expression by examining cytoplasmic staining intensity and prevalence of IL-4Ra in tumor cells in a blinded fashion using a semi-quantitative scale of 0, 1+, 2+ and 3+. **Results:** Fourteen matching pairs of newly diagnosed (ND) and rGB biopsies from the same patient were analyzed for IL-4Ra expression. 12 out of the 14 rGB showed either same or higher levels of IL-4Ra expression compared with the matching ND pair, with a majority of rGB having a staining intensity ≥ 2+ indicating that IL-4Ra expression is maintained in the recurrent setting and that this patient population could be susceptible to IL-4R directed therapy. These results are in sharp contrast to data published on IL-13Ra2 (decoy receptor targeted by IL13-PE) where expression of IL-13Ra2, as determined by RT-PCR, was significantly decreased by 15-fold in GB biopsy samples of first recurrence compared to ND GB. Biopsy samples of bowel, kidney and breast tumors metastasized to the brain were also analyzed for IL-4Ra expression. Most (20 out of 27) of the tumors tested had a staining intensity of at least 1 +,

with 7 out of 10 of the breast tumors showed a staining intensity of ≥ 2+. **Conclusion:** These results suggest rGB and tumors that metastasize to the CNS may be susceptible to MDNA55 treatment.

437 **CPRIT Grantee**

Gene Signature Shows that Phase I Liposomal MicroRNA Replacement Cancer Therapy MRX34 Represses Target Genes in Human White Blood Cells *H. Peltier, Mirna Therapeutics, Inc.; K. Kelnar, Mirna Therapeutics, Inc.; J. Stoudemire, Mirna Therapeutics, Inc.; A. Bader, Mirna Therapeutics, Inc.*

Introduction: Mirna Therapeutics is developing a potential first in class, first in clinic cancer therapy, known as microRNA replacement therapy, by delivering mimics of naturally occurring microRNAs that are under expressed in cancer cells. In contrast to many of today's cancer treatments that target only one or two oncogenes or pathways, microRNA replacement therapy is designed to regulate the expression of multiple important oncogenes across key oncogenic pathways. Mirna's lead product candidate is MRX34, a mimic of naturally occurring microRNA-34 (miR-34) encapsulated in a liposomal nanoparticle formulation. It is currently the subject of a multicenter, open label dose escalation Phase 1 clinical trial in patients with unresectable primary liver cancer, solid tumors and hematological malignancies. MRX34 was given daily for five days (QD x 5) in 21-day cycles. Here, we show that intravenous dosing of MRX34 leads to miR-34 specific gene knock-down of several direct miR-34 target genes in patient-derived human white blood cells (hWBCs). **Methods:** Whole blood was collected from subjects prior to MRX34 dosing and at incremental time points after the first and during subsequent administrations. White blood cells were fractionated from whole blood using the LeukoLOCK fractionation method followed by RNA isolation using the mirVana RNA isolation kit. RNA quality was assessed via Agilent's 2100 Bioanalyzer. Gene expression levels were measured via quantitative reverse transcription-polymerase chain reaction (qRT-PCR) comparing relative gene expression of pre- and post-dose samples. **Results:** Our data show miR-34 target genes are repressed in hWBCs 24 hours after initiation of MRX34 dosing. In addition, the downregulation of target genes is dose-dependent such that the number of downregulated genes increases at higher MRX34 dose levels. C**onclusion:** Based on our qRT-PCR analysis, the down regulation of miR-34 target genes appeared to be dose-dependent and inversely correlated with MRX34 drug exposure in whole blood. In contrast, expression levels of p21 (CDKN1A), a tumor suppressor gene specifically induced by miR-34, were increased. The hWBC data suggest that MRX34 is delivering active miR-34 mimics in cancer patients and may prove useful for evaluating responses in the tumor.

438 **CPRIT Grantee**

MicroRNA-34 Mimics Synergize with Small Molecule Inhibitors in Hepatocellular Carcinoma Cells *J. Zhao, Mirna Therapeutics, Inc.; A. Guerrero, Mirna Therapeutics, Inc.; C. Daige, Mirna Therapeutics, Inc.; J. Wiggins, Mirna Therapeutics, Inc.; L. Priddy, Mirna Therapeutics, Inc.; T. Muenzer, Mirna Therapeutics, Inc.; D. Brown, Mirna Therapeutics, Inc.; A. Bader, Mirna Therapeutics, Inc.*

Introduction: Sorafenib (Nexavar®) is an FDA-approved therapy for patients with hepatocellular carcinoma (HCC), and acts as a multi-kinase inhibitor targeting the Raf/Mek/Erk pathway. Tivantinib and EMD1214063 are c-Met inhibitors currently in Phase 1/2 clinical trials for the treatment of liver cancers. Due to the selective inhibitory action and observed toxicities, current treatment options for liver cancer remain limited. Mirna Therapeutics develops mimics of naturally occurring microRNAs that are designed to restore tumor suppressor activity and aid appropriate tumor immune response. The company's lead product candidate, MRX34, a naturally occurring miR-34 encapsulated in a liposomal nanoparticle formulation, is currently the subject of a Phase 1 clinical trial in patients with primary hepatocellular carcinoma, other solid tumors and hematological malignancies. Because microRNAs inhibit tumor growth by regulating multiple oncogenic pathways at once, therapeutic microRNA mimics in combination with the respective standard of care may bring this tumor suppressor activity back into tumor cells and thereby augment drug sensitivity. Here, we investigated the therapeutic activity of miR-34 in combination with sorafenib, tivantinib, or EMD1214063 in HCC cells. M**ethods:** Combination studies were carried using the microRNA mimic and the small molecule inhibitors at a single ratio and as well as at multiple ratios in a panel of HCC cells. Cells were reverse-transfected with miR-34 and incubated with targeted therapies three days after transfection for three days. Cellular proliferation was determined by AlamarBlue. Synergistic, additive, or antagonistic effects were determined according to Chou-Talalay's algorithm and expressed in combination index (CI) plots, isobolograms and curve shift analyses. The effects of liposomal miR-34a (MRX34) in combination with sorafenib were further evaluated in mice seeded with orthotopic Hep3B liver cancer xenografts. Tumor growth was measured by serum alpha-fetoprotein (AFP) levels and wet tumor weights. **Results:** Our data indicate synergistic interactions between miR-34 and sorafenib, as well as miR-34 and tivantinib in all cancer cells tested. Synergistic interaction between miR-34 and EMD1214063 was only observed in C3A and SK-Hep1 cells. Synergy was observed at multiple microRNA/drug ratios and at drug concentrations that induce 50% or greater cancer cell inhibition. The sorafenib + miR-34 combination was superior in inhibiting tumor growth in the orthotopic Hep3B liver cancer mouse when compared to the single agents, confirming the synergistic nature we observed in vitro. **Conclusion:** Our data demonstrate that tumor suppressor microRNAs can enhance the potency of other targeted therapeutics and may further support the preclinical and clinical development of microRNA-based therapies for liver cancer.

439 **CPRIT Grantee**

Convection Enhanced Delivery Of MDNA55 (cpIL4-PE) Is Safe With Promising Signs Of Efficacy In Patients With Recurrent Glioblastoma *R. Abi-Habib, Lebanese American University; R. Merchant, Medicenna Therapeutics, Inc; S. Denmeade, Johns Hopkins University; F. Merchant, Medicenna Therapeutics Inc.*

Introduction: There is clearly a need to develop effective, tumor-targeted therapies for GBM. One such therapeutic is MDNA55, consisting of a circularly permuted human IL-4 (cpIL-4) fused to Pseudomonas aeruginosa (PE) exotoxin A (cpIL4-PE) that targets the IL4 receptor (IL-4R) shown to be overexpressed in malignant gliomas, but not in normal brain. **Methods:** 32 patients with recurrent GBM were treated with a single infusion of MDNA55 at three dose levels: 90 μg (1.5 μg/mL x 60 mL), 240 μg (6 μg/mL x 40 mL), and 300 μg (3 μg/mL x 100 mL) with 1 to 3 ventricular catheters placed under stereotactic guidance. Infusion lasted for 4 to 5 days and 3 weeks post-treatment, the tumor was surgically resected. In 10 patients, pre-and post-treatment biopsies could be assessed for MDNA55 related effects. **Results:** No deaths attributable to MDNA55 were observed and no systemic toxicity or clinically significan abnormalities were found in hematology/serum chemistry. Drug-related adverse events (AEs) were primarily neurological, and generally related to cerebral edema following drug infusion. At least half of the patients in each group were still alive 26 weeks after infusion. The highest 6-month survival rate, 68.2%, was observed in the 90 μg dose group, followed by the 240 and 300 μg dose groups (60% and 50%, respectively). The 90 μg dose also showed the least number of adverse event and was selected for future trials. Seven of ten patients showed a marked reduction in tumor cellularity in the resection samples post-treatment. Five showed little to no tumor cellularity, while the remaining 2 had at least a 75% reduction in tumor cellularity, demonstrating that treatment with MDNA55 was cytolytic to GBM cells. In order to situate the efficacy of MDNA55 within the context of other approaches efficacy data was compared to similar external historical data and their background prognostic factors. Despite poorer performance status (34% of patients with KPS < 70) and a higher proportion of multiple relapses (41%) the survival outcomes were comparable. **Conclusion:** The molecular characteristics of MDNA55, along with its safety and efficacy data, support the continued development of this molecule as a selective treatment for patients with GBM. Additional trials are planned in patients with recurrent GBM overexpressing IL-4R.

440

Quantitative PCR and In-Situ-Hybridization Analysis to Determine Tissue Concentration and Localization of MRX34 *D. Martin, Mirna Therapeutics, Inc.; K. Kelnar, Mirna Therapeutics, Inc.; A. Bader, Mirna Therapeutics, Inc.*

Introduction: MRX34, a liposomal microRNA (miRNA)-based therapy for cancer, has recently entered clinical trials as a potential firs clinical candidate in its class. MRX34 is a mimic of naturally occurring microRNA-34 (miR-34) encapsulated in a liposomal nanoparticle formulation. Preclinical animal studies have shown that intravenous delivery of MRX34 can increase miR-34 levels in liver tumor cells more than 100-fold when analyzing whole-tissue RNA extracts by quantitative PCR (qPCR). MRX34-induced tumor regression has enhanced the survival of mice by inhibiting the growth of both hepatic and non-hepatic tumors. **Methods:** We have established a chromogenic in situ hybridization (CISH) method to track the cellular location of the miR-34 mimic in tissues after systemic MRX34 administration. In contrast to conventional biodistribution approaches that cannot distinguish between spatial differences in tissue accumulation, CISH in conjunction with microscopy allows the detection of the miRNA mimic on a cellular level and may provide new insights into cell-type specific accumulation. Here, we evaluated the localization and tissue concentrations of systemically delivered MRX34 in mice bearing orthotopic Huh7 tumors by CISH followed by image correlation to a formalin-fixed and paraffin-embedde tissue equivalent, and isolation-free qPCR analysis. **Results:** Our results show that the CISH procedure is a reproducible and robust assay capable of over 2 logs of miR-34 detection when correlated to qPCR data from matching micro-dissected samples. Systemic MRX34 delivery leads to accumulation of miR-34 mimics in tumor cells with Cmax reached approximately 1 hour post dosing. **Conclusion:** In addition, the CISH data reveal how biodistribution data generated from whole-tissue extracts can be biased due to minute impurities and suggests that these methods should be used in combination with CISH for an accurate assessment of tissue concentrations.

441

Transcriptomic Gene Profiling by Next-Generation Sequencing (RNA-Seq) Reveals Therapeutic Delivery to Patient-Derived White Blood Cells by MicroRNA Replacement Therapy MRX34 *K. Kelnar, Mirna Therapeutics, Inc.; H. Peltier, Mirna Therapeutics, Inc.; A. Bader, Mirna Therapeutics, Inc.*

Introduction: Mirna Therapeutics has recently entered clinical trials with MRX34 as a potential first in class, first in clinic microRNA replacement therapy. MRX34, a mimic of naturally occurring microRNA-34 (miR-34) encapsulated in a liposomal nanoparticle formulation, is currently being tested in a multicenter, open label dose escalation Phase 1 clinical trial enrolling patients with unresectable primary hepatocellular carcinoma, other solid tumor and hematological malignancies. Although the primary endpoints of this Phase I study relate to safety and tolerability, we also aimed to evaluate the biological activity of MRX34 in patients, particularly in human white blood cells. **Methods:** Since access to patient tumor biopsies can be limited, we isolated human white blood cells (hWBCs) as a surrogate tissue from whole blood samples following a non-invasive, simple collection procedure at multiple time points from each patient. After the separation of buffy coat and RNA isolation, whole transcriptome RNA-Sequencing (RNA-Seq) was employed to measure the level of gene expression before and after MRX34 dosing. Expression changes of 99 direct miR-34 target genes and 443 predicted target genes were examined. **Results:** Our data show that the majority of direct and predicted miR-34 target genes are repressed in hWBCs 24 hours after initiation of MRX34 dosing. Further, the downregulation of target genes was dose-dependent such that the number of downregulated genes increased at higher MRX34 dose levels. **Conclusion:** The data suggest effective delivery of miR-34 mimics into hWBCs from patients with multiple cancer types and downregulation of key miR-34 target oncogenes.

442

Synergy Between miR-34 Mimics and Conventional Chemotherapy in Non-small Cell Lung Carcinoma Cells *A. Guerrero, Mirna Therapeutics, Inc.; J. Zhao, Mirna Therapeutics, Inc.; X. Yu, The University of Texas Health Science Center at San Antonio; A. Pertsemlidis, The University of Texas Health Science Center at San Antonio; A. Bader, Mirna Therapeutics, Inc.*

Introduction: Lung cancer is one of the leading causes of cancer deaths in the United States. Current chemotherapeutic treatment options for lung cancer include conventional therapy and targeted therapy; however, chronic conventional therapy results in intolerable adverse events, and targeted therapy is effective only in a small subset of patient populations, which highlights the critical need for improved therapies. Mirna Therapeutics develops mimics of naturally occurring microRNAs that are designed to restore tumor suppressor activity and aid appropriate tumor immune response. These tumor suppressor microRNAs are small non-coding endogenous RNAs that regulate multiple important oncogenes across key oncogenic pathways, thereby inhibiting a broad range of cancer cell types in culture and in preclinical animal studies. The company's lead product candidate, MRX34, a mimic of naturally occurring microRNA-34 (miR-34) encapsulated in a liposomal nanoparticle formulation, is currently the subject of a Phase 1 clinical trial in patients with primary hepatocellular carcinoma, other solid tumors and hematological malignancies. We hypothesize that microRNAs are able to sensitize cancer cells to conventional therapeutics (CTs) and will generate more efficacious cancer treatments with minimal toxicity in normal tissues. Here, we investigated the combinatorial effects of miR-34 mimics and conventional therapeutics in NSCLC cells. **Methods:** Effects of miR-34 mimics in combination with cisplatin, paclitaxel, gemcitabine, pemetrexed and carboplatin were evaluated in a panel of NSCLC cell lines with varying degrees of resistance to CTs (A549, H460, H1299, H2073). The combination studies were carried out at multiple ratios following the "fixe ratio" method. Cells were reverse-transfected with miR-34, incubated for one day and then exposed to drug for 72 hours. Cellular proliferation was determined by AlamarBlue. Synergistic, additive, or antagonistic effects were determined according to the Chou-Talalay method and analyzed by combination index (CI) plots, isobolograms and curve shift analyses. **Results:** The data indicate synergistic drug interactions between miR-34 and all CTs tested and in all NSCLC cell lines tested. The synergy was observed at multiple microRNA and drug ratios and at drug concentrations

that induce 50% or greater cancer cell inhibition in lung cancer cells tested. Stronger synergy was observed in H2073 cells resistant to CTs. **Conclusion:** The drug combinations will be further explored in animal models to determine the extent of efficacy and synergy in vivo and may lead to new therapeutic approaches for lung cancer.

443 **CPRIT Grantee Poster Session A**

Positive vs. Negative Appeals for Recruitment to a SMS/ Text Cessation Service: Facebook Advertisement Effects on Conversions and Enrollment *P. Chalela, The University of Texas Health Science Center at San Antonio; A. McAlister, The University of Texas Health Science Center at Houston; K. Gallion, The University of Texas Health Science Center at San Antonio; E. Muñoz, The University of Texas Health Science Center at San Antonio; C. Despres, The University of Texas Health Science Center at San Antonio; D. Akopian, The University of Texas at San Antonio; A. Perez, The University of Texas Health Science Center at San Antonio; R. Garcia, The University of Texas Health Science Center at San Antonio; A. Ramirez, The University of Texas Health Science Center at San Antonio*

Introduction: Evidence shows that variations in the degree and type of a message's emotional arousal, particularly disgust, can influence message impact on smokers' interest in quitting and likelihood of seeking help. Negative emotional appeals are widely used to attract attention of Latino audiences, but positive appeals, especially those designed to increase smokers' confidence in their ability to quit, are also recommended as a way to attract them to enroll in cessation services. **Methods:** We employed positive and negative variations in Facebook advertising appeals to study their effects on "conversions" and subsequent enrollment in a tobacco cessation service (a bilingual text messaging and mobile media service specifically targeting Latino young adults in South Texas). Differences in advertisement characteristics on conversion rate and enrollment were examined using exact Chi-squared tests for proportions and 95% confidence intervals for their corresponding odds ratios. **Results:** The negative advertisement received a higher conversion rate than the positive ad, with 1,441/130,000 vs. 1,249/130,000 clicking the ad to visit our service's home page (OR= 1.16, 95% CI 1.07, 1.25). But subsequent texted enrollments in the cessation service were received by 76 (5.76%) of those exposed to the positive message and 55 (3.82%) of those exposed to the negative message (OR= 1.54, CI1.06, 2.25). The former were 54% more likely than the latter to become enrolled. **Conclusion:** Negative-appealing advertising attracted more clicks to further information, but positive appeals were more likely to lead to subsequent enrollment. Negative advertising can gain attention and spark minor actions toward behavior change, but positive advertising—appealing to the consumer's self-confidence—is more likely to lead to an actual first step toward behavior change.

444 **CPRIT Grantee Poster Session B**

Navigating Rural Highways: Expanding Access to Breast Health Care in Rural Texas *B. Joseph, The Rose; T. Hans, The Rose*

Introduction: In partnership with the Cancer Prevention and Research Institute of Texas (a $539,144 grant over 24 months), The Rose plans to reach and potentially serve 250,000 people/professionals within 10 rural Texas counties to increase access to breast cancer screening, follow-up care and to save more lives through early detection and timely treatment. Texas rural women are more likely to have breast cancers diagnosed at later, more invasive stages, thus reflecting higher breast cancer mortality rates. M**ethods:** The Navigating Rural Highways project targeted underserved women, age 40 and over, who have never had a mammogram or have not had a mammogram within the last two years. The targeted Texas counties include Angelina, Grimes, Leon, Madison, Nacogdoches, Orange, Robertson, and Trinity counties with a secondary aim to help undeserved women in Brazos and Walker counties needing access to screening and diagnostic care. Through this project, The Rose will create two dedicated positions, a Sr. Program Manager and a Program Manager, to serve as Patient Navigators to promote services in the target counties, bringing mobile mammography screening to rural communities to increase breast cancer screening and effectively navigate roughly 230 medically underserved women needing access to a continuum of care – screening, diagnostic follow-up and timely treatment. The Program Managers will serve as community liaisons to provide a continuum of care by: 1) training collaborating partners on how to have successful mobile mammography screening events, 2) bridging gaps in breast health care by connecting community resources/providers to medically underserved women, and 3) reaching health care providers to perform clinical breast exams and to ensure the care continuum between providers for each patient, ideally near their hometown/community. **Results:** This successful project impacts rural counties by removing financial and logistical barriers to care for underserved women, strengthening healthcare safety-net systems within these communities, and providing direct access to breast cancer screening and diagnostic care that leads to the reduction of breast cancer mortality due to late-stage diagnosis. C**onclusion:** It is extrememly important that enough time and effort is made to establish and develop new partnerships and strategically work to build relationships with rural-community stakeholders (6-12 months depending on the scale). Once mutual trust and understanding have been successfully established, partners are able to effectively leverage resources to bring mobile mammography screening to these communities, which increases access to care, strengthens current safety-net systems, and reduces overall breast cancer mortality through early detection and timely treatment options.

445 **CPRIT Grantee**
Poster Session A

A Retrospective Evaluation of ABC4WT-CV Compliance with Mammography Practice Parameters *L. Ross, Angelo State University; K. Stewart, Angelo State University; S. Grelle, Angelo State University; L. Allen, Angelo State University; B. Longoria, Angelo State University*

Introduction: ABC4WT-CV is a CPRIT funded breast cancer prevention project serving 14 counties in the Concho Valley region of West Texas. The project recently completed a three-year funding period (2012-2015) to deliver prevention and diagnostic services to vulnerable, high-risk women in the Concho Valley. The program served 893 unduplicated clients over the three-year performance period. Of these, 539 (60.4%) received prevention screening services only and 354 (39.6%) received one or more diagnostic services. The diagnostic services detected 22 cancers yielding a detection rate of 6.2%. **Methods:** The ABC4WT-CV poster reports the results of a retrospective evaluation of prevention screening and diagnostic services provided over the completed three-year performance period. Two core hypotheses are tested. The first asserts that patient data collected prior to service by ABC4WT will reveal unduplicated patients who eventually received diagnostic services had significantly more indicators of cancer risk than patients who received only prevention screening. The second core hypothesis holds that the magnitude of cancer risk indicators was significantly higher among unduplicated patients who received diagnostic services than patients who received only prevention screening. Each core hypothesis, if confirmed by statistical testing, will constitute evidence that services provided by ABC4WT-CV over its 2012-2015 funding period were compliant with the Practice Parameter for the Performance of Screening and Diagnostic Mammography as amended by the American College of Radiology in 2014. **Results:** The independent groups t-test is used to test the core hypotheses. Cohen's d is used to assess the effect of cancer risk indicators in screening (prevention or diagnostic) decisions. **Conclusion:** It is expected that the outcome of the analysis described above will demonstrate compliance between ABC4WT-CV mammography services and the practice standard. It is further anticipated that comparison data with regional hospital services will reveal that CPRIT-sponsored ABC4WT-CV services meaningfully augmented the benefits of quality mammography services to uninsured or underinsured younger, Hispanic, and rural resident women in this region of Texas.

446 **CPRIT Grantee**
Poster Session B

A Su Salud Breast Health Program *R. Villarreal, University Health System; L. Meraz, University Health System; L. Fornos, University Health System; V. Mika, University Health System; E. Carlson, University of North Texas; D. Gordon, University Health System; M. Martinez, University Health System*

Introduction: Access to health care is a barrier for many Bexar County, Texas residents. Access to mammography services is particularly difficul for uninsured and underinsured women. These women arrive late to care, have a high incidence of breast cancer, late stage diagnoses, and higher mortality rates. Consequently, there is a great need for increased education about breast cancer highlighting the importance of regular screening and early detection, and how to navigate the Health System to obtain a mammogram. **Methods:** The A Su Salud Breast Health Program is a culturally competent, breast cancer education, social marketing, and services program. It includes small media (newsletters), mass media (television and radio Public Service Announcements), social media (Facebook, You Tube), and telephone educational reminders. Health promotion supports behavior change and reshapes what the target population sees as public health concerns. In addition, we provided two patient navigators to motivate women socially and emotionally, and remove specific barriers to screening. Navigation services help save lives, help patients overcome barriers, and are tailored to meet specific needs of the patient. Women who receive abnormal screening results are referred to patient navigators in the Women's and Preventive Health Department that help with biopsy appointments and treatment, if necessary. **Results:** From start of screening services on June 1, 2014 through June 30, 2015, 615 women completed screening appointments: 609 (99%) had normal results and 16 (1%) were identified with abnormal results. Through outreach and education we intend to reach over 100,000 community members while developing a sustainable breast cancer screening model. **Conclusion:** The A Su Salud Breast Health Program is tailored to Hispanic and women that are part of the high risk zip codes populations. Those who have never had a mammogram receive the most intensive intervention consisting of navigation, barrier assessment, and personal follow□ups to ensure appointments are scheduled and kept. This navigation program improves upon current services by: 1) increasing awareness of breast cancer and access to mammograms, 2) improving screening service coordination, and 3) reducing individual and system barriers to mammograms. University Health System is committed to sustaining this primary and secondary breast cancer prevention program. By serving an additional 6,800 women with mammograms and navigation services, we expect to see an improved mammography screening rate of 75%, a decreased incidence of breast cancer (due to early detection), and a reduction in breast cancer mortality for uninsured, minority women.

447 **CPRIT Grantee**
Poster Session A

Mobile Cancer Survivorship Care: Improving Surveillance and Quality of Life in Rural Communities *K. Argenbright, The University of Texas Southwestern Medical Center at Dallas; T. Mazour, UT Southwestern Moncrief Cancer Institute; B. Rose, UT Southwestern Moncrief Cancer Institute; K. Hatfield, UT Southwestern Moncrief Cancer Institute; E. Berry, UT Southwestern Moncrief Cancer Institute*

Introduction: Advances in early detection and treatment have increased the number of cancer survivors living in the United States. Many experience devastating physical, psychosocial, and economic effects of their cancer and treatment as they transition out of active treatment into extended survival. The underserved often face even greater challenges, including lower functional health, life changing financial burden, and disparities in treatment. These cancer survivors do not have the opportunity to receive post-treatment services, as evidence-based cancer survivorship programs are typically only found in large cancer centers and limited in scope. As a result, UT Southwestern Moncrief Cancer Institute (UTSW-MCI) established a community-based survivorship program with funding from the Cancer Prevention Research Institute of Texas (CPRIT PP110097). The program has since been expanded to include a nine county service area using innovation funds available through the 115 Waiver. The region covers over 7,000 square miles, with Fort Worth at the urban center and is surrounded by rural ranching communities. This program expansion provides access to multidisciplinary survivorship services and follow-up cancer screening and surveillance to the nearly 5,000 uninsured and Medicaid-enrolled cancer survivors within the region. **Methods:** Using a custom-built mobile health unit, USTW-MCI is delivering survivorship specialty care to Medicaid and uninsured populations in geographically remote areas. Like the in-house ambulatory clinic, the mobile unit is staffed with an oncology certified team to provide follow-up cancer screening and surveillance as well as the following services:

- Physical and overall needs assessment including cancer treatment history/survivorship care plan
- Evaluation of dietary behaviors, assistance choosing nutritious foods, and healthy lifestyle education
- Navigation to financial assistance, psychosocial evaluation an support, care coordination
- Consultations for survivors and families for psychosocial distress, anxiety and depression

• Recommendations for safe physical activity post-treatment, assessment for balance, immobility, range-of-motion, and injury prevention

Results: During the initial 18 months of the program expansion, 645 Medicaid and low-income uninsured and underinsured cancer survivors have enrolled and completed 2,706 clinical encounters. Baseline values have also been established for breast (28.93%) and colorectal cancer screening (20.28%) compliance, follow-up to clinical resolution (80%), and quality of life (66.09) measured using the Functional Assessment of Cancer Therapy – General (FACT-G) tool. **Conclusion:** The Mobile Cancer Survivor Clinic model addresses geographic and economic barriers that impede access to care to improve both the health of medically underserved cancer survivors and the experience of care, while reducing cost of care without compromising quality.

448 **CPRIT Grantee**
Poster Session B

Alliance for Colorectal Cancer Testing (ACT) in Southeast Texas

E. Furlan, The University of Texas M.D. Anderson Cancer Center; L. Foxhall, The University of Texas M.D. Anderson Cancer Center

Introduction: Colorectal cancer (CRC), the second leading cause of cancer deaths in the United States and the State of Texas, is curable if detected in its early stages, but often fatal when diagnosed later. Studies have shown that CRC screening (CRCS) for the detection and treatment of early cancer, as well as the removal of precursor adenomatous polyps, is effective in reducing CRC incidence and mortality. According to the Agency for Healthcare Research and Quality (AHRQ), only half of all adults aged 50-75 have ever received age-appropriate CRCS, and in minority populations, that number drops to 30%. The project will utilize an evidence-based approach to increase CRCS through clinical practice changes that engage primary care clinicians to discuss the importance of CRCS with patients. Practices will be encouraged to pair CRCS with the annual flu shot, an approach with demonstrated effectiveness (Flu FIT) as a Research Tested Intervention Program (RTIP). Our goal is to increase adherence to CRCS recommendations in CPRIT's priority populations served through primary care clinics, thus reducing colorectal incidence and mortality disparities. M**ethods:** We will implement the Alliance for Colorectal Cancer (ACT) Testing, a CRCS coalition involving MD Anderson and community clinics serving the RFA priority population. The coalition will support delivery of a program offering take-home fecal immunochemical tests (FIT) to CPRIT priority populations in north, east and southeast Texas. These priority populations include, but are not limited to, underinsured and uninsured individuals, those in rural areas, medically unserved or underserved, racial, ethnic, and cultural minority populations and those with low screening rates, high incidence rates and high mortality rates. **Results:** The project will partner with FQHCs and community clinics to distribute 5,000 FIT tests annually for three consecutive years covering 21 counties. We will achieve a 50% adherence rate to ACS/NCCRT screening guidelines in year one, with a 10% increase each subsequent year. We anticipate that with the distribution of 5,000 FIT tests, we should receive 3,500 stool specimens for processing, of which we estimate approximately 10% or 350 will be positive and be referred to colonoscopy. We will navigate patients with positive FIT outcomes for colonoscopy with gastroenterologists in the patients' local community. Those diagnosed with cancer will be navigated to treatment for which they qualify. C**onclusion:** This project could provide a means to improve adherence with CRCS recommendations by increasing awareness and utilization.

449 **CPRIT Grantee**
Poster Session A

Characteristics of HPV Vaccination Initiation and Completion Among 11-26 Year Olds at a Large Urban FQHC

S. Rodriguez, The University of Texas Health Science Center at Houston; L. Savas, The University of Texas Health Science Center at Houston; T. Megdal, Legacy Community Health Services; K. Bundage, Legacy Community Health Services; M. Khan, The University of Texas Health Science Center at Houston; M. Fernandez, The University of Texas Health Science Center at Houston

Introduction: Despite FDA and CDC recommendations, HPV vaccination initiation and 3-dose series completion remain below national targets. To increase vaccination among vulnerable populations in Houston, an academic-community collaborative effort is underway to develop, implement, and evaluate a multi-level HPV vaccination intervention. This presentation describes baseline characteristics and patterns of HPV vaccination initiation and series completion among 11-26 year old patients at 20 FQHC clinics. **Methods:** We examined medical record data to determine HPV vaccination initiation and series completion for patients ages 11-26 years between April 1, 2014 and March 31, 2015. We also collected data on missed opportunities for HPV vaccine-eligible patients who sought care at a clinic but did not receive the vaccine. Other data included: age, gender, race, ethnicity, insurance type, language, and meningococcal conjugate vaccine status. **Results:** We identified 9,445 patients aged 11-26. Among these, 11.2% were non-Hispanic white, 25.4% were black, and 58.8% were Hispanic. The majority (66.7%) used public insurance, 13.3% used private insurance, and 20% were self-pay. Among patients ages 11-18 years (n=6,178), 14.6% had initiated HPV vaccination, and 1.8% completed the vaccination series. Among 3,276 patients ages 19-26 years, 4.0% had initiated vaccination, and 0.6% had completed the series. Initiation at ages 11-12 was higher compared to ages 13-26 (22.7% vs. 8%); completion rates were low for both age groups (3.1% vs. 1.5%). Overall, initiation was higher among males compared to females (12.8% vs. 9.4%); completion rates were similarly low in both males and females (1.6% vs. 1.3%). Publicly insured patients had higher initiation compared to those with private insurance and self-pay patients (11.6% vs. 8.6% vs. 10.6% respectively); all groups had low completion (<2% each). **Conclusion:** These data illustrate extremely low levels of vaccination initiation and completion in an underinsured population. To increase HPV vaccination initiation and series completion rates this

CPRIT-funded multi-level intervention delivers patient-, provider-, and systems-level targeted strategies. The intervention includes two phases across twenty clinics in Harris and Jefferson Counties. Phase One delivers systems-level strategies including opt-out HPV vaccinations, standing orders, reduction of patient out-of-pocket costs, implementation of patient reminders, and provider reminders. Phase Two targets providers and patients including provider assessment and feedback, provider training, and patient education (clinic- and community-based). We will examine intervention effect by comparing post-intervention vaccination rates with baseline rates. The goal of this prevention project is to increase vaccination and provide evidence-based intervention approaches to guide future interventions to improve vaccination outcomes in other clinic systems.

450 **CPRIT Grantee**
Poster Session B

High rate of Adenomatous Polyps Detected in Rural East Texas *C. Allen, The University of Texas Health Science Center at Tyler; S. Tison, The University of Texas Health Science Center at Tyler; M. Ross, The University of Texas Health Science Center at Tyler; B. Olusola, The University of Texas Health Science Center at Tyler; J. Morrison, The University of Texas Health Science Center at Tyler; W. Sorensen, The University of Texas at Tyler; E. Sauter, The University of Texas Health Science Center at Tyler*

Introduction: Compared to Texas as a whole, East Texas has higher incidence and mortality rates from colorectal cancer (CRC). CRC screening rates in Texas are lower for individuals without compared to those with health insurance (6.8% vs. 9.1% for stool blood testing, 28.1% vs. 57.8% for endoscopy). Screening serves to: 1) prevent CRC by polypectomy, 2) find early-stage cancers, leading to treatment with a high chance for long term survival, and 3) identify families at increased risk. **Methods:** We engaged primary care clinicians to assist with recruitment to the program which empowers individuals in deciding which method of screening is best for them. Participants with a FIT test negative for blood or whose colonoscopy revealed no polyps or benign polyps were scheduled for follow-up as appropriate based on US Preventive Services Task Force guidelines. Participants with a FIT test positive for blood were scheduled for a colonoscopy. Participants with a colonoscopic biopsy demonstrating a precancerous polyp or cancer were scheduled for clinical follow-up and intervention, as appropriate, based on National Comprehensive Cancer Network guidelines. **Results:** The study has been recruiting participants for just over 6 months. Thus far we have reached 57,620 individuals and have served 2,239 individuals. We performed 937 CRC screenings (910 colonoscopies and 27 FIT screens). A total of 91% of the colonoscopies identified one or more lesion(s) requiring biopsy. A total of 336 (37%) participants who underwent biopsy were found to have one or more adenomatous polyps. Five (0.55%) were found to have CRC. Of the cancers diagnosed, there have been stages 2, 3, and 4 identified There have been 80 (9%) normal colonoscopies completed thus far. For our population, a disproportionate number of patients have opted for a colonoscopic examination versus FIT. **Conclusion:** Most individuals who underwent colonoscopy had one or more lesions identified that prompted a biopsy. More than a third of individuals were found to have a precancerous lesion compared to a 10% frequency among participants in another CPRIT funded CRC screening project and a national frequency that generally ranges from 15-25%. The high rate of precancerous lesions identified on routine screening correlates with the high incidence of CRC in East Texas. Recent reports document that a higher adenoma detection rate correlates with lower long term mortality from CRC. Our program has been successful in identifying precancerous lesions and CRC in uninsured and underinsured individuals in East Texas.

451 **CPRIT Grantee**
Poster Session A

Initiation of a Human Papillomavirus Vaccination Program among Young Postpartum Women *A. Berenson, The University of Texas Medical Branch at Galveston; M. Rahman, The University of Texas Medical Branch at Galveston; J. Hirth, The University of Texas Medical Branch at Galveston; R. Rupp, The University of Texas Medical Branch at Galveston; K. Sarpong, The University of Texas Medical Branch at Galveston*

Introduction: Human papillomavirus (HPV) vaccine uptake is low among US adult women compared to other adult vaccines. To improve this situation in Texas, we initiated a HPV vaccination program which offered initial and follow-up doses free of charge to postpartum patients from Galveston County. The program's effectiveness was evaluated by examining rates of HPV vaccine initiation (≥1 dose) and completion (3-dose) among eligible women approached and offered HPV vaccination postpartum before and after the program was initiated along with barriers encountered. **Methods:** Women ≤26 years of age who delivered a liveborn infant at a public hospital in Galveston, Texas, were approached/counseled about HPV vaccination between November 2012 and June 2014. Those who had not completed the HPV vaccine series were offered the vaccine postpartum. In addition, HPV vaccine initiators were assisted with scheduling follow-up injections which were coordinated with postpartum or well-child visits. Medical charts of participants were reviewed to extract socio-demographic information. **Results:** Overall, 1,039 women ≤26 years of age from Galveston County were approached on the postpartum unit regarding HPV vaccination. Among them, only 161 (15.5%) had completed the 3-dose series prior to this pregnancy. Of the remaining 878, 575 (65.5%) initiated the vaccine postpartum, 86 (9.8%) received their 2nd or 3rd injection postpartum, 193 declined (22.0%) and 9 women were ineligible for other reasons. Overall, 75.8% (436/575) of new initiators completed the series by April 2015. This resulted in an improvement from baseline initiation rates of 25.4% to 80.8% and baseline completion rates of 15.5% to 65.1% among eligible women who delivered during this time period. Ethnic differences were noted with Hispanic women more likely to both initiate (odds ratio [OR] 2.33, 95% confidence interval [CI], 1.50-3.61) and complete (OR 2.15, 95% CI 1.47-3.14) the vaccine compared to non-Hispanic white women. Uptake of influenza vaccine during the same pregnancy also predicted both vaccine initiation (OR 1.88, 95% CI 1.30-2.71) and completion (OR

1.74, 95% CI 1.27-2.37) while those 21-26 years old were more likely than those ≤20 years to complete the series (OR 1.60, 95% CI 1.09-2.37). Reasons cited by women who declined vaccination (n=193) included "not interested" (60.6%), "need to talk to my primary physician" (12.4%), "married and not needed" (6.7%), "afraid of needles/painful" (6.2%) and "against any vaccine" (5.2%). C**onclusion:** HPV vaccine uptake rates can be substantially increased among young adult women if they are offered the vaccine postpartum and subsequently tracked and supported for the follow-up injections.

452 **CPRIT Grantee**
Poster Session B

Media-Rich Mobile Dissemination of a Dysphagia Prevention Program for Head and Neck Cancer Patients during Radiation
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Introduction: While cancer of the throat is highly curable, 39% of survivors experience serious permanent swallowing problems such as silent aspiration, strictures, or dependence on tube feeding. To prevent dysphagia, targeted swallowing exercises performed during radiation have been shown to be an effective in preventing radiation-induced fibrosis of the swallowing musculature. Head and neck cancer patients at the Texas Health Care Otolaryngology & Facial Plastic Surgery Associates in Fort Worth will receive specialized speech pathology services. In addition, because past research has shown that patients find the preventive swallowing exercises to be extraordinarily difficult due to significant side effects from radiation, we will also provide an effective adherence intervention program (called "PREPARE") via a mobile health technology application. **Setting:** Approximately 700 nonrecurrent pharyngeal or laryngeal patients are seen per year at this specialty community practice serving a 15-county radius; 12-15% of the patients seen at this high-volume oncologic and reconstructive surgical practice are uninsured and an additional 25% have Medicaid or Medicare insurance. **Methods:** The proposed prevention program will provide in-person speech pathology services at the proposed site. To address nonadherence, we will deliver a 10-session weekly behavioral program which provides timely coping strategies, practical side-effect information, and psychological skills training during radiation and during the four week post-radiation period. **Innovation:** We are developing the software using *GuideVue*, a mobile interactive medical software technology to deliver the behavioral adherence program. Once downloaded onto a user's smartphone (Android, iPhone compatible), GuideVue software can be accessed and executed at any time, even without internet access. Thus, the patient is able to reliably and conveniently access high-quality program images, audios, and video content from *PREPARE*. **Results:** *PREPARE* has been previously tested with 266 nonrecurrent late-stage pharyngeal and laryngeal cancer patients undergoing radiation to the throat. Compared to age-, sex-, and tumor-size matched controls, patients who underwent *PREPARE* were more likely to self-report adherence to swallowing exercises (p=.0017) and were more likely to report being able to eat a normal diet 2 years after radiation (p=.04). Pilot testing of the *PREPARE* app with 20 head and neck cancer survivors demonstrated overwhelmingly positive patient response. **Conclusion:** We believe that the proposed program will significantly decrease the likelihood of devastating long-term dysphagia in Texan head and neck cancer survivors.

453 **CPRIT Grantee**
Poster Session A

County-Level Outcomes of Rural Community Partnerships to Increase Breast Cancer Screening and Patient Navigation: A Decentralized Hub-and-Spoke Model (BSPAN2)
S. Lee, The University of Texas Southwestern Medical Center at Dallas; R. Higashi, The University of Texas Southwestern Medical Center at Dallas; S .Inrig, Mount St. Mary's University; J. Sanders, The University of Texas Southwestern Medical Center at Dallas; K. Argenbright, The University of Texas Southwestern Medical Center at Dallas; J. Tiro, The University of Texas Southwestern Medical Center at Dallas

Introduction: Rural breast cancer screening rates remain suboptimal despite programs such as NBCCEDP, which provides funding for no-cost mammography to uninsured women. Virtual clinical provider networks may be effective in overcoming limited rural infrastructure, but little is known about how to best implement patient navigation in rural healthcare settings. **Methods:** We conducted a mixed-methods evaluation of the program using EMR-driven service delivery data and qualitative data from interviews and site visits with patients and rural community partners. We sampled six of the rural counties for in-depth qualitative data collection, including 73 interviews at 51 organizations and brief surveys with 92 program participants, leading to 30 additional interviews. **Results:** The hub-and-spoke strategy succeeded in increasing comprehensive screening services among the target population of uninsured rural-residing women. For the two-year evaluation period, the program screened 4,780 unique women, diverse in age, race/ethnicity, poverty level, insurance, and screening status, across 17 rural counties. Quantitative data indicates that counties in which local "spoke" partners led outreach activities achieved comparable levels of screening to those in which the "hub" managed outreach. Qualitative findings demonstrate that greater county infrastructure did not necessarily correlate with enhanced ability to implement the program; we describe local contextual factors that facilitated and constrained outreach. **Conclusion:** Leveraging rural county resources to conduct outreach is a feasible approach for expanding programs that deliver health services to rural areas. However, increasing expansion may be limited by the hub's capacity to act on behalf of rural counties that lack the infrastructure or willingness to implement outreach and patient navigation. Using mixed-methods to assess program expansion advances implementation science by providing insight into contextual influences that contribute to program adoption and uptake.

Subsequent analyses of the program hub should assess the impact of regional expansion on service volume to better understand strengths and limitations of this de-centralized model.

454 **CPRIT Grantee**
Poster Session B

De Casa En Casa: Preventing Cervical Cancer in El Paso County and Hudspeth County *N. Shokar, Texas Tech University Health Science Center at El Paso; T. Byrd, Texas Tech University Health Sciences Center; E. Penaranda, Texas Tech University Health Science Center at El Paso; J. Molokwu, Texas Tech University Health Science Center at El Paso; S. Flores, Texas Tech University Health Science Center at El Paso; A. Franck, Texas Tech University Health Science Center at El Paso; J. Calderon-Mora, Texas Tech University Health Science Center at El Paso; A. Dwivedi, Texas Tech University Health Science Center at El Paso*

Introduction: Women on the US-Mexico border have a higher cervical cancer incidence rate, are diagnosed at later stages, and have higher mortality compared to non-Border women in the US. In addition, Hispanic women have almost double the incidence of all race/ethnicities and are twice as likely to die from cervical cancer compared to non-Hispanic women. We identified key barriers to screening through an analysis of local data, key informant interviews and focus groups and have designed a program that addresses the needs of our community, addresses gaps in services, and creates a coordinated program of education, outreach, service delivery, navigation and capacity building for the future. M**ethods:** A multicomponent, culturally tailored, bilingual, evidence-based cervical cancer screening program was developed at Texas Tech University Health Sciences Center (TTUHSC)- El Paso, Paul L Foster School of Medicine. The program is being implemented in two Border counties (El Paso and Hudspeth County), in partnership with multiple community and university partners. Key program components are: 1) theory-based and culturally tailored cervical cancer education delivered by bilingual, certified promotoras; 2) Provision of no-cost pap and HPV screening to eligible women; 3) On-site diagnostic and treatment colposcopy; 4) Patient navigation and tracking to facilitate screening, diagnosis, health insurance coverage, access to a PCP, and treatment, and 5) Enhanced resident and faculty colposcopy training to increase colposcopy capacity. A rigorous program evaluation is in process. **Results:** As of July 17, 2015, 1,043 women were recruited into the program and offered services. 855 women were eligible for screening: mean age 45.8 years. 4.7% (n=40) had never had a pap smear and 42.2 % (n=361) last received a pap over 5 years previously. So far, screening uptake is 71% (n=605); 4.0% (n=24) of screening tests required follow up with colposcopy, and 96% (n=23) have been completed so far. We have had 5.0% (n=30) positive for high risk HPV and one cancer has been diagnosed. As part of the evaluation, 301 control group surveys and 160 intervention group surveys have been completed. C**onclusion:** A comprehensive cervical cancer screening program can achieve significant screening uptake rates in a high risk population with historically low screening uptake and has the potential to significantly impact cervical cancer incidence and mortality in this border region.

455 **CPRIT Grantee**
Poster Session A

The El Paso and Hudspeth County Breast Cancer Education, Screening and Navigation Program (BEST) *N. Shokar, Texas Tech University Health Science Center at El Paso; C. Martin, Texas Tech University Health Science Center at El Paso; S. Flores, Texas Tech University Health Science Center at El Paso; T. Byrd, Texas Tech University Health Sciences Center; A. Alomari, Texas Tech University Health Science Center at El Paso; J. Gupta, Texas Tech University Health Science Center at El Paso; R. Salaiz, Texas Tech University Health Science Center at El Paso*

Introduction: Breast cancer is the leading cause of cancer among women in the US (excluding non-melanotic skin cancers), is the second commonest cause of cancer death in women, and is the leading cause of cancer death among Hispanic women. Rates of late stage diagnosis and mortality are higher among Hispanic women on the border, compared to Hispanics elsewhere in the US. Screening for breast cancer is widely recommended, yet rates among border women are low. We sought to address this through the development of a program that targets barriers to screening identified from analysis of local data, key informant interviews and focus groups. **Methods:** We developed a culturally tailored, bilingual evidence-based breast cancer screening program that is led by Texas Tech University Health Sciences Center, El Paso, Paul L Foster School of Medicine and is being implemented in El Paso County and Hudspeth County, two border counties in far west Texas. Program components include: 1) Community education by bilingual, certified promotoras delivering theory-based and culturally tailored group breast cancer education; 2) Outreach through targeted media and a network of geographically dispersed community partners serving El Paso and Hudspeth Counties; 3) Provision of no-cost mammography and diagnostic testing to eligible women; 4) Creation of an enhanced access mammography network for program participants; 5) Patient navigation to facilitate screening, diagnosis, health insurance coverage, access to a PCP, and treatment; and, 6) A rigorous process and outcomes evaluation. **Results:** As of July 17, 2015, 901 women were recruited into the BEST program and offered services. 814 women were eligible for screening: mean age 57.2 years; 11.5% (n=94) had never had a mammogram, and 53.0% (n=430) had a mammogram greater than 3 years previously. To date, 85.0% (n=689) have completed screening and 16.0% (n=110) of those completing a screening mammogram required further testing. Of

170 indicated tests, 91.2% (n=155) have been completed. 3.6% (n=4) of women requiring follow up testing have had cancers diagnosed and all have been navigated into treatment. For the program evaluation, 301 control group surveys and 205 intervention group surveys have been completed. **Conclusion:** A comprehensive breast cancer screening and diagnosis program has achieved a high screening completion rate in a Border population with low prior test completion rates and has the potential to significantly impact breast cancer outcomes in a border popu ation.

456 **CPRIT Grantee**
Poster Session B

Against Colorectal Cancer In Our Neighborhoods (ACCION) Outcomes *N. Shokar, Texas Tech University Health Science Center at El Paso; T. Byrd, Texas Tech University Health Sciences Center; R. Salaiz, Texas Tech University Health Science Center at El Paso; J. Calderon-Mora, Texas Tech University Health Science Center at El Paso; S. Flores, Texas Tech University Health Science Center at El Paso; M. Chaparro, Texas Tech University Health Science Center at El Paso; M. Ortiz, Texas Tech University Health Science Center at El Paso; A. Dwivedi, Texas Tech University Health Science Center at El Paso*

Introduction: In the US, colorectal cancer (CRC) screening is universally endorsed, yet screening rates remain relatively low, particularly among the poor, the uninsured, recent immigrants and Hispanics. In order to address this disparity, we developed, implemented and evaluated a bilingual comprehensive, theory-based CRC screening intervention in a predominantly Hispanic county with low prior screening. **Methods:** Design: Prospective intervention. Setting: Over 150 community and clinical sites throughout the County. Participants: 50-75 year old uninsured Texas residents due for screening. Exclusion criteria: past history of CRC. Intervention: Theory-based, multi-level, bilingual, culturally tailored intervention delivered by community health workers. Components of the intervention included outreach, theory-based and culturally tailored education, no-cost screening and diagnosis, and navigation services. Average risk individuals qualified for screening with the fecal immunochemical test: above average risk individuals and those with a positive FIT qualified for no-cost colonoscopy. Navigation services included tracking, reminders, scheduling and case management for cancer treatment. Analysis: qualitative and quantitative evaluation of process measures and outcomes. **Results:** 8,312 eligible participants were recruited. Overall screening completion was 74.9%. 5991/7986 (75%) completed the FIT test; the FIT positive rate was 6.1%; 68.1% (222/326) completed screening colonoscopy and 79.2% (290/367) completed a diagnostic colonoscopy. 164 patients were diagnosed with adenomatous polyps, 10 with colorectal cancer, 1 with anal cancer and 3 with carcinoid tumors. All patients were navigated into treatment. Process evaluation measures indicated high intervention fidelity and participant satisfaction. Bivariate predictors of CRC screening uptake were female gender, married/ living with a partner, clinic recruitment, higher income, higher education, prior awareness of CRC , having a regular doctor, prior

CRC screening and receiving a doctor recommendation (all P<0.0001, except for education -p<0.05). **Conclusion:** The ACCION program was successfully implemented within a Hispanic population and is currently being implemented in 20 other Texas Counties.

457 **CPRIT Grantee**
Poster Session A

Tiempo de Vacunarte - Time to get vaccinated!: Cervical cancer prevention program through HPV immunization in El Paso County *E. Penaranda, Texas Tech University Health Science Center at El Paso; J. Molokwu, Texas Tech University Health Science Center at El Paso; N. Shokar, Texas Tech University Health Science Center at El Paso; A. Dwivedi, Texas Tech University Health Science Center at El Paso; M. Reyes, Texas Tech University Health Science Center at El Paso*

Introduction: El Paso is situated on the US-Mexico border and the majority of its population is Hispanic of Mexican descent and has a high poverty rate, low educational attainment and a high proportion of residents without health insurance. Women on the US-Mexico border have one of the highest cervical cancer incidence and mortality rates in the country. Cervical cancer is caused by a persistent infection with oncogenic genotypes of the Human Papillomavirus (HPV) which is a very ubiquitous virus usually acquired during sexual activity. Cervical cancer is preventable through screening and through immunization against the most oncogenic types of HPV. Three vaccines have been approved in the US for males and females aged 9 – 26 years in three doses (0, 2 and 6 months). Despite their proven efficac , rates of initiation and completion of the immunization series have been low across all racial/ethnic groups. Main barriers to immunization uptake are lack of knowledge, lack of physician recommendation of the vaccine, lack of health insurance/ access to care. " Tiempo de Vacunarte – Time to get vaccinated" is a multicomponent evidence-based program designed to reduce cervical cancer burden among a predominantly Hispanic, low-income population in El Paso County. **Methods:** We aim to provide education to over 5,000 individuals and to administer over 1,650 3-dose vaccine series. Key program components are: 1.) Outreach and community- based recruitment to primarily reach individuals with access to care barriers, 2.) evidence-based educational intervention 3.) navigation services provided by a bilingual navigator, 4.) physician education and introduction of prompts to remind physicians to recommend the vaccine, 5.) provision of no-cost HPV vaccine, 6) A thorough evaluation of process and outcomes to facilitate replication. **Results:** We are currently in the first year of the grant and have developed the educational intervention, the e protocols for the vaccine, all evaluation materials and have hired a program coordinator, a certified medical assistant/navigator and three outreach

workers or promotoras. Recruitment began in July 2015. So far we have approached 919 individuals and have provided education to 83 and have administered 23 first –dose vaccines (16 children, 7 adults, 13 males, and 10 females). **Conclusion:** A comprehensive program that addresses key barriers to HPV vaccination uptake in a population at higher risk for cervical cancer has the potential to decrease the burden of this disease in the intermediate and long term.

458 **CPRIT Grantee**
Poster Session B

The Community Network for Cancer Prevention: Developing a Multi-Level Intervention to Improve Cancer Screening and Follow-Up among the Medically Underserved *M. Jibaja-Weiss, Baylor College of Medicine; J. Montealegre, Baylor College of Medicine; L . Hanser, Harris Health System; M. Daheri, Harris Health System; R. Chenier, Baylor College of Medicine; I. Valverde, Baylor College of Medicine; G. Chauca, Baylor College of Medicine; L. Rustveld, Baylor College of Medicine; M .Anderson, Baylor College of Medicine; L .Ramondetta, The University of Texas M.D. Anderson Cancer Center; M. Gould-Suarez, Baylor College of Medicine; B. Musher, Baylor College of Medicine; L. Scott, The University of Texas Health Science Center at Houston; J. Nangia, Baylor College of Medicine; B. Reed, Baylor College of Medicine; J. Hoagland-Sorensen, Harris Health System; A. Rieber, The University of Texas M.D. Anderson Cancer Center*

Introduction: Screening for cervical, colorectal, and breast cancer is an evidence-based strategy to reduce the morbidity and mortality from these cancers. However a large proportion of medically underserved individuals do not obtain regular screening. Using the Quality in the Continuum of Cancer Care (QCCC) framework, we developed and implemented a comprehensive systems design intervention to improve the delivery, uptake, and follow-up of cervical, colorectal, and breast cancer screening within a network of healthcare institutions that serve the medically underserved in Harris County, Texas. **Methods:** An academic-community partnership (the Community Network for Cancer Prevention, CPRIT grants PP100201, PP130084, PP140028) was established between the Dan L. Duncan Cancer Center, the Harris Health System (the county's safety net health provider), and several academic and community-based healthcare institutions. Clinical advisory boards, comprised of physicians, nurses, and public health professionals, were established for each cancer line. The QCCC framework was used to identify system-level failures that impede processes and transitions in the continuum of care from risk assessment to detection and from detection to diagnosis. Project components were developed to address the identified failures. **Results:** System failures identified at the risk assessment to detection phases included 1) failure to identify individuals in need of screening and inadequate capacity to screen, 2) inadequate access to care. Failures identified at the detection to diagnosis phases included 1) failures in the notification system, 2) failures in inter-provider communication, 3) failures in inter-institutional referrals, 4) patient non-adherence, 5) inadequate access to care. Project components to address the identified failures include community outreach, patient education, and patient navigation. Community outreach involves a community theater program aimed to increase awareness of cancer risk and cancer screening guidelines among the medically underserved and facilitate access to healthcare services. Patient education involves using the electronic medical record to identify patients due or past due for screening and targeting them for video-delivered education at the point of care. Patient navigation involves the development of a tracking database of patients with abnormal screening tests and a team of navigators who actively communicate with patients and providers. **Conclusion:** The QCCC provides a systematic approach for assessing factors that influence cancer care processes at the risk assessment, screening, detection, and diagnosis phases, as well as transitions between them. Focusing on transitions between phases is particularly effective for developing systems-level interventions to improve the delivery, uptake, and follow-up of cancer screening.

459 **CPRIT Grantee**

Colorectal Cancer Screening Using Organized Outreach to Reach an Underserved Population *K. Argenbright, The University of Texas Southwestern Medical Center at Dallas; S. Gupta, Moores Cancer Center University of California San Diego; S. Miller, John Peter Smith Health Network; B. Balasubramanian, The University of Texas Health Science Center at Houston; M. Koch, John Peter Smith Health Network; E. Carter, John Peter Smith Health Network; P. Anderson, The University of Texas Southwestern Medical Center at Dallas; E. Berry, UT Southwestern Moncrief Cancer Institute*

Introduction: Screening reduces colorectal cancer (CRC) incidence and mortality, but participation is low among underserved populations, resulting in advanced stage at diagnosis and poor outcomes. Through a previous randomized controlled trial, this program demonstrated system-level mailed outreach invitation to use and return an enclosed fecal immunochemical test (FIT) was more effective than both mailed invitation to complete colonoscopy, and usual visit-based screening offers in obtaining screening completion among an uninsured, racially/ethnically diverse population served by the safety-net health system for Tarrant County and Fort Worth, Texas (PP100039). This intervention was scaled up to offer FIT outreach to all individuals not up-to-date with screening served by the health system (PP120229). **Methods:** Uninsured individuals age 50 to 64 not up-to-date with screening are identifie using system-level electronic health record (EHR) data. Outreach efforts include: 1) a 1-page informational invitation letter in English and Spanish, 2) a FIT kit, 3) 2 automatic and up to 2 "live" reminder phone calls, and 4) telephone-based navigation to promote colonoscopy completion after positive FIT. The screening outreach team is based at the health system and includes a nurse and two medical assistants. **Results:** In year one, 8,565 individuals not up-to-date were identified within the safety-net system and invited to screening through five rounds of invitations; 61.6% were female; 37.4% white, 24.2% black, 29.1% Hispanic, 1.9% Asian, and 7.5% other race/ethnicity. The overall screening participation rate in response to the intervention was 36.0%; 30.6% for whites, 41.6% for blacks, 44.11% for Hispanics, 47.7% for Asians, and 38.6% for others. Four-hundred twenty-seven of 3,124 FIT returners (13.6%) had an abnormal FIT. 318 abnormal FIT patients have been scheduled, and 243 have completed diagnostic colonoscopy. To date, 7 individuals with CRC, and 67 with >1 adenoma(s) have been diagnosed. Factors challenging program implementation have included hiring and training of new JPS

staff to support scale up, determining electronic medical record data fields to identify patients not up-to-date with screening, and increasing health system capacity to perform timely colonoscopies after positive FIT. **Conclusion:** Having successfully implemented an organized, system-based mailed FIT outreach program to promote CRC screening in an underserved population within a closed system, the project team will begin transitioning the program to expand screening efforts to include a 20 county service area with additional CPRIT funding (PP150061). The project team will evaluate the challenges to program effectiveness associated with this transition, including staffing, EMR data mining, and expanding colonoscopy capacity.

460 **CPRIT Grantee**
Poster Session B

Expanding Access to Colorectal Cancer Screening Services: A Case for Primary Care Endoscopy *D. McClellan, Texas A&M University System Health Science Center; R. Pope, Texas A&M University System Health Science Center; J. Simmons, Texas A&M University System Health Science Center; C. Ojinnaka, Texas A&M University System Health Science Center; J. Bolin, Texas A&M University System Health Science Center; J. Helduser, Texas A&M University System Health Science Center; K. Fuller, Texas A&M University System Health Science Center; A. Richardson, Texas A&M University System Health Science Center; M. Ory, Texas A&M University System Health Science Center*

Introduction: The Texas A&M Cancer Screening, Training, Education and Prevention program (Texas C-STEP) was originally funded in August, 2011, to address barriers to colorectal cancer (CRC) screening, while concurrently increasing the number of family physicians trained in colonoscopy. The project enhanced the ability of the Texas A&M Physicians Family Medicine Center, clinical home to a family medicine residency program, to provide accessible, affordable, culturally relevant screenings to uninsured/underinsured area residents using a community health worker model (CHW) for outreach and education. The purpose of this paper is to summarize findings from the 42-month project, including quality indicators from colonoscopies performed as part of this CRC screening program targeting uninsured low-income individuals. M**ethods:** Our outreach population were residents of 7 counties, with 6 of 7 counties defined as primarily rural and several with CRC incidence and mortality rates higher than Texas' state averages. Deidentifie data for colonoscopies performed between 09/2011 and 02/2015 were analyzed including: personal/family health history, demographics, and quality indicators such as cecal intubation rate, adenoma detection rate, and withdrawal time. Contingency tables for select patient characteristics by rural/urban residence were analyzed using chi-square or Fisher's exact tests for categorical variables and T-test for age, a continuous variable. Due to insufficient numbers, race/ethnicity categories were condensed as White, Black/African American, Hispanic/Latino, and Other. Statistical significance was established as $P<0.05$. **Results:** Over a 42-month period, 1,225 individuals received 1,285 colonoscopies, with 922 procedures subsidized by the prevention grant from the Cancer Prevention & Research Institute of Texas. Thirty-six percent of individuals were rural residents. Females comprised 72% of participants; males 28%. Sixty-five percent of participants self-reported race/ethnicity as Hispanic/Latino (44%) or Black/African American (21%). Seventy-one percent of colonoscopies warranted pathology; 74% had precursors detected and 11 cancers were found. Cecal intubation rate was 96.25%. Adenoma detection rates among males and females >50 years old were 38.15% and 25.96%, respectively. Among those who had a family history of CRC and who were also >age 50, compared to rural residents, urban residents were more likely to report having a previous CRC screening using colonoscopy. Twelve CHWs became state-certified under this grant, while 46 family medicine residents received training in colonoscopy via didactics, simulation, and hands-on experiences. **Conclusion:** Primary care physicians performing colonoscopies met and exceeded recommended quality indicators set forth by the American Society for Gastrointestinal Endoscopy, while expanding access to colonoscopy screening for rural, low-income individuals.

461 **CPRIT Grantee**
Poster Session A

Impacts of Targeted Population Screening Program Implementation on a Cancer Genetics Clinic *J. Huang, The University of Texas Southwestern Medical Center at Dallas; L. Kiedrowski, Pennsylvania Hospital; K. Argenbright, The University of Texas Southwestern Medical Center at Dallas*

Introduction: There has been a recent public call for broader screening for hereditary cancer. Our cancer genetics program implemented a targeted screening process to identify additional individuals at increased risk for hereditary cancer. We hypothesized that implementing this screening program would significantly increase patient volume, the number of genetic tests ordered and the number of mutations detected. **Methods:** A family history-based screening tool developed by the Centers for Disease Control and Prevention (CDC) was modified and used to identify high-risk patients receiving mammograms starting in October 2011. Our cancer genetics department staffs both private clinics (insured) and public hospitals (underserved). Since 2011, >35,000 insured and >62,000 underserved patients were screened, with those identified as high-risk being navigated to the cancer genetics clinic. The numbers of patients seen, tests ordered, and mutations detected were tracked across all sites from 2007-2014 to compare pre- and post-screening implementation. **Results:** The number of patients seen almost doubled after implementing population screening (1401 in 2011 vs. 2679 in 2014), as did the proportion of patients tested (68% vs. 80%, on average). The mutation identificatio rate across all clinics decreased, with a pre-screening program average rate of 16% versus a post-screening program average rate of 12%. These effects are observed more clearly in the underserved population. In this group, total patients seen increased over 3-fold (279 vs. 893). On average, proportion of patients tested increased after program implementation (68% vs. 78%), and mutation identification rate decreased (18% vs. 9%). **Conclusion:** Our population screening program substantially increased clinic volume and testing volume but decreased the mutation positive rate. Implementing this type of screening program will increase the number of high-risk patients receiving genetic counseling services but also likely requires the addition of both genetic counselors and ancillary staff to take on the significant increase in volume. These are important considerations for any institution designing or expanding a cancer genetics program.

462 **CPRIT Grantee Poster Session B**

Cervical Cancer Secondary Prevention and Services - A Su Salud Pap Test Program *R. Villarreal, University Health System; E. Carlson, University of North Texas; V. Mika, University Health System; L .Meraz, University Health System; L. Fornos, University Health System; T. Khalfan Mendez, University Health System; D. Winnier, University Health System*

Introduction: University Health System is a safety-net health system serving Bexar County, Texas since 1955. A Su Salud (To your Health) Cervical Cancer Screening and HPV Vaccine Program began in 2012, providing preventive services to underinsured and uninsured individuals. The cervical cancer component targets high-risk minority women, 21-64 years old to reduce the impact of cervical cancer among this group. The HPV target population, comprised of males and females ages 9-26, was designed to assist this group to either start or complete the three dose vaccination series. To achieve these goals, we developed a culturally competent, evidence-based program using the A Su Salud disease prevention model. **Methods:** We employed Patient Navigators (PN) to contact the target population, explain the program and available services, schedule their appointments at our clinics, and provide reminder calls. The Cervical PN ensured patients with abnormal results received follow-up care. The HPV PN verified that individuals completed the HPV vaccine series. PN assisted in removing barriers to receiving preventive care by being a contact person for the patient and providing access to appointments. Tailored media messages encouraged women to get their Pap test while other messages targeted males and females to receive the HPV vaccines. These media messages consisted of bilingual newsletters, newspaper ads, radio and television PSAs, billboards, a bus shelter ads, a Facebook page and a website. Media material prompted women to call "Claudia." Claudia is a pseudonym used by multiple bilingual staff and symbolizes the Mexican American cultural value of personalized social communication, or personalismo. **Results:** To date, we have completed over 26,000 Pap tests. Of those 4,407 women were navigated to their well-woman exam. We detected 201 abnormal results; of those 21 women had late stage cancer. All were referred for treatment. Without this program, these underinsured or uninsured women would not have received services. We administered over 1,000 HPV vaccine doses to males and females and navigated over 1,800 individuals to an appointment. **Conclusion:** We improved access to care by working with Health System providers and clinics to enhance the delivery of healthcare services. After conducting 16 in-depth interviews with program staff our external evaluator concluded the majority of those interviewed felt the program was effective because it provided an entry point into the Health System. Program staff hoped to use their skills to implement the program in a manner that improved patients' lives. Overall results of our program will be available later this year.

463 **CPRIT Grantee Poster Session A**

Increasing HPV Vaccination in a Network of Pediatric Clinics in Houston, TX: A Baseline Assessment *E. Lipizzi, The University of Texas Health Science Center at Houston; L. Savas, The University of Texas Health Science Center at Houston; K. Polivka, The University of Texas Health Science Center at Houston; S. Coan, The University of Texas Health Science Center at Houston; R. Shegog, The University of Texas Health Science Center at Houston; M. Fernandez, The University of Texas Health Science Center at Houston; C. Healy, Baylor College of Medicine; S. Spinner, Texas Children's Pediatrics; C. Miller, Texas Children's Pediatrics; S. Vernon, The University of Texas Health Science Center at Houston*

Introduction: Despite evidence of safety and efficac , and endorsement by professional organizations, HPV vaccination rates remain low. We are developing a suite of evidence-based interventions to increase initiation and completion of HPV vaccination among male and female patients in a pediatric clinic network with 50 clinics in the greater Houston area. A primary emphasis of our program will be encouraging initiation of the HPV vaccine series among younger adolescents, because the first dose is recommended to be given at ages 11-12. Here, we report a baseline assessment of HPV vaccination coverage in these clinics. **Methods:** Using electronic health records, we assessed initiation and completion of the HPV vaccine series among patients aged 11-17 years who visited the clinics from May 1, 2014 to April 30, 2015. Demographic characteristics of these patients were also collected. **Results:** Among 101,669 patients aged 11-17 years, 38,068 (37.4%) had initiated before the baseline visit date and 19,212 (18.9%) had completed the HPV vaccine series before the baseline visit. For ages 11-12, rates of new initiation during the baseline year varied widely among the 50 clinics (range 0% to 66.6%). New completion rates during the baseline year among those ages 11-12 who were eligible for the 3rd HPV vaccine (received the 2nd HPV vaccination at least 12 weeks before the visit date and had not completed the HPV vaccine series before the baseline visit) ranged from 60% to 100%. Among those with no previous HPV vaccinations, patients were more likely to initiate at ages 11-12 years compared to ages 13-17 years (28.5% vs 19.5%). Females were more likely to initiate at ages 11-12 compared to males (30.6% vs 26.8%); and patients with public insurance were more likely to initiate at ages 11-12 compared to those with private insurance (41.0% vs 24.9%). Completion at ages 11-12 was higher among females compared to males (78.7% vs 74.9%); but completion among patients with public insurance was lower compared to those with private insurance (71.2% vs 79.0%). **Conclusion:** Baseline data suggest the need for targeted efforts to improve initiation and completion of the HPV vaccine series among adolescents in a large pediatric clinic network. Our program will employ strategies shown to be effective at increasing vaccination rates in children and adults, such as provider assessment and feedback, provider reminders during clinic encounters, and patient reminder systems. It will extend the evidence on effectiveness of these strategies to HPV vaccination.

464 **CPRIT Grantee**
Poster Session B

How Good is Community Outreach? Perceptions of The Most Important Stakeholders – The Patients! *E. Wischkaemper, Texas Tech University Health Science Center at Amarillo; P. Siwawa, Texas Tech University Health Science Center at Amarillo; S. Felts, Texas Tech University Health Science Center at Amarillo; J. Pettiford, Texas Tech University Health Science Center at Amarillo; R. Layequr Rahman, Texas Tech University Health Science Center at Amarillo*

Introduction: Texas Panhandle (COG-1) is largely comprised of medically underserved communities facing ethnic (specifically Hispanic), socio-economic and geographic barriers comprising access to healthcare. Women have a specific disadvantage as seen in higher rate of breast cancer mortality, and higher cervical cancer incidence and mortality. Addressing access barriers for this population can be difficult particularly in reference to the patient perception of interaction with the healthcare system. Access to Breast and Cervical Care for West Texas (ABC24WT) project leverages the Cancer Prevention and Research Institute of Texas (CPRIT) funding for screening to provide free consults funded by the Delivery of Services Reform Incentive Plan (DSRIP) for this underserved population. This study reports the patient perception of healthcare access as measured by the validated PSQ-18 survey tool. **Methods:** ABC24WT project patients that were seen in the no-cost specialty consult clinic were given the PSQ-18 survey after each appointment. The survey evaluates the following domains: General Satisfaction, Technical Quality, Interpersonal Manner, Communication, Financial Aspects, Time Spent with Doctor, and Accessibility and Convenience. Each item is scored from 1 to 5 (1 Strongly Agree to 5, Strongly Disagree). These surveys are uploaded in the pre-designed database which is programmed to calculate patient satisfaction for each of the seven domains. **Results:** During the baseline period (April to September 2014) there were 146 clinic appointments funded by DSRIP/CPRIT with 114 (81%) surveys completed. During this period, the Interpersonal Manner domain had the highest score mean(SD) of 4.11(0.83) followed by Communication 4.03(0.92); the lowest value was Accessibility and Convenience with 3.58(0.96). During the second period (October 2014 to June 2015) there were 200 clinic appointments and 101(51%) surveys. For this period the domain Time Spent with Doctor scores increased by 11.86% (from (3.71(1.03) to 4.15(0.78)) , Communication by 10.42% (from 4.03(0.92) to 4.45(0.62)); the Financial Aspect had a decrease by 3.01% (from 3.65(0.98) to 3.54(1.08)); Interpersonal Manner, Technical Quality, General Satisfaction and Accessibility and Convenience scores increased between 7.06% (from 4.11(0.83) to 4.4(0.67)) and 9.78% (from 3.58(0.96) to 3.93(0.80)). **Conclusion:** PSQ-18 questionnaire reflect good to excellent quality of patient-physician interaction as perceived by patients from our underserved targeted outreach in most domains except “financial aspect”. This highlights the impact of buying power on patient satisfaction, which is unlikely to be addressed despite the focus on enhancing accessibility.

465 **CPRIT Grantee**
Poster Session A

Cervical Cancer Screening among Hard to Reach High Risk Women in Substance Abuse Recovery *M. Felini, The University of North Texas Health Science Center at Fort Worth; R. Qualls-Hampton, The University of North Texas Health Science Center at Fort Worth; S . Bangara, The University of North Texas Health Science Center at Fort Worth; K. Ukpaka, The University of North Texas Health Science Center at Fort Worth; O. Igenoza, The University of North Texas Health Science Center at Fort Worth; O. Jegede, The University of North Texas Health Science Center at Fort Worth; S. Gupta, The University of North Texas Health Science Center at Fort Worth; G. Voskuhl, AIDS Arms, Inc.*

Introduction: Women in residential and out-patient substance abuse treatment programs represent a broad range of criminally affected uninsured women and sex workers at highest risk of cervical cancer. Using preliminary data collected through a nontraditional partnership with law enforcement and over 30 community organizations (Dallas Prostitute Diversion Initiative), we created a cancer screening prevention program within substance abuse treatment centers targeting this previously hard to reach population that is more likely to not be adhering to recommended screening guidelines. This prevention services project was designed to fill the gap left from jail diversion programs that have the unintended consequence of diverting women away from jails where accessible healthcare services are available. Project aims were to provide 3360 women in substance abuse recovery with a trauma-informed cancer prevention education in easy to understand language, and provide 1220 women with a cervical cancer screen. This study examines progress over the 18 months of screening activities (February 2014 – July 2015). **Methods:** Focus groups conducted among the target population informed the development of an evidence-based and trauma sensitive cancer prevention education that was subsequently integrated into Nexus Recovery Center, the largest female substance abuse center in North Texas, and the Dallas Salvation Army. Cervical screenings were provided at AIDS Arms clinic which specializes in providing trauma-informed medical care to poor and vulnerable populations. Other screenings provided included anal, breast, HIV / sexually transmitted infections, and hepatitis. A follow-up clinic visit is provided 7 days from the initial screen as a second opportunity to educate, explain screen findings, and navigate positives to diagnostic care. **Results:** A total of 1451 women from 73 Texas counties participating in substance abuse treatment have been served through this program. Recruitment is ongoing through August 2016. Over 80% of women reached were uninsured, smokers, with a history of trauma. Nearly half reported 50+ sex partners. Of the 648 women provided an opportunity for a cervical cancer screen, 607 (94%) participated in the screen and 66% returned for the follow-up clinic visit. One quarter had never had a PAP smear or had not had one in 5+ years. One out of every four women screened had an abnormal PAP smear. Unexpected was the proportion of abnormal anal PAP smears, with more testing positive for anal high risk HPV than cervical high risk HPV. **Conclusion:** Substance abuse treatment centers provide an exceptional window of opportunity to engage high risk indigent women in cancer screening programs.

466 **CPRIT Grantee Poster Session B**

Empower Her® to Care Expansion Project: Increasing Access to Breast Health Care *B. Joseph, The Rose; T. Hans, The Rose*

Introduction: Rural Texas counties have a greater cancer burden than their urban counterparts due to access-to-care barriers (being uninsured, poor and with limited transportation) and lack of infrastructure to carry out prevention programs. With more than 80% of Texas designated rural by federal government standards, our rural population represents more than 3.3 million people. Compounding concerns, Texas still has the highest number of uninsured adults in the nation at between 20-23% despite healthcare reform efforts. The Empower Her® to Care project will continue to increase the delivery of breast cancer screenings, diagnostic procedures and patient navigation services to 3,700 underserved women over 24 months who live in 34 southeast Texas. **Methods:** The two-year project will serve 3,700 underserved/uninsured women, age 40 and above, who have not had a mammogram within the last five years or are not regularly receiving mammograms and live within 34 Texas counties. Services will be provided through The Rose's digital Mobile Mammography Program and partnerships with established community clinics, physicians and other organizations that offer education and outreach efforts and clinical breast exams to recruit eligible women in need of screening. Diagnostic testing, coordinated care and access to breast cancer treatment will be provided at either of The Rose's two locations. To address barriers, The Rose will apply the following evidenced-based models to promote cancer prevention and control: 1) reduce structural barriers through mobile mammography, 2) reduce out-of-pocket costs for services and transportation (CPRIT-sponsored care), 3) apply client reminders for appointments; 4) reducing structural barriers through patient navigation services to ensure timely access to diagnostic testing and treatment. **Results:** By addressing transportation, financia and system barriers, the Empower Her® to Care project will increase access to breast health services while offering a continuum of care unique to these communities. Over three months (March and May 2015), this project served 544 women and 42.5% (231) of those women recieved their first-ever (baseline) mammograms. Seven women were diagnosed with breast cancer from this group -- four of which had just received their first-ever screening mammogram (ages 41-78). C**onclusion:** The Empower Her® to Care project is making a significant impact in Texas, saving lives and improving safety-net systems for long-term community health. Collaborative partnerships are key to reaching service-delivery goals and improves patient trust to more effectively provide coordinated care that truly saves lives.

467 **CPRIT Grantee Poster Session A**

Building a Healthy Temple Cancer Primary Prevention Program amongst Hispanics *S. Wilmoth, The University of Texas at San Antonio; L. Correa, The University of Texas at San Antonio; E. Martinez, The University of Texas at San Antonio; M. Pan, The University of Texas at San Antonio; R. Mendoza, The University of Texas at San Antonio; D. Parra-Medina, The University of Texas Health Science Center at San Antonio; L. Neira, San Antonio Food Bank; E. Sosa, The University of Texas at San Antonio; Z. Yin, The University of Texas at San Antonio; M .He, The University of Texas at San Antonio*

Introduction: The Building a Healthy Temple (BHT) Primary Cancer Prevention Program is a translational research program adapting and implementing "Body and Soul" (B&S), an evidence-based cancer prevention program created for African-American churches, into Hispanic churches. BHT aims to reduce cancer risks through the promotion of healthy lifestyles amongst Hispanic congregants. BHT has 4 specifi goals: 1) To build Hispanic faith-based community's capacity in cancer primary prevention; 2) To reduce cancer risks through the promotion of healthy lifestyles amongst Hispanics; 3) To foster the maintenance and adoption of the BHT program; and 4) To scale up the BHT program at local and state level. **Methods:** BHT is a 4-month program adapting the four pillar model of B&S. BHT intervention components include: Health Sermons, Health Bible Study, Nutrition Education and Cooking Demonstrations, Active Living Competition, Health Ministry Committee, church health-conducive environmental changes, and Peer Counseling by trained health lay leaders. The program is to be implemented in up to 18 faith-based communities and reach approximately 3600 individuals in San Antonio's low income neighborhoods between 2014 and 2017. Using a one group pre/ post- test design, BHT measures both congregational and individual level changes. Congregational level outcomes (i.e. nutrition & physical activity environment) are to be measured by the Congregational Health Index tool. Individual level eating and physical activity behavior will be measured using the Dietary Screener Questionnaire by the National Cancer Institute and the International Physical Activity Questionnaire short form, respectively. Self-reported body mass index and waistline, alcohol consumption, religiosity, and social demographics will also be examined. Data are collected at baseline, 4 months and 12 months. **Results:** Data collection is currently underway. It is expected that at the end of the intervention, participating churches will have significant increases in their congregation's nutrition and physical activity environment scores. Additionally, individuals will increase fruit, vegetable, whole grain and dairy consumption as well as their level of physical activity, while consumption of processed meat and sugar will decrease. C**onclusion:** The BHT program targets three preventable cancer risk factors, i.e., poor nutrition, physical inactivity and obesity, among Hispanics. The program will lead to the adoption of an evidence-based cancer prevention program appropriate in Hispanic faith community settings. The program has the great potential to be disseminated on a broad scale to meet community needs, impact practice and policy, and ultimately lead to the reduction in cancer risks among underserved Hispanics.

468 **CPRIT Grantee**
Poster Session B

Use of a FIT-Flu Intervention to Increase Colorectal Cancer Screening Rates in the Houston Metropolitan Area *D. Vining, The University of Texas M.D. Anderson Cancer Center; P. Fernandez, The University of Texas M.D. Anderson Cancer Center; E. Furlan, The University of Texas M.D. Anderson Cancer Center; D. Pacheco, The University of Texas M.D. Anderson Cancer Center; S. Peterson, The University of Texas M.D. Anderson Cancer Center; G. Raju, The University of Texas M.D. Anderson Cancer Center; E. Hawk, The University of Texas M.D. Anderson Cancer Center*

Introduction: Adherence to the use of fecal stool tests for colorectal cancer (CRC) screening in underserved and underinsured populations is approximately 20%. To address this deficienc , we instituted a FIT-Flu screening program to distribute fecal immunochemical tests (FIT) annually to eligible patients at the time that they received flu inoculations with the expectation of doubling the CRC screening adherence with this intervention. **Methods:** We implemented a FIT-Flu intervention in the Houston metropolitan statistical area in partnership with eight local Federally Qualified Health Centers (FQHCs). FIT stool tests, along with CRC screening education, were distributed for two consecutive years to qualified patients in conjunction with annual flu inoculation programs. The adherence rate was calculated as the percentage of distributed FIT tests returned by patients for laboratory processing. Patients with positive FIT results (i.e., occult blood in stool) were offered colonoscopy examinations. **Results:** A total of 900 FIT tests were distributed over two years, with 576 returned for laboratory processing, resulting in an adherence rate of 64%. Twenty-two (3.65%) of the FIT tests were positive for detecting fecal blood which necessitated further evaluation with colonoscopy. Of these twenty-two patients, four were lost to follow up and one was still pending colonoscopy at the time of this abstract submission. Two of the patients lost to follow up could not be contacted by telephone or registered letters and two completed colonoscopy at outside facilities without returned results. The following results were reported for the 17 patients completing colonoscopy at our facility: one cancer diagnosis; one sigmoid colon mass due to diverticulosis without evidence of cancer; eight patients diagnosed with adenomas requiring future surveillance; 7 patients with a negative colonoscopy or benign hyperplastic polyps. Lessons learned included: (1) the transience of the underinsured/underserved population in Houston results in patients being lost to follow up as there is rarely a forwarding address or reliable telephone contact, (2) using a clinic's existing laboratory provider, rather than a third-party source, proved more effective in communicating results to the provider, (3) obtaining treatment for cancer care for this population of patients enrolled in cancer screening programs can be challenging due to limited available resources, both financial and clinical, as well as bureaucratic hurdles. **Conclusion:** The FIT-Flu intervention proved to be a successful method for increasing patient adherence to the use of stool tests for CRC screening. We were able to increase adherence from a baseline of 20% to 64%.

469 **CPRIT Grantee**
Poster Session A

Increasing the Number of Rural and Border Women Obtaining Breast and Cervical Cancer Screening and Diagnostics with Patient Navigators *C. Rice, Texas Agrilife Extension Service; C. Cubbin, The University of Texas at Austin*

Introduction: Women living in rural and border areas of Texas are less likely than their urban counterparts to have had a mammogram or Papanicolau (Pap) test within the past three years. Screening and diagnostic services tend to be "disconnected," and not easy to locate or access in rural and border areas of Texas, especially for uninsured women. As a result, uninsured women in these areas tend to be diagnosed in later stages of breast and cervical cancer, making treatment more difficult and costly and impairing future quality of life. Friend to Friend, an evidence based program, was adapted for use in rural Texas. Cancer Prevention and Research Institute funding was awarded to Texas A&M AgriLife Extension to hire regional cancer prevention specialists and patient navigators, gather women with a Friend to Friend event, find screening services, secure clinical sub-contracts, pay for the screening and diagnostic services, provide transportation, and support women through the process of obtaining needed screening, diagnostic services and treatment, if needed. Regional staff members are representative of the women being served by the project: 5 are bi-lingual Latinas, 2 are African American and one is Caucasian. Fifty counties were served. **Methods:** Four regional specialists and 4 patient navigators work with Extension agents in target counties to form community volunteer task forces which gather women at a Friend to Friend event. Women coming to the event are provided a presentation by a local health care provider urging them to get screened. The women are then given an opportunity to request help. Navigators follow up with those requesting help, supporting them through the process of screening, diagnostics and treatment, if needed. Not all women request navigation help. **Results:** To date, AgriLife staff conducted follow-up phone surveys with 5,283 women who have attended a Friend to Friend event. Three hundred and forty (340) non-Patient Navigation (PN) participants (23%) and 2,244 PN participants (59%) were screened for breast cancer after a Friend to Friend event; 225 non-PN participants (15%) and 1,756 PN participants (46%) were screened for cervical cancer after attending a Friend to Friend event. **Conclusion:** Patient navigators significantly increase the number of uninsured women living in rural and border areas of Texas who obtain screening and diagnostic services for breast and cervical cancer.

470 **CPRIT Grantee Poster Session B**

Active Living After Breast Cancer: Combining Physical Activity and Survivorship Navigation to Improve Quality of Life of Breast Cancer Survivors *K. Basen-Engquist, The University of Texas M.D. Anderson Cancer Center; E. Shinn, The University of Texas M.D. Anderson Cancer Center; L. Martinez, The University of Texas M.D. Anderson Cancer Center; A. Rieber, The University of Texas M.D. Anderson Cancer Center; P. Niravath, Baylor College of Medicine; S. Bonilla, Kelsey Research Foundation; N. Gonzalez, Kelsey Research Foundation; S. Scruggs, MD Anderson*

Introduction: Physical activity is associated with improved quality of life and with increased disease-free survival in breast cancer survivors. Active Living after Breast Cancer (ALABC) is a CPRIT-funded prevention program to improve breast cancer survivors' quality of life through increasing physical activity and providing survivorship information. The evidence-based program was developed and tested at MD Anderson, and adapted for delivery in the Houston community. We will describe participant characteristics and the changes in participants' physical activity, physical functioning, and quality of life. **Methods:** Participants for ALABC are recruited from throughout the Houston community. The program is delivered in 12 group sessions over a 16-week period. Each session covers behavioral skills for increasing physical activity (40-50 minutes), 10 minutes of physical activity, and 30 minutes addressing a survivorship topic. The program emphasizes increasing physical activity through incorporating short bouts of activity throughout the day. At the first and last sessions, participants complete questionnaires, performance tasks (6-minute walk, 30-second sit-stand), and anthropometric assessments. **Results:** The first group began November, 2014. We have completed 3 groups (one in Spanish) and have 2 groups ongoing (one in Spanish), and 2 groups scheduled to start in the next month. We have screened a total of 121 survivors. Of those who have been screened, 45 have started a group, 24 are registered for an upcoming group, and 41 are on waiting for a group to start in their area. Of those who have started the program 21 have completed, 19 are in a current group, and 5 dropped out. Average age of participants is 59 years (SD=10.3), and average time from diagnosis is 57 months (SD=33.1). Participants are 34% Black, 30% Hispanic, 31% white, 3% Asian, and 2% multi-racial. The median educational level is some college. Participants who have completed the program report significant increases in their moderate-vigorous physical activity (p=.013) and walking time (p=.05; n=21). Changes in six-minute walk and sit-stand tests improved, but changes were not statistically significant (p=.094 and p=.087, respectively). The physical health subscale of the QOL questionnaire improved (p=.003) but there was no change in the mental health subscale (p=.406). There were no significant changes in waist circumference or BMI. **Conclusion:** Preliminary data from the ALABC program evaluation indicate that it is effective at increasing physical activity and improving physical quality of life. Furthermore, it is feasible to deliver to a diverse survivor population.

471 **CPRIT Grantee Poster Session A**

Increasing Screening Prevention to Reduce Disparities in Underserved Hispanic Women *L. Gatus, The University of Texas Health Science Center at Houston; E. Figueroa, The University of Texas Health Science Center at Houston; L. Savas, The University of Texas Health Science Center at Houston; M. Fernandez, The University of Texas Health Science Center at Houston; P. Morales, Cancer & Chronic Disease Consortium; A. Valdez, Cancer & Chronic Disease Consortium; K. Hernandez, Cancer & Chronic Disease Consortium*

Introduction: In El Paso County, the breast and cervical cancer mortality rates are higher than state rates, reflecting a particularly strong need for interventions delivering comprehensive screening education and navigation services. The overall goal of this CPRIT-funded program is to increase breast and cervical cancer screening among low-income Hispanic women in El Paso. The Cancer and Chronic Disease Consortium (CCDC) and University of Texas SPH-Houston collaborated to adapt, implement, deliver and evaluate a culturally-tailored and evidence-based prevention program. **Methods:** Promotoras recruited Hispanic women living in low-income housing units from December 2014 to July 2015. Eligibility criteria include the following: Hispanic, 21-40 years of age and had no Pap test within the previous 3 years, or 40 years of age and older and had no mammogram within the previous 2 years or no Pap test within the previous 3 years. Bilingual data collectors conducted baseline surveys using RedCap, a web-based application accessed through secured laptops and hotspots. Baseline surveys measured women's demographics, cancer history, knowledge about cancer and screenings, cancer awareness, intentions and behaviors to receive screenings. **Results:** A total of 913 women were screened for eligibility and 660 women consented to participate, and completed baseline surveys. Promotoras provided education sessions to 330 women in the intervention group and assisted them in scheduling appointments for a cancer screening. Among the 660 participants, 74.54% were over 40 years old. Among the 40 year olds and older, 66.26% were non-adherent to both Pap test and mammography screening, 26.42% were adherent to Pap test screening, but non-adherent to mammography screening, and 7.32% were adherent to mammography but not Pap test screening. A total of 167 21-40 year olds needed a Pap test screening. Promotoras referred 212 women for mammograms, 252 for Pap tests and 115 for clinical breast exams. **Conclusion:** The program has successfully identified and served a large number of hard to reach low-income women in need of breast and cervical cancer screening services and navigation services. Promotoras continue to deliver the program to women in the delayed intervention group, as well as women identified in the community and using CCDC records to identify previous clients who are overdue for a screening exam. This presentation will describe outreach, participant characteristics, and program implementation.

472 **CPRIT Grantee**
Poster Session B

Impact of a Comprehensive Program Focused on Cervical Cancer Screening and Follow up Within a Texas Safety Net Health System *M. Anderson, Baylor College of Medicine; Y. Cui, Baylor College of Medicine; M. Daheri, Harris Health System; C. Bailey-Delesbore, Baylor College of Medicine; J. Montealegre, Baylor College of Medicine; A. Ogunwale, Baylor College of Medicine; L. Ramondetta, The University of Texas M.D. Anderson Cancer Center; H. Sangi-Haghpeykar, Baylor College of Medicine; L. Hanser, Harris Health System; M. Jibaja-Weiss, Baylor College of Medicine*

Introduction: The ability of patient navigation to improve health outcomes when implemented across large scale health systems has not been previously documented. The purpose of this study was to examine the impact of a comprehensive, multi-modal program designed to improve rates of cervical cancer screening and follow up among medically underserved women in Harris County, Texas by intervening at key transitions in the continuum of care provided by Harris Health System (HHS), the nation's 3rd largest safety net health system. M**ethods:** Routine use of culturally sensitive educational videos was implemented at points of patient contract at HHS community health centers. Medical homes for cervical cancer screening and colposcopy were implemented at multiple HHS clinical sites with low rates of screening. Multilingual patient navigators were cross trained as community health workers and utilized to identify and resolve barriers to care, educate patients, resolve issues with financial eligibility, facilitate Medicaid enrollment, prompt timely patient notification of results and colposcopy referral. Navigation activities were supported by a comprehensive tickler file to track all women diagnosed with abnormal cytology. **Results:** Total numbers of screen-eligible women enrolled in HHS increased from 108,232 in 2011 (baseline) to 156,939 in 2014. During this interval, an estimated total of 35,000 women viewed educational videos at points of contact. Concomitant with program activities, the proportion of HHS enrollees noncompliant with current screening recommendations decreased from 17% to 11%. The number of women diagnosed with abnormal cervical cytology increased from 2,903 to 3,436. Diagnostic resolution was reduced from 19 to 7 days for paps suspicious for carcinoma ($p<0.007$), from 54 to 44 days for high-grade cytologic abnormalities ($p=0.1$) and from 79 to 58 days for low-grade cytologic abnormalities ($p=0.0001$). By year 3 of program activity, only colposcopy follow-up of high-grade cytology remained outside the nationally recommended window. Overall, there was a system-wide shift towards earlier stage of disease at diagnosis. Numbers of women identifie with cervical precursors (AIS, CIS, CIN III) increasing from 186 to 216. Similarly, the proportion of early stage cervical cancers identified among HHS enrollees increased from 50% to 64%; the proportion of Stage IV cancers decreased from 22.9% to 8.5%. C**onclusion:** Implementation of a multi-modal, comprehensive approach to targeting key transitions along the continuum of cervical cancer screening can be successfully scaled for use within a high volume safety net health system and improves rates of cervical cancer screening and follow up among medically underserved women.

474 **Poster Session B**

A Multi-Layered Smoking Cessation Program for Cancer Patients: First Year Outcomes *V. Rabius, The University of Texas M.D. Anderson Cancer Center; J. Robinson, The University of Texas M.D. Anderson Cancer Center; J. Blalock, The University of Texas M.D. Anderson Cancer Center; M. Karam-Hage, The University of Texas M.D. Anderson Cancer Center; D. Beneventi, The University of Texas M.D. Anderson Cancer Center; P. Cinciripini, The University of Texas M.D. Anderson Cancer Center*

Introduction: Tobacco plays a causal role in at least 18 types of cancer, accounts for almost one-third of all cancers, and has deleterious consequences on cancer treatment outcomes. Cancer patients who continue to smoke are also at increased risk of cancer recurrence and developing additional types of cancer. Since 2006, the Tobacco Treatment Program (TTP) has provided smoking cessation treatment to patients and employees at MD Anderson Cancer Center (MDACC) free of charge. TTP provides a program of behavioral counseling and pharmacotherapy for smoking cessation, in combination with psychotherapy and/or psychiatric treatment for conditions directly affecting a cessation attempt (TTP1). **Methods:** In early 2012, an electronic screening was put in place for all MDACC patients to conform to meaningful use of electronic health record (EHR) standards. This resulted in the automatic electronic referral (AER) to TTP of all patients who self-identified as tobacco users or recent quitters. We also developed a multi-layered series of options to serve all patients. In September 2012, we added two educational/motivational services (EMS): (1) the minimum provision of educational materials and a follow-up phone call and (2) a motivational call, educational materials and follow-up call. In May 2013 we began offering a phone-counseling only option (PO), which provides behavioral counseling for smoking cessation, but not psychotherapy, psychiatric support, or pharmacotherapy. Here we report the impact of enhancing provider-driven referrals by adding AERs based on self-reported tobacco use in a patient's EHR and the first year outcomes of expanding our services to reach all patients who smoke. **Results:** In the first year of our multilayered program we provided service to 5,613 patients. We attempted to contact all patients at 3 months following their referral. Follow-up rates (FR) and 7-day point prevalence abstinence (PP7), assuming non-responders are still smoking, are as follows: EMS1 – FR=26%, PP7= 6% (74/1210; EMS2 – FR=24%, PP7 = 7% (223/3392); PO – FR=83%, PP7=16% (31/193); TTP1 – FR=97%, PP7=38% (312/818). **Conclusion:** AER's, combined with a multi-layered service offering significantly extended TTP's reach. Higher abstinence rates were associated with counseling services and the highest rate was associated with counseling plus pharmacotherapy.

475 **Poster Session A**

HPV Vaccination among Young Women (19-26 years old) and Guideline-Adherent Cervical Cytology Screening *J. Hirth, The University of Texas Medical Branch at Galveston; Y. Lin, The University of Texas Medical Branch at Galveston; Y. Kuo, The University of Texas Medical Branch at Galveston; A. Berenson, The University of Texas Medical Branch at Galveston*

Introduction: Women who have received the human papillomavirus (HPV) vaccine are recommended to receive regular screening for cervical cancer. Although a high proportion of vaccinated young women report intending to continue screening, it is unknown whether they have followed through, and whether the number of vaccine doses affects later screening behavior. **Methods:** This retrospective cohort study used administrative insurance claims records to examine the behavior of 19-26 year old women who received at least 1 injection of the HPV vaccine between January 2006 and November 2009, with follow-up data available through December 2012. HPV vaccinated young women continuously enrolled in a nationally-representative private insurance plan for 6 months prior to and 37 months after HPV vaccine administration were included. We evaluated the association of the number of HPV vaccine injections and the specialty of the HPV vaccine administrator with cervical cytology screening within 3 years after initial vaccination. Interactions between the number of doses and year of vaccine initiation were also evaluated. **Results:** A population-based selection of 24,964 young women who received 1 or more HPV vaccine doses were included. In this sample, 79.3% had a Papanicolaou (Pap) test within 3 years following vaccination. Receiving 1 (aOR: 0.60, 95% CI 0.55-0.65) or 2 (aOR: 0.80, 95% CI 0.74-0.87) doses was associated with decreased odds of Pap testing after vaccination. Patients vaccinated by non-obstetrician/ gynecologist provider types were less likely to get a Pap test following vaccination. There were significan interactions between the number of doses and the year that vaccination was initiated. The proportion of young women who did not complete the series and who received a Pap test remained steady, regardless of the year that they initiated vaccination. The proportion of young women who received Pap tests after completing the series, however, grew smaller across time (from 86% of those who initiated in 2006 to 79% of those who initiated in 2009). **Conclusion:** Young women who received less than 3 doses of the HPV vaccine need to continue to receive guideline-adherent Pap tests, and more effort should be made to educate them about this need. Vaccinating providers, particularly those specializing in pediatrics, need to educate patients about the necessity of continuing Pap testing after vaccination.

476 **Poster Session B**

LIVESTRONG Cancer Navigation: Overview of Needs and Services of Cancer Survivors in Texas *B. Hemingway, LIVESTRONG Foundation; E. Eargle, LIVESTRONG Foundation; S. Arvey, LIVESTRONG Foundation; R. Rechis, LIVESTRONG Foundation*

Introduction: With more than 500,000 cancer survivors currently living in Texas, the delivery of effective care for late and long-term effects caused by cancer and its treatment is crucial. LIVESTRONG Cancer Navigation (LCN) offers services designed to address survivors' needs at all stages of the cancer experience. We present data from 2014 about the LCN program. The data includes aggregate data about clients from Texas that utilized LIVESTRONG's Navigation services including: number of individuals served, top needs reported, services provided and client satisfaction with services. M**ethods:** LCN uses a customized HIPAA-compliant client management system (CMS), which disseminates surveys and collects and tracks client data including: Client demographics, needs identified through the intake form, number and type of client interactions and services provided, case notes summarizing interactions, and number and type of referrals to partner organizations. Participants (n=756) received a survey six weeks following their initial intake that ask about their level of satisfaction with the services they received. **Results:** Of the 8,893 individuals served nationally, 756 (9%) individuals from Texas accessed LCN services. Texas clients are similar to national clients demographically, as most were diagnosed with cancer themselves (75%) (as opposed to a caregiver), White (34%), between the age of 26-39 (27%) and currently in treatment (26%). However Texas clients were more likely to identify as Hispanic and more likely to report a need for emotional support services than national clientele. Both groups reported financia assistance as a top need, therefore Texas clients were most commonly referred to services to help with insurance and financial concerns (38%). Texas clients reported more emotional support needs and were referred to LIVESTRONG Emotional Counseling more often (23%). The poster will further explore the data regarding Texas clients that accessed Navigation services including referrals and resources. **Conclusion:** Understanding the various challenges experienced by cancer survivors in Texas allow us to continue to identify key barriers to care delivery. In addition, understanding the type of support survivors are seeking can inform service design in clinical and community-based settings that can continue to support clients through survivorship.

477 **Poster Session A**

Using a Customized Vaccination Provider Mail Out to Encourage Strong Recommendation of HPV Vaccine *L. Palenapa, Texas Department of State Health Services; E. Gardner, Texas Department of State Health Services; K. Guerrero, Texas Department of State Health Services*

Introduction: The Advisory Committee on Immunization Practices recommends three adolescent vaccines for boys and girls aged 11-12 years: one dose of tetanus, diphtheria, and acellular pertussis (Tdap) vaccine, two doses of meningococcal vaccine (MCV4), and three doses of HPV vaccine. Preventing HPV infection through immunization protects against HPV-associated cancers in males and females, including cancers of the cervix, vulva, vagina, penis, anus, and oropharynx. In Texas, while immunization coverage levels for Tdap and MCV4 vaccine have increased significantl , HPV coverage has not. Despite the safety and effectiveness of the HPV vaccine, less than one third of teens in Texas are fully vaccinated against HPV. The CDC has found that healthcare provider recommendation is the single best predictor of vaccination. Stronger provider recommendation at Texas Vaccines for Children (TVFC) clinics could lead to increased HPV immunization rates in Texas adolescents. Ideally, providers should be ordering and administering three doses of HPV vaccine for every one dose of Tdap. M**ethods:** On June 2, 2015, a customized adolescent vaccine ordering profile email was distributed to approximately 2,400 TVFC providers who serve adolescent populations. The purpose of the email was to encourage TVFC providers to make a strong recommendation for HPV vaccine and also make providers aware of their recent ordering history for vaccines in the adolescent platform. Provider letters reflected two data tables including Tdap to HPV ordering ratios as well as total doses ordered in 2013 and 2014 for each adolescent vaccine with a percent increase or decrease illustrated. **Results:** The results of the analysis indicate that, on average, TVFC providers order 1.4 doses of HPV vaccine for every dose of Tdap. By June 24, 2015, over 600 TVFC providers had increased their HPV vaccine orders for June of 2015 compared to their HPV orders in June of 2014. **Conclusion:** Prior to mailout, TVFC providers were ordering far less HPV vaccine as indicated by a ratio of HPV:Tdap of only 1.4:1. The ideal ratio of HPV:Tdap is 3:1. Provider mail outs with customized vaccine ordering information and strategies for giving stronger recommendation for HPV vaccine can increase HPV vaccine ordering and uptake. Increased HPV immunization levels among adolescents in Texas will lead to lower burden of HPV-associated cancers.

478 **CPRIT Grantee**

SMS Cessation Service for Young Adult Latinos in South Texas: Program Protocol and Preliminary Results *A. Ramirez, The University of Texas Health Science Center at San Antonio; P. Chalela, The University of Texas Health Science Center at San Antonio; K. Gallion, The University of Texas Health Science Center at San Antonio; E. Muñoz, The University of Texas Health Science Center at San Antonio; C. Despres, The University of Texas Health Science Center at San Antonio; D. Akopian, The University of Texas at San Antonio; A. Perez, The University of Texas Health Science Center at San Antonio; R. Garcia, The University of Texas Health Science Center at San Antonio; A. McAlister, The University of Texas Health Science Center at Houston*

Introduction: Smoking among Latino young adults (18-29) in South Texas is high (23.2% to 25.7%), representing a serious public health problem. Yet few are reached by services to help them quit smoking. Young adult Latinos are heavy users of mobile devices for texting and access to mobile media. These have an extraordinary theoretical potential for assisting smoking cessation by providing peer modeling and eliciting social reinforcement for behavior change. Thus, we are developing bilingual text messaging cessation services tailored to Latinos. **Methods:** After a preparatory phase of work to finalize promotional plans, develop and pretest evidence-based, culturally tailored English and Spanish SMS cessation services, we will launch an intensive social and mobile media promotional campaign to recruit 3,000 young adult smokers to this service. This project's innovative features include attention to a population that has not been served with efforts to promote smoking cessation. We will employ social media (Facebook, Twitter, Instagram and YouTube) for outreach to young adults who smoke. Messages include links to web pages with additional content and YouTube videos with peer modeling of reasons and skills to quit smoking. We are transforming evidence-based SMS cessation assistance methods that have previously been available only in English or with generic Spanish translation, to fit the language use and cultural milieu of young Spanish and English speakers. **Results:** Results of our initial pilot test showed that the Facebook advertising yielded 481,601 impressions and 1,534 unique users clicked "join" to view the service's home page. The invitation to text a code there resulted in 147 enrollments in the service, at a cost of $37 for each user recruited. Users were all men, with a mean age of 28 and 63% reporting that they were Hispanic or Latino. These users received texts with links to mobile pages addressing reasons for quitting, social support, nicotine replacements, breathing exercises, getting active, things to do instead of smoking, talk yourself out of smoking and binge drinking. On average these service users spent 7.75 minutes consuming the graphic, text and video content on these pages. **Conclusion:** Preliminary results provide evidence that young adult Latino smokers can be reached via mobile media service. The anticipated outcome is a scalable, culturally relevant, evidence-based and cost-effective service with broad national reach to help young adults of Latinos stop smoking, with the potential to reduce health care costs, reduce chronic disease burden and improve quality of life among this young, fast-growing, at-risk population.

479 **CPRIT Grantee**

CPRIT and the 1115 Medicaid Waiver DSRIP Partnership *C. Allen, The University of Texas Health Science Center at Tyler; S. Tison, The University of Texas Health Science Center at Tyler; M. Ross, The University of Texas Health Science Center at Tyler; B. Olusola, The University of Texas Health Science Center at Tyler; J. Morrison, The University of Texas Health Science Center at Tyler; P. McGaha, The University of Texas Health Science Center at Tyler; W. Sorensen, The University of Texas at Tyler; E. Caldwell, The University of Texas Health Science Center at Tyler; M. Gaona-Bustos, The University of Texas Health Science Center at Tyler; M. Barnett, The University of Texas Health Science Center at Tyler; E. Sauter, The University of Texas Health Science Center at Tyler*

Introduction: Our team has launched an innovative and ambitious educational campaign to provide increased access to colorectal cancer (CRC) services in East Texas among the un- and under-insured. The project has a complementary, non-overlapping partnership between a federal project, the Texas Medicaid Transformation Waiver Delivery System Reform Incentive Payment (DSRIP) project, and a state based Cancer Prevention & Research Institute of Texas (CPRIT) project. In a seven county region of East Texas, the DSRIP project focuses on increasing awareness (through social marketing and education) on the benefits of CRC screening. **Methods:** The approach to social marketing and education utilized by DSRIP includes the development of focus groups from rural Caucasian, African American and Hispanic communities. Informational materials, including a CRC screening decision aid-tailored to the needs of each group, are shared with the groups. Working with the DSRIP project, we are utilizing their large inflatable colon as an educational tool for community outreach programs. The limitation of the DSRIP program is that it does not provide funding for CRC screening which includes neither fecal immunochemical testing (FIT) nor colonoscopy, while our CPRIT project does. Our CPRIT project has leveraged the work of DSRIP by providing a source of funding for people educated in the community by the DSRIP project who wish to undergo screening but lack the financial resources and/or the transportation necessary to do so. **Results:** In less than a year we have reached 57,620 and served, through interactive public education and outreach activities, 2,239 individuals. Of the 1,760 individuals engaged through community outreach by DSRIP since October 2014, 408 wanted more information. Half of these people were without insurance which was a barrier to their undergoing CRC screenings. A total of 77 (38%) of the individuals lacking insurance have undergone CRC screening through CPRIT funding. **Conclusion:** This program leverages two complementary, non-duplicative programs to increase CRC screening in a seven county area of East Texas. Our combined programs are designed to provide education and to deliver CRC screening services to individuals who are most in need, because of limited resources, lack of insurance or inadequate insurance, and the transportation barriers faced by many rural residents. CPRIT support has allowed us to remedy barriers to CRC screening. The synergistic application of the CPRIT and DSRIP CRC funding sources is allowing East Texans increased access to a full complement of CRC educational outreach and screening services.

480 **CPRIT Grantee**

Increasing Access to Colorectal Cancer (CRC) Screening in Rural East Texas *C. Allen, The University of Texas Health Science Center at Tyler; S. Tison, The University of Texas Health Science Center at Tyler; M. Ross, The University of Texas Health Science Center at Tyler; B. Olusola, The University of Texas Health Science Center at Tyler; J . Morrison, The University of Texas Health Science Center at Tyler; W . Sorensen, The University of Texas at Tyler; E. Caldwell, The University of Texas Health Science Center at Tyler; M. Gaona-Bustos, The University of Texas Health Science Center at Tyler; M. Barnett, The University of Texas Health Science Center at Tyler; E. Sauter, The University of Texas Health Science Center at Tyler*

Introduction: CRC death rates are higher in rural than in urban counties. In Texas, only 39.5% of eligible rural Texans had colonoscopy in the past five years vs. 46.2% in urban counties. Our team is increasing access to CRC screening services to un- and under-insured in a seven county area of rural East Texas. Delivery of services to our mostly rural population poses unique challenges related to access, delivery of education and CRC services. We have developed a unique integrated public health and clinical model to optimize success. **Methods:** To achieve our objectives we have established multiple partnerships with existing programs in the community which are visible, effective and trusted by the region. We have used social marketing to reach a large pool of potential participants. We have provided focus groups to eligible candidates in our rural Caucasian, African American and Hispanic communities to tailor informational materials for each group. We have engaged clinical colleagues in primary care to assist with recruitment to the program. We are working with the American Cancer Society to optimize provider and participant education. **Results:** The study has been recruiting participants for just over 6 months. Thus far we have reached 57,620 individuals and, through interactive public education and outreach activities, have served 2,239 individuals. A total of 937 CRC screenings, of which 910 have been colonoscopies, have been performed since the inception of the grant. Of the 910 colonoscopies performed, 336 (37%) individuals have had precancerous polyps removed. This compares with a national average of 15-25% and 10% frequency for a CRC screening project performed in a predominantly urban-suburban area of Texas. **Conclusion:** The reasons for the high incidence and mortality rates from CRC in East Texas are likely multifactorial. The rural setting presents unique challenges, including relatively low population density and distance. The high adenoma detection rate suggests we are screening a population at increased risk of CRC. Our program is designed to educate and deliver CRC screening services to individuals who are most in need. Recent reports in the New England J of Medicine and in JAMA document that a higher adenoma detection rate correlates with lower long term mortality from CRC. It is our belief that the removal of adenomatous polyps among our participants will decrease their risk of developing CRC, and that the detection of CRC in the screened individuals will improve their chance of survival.

481 **CPRIT Grantee**
Poster Session A

Impact of Modest Financial Incentives on Colorectal Cancer Screening Participation with Mailed Fecal Immunochemical Tests: a Randomized, Comparative Effectiveness Trial *S. Miller, John Peter Smith Health Network; S. Gupta, Moores Cancer Center University of California San Diego; E. Berry, UT Southwestern Moncrief Cancer Institute; M. Koch, John Peter Smith Health Network; P .Anderson, The University of Texas Southwestern Medical Center at Dallas; E .Borton, The University of Texas Southwestern Medical Center at Dallas; S . Pruitt, The University of Texas Southwestern Medical Center at Dallas; A. Hughes, The University of Texas Southwestern Medical Center at Dallas; E. Carter, John Peter Smith Health Network; B. Balasubramanian, The University of Texas Health Science Center at Houston; K. Argenbright, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in the United States. Screening can prevent CRC mortality, but participation is limited, especially among the uninsured. Prevention programs commonly offer financial incentives to participants, but the impact on screening completion is incompletely understood. Our aim was to compare impact of offering modest financial incentives vs. no incentives on screening completion as part of a large-scale implementation of a CRC screening outreach program offering mailed invitation to complete a fecal immunochemical test (FIT). **Methods:** We conducted a randomized, comparative effectiveness trial of financial incentives for increasing participation in CRC screening with FIT. The trial was nested within a larger outreach program offering mailed FIT invitations to promote screening for uninsured individuals served by a large safety-net health system initiated in 2013. All individuals not current with CRC screening, age 50 to 64, uninsured but participating in Tarrant County's safety-net health system medical assistance program with a primary care visit in the last year were included. Individuals were randomly assigned to mailed FIT outreach (n=6,565), outreach plus $5 incentive (n=1,000), or outreach plus $10 incentive (n=1,000). Outreach included: 1) mailed invitation in English and Spanish to complete and return a FIT; 2) a single sample Polymedco OC Sensor FIT test; 3) automated telephone reminders in English and Spanish to encourage test completion, delivered at time of invite and within 1 week of invitation, 4) up to two "live" telephone reminders delivered within four weeks post-invitation. The primary outcome was FIT completion. The primary comparison was FIT completion for individuals receiving outreach alone vs. any incentive, using a chi-square test of proportions; in secondary analyses we compared outreach vs. the $5 and $10 groups separately, and evaluated results stratified by sex and race/ethnicity; p <0.05 was considered statistically significant. **Results:** No clinically significan differences in age, sex, or race/ethnicity were observed across groups. Among individuals not current at baseline, participation ranged from 34.6 to 39.2% across intervention groups, and was not statistically significantl different. Specificall , individuals receiving any incentive, were not more likely to complete FIT than individuals receiving no incentive; confidenc intervals around point estimates for FIT participation were overlapping (p>0.05 for all comparisons). In subgroup analyses stratified by sex and race/ethnicity, no clinically significant differences across intervention groups were observed. **Conclusion:** FIT outreach results in clinically significant CRC screening participation among the uninsured. Modest financial incentives do not impact screening completion

482 **CPRIT Grantee**

Assessing Local County Capacity to Expand Rural Breast Cancer Screening and Patient Navigation: A Decentralized Hub-and-Spoke Model (BSPAN2) *S. Inrig, Mount St. Mary's University; R. Higashi, The University of Texas Southwestern Medical Center at Dallas; J. Tiro, The University of Texas Southwestern Medical Center at Dallas; K. Argenbright, The University of Texas Southwestern Medical Center at Dallas; S. Lee, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Federal funding for state programs like the Texas Breast and Cervical Cancer Services (BCCS) has increased mammography utilization among uninsured women. However, rural-residing women continue to experience suboptimal screening rates because access is limited by local county capacity and lack of coordinated care along the screening continuum. We describe development and application of a mixed-method, iterative assessment tool to determine county needs and capacity to participate in a decentralized regional "hub-and-spoke" service delivery program. **Methods:** To assess the capacity of each of the 17 rural counties ("spokes"), we ("hub") developed a mixed-methods, iterative assessment tool to triangulate quantitative data, such as the number of mammography units and number of women over age 40, with qualitative data derived from observations and semi-structured interviews among county stakeholders. We operationalized county "capacity" as the ability and potential to collaborate, conduct outreach, and adopt implementation strategies to systematically improve access to breast health services. Assessment and the tool itself were developed in an iterative process to determine each county's capacity to implement the components of the screening service delivery model. **Results:** Among ten of 17 counties, capacity level assignment changed between the initial point of assessment (quantitative only) to the commencement of service delivery (quantitative and qualitative analysis). We determined one county had capacity to lead both clinical navigation and outreach components of the program; 7 counties adopted local outreach efforts but relied on the hub for navigation; and 9 counties relied on the hub for both outreach and navigation. We present three case examples to illustrate how our tool captured changes in county capacity. **Conclusion:** The mixed-method, iterative assessment tool enabled hub leaders to establish effective partnerships with spokes by tailoring program support to local capacity and needs. In the absence of vertical integration of health services in rural areas, BSPAN2 created a virtually-integrated regional system that effectively extended federal funding for breast cancer screening to rural women.

483 **CPRIT Grantee**

Adapting Rural Outreach and Service Delivery to Meet Local Demand for Mammography among Hispanic and Spanish-speaking Women (BSPAN2) *S. Lee, The University of Texas Southwestern Medical Center at Dallas; R. Higashi, The University of Texas Southwestern Medical Center at Dallas; S. Inrig, Mount St. Mary's University; J. Sanders, The University of Texas Southwestern Medical Center at Dallas; A. Hughes, The University of Texas Southwestern Medical Center at Dallas; W. Bishop, The University of Texas Southwestern Medical Center at Dallas; K. Argenbright, The University of Texas Southwestern Medical Center at Dallas; S. Pruitt, The University of Texas Southwestern Medical Center at Dallas*

Introduction: Texas is experiencing significant population growth and demographic diversification, especially among Hispanics. These changes impact public health planning vital to adapt service delivery systems and develop new interventions. Unfortunately, federal and state population health survey estimates for rural and other underserved counties may be inadequate or unstable due to small sample sizes and may not reflec recent demographic changes. **Methods:** Drawing from a de-centralized regional program for breast cancer screening and patient navigation for under- and uninsured women (BSPAN2), we compare mammography utilization data for Hispanic and Spanish-language preferring women against US Census county estimates. We used Chi-square tests to compare these groups in 13 BSPAN2 rural and underserved counties. We sampled six of the rural counties for in-depth qualitative data collection, including 73 interviews at 51 organizations and brief surveys with 92 program participants, leading to 30 additional interviews. **Results:** Ten of 13 counties served a higher proportion of Hispanic women; 9 of 13 served a higher proportion of Spanish-language speakers. In the county with the greatest observed difference ($p < .001$), 53% of women screened were Hispanic (vs. 12% census), and 45% were Spanish-speaking (vs. 10% census). We explore contextual data understand uptake of outreach efforts among Hispanic and Spanish-speaking communities across the expanded service area. **Conclusion:** Adapting program services to heterogeneous rural contexts requires ongoing assessment, flexibilit to respond to challenges as they arise, and strengthening shared commitment with community partners to reach underserved populations. For rural areas experiencing rapid population growth where federal and state health survey estimates are unstable due to small sample size, local service delivery program data reflect more proximal experience of rural area need and uptake of preventive health services.

484 **CPRIT Grantee**

Factors Associated with Breast Cancer Screening Guidelines Adherence among Low-income Women *J. Bolin, Texas A&M University System Health Science Center; A. Lichorad, Texas A&M University System Health Science Center; D. McClellan, Texas A&M University System Health Science Center; C. Ojinnaka, Texas A&M University System Health Science Center; J. Helduser, Texas A&M University System Health Science Center; R. Hernandez, Texas A&M University System Health Science Center; B. Hernandez, Texas A&M University System Health Science Center; M. Ory, Texas A&M University System Health Science Center*

Introduction: The Texas A&M Health Science Center, through its Family Medicine Residency in Bryan, Texas, received grant funding in December, 2013, to provide breast and cervical cancer screening and diagnostic services to uninsured and low-income women living in a 9-county region of central Texas known as the Brazos Valley. This prevention grant takes an interdisciplinary approach to training family medicine resident physicians, nursing students, and community health workers in clinical procedures and culturally appropriate patient care. The purpose of this paper is to analyze potential associations between breast cancer knowledge, attitudes and perceived susceptibility, and screening guidelines adherence among low-income women. **Methods:** Deidentified survey responses of patients who received financial assistance for breast cancer screening were analyzed. Descriptive statistics for demographic characteristics were conducted. Factor analysis of 19 survey items measuring breast cancer awareness, attitudes, and perceived susceptibility was conducted. Multivariate logistic regressions were estimated with factors measuring knowledge, attitude and perceived susceptibility as independent variables of interest. Demographic characteristics were covariates. **Results:** The study sample was comprised of approximately 39%, 20% and 41% Whites, Blacks and Hispanics, respectively. About 61% of recipients were urban residents, while 39% were rural residents. Factor analysis revealed that three factors were associated with improved recognition of screening recommendations as shown by the (1) awareness, (2) attitude, and (3) susceptibility survey items. Multivariate analysis revealed that negative attitude towards mammogram was associated with decreased likelihood of being within screening guidelines (OR=0.40; 95% CI=0.22-0.72). An increase in age was also associated with increased likelihood of being compliant with screening guidelines (OR=1.07; 95% CI=1.03-1.11). **Conclusion:** In addition to increased access to subsidized screening

tests, educational interventions aimed at improving attitudes toward mammograms among low-income women should be explored as a means of improving adherence to screening guidelines.

485 **CPRIT Grantee**

Factors Impacting Non-utilization of PAP Testing Among Medically Underserved Women Enrolled in a Texas Safety Net Health System *A. Ogunwale, Baylor College of Medicine; H. Sangi-Haghpeykar, Baylor College of Medicine; Y. Cui, Baylor College of Medicine; J. Montealegre, Baylor College of Medicine; M. Jibaja-Weiss, Baylor College of Medicine; M. Anderson, Baylor College of Medicine*

Introduction: Despite world-class medical resources, the State of Texas is characterized by an incidence of cervical cancer that exceeds not only many other parts of the United States but also the world. Rates of cervical cancer are highest among medically underserved women, women who have never been screened for this disease and recent immigrants. However, factors determining non-utilization of PAP testing among medically underserved women remain poorly understood, particularly once issues related to health access have been resolved. The purpose of this study was to delineate factors determining whether medically underserved women in Harris County, Texas at greatest risk for cervical cancer have ever been screened for this disease. **Methods:** Subjects presenting for routine primary care at two community health centers operated by Harris Health System (HHS) were invited to complete a self-administered questionnaire. The goal of this questionnaire was to identify beliefs, perceptions and attitudes affecting cervical screening decisions as well as the impact of physician and male partner support on Pap utilization. Permission to conduct all study activities was obtained from the Institutional Review Board for Baylor College of Medicine (BCM) and HHS. Multivariate logistic regression models were constructed and used to identify independent risk factors associated with Pap test non-utilization. **Results:** A total of 983 of the 1065 women invited to participate in this study returned completed questionnaires. Nearly 11% of subjects reported that they had never previously been screened for cervical cancer, despite a mean of nearly 4 clinic visits with an HHS primary care provider in the preceding 12 months. Never screeners were significantl younger, more likely to be Hispanic, non-U.S. born and less likely to have healthcare continuity. In multivariable analysis, odds for never screening was independently lower among women with male partner support for screening (aOR 0.29, $p<0.05$) and physician's recommendation for screening (aOR 0.34, $p<0.05$), whereas never use was more likely among women who believed screening visits are too long (aOR 2.53, $p<0.05$). **Conclusion:** In addition to well-recognized situational barriers related to interactions with the health care system, perceptions of male partners play an important role in determining whether Hispanic or immigrant women have ever been screened for cervical cancer. Efforts should be made to better understand the role that men play in determining whether or not their female partners are screened for cervical cancer and develop targeted educational tools and other strategies to favorably engage male partners and encourage regular screening.

486 **CPRIT Grantee**

Predictors of Cervical Cancer Screening among Medically Underserved Women Living with HIV-AIDS *M. Coleman, Baylor College of Medicine; A. Ogunwale, Baylor College of Medicine; H. Sangi-Haghpeykar, Baylor College of Medicine; I. Valverde, Baylor College of Medicine; J. Montealegre, Baylor College of Medicine; M. Jibaja-Weiss, Baylor College of Medicine; M. Anderson, Baylor College of Medicine*

Introduction: Recent evidence suggests that the integration of dedicated women's health services into free standing specialty clinics designed to address the needs of women living with HIV-AIDS (WLHA) improves cervical cancer screening compliance. However, very little is currently known about the personal beliefs, attitudes and perceptions that impact cervical cancer screening among medically underserved WLHA. To better understand these issues, we surveyed low income, medically underserved women receiving subsidized gynecologic care through an integrated HIV clinic. M**ethods:** The study instrument consisted of 39 items drawn from previously validated surveys used to delineate factors impacting cervical cancer screening. Survey items broadly covered psychosocial factors, beliefs, attitudes and perceptions potentially impacting PAP test utilization. A target of 210 WHLA was set with goal of 80% power to detect a difference of 25% between study groups on measured parameters (alpha = .05, two-side Chi square test). Multiple logistic regression models were constructed to delineate factors independently associated with screening compliance. **Results:** A total of 310 of the 346 women invited to participate completed the study instrument. Of the 220 subjects who self-identified as WHLA, 179 (85.7%) reported having had a Pap test in the last three years. The majority of WLHA (95%) knew that the Pap test screens for cervical cancer. However, overall knowledge of cervical cancer risk factors, such as multiple sexual partners or sex with a man with multiple partners was low (43% and 35%, respectively). Consistent with this observation, unscreened women were more likely to be single with multiple current sexual partners compared to the screened group. They were also more likely to younger. In multivariable analyses, the only factors associated with Pap testing were a woman's perception that her partner wants her to receive regular screening (aOR 4.64; 95% CI: 1.15, 23.76; p=0.04), number of clinic visits during the past year (aOR 1.36, 95% CI: 1.05, 1.94; p = 0.04) and knowledge that the need for a Pap test does not depend on whether or not a woman is experiencing vaginal bleeding (aOR 6.52, 95% CI: 1.04, 49.71; p = 0.05). C**onclusion:** We conclude that support from male partners in addition to effective contact with the

health system and knowledge of cervical cancer risk factors influenc Pap utilization among low income WLHA. Future measures to improve the care for this population should increase knowledge of cervical cancer risk factors and encourage social support for cervical cancer screening among WLHA.

487

Financial Burden Experienced by Young Texas Survivors: Results from the LIVESTRONG Survey *S. Nutt, LIVESTRONG Foundation; C. Bann, RTI International; L. Squiers, RTI International; S. Arvey, LIVESTRONG Foundation; R. Rechis, LIVESTRONG Foundation*

Introduction: With improved early detection and treatment for cancer, the number of cancer survivors in the U.S. continues to grow with survivors living longer than ever before. Survivors face many challenges during and after cancer including coordinating medical care, managing late and long-term effects, and monitoring for recurrences or new cancers. In particular, previous studies have shown that survivors experience significant economic burden due to rising medical costs compared to people without a history of cancer. **Methods:** The LIVESTRONG Foundation (LIVESTRONG) conducted an online survey of cancer survivors from June to December 2012 to better understand the financia concerns that survivors encounter during and after diagnosis. The survey was based on the Experiences with Cancer Survivorship supplement of the Medical Expenditures Panel Survey. **Results:** Of more than 5,000 U.S. survivors who responded, 492 survivors reported living in Texas at the time of the survey. This analysis focuses on these 492 individuals. Most respondents were female (64%) and between the ages of 40 and 59 (56%). Additionally, the largest percentage of respondents had breast cancer (30%). In terms of the impacts that cancer had on survivors' finances, the majority worried about paying large medical bills (68%).

Stratifying by age at diagnosis, survivors diagnosed before age 40 (young survivors) were more likely to experience financial burden and concerns than adults diagnosed at an older age. For instance, 75% of young survivors reported worrying about paying large medical bills compared to only 39% of survivors over the age of 60 (older survivors)*. Moreover, young survivors were more likely than older survivors to report making financial sacrifices (49% vs. 26%)*, borrowing money (44% vs. 18%)* and being unable to cover costs of medical care visits (41% vs. 16%)*. Financial impact among young survivors appears to be increasing over time with those diagnosed in 2008 or later reporting greater worry than those diagnosed before 2008 (86% vs. 68%)*. * $p < 0.05$ **Conclusion:** Many young survivors in Texas experience financial impacts, and they are more likely to experience these impacts than their older counterparts. This finding is consistent with the national sample of survivors as well. Given that nearly 80,000 people under 40 are diagnosed with cancer each year, it is imperative that the cancer community considers the unique burden they experience and reduce financial hardship when possible to ensure that these survivors can live full lives without derailing their financial future

488

Estimating HPV Vaccine Coverage in Texas Adults Using the Behavioral Risk Factor Surveillance System, 2013 *E. Gardner, Texas Department of State Health Services; L. Palenapa, Texas Department of State Health Services; K. Guerrero, Texas Department of State Health Services*

Introduction: Human papillomavirus (HPV) causes approximately 33,200 new cancers in the US each year, including cancers of the cervix, vulva, vagina, penis, anus, and oropharynx. It is estimated that 79 million Americans are currently infected with HPV. The Advisory Committee on Immunization Practices (ACIP) recommends three doses of HPV vaccine through age 26 for females and through age 21 for males who are not at increased risk. HPV vaccine is recommended through age 26 for men who have sex with men or are immunocompromised. The objective of this study was to assess adult HPV immunization coverage in Texas and identify disparities. M**ethods:** HPV immunization coverage was assessed using the Behavioral Risk Factor Surveillance System (BRFSS), a nationwide telephone survey which obtains state and local level estimates of health indicators. Adults aged 18-49 years sampled for Texas were asked "A vaccine to prevent the human papillomavirus or HPV infection is available and is called the cervical cancer or genital warts vaccine, HPV shot, Gardasil, or Cervarix. Have you ever had an HPV vaccination?" Data is composed of weighted immunization coverage levels by race/ethnicity, insurance status, education levels and geographic area for adults eligible to be vaccinated with the HPV vaccine. **Results:** Among Texas adults, 17.0 percent of females and 3.5 percent of males had ever received an HPV vaccination. Coverage was higher in Hispanics, those with lower incomes, and those with some college education. C**onclusion:** The majority of Texas adults are unvaccinated against HPV, largely because ACIP only recommends immunization through age 26 for females and age 21 for males, and health care utilization among this group is low compared to other age groups. Strong healthcare provider recommendations for HPV vaccine are necessary in order to improve coverage among eligible young adults aged 18-26. Uninsured adults can receive all ACIP-recommended vaccinations, including the HPV series, at no cost through the Texas Adult Safety Net Program (ASN).

489

Estimating HPV Vaccine Coverage in Texas Teens Using the National Immunziation Survey – Teen, 2014 *L. Palenapa, Texas Department of State Health Services; E. Gardner, Texas Department of State Health Services; K. Guerrero, Texas Department of State Health Services*

Introduction: Human papillomavirus (HPV) causes approximately 33,200 new cancers in the US each year, including cancers of the cervix, vulva, vagina, penis, anus, and oropharynx. It is estimated that 79 million Americans are currently infected with HPV. The Advisory Committee on Immunization Practices recommends three doses of HPV vaccine at age 11-12 years for both males and females. Despite the safety and effectiveness of the HPV vaccine, state and national coverage levels for children in this age group fall far below those of the other adolescent vaccines. The objective of the study was to assess adolescent HPV immunization coverage in Texas. M**ethods:** HPV immunization coverage in Texas adolescents was assessed using the National Immunization Survey – Teen (NIS-Teen), a nationwide survey of parents and guardians used to monitor vaccination coverage among teens aged 13-17 years at state and local levels. Vaccines and dates of administration are verifie and collected from the child's vaccination provider(s). Local level data included coverage estimates for: Bexar County, El Paso County, City of Houston, and the Rest of the State (excluding the aforementioned local areas). **Results:** In 2014, one-dose HPV coverage among females in Texas was 50.7 percent, a 4.8 percentage point decrease from 2013. This was lower than the national level of 60.0 percent. Among males in Texas, one-dose HPV coverage was 36.6 percent. This was a 2.9 percentage point increase from 2013, but was below the national average of 41.7 percent. Three-dose coverage among females in Texas was 33.9 percent. This was a 3.9 percentage point decrease from 2013 that was below the national average of 39.7 percent. Among males in Texas, three-dose coverage was 17.7 percent, which was below the national average of 21.6 percent but increased 2.8 percentage points from 2013. Among local areas in Texas, El Paso County had the highest levels of HPV coverage and exceeded national coverage levels. City of Houston also exceeded national levels for all measures of HPV coverage. C**onclusion:** HPV coverage levels continue to increase on the national level, and among males in Texas. Progress towards national goals for HPV coverage must continue each year. Females in Texas experienced a drop in coverage levels in 2014, and though this drop was not statistically significant, it must be addressed. DSHS continues to develop and implement strategies to increase HPV coverage levels in Texas adolescents, including media campaigns, healthcare provider communications, and collaboration with various stakeholders.

490 **CPRIT Grantee**
Poster Session B

Adapting Colorectal Screening Education for the Rural West Texas Population *T. Byrd, Texas Tech University Health Sciences Center; N .Shokar, Texas Tech University Health Science Center at El Paso*

Introduction: A successful intervention for CRC screening (ACCION) was developed for the US/Mexico border region of El Paso, Texas. Because the rural population of west Texas has high rates of CRC and lower rates of screening than other regions, we are developing a similar program in the Lubbock and eight county surrounding area. Because the educational materials used in ACCION were mostly in Spanish and included Hispanic actors in the video, we will adapt the current educational tools for the rural west Texas population. **Methods:** We are completing community focus groups in our 9 rural counties to better understand the issue in this region. Focus groups will be completed in July, and we intend to re-shoot the ACCION video using local rural resdients and focusing on the issues here. The new footage will be edited into the current ACCION video. **Results:** The new video should be completed by September of this year. We expect that using focus groups to gather information about the community and using community residents as actors will increase the efficacy of the education and will help us get the same good screening results as we did in the original ACCION program. **Conclusion:** We expect to use the ACCION video with the Hisapnic population in rural west Texas, and to use the adapted version with other populations Assuming that our education is successful in increasing CRC screening, we will show how to adapt existing educational interventions for new populations.

491 **CPRIT Grantee**
Poster Session A

MHMR of Tarrant County Smoking Cessation Program Within Behavioral Health *G. Hollis, MHMR of Tarrant County; K. Curry, MHMR of Tarrant County*

Introduction: MHMR of Tarrant County's No Butts: New Opportunities Smoking Cessation Program model was developed with assistance from the Smoking Cessation Leadership Center and the Robert Wood Johnson Foundation and based on a review of evidence-based best practices. No Butts, New Opportunities (NBNO) encompasses five components : (1) the National Association of State Mental Health Program Directors (NASMHPD) Tobacco-Free Living Model, (2) the Fagerstrom Nicotine Dependence Questionnaire (FTND), (3) the 5 A's of Prevention, (4) the Texas Youth Tobacco Awareness Program (TYTAP), and (5) social marketing to reinforce tobacco cessation. CPRIT funding allowed for service expansion of NBNO to MHMR's Mental Health division, offering services in all Mental Health Clinics. In addition, with the help of partner agency Tarrant County Challenge, NBNO provides smoking cessation treatment to youth in Tarrant County and uses social marketing to improve the outcomes of all No Butts clients. M**ethods:** As part of the infrastructure changes, the required online training of the Center of Tobacco Treatment Research Training (CTTRT) was expanded to the mental health clinicians. Clinicians administer a tobacco screening measure, the Fagerstrom Test for Nicotine Dependence (FTND), to individuals who reported the use of tobacco products which initiates conversation with individuals to determine if they are interested in quitting tobacco products. Individuals who wanted to make a "Quit Attempt" are invited to participate in the program which includes options such as individual counseling, nicotine replacement treatment (NRT) and group counseling. Groups are open-ended so individuals could join at any time. Program completion is determined by the individual's participation in all four sessions. Individuals are also given the option to continue to participate in group after completing the four required sessions as a support tool. **Results:** Since the program expansion there are 19 groups being held at the various MHMR Tarrant clinics on a weekly basis. Since the expansion of the program 1966 individuals have attended group sessions and 619 attended individual sessions. At this time 933 people have completed the program with success in quitting or reduced smoking. C**onclusion:** Continuous modifications have been made to enhance the programs' effectiveness such as additional teaching visual aids, guest speakers, etc. The program has shown great response through the social marketing. The program has been able to assess 97% of the new individuals that enter the MH clinics with the FTND. This allows clinicians to make follow up contact to those that show interest in quitting smoking.

492 **CPRIT Grantee**
Poster Session B

Interprofessional Training of Nursing Students and Family Medicine Residents through Clinical Simulation Activities *D. Holland, Texas A&M University System Health Science Center; L. Livingston, Texas A&M University System Health Science Center*

Introduction: The Texas A&M Health Science Center, through its Family Medicine Residency in Bryan, Texas, received grant funding in December, 2013, to provide breast and cervical cancer screening and diagnostic services to uninsured and low-income women living in a 9-county region of central Texas known as the Brazos Valley. The prevention grant takes an interprofessional approach to training family medicine physician residents, nursing students, and community health workers in clinical procedures and culturally appropriate patient care. The purpose of this paper is to describe the clinical simulation technology utilized in their training. **Methods:** In the fall of 2014 and the spring of 2015, nursing students and residents participated in interprofessional clinical simulations utilizing simulation technology, e.g., standardized patients (SP) representing a diverse patient population, a simulated female breast model, and a simulated vaginal vault made of plastic plumbing fittings with a chicken breast simulating the cervix. Paired residents and nursing students participated in a scenario with a SP portraying a female patient meeting the parameters for a breast biopsy or colposcopy. The interprofessional team conducted an initial consultation with the SP to explain the procedure, the rationalale for performing the procedure, and obtained informed consent from the SP. The scenario then moved to performing the simulated breast biopsy or colposcopy. The simulation technology provided practice in performing a breast biopsy of a detected breast mass utilizing a simulated female breast model and practice performing a colposcopy on a simulated cervix. Faculty instructed and assessed the residents in using the diagnostic equipment. Each interprofessional team then provided post-procedure consultation to the SP on the results of the diagnostic procedure. The residents and nursing students completed a pre/post-scenario survey on their knowledge and competence in performing breast biopsy and colposcopy procedures. **Results:** Participation in interprofessional clinical simulation training aided in providing 687 grant-funded procedures during the first 18 months of the grant. Results of the pre/post-scenario survey are pending completion of the grant, but early results appear to show high satisfaction with simulation technology utilized in interprofessional clinical simulation scenarios and a perceived increase in knowledge

and skills related to breast and cervical cancer screening and diagnostic procedures. **Conclusion:** Simulation technology and interprofessional clinical simulation scenarios are an acceptable methodology for training residents and nursing students on breast and cervical cancer screening procedures and culturally appropriate patient care.

493 **CPRIT Grantee Poster Session A**

Getting To the Root: Cancer Prevention that Addresses the Social Determinants of Health *M. Arjona, MHP Salud*

Introduction: According to the American Cancer Society, Hispanics are more likely to die from cancer than are non-Hispanic whites. These disparities begin in communities with existing social determinants of health long before a diagnosis is ever reached. Disparities such as these are particularly acute in the Rio Grande Valley, a four-county region located in the southernmost tip of Texas with high levels of Hispanic residents. Incidences and mortalities related to cancer in this region have exceeded state averages, indicating a significant need for outreach, education, screening and diagnostic services. **Methods:** Organizations have struggled to adequately provide these services due to their inability to reach the source of the problem; the distinct social, behavioral, and environmental determinants of health unique to this region. To address these issues at their source, resources must first be channeled to respond to community level determinants of health, a key ingredient most clinical interventions lack. Recognizing that this cannot be done without an expert in the local area and culture, the Cada Paso del Camino project utilizes Promotores(as), or Community Health Workers, as a bridge from the community to clinical services. The Promotores(as) offer outreach, one-on-one education, referrals, and follow-up, but they are only part of the overall strategy of this project. Cada Paso del Camino is a unique collaboration between a nonprofit organization and local area providers that provides holistic preventive, diagnostic, and treatment services to the underserved, uninsured residents in this region. The Promotores(as) provide the previously mentioned social services, and the clinical partners provide screening, diagnostic, and treatment services. The true innovation that distinguishes this project comes from the direct connection forged by Promotores(as) between the project and a program that offers enrollment assistance for the Health Insurance Marketplace. **Results:** Although this project is only in its beginning phases, the anticipated results are an increase in cancer screening and rescreening at RGSC from a baseline of 0 by 1,705 screenings and to offer affordable diagnostic services, and when needed, treatment to 100% of clients whose initial screening results are abnormal, serving an estimated total of 11,852 people. Furthermore, the collaborative approach will provide participants with long-lasting tools and sustainable solutions to healthcare access. **Conclusion:** Cada Paso del Camino offers a unique partnership model that could be used to inform similar future efforts within and beyond Texas's Rio Grande Valley.

494 **CPRIT Grantee Poster Session B**

The Community Network for Cancer Prevention: Using the RE-AIM Framework to Develop and Implement a Cancer Screening Educational Too *J. Montealegre, Baylor College of Medicine; L .Hanser, Harris Health System; R. Chenier, Baylor College of Medicine; I. Valverde, Baylor College of Medicine; G. Chauca, Baylor College of Medicine; M. Daheri, Harris Health System; L. Rustveld, Baylor College of Medicine; B. Reed, Baylor College of Medicine; M. Anderson, Baylor College of Medicine; L. Ramondetta, The University of Texas M.D. Anderson Cancer Center; M. Gould-Suarez, Baylor College of Medicine; B. Musher, Baylor College of Medicine; L. Scott, The University of Texas Health Science Center at Houston; J. Nangia, Baylor College of Medicine; J. Hoagland-Sorensen, Harris Health System; A. Rieber, The University of Texas M.D. Anderson Cancer Center; M. Jibaja-Weiss, Baylor College of Medicine*

Introduction: Cancer screening to detect pre-cancerous or early stage lesions is a fundamental process within the Continuum of Cancer Care. However, health clinics often face budgetary constraints and competing health issues that impede their ability to deliver cancer screening education to their patients. As part of a comprehensive systems design intervention (CPRIT grants 100201, 130084, 140028), we developed and implemented linguistic- and culturally-targeted educational videos to promote the uptake and delivery of cervical, colorectal, and breast cancer screening within a high-volume public healthcare system. Here we discuss our use of the RE-AIM implementation science framework to develop and implement the intervention. **Methods:** The Reach, Effectiveness, Adoption, Implementation, Maintenance (RE-AIM) framework was used to develop and implement a multimedia tool to facilitate patient education within a clinical setting. **Results:** The intervention was developed and implemented using all factors in the RE-AIM model. Reach considerations included creating linguistic- and culturally-tailored videos for the African American, White, Hispanic, and Vietnamese patient populations; using the intranet to make videos accessible on desktop computers in all exam rooms within the health system; and harnessing the electronic medical record (EMR) to identify all patients due for a screening test. Effectiveness considerations included using an evidence-based intervention strategy (one-on-one education with small media) and theory-based messaging to encourage screening utilization and foster effective patient-provider communication. Adoption considerations included in-service trainings of clinical staff and developing strategies to incorporate the intervention within the clinic workflow (e.g., playlist features to select multiple videos, documentation of video-viewing in the EMR as a quality measure). Implementation considerations included administrative reporting features to evaluate clinic-level performance and outcomes. Maintenance considerations included integration within clinic workflow and features to promote its use as a quality measure. To date, the videos have been implemented at 13 community health centers, 10 homeless clinics, and 3 outpatient and specialty clinics. **Conclusion:** Guided by the RE-AIM framework, this education tool has become an integral aspect of cervical, colorectal, and breast cancer screening in a high-volume safety net healthcare system. The videos may contribute to improved cancer screening by encouraging screening utilization and enhancing patient-provider communication regarding screening. Employing the RE-AIM framework ensured the integration of key features that make the intervention feasible in a busy clinical setting, foster its adoption as a quality measure, and promote its sustained use in diverse clinical settings.

495 **CPRIT Grantee Poster Session A**

Developing an Interactive Training on Cancer and Fertility for Healthcare Professionals: Lessons Learned *S. Arvey, LIVESTRONG Foundation; E. Eargle, LIVESTRONG Foundation; A. Narayan, LIVESTRONG Foundation; S. Nutt, LIVESTRONG Foundation; R. Rechis, LIVESTRONG Foundation*

Introduction: Each year, >150,000 Americans are diagnosed with cancer during their reproductive years (<45 years). With a national trend in delayed childbearing, more patients diagnosed <45 years old still wish to bear biological offspring. Despite guidelines such as ASCO's Quality Oncology Practice Initiative concerning disclosing risk of infertility, providers are still not informing nor referring patients to fertility preservation services at an acceptable rate. In addition to fulfilling clinical quality standards, healthcare professionals (HCPs) have a moral imperative to provide patient-centered care that includes addressing cancer-related fertility needs. CPRIT awarded the LIVESTRONG Foundation funds to create a cancer and fertility training for healthcare professionals, which was launched in 2015. This abstract describes the process of developing the training and lessons learned in its development and initial dissemination phase. **Methods:** LIVESTRONG staff and fertility experts established seven recommended practices to systematically address cancer and fertility at an institutional level based on existing cancer and fertility programs. LIVESTRONG Navigation staff designed algorithmic conversation simulations for effective patient/provider communications and updated online tools to assess the risks of infertility and options for family-building. LIVESTRONG contracted with educational developers to deliver the content in an online format, and experts in fertility reviewed the content throughout. **Results:** Over 12 months, four lessons were created that introduce the topic to the learner; teach the learner how to use the online risks/options calculators; lead the learner through simulated conversations and provide feedback; introduce the learner to the importance of adopting a systematic approach to cancer and fertility at an institutional level. Utilizing the learning management system (LMS) to deploy the training initially posed the biggest challenge due to difficulties collecting necessary data. We switched vendors soon after the training launched to ensure the delivery of accurate metrics, improve user experience, and decrease costs. Another challenge has been dissemination of the training. Numbers of learners are low, despite widespread, Texas-focused outreach efforts. We are exploring opportunities to improve uptake, including offering continuing education units and partnering with individual cancer clinics and hospital systems to disseminate the training to their staff. **Conclusion:** While LIVESTRONG had the expertise and resources to create the training content, the two biggest challenges to the project have been finding technological solutions best suited to the training and trying to disseminate the training broadly. We have had to come up with innovative solutions which we are pursuing now and look forward to reporting on at the next CPRIT conference.

496 **CPRIT Grantee Poster Session B**

Surveying Quality of Life, Nutrition and Health Practices of Members of the West Texas Cancer Survivors Network *K. Chauncey, Texas Tech University Health Sciences Center; B. Pence, Texas Tech University Health Sciences Center; J. Basom, Texas Tech University Health Sciences Center*

Introduction: The West Texas Cancer Survivors Network – Phase 2 (WTCSN-2) is funded by the Cancer Prevention and Research Institute of Texas (CPRIT) to provide nutrition and lifestyle advice and to promote a better quality of life (QoL) among cancer survivors in the Texas Tech University Health Sciences Center (TTUHSC) catchment area known as west Texas. We are evaluating improvements in QoL and health behaviors, as a result of the network intervention, among these cancer survivors. Health-Promoting Lifestyle Profile II (HPLP II) and the Nutrition and Health Practices Surveys are tools utilized to assess the QoL and health practices of the cancer survivors who have enrolled in the WTCSN at baseline and then again in 12-18 months, to assess WTCSN intervention of providing targeted health messages tailored for improving QoL and health practices of all cancer survivors. **Methods:** 1000 adult (age range 21 to 80 years) cancer survivors residing in West Texas, enrolling in WTCSN, are invited to participate in the QoL and Health Practices evaluation. Completion and return of the enclosed surveys, in the provided envelope, acknowledges participation. Return of blank documents acknowledges declining participation. Participants will avail themselves of targeted health messages via website (www.ttuchsc.edu/wtcsn), monthly newsletters, meetings, fact sheets, and other mailings. Approximately 12-18 months following completion of initial QoL and Health Practices Survey, participants will be sent a second set of surveys. Surveys will be scored, comparing the initial and follow up surveys, for any QoL and health behavior improvements attributable to WTCSN interventions. Statistical Analysis: We anticipate inviting a minimum of 500 and a maximum of 1000 enrollees to participate. Primarily, the appropriate paired testing procedure (likely paired t-test) will be employed to test if there were any significant changes in the HPLPII Survey or the Nutrition and Health Practices Survey between baseline and 1 year measurements and the associated 95% confidence interval for changes in scores will be reported. **Results:** This project is in progress and results/conclusions are not currently available. **Conclusion:** This project is in progress and results/conclusions are not currently available.

497 **CPRIT Grantee Poster Session A**

Usefulness of a Patient Advisory Panel in Planning Practice-Based Projects *W. Calmbach, The University of Texas Health Science Center at San Antonio; A. Machuca, The University of Texas Health Science Center at San Antonio*

Introduction: "Patient-centeredness" is a concept that is receiving much attention from researchers, patient advocacy groups, and funders. Active patient involvement in all phases of research are key to any successful PCORI application, and the "PCORI Rubric" on patient involvement includes key elements like "reciprocal relationships, co-learning, partnership, trust, transparency, and honesty". The purpose of this poster is to introduce CPRIT attendees to the concepts and lessons learned of a "Patient Advisory Roundtable" established by a CPRIT-funded practice-based research network (i.e., STARNet, the South Texas Ambulatory Research Network). **Methods:** STARNet is a practice-based research network (PBRN) comprised of approximately 40 local primary care practices. In order to improve the "patient-centeredness" of various STARNet projects, we created a "Patient Advisory Roundtable" (PAR). The eight physician members of the STARNet board of directors were each invited to nominate 3 patients whom they believed could effectively represent patient needs and interests on this panel. The STARNet Patient Advisory Roundtable meets quarterly to review current and proposed projects. The network director and coordinator prepare brief presentations to summarize projects, and highlight key points for patient input (e.g., patient-relevant research questions, outcomes important to patients, patient-friendly implementation, and patient-specific dissemination).The patient group also reviews planned patient surveys and questionnaires, helping shape them to better fi the needs of potential participants. **Results:** STARNet's CPRIT-funded project, "Teaching Behavior Change Skills to Physicians and Staff", has benefited greatly from input by the Patient Advisory Roundtable (PAR). Specificall , the patient group has reviewed planned diet and physical activity questionnaires, and a draft "Patient Toolkit" prepared for this project (e.g., diet diary, physical activity log, tips on safe weight loss, recommended websites, pedometer and instructions). "Lessons learned" from our experience with the Patient Advisory Roundtable include: a. maintaining close contact with patient members, b. using multiple means of communication (e.g., email, phone calls, quarterly in-person meetings, US mail, etc.), c. keeping patient members busy, especially

with meaningful work such as reviewing projects and/or questionnaires, d. providing positive feedback about the value of their input, e. teaching patient members about basic research principles (e.g., protecting human subjects, ethics, sample size calculations, etc.), f. allowing each member to pursue an element of the proposal that fits their interest and/or skillset, and g. sharing patient insights with STARNet board members, physicians, and practice staff. **Conclusion:** Creating and sustaining a Patient Advisory Roundtable is time-consuming and resource-intensive, but is invaluable to PBRN's and primary care researchers.

498 **CPRIT Grantee**
Poster Session B

Project ECHO Telementoring to Improve Cervical Cancer Prevention Services in Medically Underserved Areas *K. Schmeler, The University of Texas M.D. Anderson Cancer Center; M. Lopez, The University of Texas M.D. Anderson Cancer Center; E. Baker, The University of Texas M.D. Anderson Cancer Center; R. Gowen, The University of Texas Health Science Center at Houston; A. Milbourne, The University of Texas M.D. Anderson Cancer Center; A. Rodriguez, The University of Texas Medical Branch at Galveston; S. Fisher-Hoch, The University of Texas Health Science Center at Houston; M. Mallory, Su Clinica; P. Toscano, The University of Texas Health Science Center at Houston; C. Carey, Su Clinica; E. Hawk, The University of Texas M.D. Anderson Cancer Center*

Introduction: Project ECHO, Extension for Community Healthcare Outcomes, is an established telementoring model proven to increase capacity and expand access to specialty medical care for underserved areas. ECHO uses videoconferencing and clinical tools to build capacity among clinicians via case-based learning and co-management of patients. We have adapted the ECHO model to support physicians and providers in the management and treatment of cervical dysplasia in medically underserved areas. **Methods:** Regular multi-disciplinary videoconferences are held connecting specialist teams at MD Anderson Cancer Center in Houston and the University of Texas Medical Branch (UTMB) in Galveston, with colleagues in medically underserved areas along the Texas-Mexico border. Partnering sites include Su Clinica, a FQHC system with clinics in Brownsville, Harlingen and Raymondville, the University of Texas Mobile Health Clinic in Brownsville, and the UTMB Stop Cervical Cancer Clinic in McAllen. The 1-hour videoconferences are held bi-weekly using a free, internet-based application and include 45 minutes of case presentations by local providers with discussion, feedback and mentoring from specialists, followed by a 15 minute didactic lecture. Additional hands on training has been provided to local clinicians, with continued mentoring and skill development supplemented through ECHO conferences. **Results:** The Cervical Cancer Prevention ECHO program began as a pilot project in April, 2014. To date, 32 ECHO telementoring conferences have been held, with an average of 17 providers participating per session. More than 137 CME and 66 CNE credits have been issued. Additionally, two providers have been trained in colposcopy and one in performing loop electrosurgical excision procedures (LEEPs), resulting in increased care delivery capacity in the region. Workshops and visits by teams in all locations supplement regular videoconferences and provide further mentoring and support. Building on the success of this program, the MD Anderson team has expanded its reach, using the same ECHO model for management and treatment of cervical dysplasia and invasive cervical cancer with providers in Latin America (Guatemala, El Salvador, Colombia, Uruguay and Brazil) and Africa (Zambia and Mozambique). **Conclusion:** Our initial experience suggests that Project ECHO is an effective platform to provide dissemination of best practices in the management of cervical dysplasia in settings that lack access to colposcopy and oncology specialists.

499 **CPRIT Grantee**
Poster Session A

Innovative Methods to Increase Community Health Workers Available for Outreach *S. Misra, Texas Tech University Health Science Center at Amarillo; N. Ray, Texas Tech University Health Science Center at Amarillo; M. Marsh, Texas Tech University Health Science Center at Amarillo; M. Guzman, Texas Tech University Health Science Center at Amarillo; D. Gregory, Texas Tech University Health Science Center at Amarillo; A. Mishra, Texas Tech University Health Science Center at Amarillo*

Introduction: As part of the grant application process to increase colorectal cancer (CRC) screening rates, we had budgeted for two half time Community Health Workers (CHWs), but realized this would limit the time available for outreach. Since the grant covers 10 counties over approximately 25,000 square miles, the cost of increasing staffing and travel would be higher. Given the diverse and widespread area our team targets, there was a need to increase the numbers of CHWs to reach our goals without increasing costs. We adapted innovative methods of increasing the number of available CHWs by partnering with an established home health care organization that already has state certifie CHWs. M**ethods:** Comprehensive review of the grant budget to reallocate funds for CHW costs was preformed. Satisfaction Surveys from education sessions were analyzed to assess quality measures. **Results:** Using this innovative approach, we were able to increase the number of CHWs from two to eight. Since the CHWs are on a contractual basis, we were able to save costs of $9,050 for fringe benefits, $5,000 for travel and $9,800 on CHW salary by only paying for hours worked, bringing the reduction in cost to $23,850 over five months. This has also provided the availability to host events in multiple counties on the same day and to have multiple CHWs on site to answer questions and assist with paperwork. To ensure education sessions are of high quality and consistent despite having more CHWs post education participant satisfaction surveys were analyzed. 98.7% thought the education sessions were a good use of time and provided enough information to make decisions about CRC screening. 97.4% agreed the information presented was easy to understand and the CHW provided reassurance about procedures discussed. 93.6% thought the CHW provided an adequate explanation of confusing/complex information and the amount of information provided was "about right". 11.7% rarely felt embarrassed about the information presented, while 84.4% were not embarrassed at all. **Conclusion:** Limiting the hiring to

key personnel while contracting with local partners has provided cost reduction with respect to travel, fringe benefits, and only paying CHWs for hours worked. This has led to better coverage and flexibility to host outreach events in multiple counties on the same day. Satisfaction surveys show the education provided is of high quality and participants appreciate the program and find it beneficia

500 **CPRIT Grantee**
Poster Session B

Implementation of a Tobacco-Free Workplace Program in Texas Community Mental Health Clinics: Barriers and Solutions as Identified by Clinic Leaders *L. Reitzel, University of Houston; E .Paredes, University of Houston; P. Agrawal, University of Houston; V. Correa-Fernández, University of Houston; T. Williams, Austin/Travis County Health and Human Services Department; W. Wilson, Austin/Travis County Health and Human Services Department; B. Kyburz, Austin/Travis County Health and Human Services Department; T .Stacey, Austin/Travis County Health and Human Services Department; J. Goode, Betty Hardwick Center; S. Broussard, Spindletop Center; M. Howard, Heart of Texas Region MHNR; B. Alderman, Austin/Travis County Health and Human Services Department; C. Lam, Rice University*

Introduction: Tobacco use rates are high among individuals with mental illness; thus, tobacco-related cancer incidence is elevated for this group relative to the general population. Comprehensive tobacco free workplace policies are effective for reducing smoking rates among mental health consumers and clinic employees. The Taking Texas Tobacco Free (TTTF) project is a CPRIT-funded prevention effort (12/01/13 – 11/30/16) to disseminate and implement a sustainable, comprehensive tobacco free workplace program to local mental health authorities (LMHA) across Texas. TTTF provides education, policy guidance, resources (e.g., a starter supply of Nicotine Replacement Therapies (NRT)), and clinician training to reduce tobacco use rates among consumers and employees, and has been fully implemented in 7 LMHAs to date. In July 2015, we evaluated the experiences of associated clinic leaders to identify obstacles experienced and potential solutions to enhance implementation for the remaining 11 targeted LMHAs. **Methods:** Individual interviews were conducted with LMHA's leadership using a standardized interview script, and brief surveys were administered. **Results:** Identified barriers included a need for additional trained Certified Tobacco Treatment Specialists (CTTS), unexpectedly high demand and insufficient planning/budgeting for sustained availability of NRT, inability to provide consumers' relatives with free NRT, the absence of a tracking mechanism for NRT distribution, a lack of consumer involvement in the initial planning of the tobacco-free workplace policy change, a need for additional training and engagement of staff, challenges with tobacco-free policy compliance within residential settings, and a lack of readily available educational materials for consumers to take home. **Conclusion:** As a result of the information learned and in collaboration with the LMHAs, we plan to increase CTTS training capacity, provide supplementary NRTs, and offer additional printed materials. Clinic leaders emphasized that the sustained availability of NRT increased consumers' and employees' support for the tobacco-free program, highlighting the importance of this program component to policy implementation. Per the leaders' feedback, we recommend that the remaining 11 LMHAs seek additional funding (e.g., governmental contracts) to procure and offer NRT to their consumers and staff beyond the amount provided by TTTF. Additionally, we recommend that LMHAs consider consumer feedback and policy planning involvement to facilitate buy-in. The presentation will also describe other themes that emerged from the interviews, including the usefulness of having a staff committee involved in policy roll-out. Together, this information will impact our activities during the remainder of the CPRIT funding period, and can help inform the implementation of other tobacco-free workplace programs.

501 **CPRIT Grantee**
Poster Session A

The Development of a Cancer Genetic Evaluation Referral Training Program for Texas Health Educators *L. Chen, Texas A&M University; S. Dhar, Baylor College of Medicine; T. Eble, Baylor College of Medicine; S. Zhao, Texas A&M University; D. Talwar, Texas A&M University*

Introduction: Genomics, a part of personalized medicine, is an emerging trend in the field of cancer prevention and control. Leading health agencies advocate the need for non-genetic professionals to assess cancer family histories and refer high risk clients to genetic professionals for genetic evaluation and testing to understand their risk of developing diseases. As health educators provide education on health topics and deal with vast and different communities, training this professional group to establish those genomic competencies is needed. **Methods:** We assembled an interdisciplinary research team with geneticists, genetic counselors, community health workers, public health practitioners and certified health education specialists to develop the first cancer genetic evaluation referral training program for Texas public health educators. This training program is currently funded by the Cancer Prevention and Research Institute of Texas. **Results:** This cancer genetic evaluation referral training program was based on health behavior theoretical framework, including the Theory of Planned Behavior, the Social Cognitive Theory and the Diffusion of Innovations Theory. It included four main modules and eleven learning objectives. The context covered a variety of topics, including the definitions of genetic evaluation and testing, ACCE model to evaluate genetic testing, cancer family history assessment, genetic professionals search and genetic evaluation referral. **Conclusion:** This first theory-based cancer genetic evaluation referral training program is anticipated to increase Texas health educators' knowledge and skills about 1) genetic evaluation and testing and 2) the identification and referral of high risk clients to genetic professionals for genetic evaluation and testing.

502 **CPRIT Grantee Poster Session B**

Entre Familia: Educating Hispanic Adolescents and their Families on Cervical Cancer and HPV Vaccination *D. Morales-Campos, The University of Texas Health Science Center at San Antonio; D. Parra-Medina, The University of Texas Health Science Center at San Antonio; M. Morales, The University of Texas Health Science Center at San Antonio*

Introduction: Women in the LRGV experience higher incidence and mortality from cervical cancer compared to the state and nation. Prevention of cervical cancer is possible through use of two vaccines, which protect against two strains of HPV (16, 18) that cause 70% of cervical cancer cases. The HPV vaccine is recommended for women/men (aged 18-26) and girls/boys (aged 11-17). A smaller proportion of Hispanic girls in Texas aged 13-17 compared to Hispanic girls nationwide receive ≥1 dose of the vaccine (57% vs. 63%), and fewer receive all three doses (31% vs 36%). This proportion falls short of the Healthy People 2020 target of 80% of girls aged 13-15 receiving all three doses. Additionally, only 13% of U.S. Hispanic males age 13-17 have completed the vaccine series, compared to just 10% of males in Texas. Infrequent healthcare visits, missed opportunities for vaccination during urgent visits, and lacking health insurance and/or a usual source of care, have been identifie as barriers to receiving timely vaccinations. Our program used trained promotoras to deliver health education to families and collaborations with local community organizations and clinics to overcome these barriers. **Methods:** Promotoras delivered Entre Familia (EF), a cervical cancer prevention, education, and outreach program to Hispanic families. Eligible program participants were Hispanic adults with boys and/or girls ages 11-17 years old who lived in Hidalgo County. Promotoras provided health education sessions to adults in two formats: group with a flipchar or one-on-one with a print brochure. They also provided referrals and navigation support for parents who requested assistance with vaccinating their children. Our clinical partner provided vaccinations to our program participants, idenitified vaccine eligible clinic patients, and reported vaccine status of program participants based on medical charts. **Results:** The program is currently underway. We expect EF will increase HPV immunization rates (initiation and completion) using public education and clinic in-reach strategies among Hispanic adolescent males and females in Hidalgo County. From 8/2014 to 8/2016, we estimate (1) reaching 3,000 adult residents of Hidalgo County through outreach; (2) educating 1,500 adult residents of Hidalgo County using EF's evidence-based education sessions; (3) meeting or exceeding Texas' vaccine initiation (33%) and completion (10%) rates for males; and (4) meeting the vaccine initiation (88%) and completion (46%) rates for girls exposed to the initial EMH program. **Conclusion:** By increasing vaccine initiation and completion, EF has the potential to reduce cervical cancer incidence and mortality among Hispanic women in Texas' LRGV.

503 **CPRIT Grantee Poster Session A**

Cancer Genetic Risk Assessment, Counseling, and Screening of High-risk Individuals in Greater San Antonio and South Texas: Education and Implementation *G. Tomlinson, The University of Texas Health Science Center at San Antonio; L. Mette, The University of Texas Health Science Center at San Antonio; I. Torres, The University of Texas Health Science Center at San Antonio; A. Pulido Saldivar, The University of Texas Health Science Center at San Antonio; N .Poullard, The University of Texas Health Science Center at San Antonio; H . Parsons, The University of Texas Health Science Center at San Antonio; B .Pollock, Univ California Davis; E. Marin, The University of Texas Health Science Center at San Antonio; S. Gomez, The University of Texas Health Science Center at San Antonio; N. Esquivel, The University of Texas Health Science Center at San Antonio; D. Seigler, The University of Texas Health Science Center at San Antonio; I .Jatoi, The University of Texas Health Science Center at San Antonio; J .Tysinger, The University of Texas Health Science Center at San Antonio*

Introduction: It is well recognized that an appreciable portion of breast and colon cancer is hereditary in nature. The USPSTF recommends screening patients' family history to identify those with a predisposition to cancer with subsequent referral to for genetic counseling and testing. Limitations in South Texas have been the lack of professional education in cancer genetic risk assessment and the absence of cancer genetic counselors. Our project served two purposes: 1) to educate the South Texas lay and professional population in the importance of family history and genetic risk factors for cancer and 2) to provide access to cancer genetic counseling services in underserved regions of South Texas. Barriers in our population include lack of resources and health insurance, high poverty and illiteracy rates and distance to tertiary care centers. M**ethods:** We reviewed family histories at multiple venues including mammography and primary care centers, provided a presence in primary care clinics at a FQHC and established a weekly clinic at a primary care clinic in downtown San Antonio. We established hubs for videoconferencing in three regional border facilities providing weekly access to San Antonio-based genetic counselors. We facilitated the establishment of a high-risk cancer screening clinic at our university cancer center. **Results:** We provided direct education to 3,137 professionals through formal presentations and small group discussions. Within trainee programs, we evaluated learner knowledge through pre-test and identified specific weak sses in communication approaches. We have provided clinical services including family history review to over 3000 individuals and genetic counseling and testing to 640 individuals. We observed 125 genetic abnormalities including 41 different BRCA1/2 mutations in Hispanic women, five of which were previously unreported and identified a new Hispanic founder mutation. Genetic counseling by videoconferencing was found to be highly satisfying (4.7 on 5.0 scale) with universally very positive comments. We provided 215 cancer screening services and identified four invasive breast cancers. Multiple patients were found to have colonic polyps which were successfully removed. We observed improvement in the appropriateness of provider services for cancer genetic counseling over the course of the project. Although we anticipated treatment barriers for newly discovered cancers, all patients with cancer diagnoses were successfully navigated to receiving treatment services. **Conclusion:** Through disseminated lay and professional education a highly effective cancer risk assessment and intervention program was established in a previously underserved region. Cancer genetic counseling via videoteleconferencing is well accepted and effective in underserved areas of South Texas.

504 **CPRIT Grantee**
Poster Session B

Adapting and Implementing Cancer Education Among Refugee Families *A. Raines Milenkov, The University of North Texas Health Science Center at Fort Worth; L. Smith, The University of North Texas Health Science Center at Fort Worth; E. Baker, The University of North Texas Health Science Center at Fort Worth; R. Qualls-Hampton, The University of North Texas Health Science Center at Fort Worth*

Introduction: Cancer prevention education and screening among refugees are not standard services provided by resettlement agencies. Services exist that could address the health of refugees, but complex barriers exist that prevent their use. Building Bridges is a program that provides breast, cervical and liver cancer education to refugee women in the community setting and links them into appropriate health services. This presentation aims to describe individual and group education adapted for Bhutanese, Burmese (Karen ethnic group), Somalian-Bantu, and Congolese refugees and highlights different approaches used for each group. **Methods:** Refugee community leaders/community experts provided consultation during the adaptation of research tested intervention programs (RTIPs). This process included reviewing and discussing existing materials, incorporating cultural beliefs and norms, and discussing with community experts best approaches for presenting the information. Community experts also reviewed translated materials for accuracy. Adaptation continues with the Lay Health Educators in their specific communities. **Results:** The result of this process was the development of four culturally and linguistically appropriate cervical, liver and breast cancer education materials and a culturally sensitive approach to outreach, education and cancer screening. **Conclusion:** RTIPs are effective in increasing cervical, liver, and breast cancer screenings. Adapting these educational interventions for other populations, including recently arriving refugee populations, expands the ability to reach underserved populations. The process of partnering with community leaders conveys respect for their culture, increases the ability to reach the target population and support for the intervention. The use of lay health educators from each community provides insights into effective development and implementation of education materials.

505 **CPRIT Grantee**
Poster Session A

A Novel Online Patient-Centered Decision Support Service to Empower Childhood Cancer Survivors in Managing Screening and Prevention of Late Effects *F. Bonaduce de Nigris, Baylor College of Medicine; P. Lupo, Baylor College of Medicine; M. Scheurer, Baylor College of Medicine; E. Shohet, Baylor College of Medicine; J. King, Baylor College of Medicine; M. Horowitz, Texas Children's Cancer Center; D. Poplack, Texas Children's Cancer Center; M. Fordis, Baylor College of Medicine*

Introduction: This project builds upon earlier work in which the Passport for Care (PFC) was developed to provide clinicians with follow-up screening recommendations for potential late effects (PLEs) based upon a survivor's history of cancer therapy. To increase survivor participation in follow-up screening/preventive services the current project creates a Survivor-Centered Service System (SCSS) that includes pediatric cancer treatment centers, the Texas Cancer Registry (TCR), survivor navigation services, and a novel PFC Survivor Website (PFC-S) that provides survivors with direct access to treatment summaries and individualized care recommendations in lay language. Collaboration with the TCR allows the SCSS to identify survivors who may be lost to follow-up screening. Project aims are to increase survivors' knowledge of risks of late effects and involvement in their care; provide portable access to treatment summaries and recommended assessments for use by survivors and their clinicians; and address system fragmentation by providing navigator guidance. **Methods:** Development of the PFC-S was guided by qualitative data obtained from interviews and focus groups with survivors and caregivers, usability testing, and field testing. The user feedback prompted a number of improvements to the organization and design of the interface and information provided, including expanded use of lay language and translation of the website, PLEs, and individualized recommendations into Spanish. User surveys were designed to measure attitudinal, knowledge, and behavioral change, and to include the Patient Activation Measure (PAM) items. Survivors and caregivers are invited to participate and complete a survey during initial PFC-S log in (baseline) and one year later (post). **Results:** To date, treatment histories from over 1,000 Texas-based survivors across 13 clinics have been entered into the PFC-S, and about 400 survivors/parents have logged in to access their profiles. Of those accessing the PFC-S, 30% have completed baseline surveys. Preliminary analyses indicate that less than half of respondents have High or Very High Knowledge of prior cancer treatments; one third find it Difficult to describe their prior treatments when seeing a new clinician; and one quarter have High or Very High Knowledge of potential late effects **Conclusion:** These early survey findings are consistent with findings from initial qualitative research and suggest the critical need for this intervention. The project will assess if and how the PFC-S empowers survivors in understanding and managing risks and participation in screening in partnership with their clinicians whether they be primary care clinicians, pediatric or adult oncologists.

506 **CPRIT Grantee**
Poster Session B

Loteria de Comadres Contra el Cancer *F. Garcia, Mercy Ministries of Laredo; R. Welsh, Mercy Ministries of Laredo; R. Ramirez, Mercy Ministries of Laredo; M. Trejo, Mercy Ministries of Laredo; S. Ramirez, Mercy Ministries of Laredo*

Introduction: Mercy Ministries of Laredo Clinic (MML) serves some of the poorest neighorhoods and colonias in the United States. MML serves 2,500 unduplicated patients and sees 30,000 encounters every year. Communities reached through the Loteria de Comadres Contra el Cancer project suffer from deep disparities including ethinic composition (essentially 100% of the families served are of Hispanic origin),per capatia income ($10,759),and percentage of families below poverty level(88% of the population served by MML) (US Census Bureau,2006). **Methods:** MML implemented an innovative and highly participatory outreach and educational program including: 1) promotora (community educator-based) outreach through platicas (informal talks) and traditional Loteria (Mexican Bingo) games; 2) mailed reminder cards and telephone calls to patients when the date for their screening tests approaches or when they are already past due; 3) screening appointments scheduled at point and time of contact in the community; 4) peer-to-peer education and motivation using a snow-balling strategy where individuals, who have been contacted by MML, bring another person from their family or community who needs cancer screening services with them; 5) partnering with local media to share cancer screening messages; and 6) messages from clinical nurse practitioners and promotoras reinforced at every point of contact, using evidenced based, culturally appropriate bilingual educational materials on breast, cervical, and colorectal cancers. **Results:** A total of 2,907,520 people have been reached and given information on cancer prevention during the past 3 years. The majority of the contacts were through radio, television and newspaper along with breast cancer walks and a trail ride that promoted cancer prevention. Loteria (bingo-style) games were developed on cancer prevention and used throughout the project period. Seven hundred and thirty one people were impacted during the Loteria games. The number reached in the platicas totaled 2,984. All who attended the project activities were given an appointment for screening. Thirty-three to thirty-eight percent of the patients scheduled kept their appointments. A total of 1,300 persons were screened in the three year period. C**onclusion:** The project demonstrated the effectiveness of a community-based and culturally relavant approach

to cancer screening. Barriers and challenges were addressed and questions answered as they surfaced during the interactive sessions. A high rate of missed appointments led to team discussions and plans to address contributing factors. Overall, the project demonstrated a culturally sensitive, economically and linguistically appropriate way to promote cancer screening.

507 **CPRIT Grantee Poster Session A**

Barriers to HCV Screening and Care in South Texas Baby Boomers *K. Craig, The University of Texas Health Science Center at San Antonio; L. Tenner, The University of Texas Health Science Center at San Antonio; B. Turner, The University of Texas Health Science Center at San Antonio*

Introduction: The United States Preventative Services Task Force has recommended a one-time screening of hepatitis C virus (HCV) for individuals born between 1945 and 1965 (baby boomers). Despite the fact that south Texas has a significantly higher than average prevalence and incidence of hepatocellular carcinoma and HCV, physicians and communities have been slow to adopt these recommendations. This qualitative study explores the personal beliefs of patients with HCV and their perceived barriers to screening and care through the Health Belief Model (HBM). **Methods:** Sixteen HCV positive persons born 1945-1965 were recruited into three focus groups between December 2014 and February 2015. Open-ended questions were used to facilitate discussion on perceived severity, susceptibility, benefits and barriers to HCV screening and care. Each focus group discussion was transcribed and subsequently coded by four separate coders that identified the number of times a theme from the HBM was mentioned. The responses were characterized into twenty-six categories grouped according to the six HBM tenets. The scores were averaged across all four coders and the 26 categories were scored according to quartile. **Results:** The categories that scored in the top quartile in three of three focus groups were a perceived severity of HCV causing fear for oneself and a perceived barrier to care because of embarrassment and stigma about having HCV. The themes that scored in the top quartile in two of the three focus groups are the following: the perceived susceptibility of infecting family members, the perceived barriers of both lack of personal knowledge about HCV and having distracting comorbidities that take priority over HCV screening and care, and the perceived difficulty with the cue to action of acknowledging or starting to reduce or stop alcohol use. C**onclusion:** The most significan barriers to HCV screening and care were the emotional stigma of disease as well as educational deficits. Future ongoing projects are working on education tools for the community to help overcome the knowledge gaps of HCV and lessen the fear and embarrassment individuals may experience with this diagnosis.

508 **CPRIT Grantee Poster Session B**

Evaluation of the CPRIT 2-1-1 Program to Increase Cancer Control Behaviors Among Underserved Texans *M. Fernandez, The University of Texas Health Science Center at Houston; L. Savas, The University of Texas Health Science Center at Houston; R. Arya, The University of Texas Health Science Center at Houston; G. Dawson, The University of Texas Health Science Center at Houston; R. Wood, The University of Texas Health Science Center at Houston; M. Khan, The University of Texas Health Science Center at Houston; D. Jobe, United Way of Greater Houston 211 Texas/United Way HELPLINE ; S. Vernon, The University of Texas Health Science Center at Houston*

Introduction: Increasing utilization of cancer control and prevention services is an opportunity to improve survival and decrease mortality disparities in minorities, uninsured peoples, and the poor. Health behaviors such as cancer screening and vaccination can reduce colorectal, breast, and cervical cancer incidence and mortality. To reach large numbers of underserved Texans, the University of Texas School of Public Health (UTSPH) and the 2-1-1 Texas Helpline formed a unique partnership to deliver this CPRIT 2-1-1 Prevention Program. 2-1-1 is a nationally designated Helpline that connects low-income and minority callers with health and social services. The goal of this program was to link callers to cancer control and prevention services in the Houston area and the Lower Rio Grande Valley and to evaluate program effect on increasing cancer prevention services. **Methods:** To evaluate the effectiveness of a Cancer Control Navigation (CCN) intervention delivered by phone on increasing use of cancer control services among 2-1-1 callers we used a randomized comparison group design. We assessed the impact of the program on increasing cancer screening behaviors, HPV vaccination, and smoking cessation among a sample of 2-1-1 callers. Trained 2-1-1 Information Specialists invited callers to complete a risk assessment after receiving usual 2-1-1 service. They offered eligible callers referrals and invited them to participate in the program. Callers that agreed to participate were randomized to either the CCN condition or referral only condition. We conducted follow-up at three and six months to assess whether participants obtained the needed cancer control service. **Results:** When considering any needed cancer control outcome, CCN significantly increased completion of the cancer control behavior by 25% ($p < .05$). CCN also significantly increased colorectal screening by 59% ($p < .05$). For other specific behaviors, while not statistically significant, results indicated that CNN increased Pap test completion by 31%, mammography by 23%, HPV vaccination of daughters by 42%, and participation in smoking cessation programs by 90%. **Conclusion:** Evaluation results demonstrate program feasibility to reach 2-1-1 callers and deliver navigation to cancer control services. Outcome evaluation results also demonstrate effectiveness regarding increasing cancer control behaviors among underserved Texans. This 2-1-1-delivered program has great potential to connect large numbers of medically underserved Texans to cancer prevention services and decrease the burden of cancer in hard-to-reach ethnic minorities, rural, and poor subgroups.

509 **Poster Session A**

Chronic Disease Prevention & HIV Programs: A Synergistic Partnership *J. Hyde, Texas Department of State Health Services*

Introduction: People living with HIV (PLWH) who smoke lose more years of life to tobacco use than to HIV infection if undergoing treatment. The smoking rate among PLWH is 2 to 3 times higher than that of the general population. Smoking makes it more difficult to fight off opportunistic infections, leaving PLWH particularly vulnerable to developing serious chronic illnesses. Tobacco use can also complicate HIV treatment by decreasing the effectiveness of antiretroviral therapy. Smoking is a cross-cutting risk factor for developing chronic disease and exacerbating acute illness; therefore, chronic disease prevention and HIV programs are in a unique position to collaboratively leverage resources to reduce smoking rates among PLWH. **Methods:** The Texas Comprehensive Cancer Control Program, the Tobacco Prevention & Control Branch, and the TB/HIV/STD/Viral Hepatitis Unit of the Texas Department of State Health Services have come together with the common goal of improving the health of PLWH by reducing their rate of tobacco use. Collaboration to date has included the promotion of Ask, Advise, Refer (AAR), an evidence-based smoking cessation protocol for clinicians that encourages referrals to the state quitline (QL); dissemination of the "Cigarettes are My Greatest Enemy" (CAMGE) advertisement; and implementation of The Last Drag, a culturally tailored smoking cessation class for lesbian, gay, bisexual, transgender (LGBT) or HIV-positive smokers. **Results:** More than 350 AAR toolkits were mailed out to HIV service providers throughout Texas, and a live AAR training webinar was offered. As a result of the webinar, 81% of attendees indicated they would make changes in their practice/service setting, generating requests for additional toolkits and assistance with integrating AAR into standard practice. The CAMGE advertisement will run from June to August 2015 in select publications in Texas' major metropolitan areas, with potential impressions exceeding 2.3 million. Implementation of The Last Drag is currently underway in San Marcos and Tyler, Texas. Currently, the QL does not collect data on HIV serostatus; however, other measures of success include an increase in the number of clinicians who submit QL referrals and the number of registrants who self-identify as LGBT, as more than two-thirds of new HIV diagnoses in Texas are among men who have sex with men. The projected impact of these activities is an overall decrease in smoking rates among PLWH in Texas. **Conclusion:** Acute and chronic disease programs can pool their resources to form effective partnerships that advance the health of marginalized populations, such as PLWH.

510 **Poster Session B**

Improving Service Delivery To Cancer Survivors In Primary Care Settings *L. Foxhall, The University of Texas M.D. Anderson Cancer Center; A. Rodriguez, The University of Texas M.D. Anderson Cancer Center; L. Palos, The University of Texas M.D. Anderson Cancer Center; P. Lewis-Patterson, The University of Texas M.D. Anderson Cancer Center*

Introduction: The aim of this project is to improve evidence based prevention service delivery to those who have survived cancer diagnosed in adulthood being cared for in primary care practices. This innovative intervention is expected to promote the adoption of changes in practice systems associated with improved coordination and delivery of recommended services. **Methods:** The project utilizes a comprehensive approach to engage cancer survivors, oncology specialists and the primary care clinical team. Practice system changes will be implemented to identify cancer survivors currently receiving general medical care in the three (3) partnering practices. The knowledge base of primary care clinicians related to survivorship care management will be assessed and any gaps addressed through an existing CPRIT funded online curriculum. The clinicians will obtain or develop treatment summaries and survivorship care plans for those patients based on best evidence and guidelines developed by recognized organizations. Procedures will be implemented to promote communication with treating oncologists or cancer centers to coordinate delivery survivorship care management to reduce duplication of effort and eliminate gaps in care. Tele-mentoring will be provided through regular interactive sessions. Led by cancer center faculty content experts and collaborating partners, this will facilitate case based problem solving, sharing of best practices provide targeted educational programming and support process improvement initiatives. **Results:** This project is expected to enhance the capabilities and self-efficacy of clinicians to address the primary domains of survivorship care and will ultimately result in reduced morbidity and mortality while maximizing the quality of life for cancer survivors. We will present the results of a process evaluation conducted using both qualitative and quantitative methods to systematically monitor the implementation of the program across participating practices. We will also provide findings from pre-and post-surveys of physicians and residents. We will provide data from the outcomes evaluation of the project on: 1) practice-level capacity; 2) effect of the project on provider knowledge, self-efficac , and practice and 3) the provision of evidence-based preventive services to cancer survivors. **Conclusion:** This project is highly innovative in addressing persistent barriers to the optimal care of cancer survivors. It establishes durable practice changes that integrate use of the Treatment Summary and Survivorship Care Plan and fosters discussion among the primary care clinicians, oncology specialists and patients to better coordinate care.

511 **Poster Session A**

The Angelina Jolie Boomerang Effect: How Are Things Different This Time Around? *J. Huang, The University of Texas Southwestern Medical Center at Dallas; L. Kiedrowski, Pennsylvania Hospital*

Introduction: The "Angelina Jolie effect" refers to the rise in awareness and uptake of genetics services after her 2013 New York Times op-ed about her decision to undergo genetic testing and (when positive for a BRCA1 mutation) preventive bilateral mastectomy (PBM). This year, Ms. Jolie wrote about her preventive bilateral salpingo-oophorectomy (BSO). We sought to explore how individuals made meaning of her decisions. We hypothesized that the public would be more supportive of her BSO than PBM and have an increased awareness of genetic counseling and improved genetics knowledge in 2015 vs. 2013. **Methods:** We performed a content analysis of the online comments to Ms. Jolie's two op-ed pieces for the New York Times (2013, N= 624; 2015, N= 544). Comments represented individuals from 42 states and 37 countries. Two study team members coded comments for emerging themes (inter-rater reliability 90%). **Results:** More people reported a personal or family history of a gene mutation, cancer, or similar surgery in 2013 than in 2015 (18% v. 14%; $p < 0.001$). More individuals expressed direct support for Ms. Jolie's PBM than her BSO (7% vs. 2%, respectively; $p<0.05$). Consistent themes across both op-eds included improved education, bravery, the importance of screening, choice, and not feeling alone or afraid anymore. More comments discussed risk ($p<0.001$) and femininity ($p<0.01$) in 2013. More comments equating knowledge with power appeared in 2015 ($p<0.001$). Although proportions of comments on problems with access to healthcare/resources were similar (13% vs. 15%), more comments expressed insurance concerns in 2015 ($p<0.001$), specifically noting the Affordable Care Act. Twenty comments mentioned gene patents in 2013, compared to 1 in 2015 ($p<0.001$). These results indicate a need for genetic counselors to be aware of overarching current events in healthcare. For each op-ed, <2% of comments mentioned genetic counseling. There were also multiple comments demonstrating inaccurate genetics knowledge. **Conclusion:** We sought to understand how the public views prophylactic risk-reducing surgeries for inherited cancer risk. The multiple themes that emerged suggests that there could be many issues that an individual considers when evaluating prophylactic risk-reducing surgeries. These may be important for healthcare providers performing genetic counseling or cancer risk assessments to address with patients. The lack of comments mentioning genetic counseling or the number of comments that including inaccurate genetics knowledge also suggests that there are still improvements to be made in educating the public about cancer genetics and the role of genetic counselors.

512 **Poster Session B**

Impacts of a faith-based obesity prevention program on congregational level nutrition and physical activity environment and policies *S. Wilmoth, The University of Texas at San Antonio; L. Correa, The University of Texas at San Antonio; E. Martinez, The University of Texas at San Antonio; M. Pan, The University of Texas at San Antonio; R. Mendoza, The University of Texas at San Antonio; L. Neira, San Antonio Food Bank; E. Sosa, The University of Texas at San Antonio; Z. Yin, The University of Texas at San Antonio; M. He, The University of Texas at San Antonio*

Introduction: Hispanics are disproportionally affected by obesity, cancer, and other obesity-related chronic diseases. In response to community needs, The University of Texas at San Antonio (UTSA) in partnership with the San Antonio Food Bank (SAFB) and Central Church of God developed and implemented Building a Healthy Temple (BHT), a lifestyle intervention program aimed to prevent obesity-related chronic diseases in low-income, faith communities, through the integration of spiritual and physical health promotion. M**ethods:** BHT was a 12-month multi-component intervention implemented in a church setting for Hispanic children and adults. Intervention core components included a Health Ministry Committee, Health Sermons, Health Screening, Sunday School Curriculum, Bible Study Sessions, Nutrition Education/Cooking Demonstrations, physical activity and nutrition environmental and policy changes. Using a train-the-trainer model, intervention activities were delivered by trained lay leaders from the participating churches. Institutional level outcomes were assessed using the Congregational Health Index (CHI) survey tool developed to measure church nutrition and physical activity environment. Data was collected at baseline, six months and endpoint. Repeated measures ANOVA were performed to detect outcome measure changes. **Results:** Eight predominantly Hispanic churches in San Antonio, TX participated in the program. Church nutrition environment scores have significantly improved since program start. The endpoint nutrition scores were almost tripled of that at baseline. Significant nutritional scores improvements included: "churches provide nutritious meals and refreshment", "churches make healthy beverages available", "churches offer meals and refreshments with low-fat items, and "church practice food purchasing and preparation to reduce fat content". Physical environmental scores also showed significant improvement. Significant physical activity (PA) environmental improvement included: "church's built environment supports PA", "PA equipment is available at church", "churches promote PA", and "church's PA facility is safe". C**onclusion:** BHT took a holistic approach by integrating and promoting spiritual and physical health that is more likely to result in lasting lifestyle changes. Environmental and policy changes in the faith communities can play a compelling role in encouraging and supporting congregation members in making healthy lifestyle choices that will keep their bodies and mind well.

513 **Poster Session B**

Dissemination and Implementation of the Sunbeatables™ Program to Reduce Sun Exposure in Preschool Children *M. Tripp, The University of Texas M.D. Anderson Cancer Center; P. Pandit Talati, The University of Texas M.D. Anderson Cancer Center; E. Winters, The University of Texas M.D. Anderson Cancer Center; A. Sintes Yallen, The University of Texas M.D. Anderson Cancer Center; C. Galvan, The University of Texas M.D. Anderson Cancer Center; M. Euresti, The University of Texas M.D. Anderson Cancer Center; S. Garrison, The University of Texas M.D. Anderson Cancer Center; E. Gritz, The University of Texas M.D. Anderson Cancer Center; S. Peterson, The University of Texas M.D. Anderson Cancer Center*

Introduction: Excessive sun exposure during childhood increases lifetime risk of skin cancer. Preschool interventions can reach large numbers of young children, and their teachers and parents, to promote the development of sun protection habits. MD Anderson has developed an evidence-based program, Ray and the Sunbeatables™: A Sun Safety Curriculum for Preschoolers, which includes a curriculum, teacher's guide and training, parent education and preschool sun protection policy guidance. Implementation of the Sunbeatables™ program launched in summer 2015 with a concurrent evaluation protocol. The goal of current research is to assess program implementation in preschool settings. Major aims include: (1) assess fidelity of implementation of core and supplemental program components; (2) evaluate whether, why and how the program is adapted; (3) assess implementation barriers and facilitators; and (4) assess technical assistance needs. **Methods:** Eligible preschools requested the Sunbeatables™ program, received training and were located in the greater Houston area. Data collection includes classroom observations of curriculum activities, playground/pool observations of sun protection, surveys and semi-structured interviews with center directors and teachers, and brief interviews with parents about program awareness. **Results:** The Sunbeatables™ program has been disseminated to 59 sites in six states. Six Houston preschools participated in the concurrent evaluation protocol. Study observations to date include 22 classroom observations, 11 playground/pool observations, 8 semi-structured center director/teacher interviews, 39 center director/teacher surveys, and 58 brief parent interviews. In 18 out of 22 (82%) observed classroom activities, teachers taught the core sun safety concepts for the activity. In 15 out of 22 (68%) observed classrooms, there were visuals of the curriculum materials displayed. In 8 out of 11 (73%) playground/pool observations, teachers applied sunscreen to children before going outside. Of 58 parents interviewed, 26 (45%) were aware of the program. Teachers indicated that sun exposure is an important issue to address and that they are confident in communicating with parents about sun protection. Teachers adapted curriculum activities to address barriers including limited preparation time and unique classroom needs. Gaps in teacher training and technical assistance needs, including web-based program resources, were identified. **Conclusion:** Additional training may be needed to address barriers observed during pilot implementation. Findings from classroom observations will guide modifications of curriculum activities. Gaps in teacher training and any technical assistance needs will be addressed prior to large-scale dissemination of the program, to enhance its fit with the target population and practice sett ng.

514 **CPRIT Grantee**

The Influence of Health-related Quality of Life and Perceived Barriers on Previous Colorectal Cancer Screening *C. Ojinnaka, Texas A&M University System Health Science Center; J. Bolin, Texas A&M University System Health Science Center; D. McClellan, Texas A&M University System Health Science Center; J. Helduser, Texas A&M University System Health Science Center; M. Ory, Texas A&M University System Health Science Center*

Introduction: The Texas A&M Cancer Screening, Training, Education and Prevention program (Texas C-STEP) was originally funded in August, 2011, to address barriers to colorectal cancer (CRC) screening in the 7-county Brazos Valley region of Texas, while concurrently increasing the number of family physicians trained in colonoscopy. The project enhanced the ability of the Texas A&M Physicians Family Medicine Center, clinical home to a family medicine residency program (FMR), to provide accessible, affordable, culturally relevant screenings to uninsured/underinsured area residents using a community health worker model for outreach and education. The purpose of this paper is to determine the association between health-related quality of life and previous CRC screening using colonoscopy, fecal occult blood test, or sigmoidoscopy. **Methods:** Deidentified survey responses of patients who received financial assistance for colonoscopy during a 42-month period between 2011 and 2015 were analyzed. Descriptive statistics for demographic characteristics were calculated. Bivariate and multivariate logistic regressions were estimated. Covariates included race/ethnicity, educational attainment, marital status, gender, age, family history of CRC or adenomatous polyps, and number of barriers identified. **Results:** About 32% of participants were White, 24% were African American and 44% were Hispanic. Eighteen percent had less than a high school education, 51% had some high school education or a high school diploma, and 31% had more than a high school education. Multivariate analysis revealed no significant associations between health-related quality of life measures and previous CRC screening. Compared to Whites, Hispanics were more likely to report previous CRC screening (OR=1.81; 95% CI= 1.01-3.25). Having a higher number of barriers identified was significantly associated with decreased likelihood of previous CRC screening (OR=0.85; 95% CI=0.76-0.95). **Conclusion:** Health-related quality of life does not appear to influence previous CRC screening among low-income populations. Most significantl , race-related disparities observed for CRC screening that is often reported may be reversed among low-income individuals. Reducing the number of perceived barriers by this population could potentially increase CRC screening rates.

515 **CPRIT Grantee**

Innovative Use of Social Media for Increasing Colorectal Cancer Awareness and Screening *S. Misra, Texas Tech University Health Science Center at Amarillo; S. Trehan, Texas Tech University Health Science Center at Amarillo; M. Guzman, Texas Tech University Health Science Center at Amarillo; M. Marsh, Texas Tech University Health Science Center at Amarillo; D. Gregory, Texas Tech University Health Science Center at Amarillo; A. Mishra, Texas Tech University Health Science Center at Amarillo*

Introduction: This grant targets the ten most populous counties in the Texas panhandle in an effort to improve colorectal cancer (CRC) awareness and screening. The screening rate in the panhandle is 41%, far below the national average of 65.4%. The population of the panhandle is 428,000 over 26,000 square miles, posing challenges in reaching remote areas. The American Cancer Society has set a goal of screening 80% of the eligible population of the United States by 2018, making the need for innovations in media vital to outreach. M**ethods:** Various media related programs are being used to disseminate information for the grant. Specificall , the website www.cancerscreeningtx.com was created to promote the program and allow us to track web traffic. Facebook and Twitter accounts were initiated to raise online awareness about CRC. Public Service Announcements (PSAs) and expert interviews were aired on various television (TV) and radio stations. Due to the multimedia nature of the campaign, we made note from where participants were referred and when they called in for information to measure effectiveness of different approaches and then tailor outreach accordingly. **Results:** Year to date the website has had 252 visits, 330 page views showing a distinct upward trend. The site is being accessed on a variety of devices. Desktop devices account for 66.5% of views and mobile devices bring in 34.6%. Facebook page has garnered 86 likes and Twitter has 9 followers. Expert interviews and several PSAs were aired multiple times on local TV and radio station reaching a total audience of 176,000 adults. Immediately after airing there was a noticeable uptick in calls to the program. Many unsolicited calls were logged from potential participants asking for more information. C**onclusion:** The innovative use of media has heightened awareness about CRC and generated support for the program. Free of charge social media platforms, such as Twitter and Facebook, have allowed us to bring CRC to the forefront of the public eye. Partnering with local TV and radio stations has helped reach out to a wide number of participants at no cost to the program and has resulted in unsolicited calls of interest in the program.

516 **CPRIT Grantee**

Reducing Tobacco-Related Cancers in Texas through Unique Community-Academic Training Opportunities for Local Mental Health Authorities *T. Stacey, Austin/Travis County Health and Human Services Department; B. Kyburz, Austin/Travis County Health and Human Services Department; W. Wilson, Austin/Travis County Health and Human Services Department; T. Williams, Austin/Travis County Health and Human Services Department; B. Alderman, Austin/Travis County Health and Human Services Department; C. Lam, Rice University; V. Correa-Fernández, University of Houston; M. Howard, Heart of Texas Region MHNR; S. Broussard, Spindletop Center; J. Goode, Betty Hardwick Center; L. Reitzel, University of Houston*

Introduction: Approximately 36% of adults with mental illness smoke cigarettes, and this is a leading cause of cancers in Texas. However, many mental health providers lack knowledge about tobacco use, the relationship between tobacco use and mental illness and cessation treatments. This lack of knowledge leads to reduced confidence in the ability to deliver cessation treatments. Through the ongoing, CPRIT funded, Taking Texas Tobacco Free (TTTF) program, a diverse set of trainings were offered to the staff of seven local mental health authorities (LMHA), covering 31 counties, to prepare them to assess and treat tobacco use amongst mental health consumers. These training opportunities were facilitated by the unique collaboration between Austin Travis County Integral Care (ATCIC) and project affiliated academic institutions. The aim of this presentation is to describe the various trainings and their reach within the targeted mental health care agencies. **Methods:** From December 1, 2013 to January 1, 2015, trainings offered to LMHAs included one day, clinician-focused, Motivational Interview (MI) Trainings conducted by psychologists from MD Anderson and University of Houston, clinician and prescriber focused, 2-day, Treating Tobacco Dependence in Mental Health Settings and 5-day Certified Tobacco Cessation Treatment Specialist Training (CTTS) conducted by Rutgers University psychiatrist, psychologists and other clinicians and 1-2 hour staff trainings on general tobacco education at each LMHA conducted by tobacco cessation specialists from ATCIC. These staff trainings were divided into two groups: trainings for staff that had direct contact with consumers (2 hours) and trainings for staff that did not have direct contact with consumers (1 hour). **Results:** At the halfway point in project implementation, 93 staff have received MI training, 22 staff have received Treating Tobacco Dependence in Mental Health Settings training, 8 staff have received CTTS training and 2,270 staff have received tobacco education trainings (615 non direct/1,655 direct contact). **Conclusion:** The collaboration between ATCIC and academic institutions allowed for a diverse set of trainings to be offered to each LMHA, more so than would have been via one agency. Similar community and academic partnerships could be implemented within other health care systems to address the problem of tobacco use among their consumers, and ultimately reduce tobacco-related cancer deaths in Texas. Tips on forming, implementing and maintaining such successful community-academic training partnerships will be highlighted.

517 **CPRIT Grantee**

Effects of the LIVESTRONG Fertility Training for Healthcare Professionals on Practice Change *A. Narayan, LIVESTRONG Foundation; S. Arvey, LIVESTRONG Foundation; E. Eargle, LIVESTRONG Foundation; S. Nutt, LIVESTRONG Foundation*

Introduction: More than 150,000 Americans under 45years are diagnosed with cancer each year. These individuals are at risk for infertility as some cancer diagnoses and treatments may negatively impact an individual's fertility. Even though the American Society for Clinical Oncology developed recommendations in 2006 and 2013 encouraging healthcare providers to inform patients about potential risks to their fertility, many patients report not receiving information prior to treatment or at all from their oncologists. CPRIT awarded the LIVE**STRONG** Foundation a grant to develop an educational training for healthcare professionals on the importance of communicating with patients about potential cancer-related fertility risks and options for fertility preservation (PP120225). The training was designed to include case studies and simulated conversations as interactive trainings have been shown to have a greater effect on practice change compared to traditional educational opportunities. This abstract presents the initial results from the immediate and eight week post-training surveys measuring practice changes. **Methods:** LIVE**STRONG** Research and Evaluation staff created two online surveys to measure participants' practice changes over eight weeks after training completion. The surveys are distributed via SurveyGizmo, and all responses are self-reported. The immediate post-training survey measures the likelihood of participants discussing cancer-related fertility risks with their patients and the confidence in their ability to have those conversations. The eight week post-training survey includes added measures to learn about whether participants have implemented the recommended behaviors in practice. **Results:** As of July 28, 2015, 70 Texas-based healthcare professionals have completed the training, of which 23% have completed the immediate post-training survey. Seventy-three percent of respondents indicated they were very likely or likely to discuss cancer-related fertility risks with patients, and 87% strongly agreed or agreed they were more confiden in their ability to know how and when to speak to patients about cancer-related fertility risks. Thirty-four percent of participants who have completed the training have received the eight week post-treatment survey, of which four have completed the survey. All respondents indicated they were very likely or likely to discuss cancer-related fertility risks with patients. Twenty-five percent (n=1) agreed they were more confident in their ability to know how and when to speak to patients about cancer-related fertility risks. Seventy-five percent (n=3) reported they had referred patients to fertility preservation resources. **Conclusion:** Initial data indicate that LIVESTRONG Fertility Training for Healthcare Professionals increases healthcare professionals' confidence in their ability to discuss and the likelihood of them discussing cancer-related fertility risks with patients.

518 **CPRIT Grantee**

Changes in Tobacco Cessation Practices among Mental Health Centers Staff Before and After Implementation of Tobacco-free Policy: An Interim Report *C. Lam, Rice University; W. Wilson, Austin/Travis County Health and Human Services Department; B. Kyburz, Austin/Travis County Health and Human Services Department; T .Stacey, Austin/Travis County Health and Human Services Department; V. Correa-Fernández, University of Houston; T. Williams, Austin/Travis County Health and Human Services Department; B. Alderman, Austin/Travis County Health and Human Services Department; S. Broussard, Spindletop Center; M. Howard, Heart of Texas Region MHNR; J. Goode, Betty Hardwick Center; L. Reitzel, University of Houston*

Introduction: As many as 69% of persons with mental illness use tobacco. Persons with mental illness are 2.6 times more likely to have cancer than those without mental illness. This heightened risk of cancer in persons with mental illness has been attributed, in part, to their higher rate of tobacco use. Thus, the prevalence of tobacco use in persons with mental illness is a serious concern with direct relevance to cancer prevention. The goal of Taking Texas Tobacco Free (TTTF) is to assist selected community mental health centers that are associated with Local Mental Health Authorities (LMHAs) across Texas to adopt comprehensive, evidence-based, multicomponent Tobacco-Free Workplace Programs that are sustainable beyond the grant funding period. **Methods:** TTTF has several major components that target centers' consumers and staff, as well as the local communities in which the selected LMHAs are embedded. The components include: 1) assistance in the implementation of tobacco-free workplace policy, 2) basic tobacco education for all centers' employees, 3) tobacco assessment and cessation intervention training for centers' clinical providers, 4) provision of nicotine replacement therapy, and 5) community education and outreach. TTTF enrolled community centers from 18 selected LMHAs (7 in the first cohort and 11 in the second cohort), representing over half of all LMHAs across the state. Centers' leaders, clinical providers, and employees from both cohorts have completed pre-implementation surveys that were designed to assess centers' readiness for change, providers' previous training and practices on tobacco use cessation, and employees' knowledge and behaviors regarding tobacco use. Post-implementation surveys have been administered to leaders, providers, and employees in the first cohort. **Results:** Similar to national data on tobacco assessment among the mentally ill, the first cohort survey found that while 72% of the clinical providers reported that they had seen at least one tobacco user in the past month, over half of them (53.9%) did not routinely assess the tobacco use status of their patients. Of the providers who reported that they had seen at least one tobacco user in the past month, 40% indicated that they had not advised their consumers to quit and 71% indicated that they did not provide any cessation treatments to assist their consumers in stopping tobacco use. **Conclusion:** Before TTTF implementation, clinical providers at community mental health centers did not routinely assess or treat tobacco use among their consumers. The poster will discuss changes observed after the project's implementation.

519 **CPRIT Grantee**

Stepping through Treatment: Using the Transtheoretical Model to Project Knowledge and Perception regarding Cancer Screening and Health *R. Qualls-Hampton, The University of North Texas Health Science Center at Fort Worth; M. Felini, The University of North Texas Health Science Center at Fort Worth; K. Ukpaka, The University of North Texas Health Science Center at Fort Worth; O. Igenoza, The University of North Texas Health Science Center at Fort Worth; O . Jegede, The University of North Texas Health Science Center at Fort Worth; S. Gupta, The University of North Texas Health Science Center at Fort Worth; S . Bangara, The University of North Texas Health Science Center at Fort Worth; S. Burns, Nexus Recovery Center*

Introduction: Individuals who use/abuse controlled substances face complex obstacles in their attempt to change behaviors. In particular, women experience greater barriers to successful treatment outcomes including exposure to trauma, co-occurring mental health issues, and lack of cultural, community and clinical support. The stages of change or Transtheoretical Model is used to assess readiness for treatment as a predictor of treatment success and relapse. Substance abuse treatment programs provide a unique opportunity to also offer health services such as cancer screening. However, some women have negative or limited experience with cancer screening and low health literacy. Project aims were to provide 3360 women in substance abuse recovery with a trauma-informed cancer prevention education. This study investigates knowledge, perceptions and attitudes among women receiving cervical cancer education with an opportunity to receive screening over the 18 months study period. **Methods:** Using findings from six focus groups conducted, investigators and health educators developed a 5-part cancer education seminar with pre- and post-assessments and 32-item questionnaire assessing cancer screening perceptions and attitudes. Seminars and assessments were integrated in a culturally appropriate and trauma-sensitive women's health education into the largest female substance abuse treatment center in North Texas. Both residential and outpatient clients participated in the weekly education seminars. Response assessments were individually scored and summarized by treatment stage change, as assessed by counselor. **Results:** Over 1100 women participated in at least one education seminar and 200 women completed the perceptions instrument. Education seminars are ongoing until August 2016. The average participant age is 33.2 years and nearly 40% reported having at least "some college" (36.5%). The most common drugs reported were methamphetamine (27.7%) and alcohol (18.6%) and heroin (16.0%) with the average age of first use being 20.6 years, ranging from 3 to 58 years old. At treatment initiation, most women were in the Contemplation stage (83.0%). By substance treatment end, 60.5% of women were in the Preparation stage. Approximately, 87.0% agreed that having a "clean bill of health is very important to me" and would "get a Pap test today" (83.2%). **Conclusion:** The Transtheoretical model evaluates substance use treatment readiness as well as current health and screening perceptions of women receiving treatment for co-occurring disorders. Substance use treatment is a critical and vulnerable time; careful consideration is important when approaching women for cancer screening opportunities.

520 **CPRIT Grantee**

Knowledge and Familiarity with Cancer Screening Among Refugee Populations in Tarrant County *R. Qualls-Hampton, The University of North Texas Health Science Center at Fort Worth; A. Raines Milenkov, The University of North Texas Health Science Center at Fort Worth; E. Baker, The University of North Texas Health Science Center at Fort Worth; M. Okaalet, The University of North Texas Health Science Center at Fort Worth; I. Liang, The University of North Texas Health Science Center at Fort Worth*

Introduction: More than 53,000 refugees resettled in Texas since 2004, majority arriving from Burma, Bhutan, Somalia, the Democratic Republic of Congo (DRC), Iran and Iraq. Despite the sophisticated US healthcare system, refugees face numerous barriers to diagnosis, treatment and screening for illnesses originating in their home country. Individual factors (language, culture, formal education), and system barriers (access to insurance) prevent refugees from accessing clinical and assistive services that would reduce human and financial costs of cancer. Currently, there is limited data on refugee groups and their awareness of cancer. This study investigates influences to refugee women's awareness and receipt of cervical cancer screening exams at baseline. M**ethods:** Four Lay Health Educators (LHEs) conducted outreach in their respective communities to engage and enroll participants in the Building Bridges Initiative (BBI). Comprehensive baseline assessments were completed measuring family characteristics, culture (languages, length in US, etc.) cancer awareness, and screening experience. Subsequently, LHE's conducted health education classes in the participants' own language to provide information about cervical, breast, and liver cancer. Data collected was obtained from baseline assessments. Means and t-tests evaluated the influences of time in the US, schooling and baseline knowledge and experience with cancer screening. **Results:** To date, approximately 230 women have participated in BBI. Majority are, on average, 40 years old with some difference by refugee group. Most participants were originally from Bhutan (29.7%), Burma (16.6%) or DRC (14.9%). On average, BBI participants left their home country approximately 11.3 years ago and have lived in Tarrant County approximately three years, with Somalian participants here longer (12.4 and 4.6 years, respectively). Nearly half (46.7%) reported having some kind of health coverage, primarily Medicaid (78.0%). Almost two-thirds reported completing formal education (61.2%), with Bhutanese (81.5%) and Rwandan (81.2%) having the greatest proportion of formal education. However, only 30.0% had ever heard of a Pap test and 36.8% had one in the past. **Conclusion:** Time in the U.S. does not influenc the rate of cervical cancer screening among individual ethnic groups of refugee women. Contrary to the US, having health insurance or formal education does not influence familiarity with cervical cancer screening. Refugee populations cannot be generalized as one group of people. Although groups of refugees share similar circumstances, different ethnic groups have their own culture, traditions, backgrounds, and healthcare beliefs that must be considered to understand barriers to screening. Refugee populations are in need of culturally and linguistically tailored cancer education prevention and intervention programs.

521 **CPRIT Grantee**

The Community Network for Cancer Prevention: Using Community Theater to Promote Cancer Screening among the Medically Underserved *J. Montealegre, Baylor College of Medicine; R. Chenier, Baylor College of Medicine; I. Valverde, Baylor College of Medicine; G. Chauca, Baylor College of Medicine; L. Hanser, Harris Health System; M. Daheri, Harris Health System; L. Rustveld, Baylor College of Medicine; B. Reed, Baylor College of Medicine; M. Anderson, Baylor College of Medicine; L. Ramondetta, The University of Texas M.D. Anderson Cancer Center; M. Gould-Suarez, Baylor College of Medicine; B. Musher, Baylor College of Medicine; L. Scott, The University of Texas Health Science Center at Houston; J. Nangia, Baylor College of Medicine; J. Hoagland-Sorensen, Harris Health System; A. Rieber, The University of Texas M.D. Anderson Cancer Center; M. Jibaja-Weiss, Baylor College of Medicine*

Introduction: Disparities in cancer screening contribute to the increased burden of cervical (CxC), colorectal (CRC), and breast cancer (BC) in medically underserved minority populations. While one-on-one education is a recommended, evidence-based interventions to promote screening test utilization, innovative strategies are needed to disseminate screening education in a culturally sensitive manner. As part of a comprehensive systems design intervention (CPRIT grants 100201, 130084, 140028), we developed a community theater program designed to promote CxC, CRC, and BC screening among medically underserved Hispanic, African American, and Vietnamese communities in Houston, Texas. **Methods:** Two plays and 9 monologues (3 for each target population) were developed. Synopses and scripts were created by professional playwrights based on theory-based messages and with oversight from clinical advisory boards. Professional actors enacted the performances. Community venues for live performances were identified within medically underserved areas with a high incidence of CxC, CRC, and/or BC. A brief survey was used to assess audience members' intentions to obtain screening before and after the performance. A community access navigator was available on site at each performance to assist audience members in applying for financial assistance from the county's safety net healthcare provider. **Results:** Between January 2014 and May 2015, 34 monologues and 6 plays were performed. Total attendance was of 1,742 individuals (average = 30/monologue and 121/play). Surveys (n = 1,301, response rate = 75%) indicate a significant increase in the proportion of audience members' reporting that they are "highly likely" to obtain a screening test following the performance ($p<0.05$). Specificall , scores increased from 44.2% to 68.6% for CxC performances, from 46.8% to 56.9% for CRC, and from 69.8 to 76.3% for BC. Feedback from community partners indicates that the monologues especially are highly regarded due to their brief duration (<20 minutes) and ability to be set-up without a formal stage and minimal need for props. **Conclusion:** Our finding suggest that community theater performances may be an effective tool to communicate health messages to promote cancer screening in medically underserved minority populations. Following an intense development phase, a repertoire of monologues and full-length plays is available for ongoing performances. Sustainability and dissemination are augmented by the use of monologues, which are brief in duration and require minimal set-up and a single actor.

522 **CPRIT Grantee**

Refugee Women's Perspectives On Cancer and Screenings *E. Baker, The University of North Texas Health Science Center at Fort Worth; A. Raines Milenkov, The University of North Texas Health Science Center at Fort Worth; H. Mudey, The University of North Texas Health Science Center at Fort Worth; E. Thein, The University of North Texas Health Science Center at Fort Worth; R. Subedi, The University of North Texas Health Science Center at Fort Worth; L. Rudasingwa, The University of North Texas Health Science Center at Fort Worth*

Introduction: The Building Bridges Initiative (BBI) is a program that uses refugee Lay Health Workers to provide culturally and linguistically appropriate breast, cervical and liver cancer education to refugee women in Tarrant County and links them into appropriate health services. Using trained lay health workers from four different refugee groups overcomes language and cultural barriers, and allows for an emic perspective of refugee women's knowledge, beliefs, and attitudes towards cancer, cancer screening and cancer prevention. This study explores these concepts from lay health worker, program participant and community leader perspectives. **Methods:** LHWs recruited participants through outreach in known social circles, community gatherings, churches and within apartment complexes with high concentrations of refugees. Following participation in group or individual educational sessions enrollees were connected to screening services. Lay health workers elicited participant's questions, comments, and perspectives regarding knowledge of cancer and cancer screening formally through an open-ended data collection tool during group education. Perspectives, concerns, and community conversations regarding the program were also gathered informally through community advisors meetings and weekly staff meetings with Lay Health Workers. Qualitative findings were coded and arranged according to themes, i.e., thematic analysis. **Results:** To date, for this analysis five broad themes emerged. These include 1) Cultural specific causes of illness, 2) Inaccurate knowledge of human anatomy, 3) Health care system barriers, 4) Role of traditional healers, and 5) Community influences on participation in education and screening efforts. C**onclusion:** The purpose of the LHW model is to reduce barriers to services for vulnerable populations. Understanding beliefs, practices, knowledge and other factors influencing cancer screening is essential to connecting communities to effective health care services. BBI has used these refugee voices to adapt and guide the implementation of the outreach, education and clinical screenings. Providing culturally and linguistically appropriate education, clinical, and assistive services to healthcare providers as well as the refugee women will reduce the human and financial costs of cancer to this population

523 **CPRIT Grantee**

Acceptability of Cervical Self-Sampling Devices among High-Risk Indigent Women in Substance Abuse Treatment *M. Felini, The University of North Texas Health Science Center at Fort Worth; R . Qualls-Hampton, The University of North Texas Health Science Center at Fort Worth; S. Bangara, The University of North Texas Health Science Center at Fort Worth; M. Shuler, The University of North Texas Health Science Center at Fort Worth*

Introduction: A history of childhood sexual abuse and intimate partner victimization is associated with low adherence to recommended cervical cancer screening guidelines. High rates of trauma have been reported among women in substance abuse treatment centers, including our study population, of whom 80% report having suffered sexual and/or physical abuse. Cervical self-sampling devices (CSSDs) have been shown to be an acceptable alternative in immigrant and indigent populations where barriers may prevent access to conventional in-office cervical screening examinations. However, few studies to date have examined whether this self-screening tool would be an option among indigent women engaging in high-risk behaviors. The objective of this study was to assess the perceptions of CSSDs among women in treatment for substance abuse and co-occurring disorders at the Nexus Recovery Center, the largest female substance abuse treatment center in North Texas. **Methods:** Six focus groups were conducted among women participating in treatment at the Nexus Recovery Center. A separate focus group was conducted among members of our project advisory board (medical professionals, social workers, and recovering addicts) who guided the research. A mixed methods approach was used to analyze the data and identify themes from participants' responses. **Results:** A total of 48 women participated in six focus groups. The average age of participants was 32 years, 42% were non-white, and 40% had less than a high school education. In assessing the participants' utilization of cervical cancer screenings, 30 (63%) reported having had a cervical PAP smear in the last two years. Over half of respondents would not use a CSSD, but were positive about the potential benefits for others with histories of trauma or without access to care. The primary reason for low personal acceptance of CSSDs was the lack of trust in test results. High value was placed on the added benefit of a visual check during a clinical cervical exam, specifically for other sexually transmitted diseases. **Conclusion:** The low acceptability of cervical self-sampling devices in our study population is due to a perceived lack of trust in the effectiveness of this tool. This finding was used to inform a trauma-informed, culturally sensitive cervical cancer education program that was integrated into substance abuse treatment centers as part of this project.

524 **CPRIT Grantee**

The Community Network for Cancer Prevention: Patient Navigation to Ensure Diagnostic Follow-Up among Patients with Abnormal Cancer Screening Results *J. Montealegre, Baylor College of Medicine; L. Hanser, Harris Health System; M. Daheri, Harris Health System; J. Hanke, Harris Health System; R. Chenier, Baylor College of Medicine; I. Valverde, Baylor College of Medicine; L. Rustveld, Baylor College of Medicine; G. Chauca, Baylor College of Medicine; B. Reed, Baylor College of Medicine; M. Anderson, Baylor College of Medicine; L .Ramondetta, The University of Texas M.D. Anderson Cancer Center; M. Gould-Suarez, Baylor College of Medicine; B. Musher, Baylor College of Medicine; L. Scott, The University of Texas Health Science Center at Houston; J. Nangia, Baylor College of Medicine; J. Hoagland-Sorensen, Harris Health System; A. Rieber, The University of Texas M.D. Anderson Cancer Center; M. Jibaja-Weiss, Baylor College of Medicine*

Introduction: Patient navigation is a patient-centered intervention model that has been advocated as a possible strategy to overcome patient-level barriers to cancer care. Patient navigation has been employed at all stages in the continuum of cancer care, most commonly to increase cancer screening rates, but also to ensure diagnostic and therapeutic follow-up and address survivorship issues. Ensuring diagnostic follow-up of patients with abnormal screening tests is of particular importance given that screening is of no use without clinical resolution. While much attention has been given to the interactions between patient navigators (PN) and patients to address individual-level barriers to follow-up, ensuring diagnostic follow-up often requires broader interaction with the larger healthcare system. Here we discuss the broader role of PNs in addressing loss-to-follow-up in a high-volume public healthcare system. **Methods:** As part of a comprehensive systems design intervention (CPRIT grants PP100201, PP130084, PP140028), we developed and implemented a patient navigation model to improve diagnostic follow-up among patients with abnormal cervical, colorectal, and breast cancer screening results. As part of a recent Community Needs Assessment commissioned by the Harris Health System, interviews were conducted with the lead PN and supervisor overseeing navigation for all three service lines. A thematic analysis was conducted to categorize tasks conducted by the PNs. **Results:** Patients with abnormal screening results are tracked using a Tickler File database that is manually populated and maintained by the PNs. Patients are identified using the electronic medical record based on their screening test results. A large portion of PNs' time is spent prompting providers to notify patients of their abnormal test results and ensuring that providers complete diagnostic referrals. A subset of patients requires additional intervention. PNs communicate with them to address individual-level barriers; most commonly 1) applying for financia assistance; 2) managing fears associated with cancer and/or diagnostic procedures; and 3) reminding them of appointments. **Conclusion:** In the present model, PNs intervene on two levels: 1) with the patient to ensure their timely flow through the continuum of care; and 2) with providers to ensure that appropriate notifications and referrals are made. The latter requires the availability of timely data and the ongoing population and maintenance of a Tickler File database to proactively identify and track patients at risk. Under this model, patient navigation goes beyond addressing patient-level barriers to more broadly address systems-level failures that otherwise allow patients to be lost to follow-up.

525

Spreading the Word About HPV: Texas Department of State Health Services Adolescent Immunization Campaigns *R. Velazquez, Department of State Health Services; A. Thompson, Department of State Health Services*

Introduction: The Texas Department of State Health Services Immunization Branch receives funding from the Centers for Disease Control and Prevention to provide education to the general public and healthcare providers and promote the importance of immunizations throughout the life cycle. As part of those promotion and marketing efforts, the Immunization Branch conducted a marketing evaluation study in 2013 and utilized the study data and findings to develop a media campaign in 2013 to promote adolescent immunizations, including the human papillomavirus (HPV) vaccine. The campaign deliverables included television and radio advertisements specifically targeting HPV as well as informational websites for the general public and healthcare providers. The adolescent immunization campaign was re-launched in 2014 and is in planning phases for 2015. M**ethods:** The Immunization Branch contracted with a marketing research firm, SUMA Social Marketing, Inc., to conduct a marketing research study to determine best practices and marketing messaging for adolescent immunizations, and specifically for the HPV vaccine, to the general public and healthcare providers. As part the qualitative study, the Immunization Branch analyzed HPV vaccination coverage rates to determine if specific areas of the state to identify areas with high and low vaccination coverage rates. Dallas and El Paso were the selected cities to conduct further qualitative research to determine best practices and barriers to the HPV vaccine. Focus groups were held with healthcare providers and partners with adolescent children were held in Dallas and El Paso. In-depth interviewers were also conducted with stakeholders within the two cities. **Results:** Based on the research and analysis conducted on the focus group responses and the best practices research, the following findings were identified: 1) Physicians indicated a higher number of parents immunizing their adolescents when the HPV vaccine was presented as a cancer prevention vaccine; 2) Education and awareness activities need to be broadened to reduce the misconception that the HPV vaccine is only for adolescent girls; and 3) the healthcare professional's strong recommendation is important for in the decision making process for parents. **Conclusion:** Utilizing the study findings the Immunization Branch developed and launched the Adolescent Immunization Campaigns. The messaging utilized in the advertisements for parents was "There's no vaccine for her drama, but there's one for HPV that helps prevent cervical cancer. Ask your doctor. Visit www.ImmunizeTexas.com for more information." For the healthcare provider campaign, the messaging utilized was "Your recommendation matters. Help preteens get all recommended vaccines." The campaign was well-received throughout Texas.

Abstract Author Index

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