2ND ANNUAL INNOVATIVE DRUG DISCOVERY AND DEVELOPMENT CONFERENCE

MAY 10 – 11, 2021

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi- institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting-edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include AI in Healthcare, Antimicrobial Resistance, Cellular and Molecular Biophysics, Innovative Drug Discovery and Development, Immunology, Mental Health, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

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Michael Mancini, Baylor College of Medicine
Margie Moczygemba, IBT TAMU
Suzanne Tomlinson, Gulf Coast Consortia
Stephen T. C. Wong, Houston Methodist Research Institute
Damian Young, Baylor College of Medicine

Day 1 Monday, May 10

8:50 **Welcome:**
Suzanne Tomlinson, Gulf Coast Consortia

Convener: **Scott Gilbertson**, University of Houston

9:00 **Keynote Address:**
Discovery and Development of GPCR Allosteric Ligands: From Concept to Clinic
Craig Lindsley, Vanderbilt University

Session 1: HT Discovery
Conveners: **Cliff Stephan**, Institute of Biosciences & Technology, Texas A&M Health Science Center
Margie Moczygemba, Institute of Biosciences & Technology, Texas A&M Health Science Center

9:40 *Assay and Screening Strategies for Chemical Probe and Drug Discovery*
James Inglese, NIH/NCATS

10:10 *High Throughput Flow Cytometry for Drug Discovery and Repurposing*
Larry Sklar, University of New Mexico

10:40 *The Evolution of a Fully Automated High Throughput Flow Cytometry Screening System to Enable Drug Discovery*
John Joslin, Novartis

11:10 Networking Break

Session 2: Advanced Imaging for Target Validation and Drug Discovery

Convener: **Mike Mancini**, Baylor College of Medicine

11:25 *Single Cell Analysis of Estrogen Receptor Actions*
Fabio Stossi, Baylor College of Medicine

11:45 *Implementing Deep Neural Network Technologies Across Experimental Scales*
Reid Powel, Institute of Biosciences & Technology, Texas A&M Health Science Center

11:55 *Microscopy-Based Identification of RNA Foci Modulators in Myotonic Dystrophy Type 1*
Sara Jane Johnson, Baylor College of Medicine

12:05 *High-throughput Imaging and a Multi-omics Approach Shed Light on Cisplatin Resistance*
Vlad Sandulace, Baylor College of Medicine

12:15 Poster Session-Odd numbered posters

12:55 Lunch and Networking Break

Session 3: Metabolism-Targeted Therapeutics
Convener: **Peter Davies**, Institute of Biosciences & Technology, Texas A&M Health Science Center

1:30 *The Role of Oxidative Phosphorylation (OXPHOS) in Metastasis and Therapeutic Resistance in Melanoma*
Mike Davies, MD Anderson Cancer Center

2:00 *IACS-6274, a Potent and Selective Inhibitor of Glutaminase (GLS1) Being Developed for KEAP1/NFE2L2 Mutant NSCLC and ASNS-low Ovarian Cancer Patients*
Nakia Spencer, MD Anderson Cancer Center

2:30 *Optical Metabolic Imaging to Quantify Heterogeneity in Anti-Cancer Drug Response*
Alexandra Walsh, Texas A&M University

3:00 Networking Break

Session 4: PK/PD and Formulation
Conveners: **Diana Chow**, University of Houston
Dong Liang, Texas Southern University

3:15 *Development of AZD5305, The Next Generation PARP1 Selective Inhibitor and Trapper*
Elisabetta Leo, Astrazeneca

3:45 *Overcoming Transport Barriers in Cancer Treatment*
Haifa Shen, Houston Methodist

4:15 *Preclinical and Clinical PK/PD/Efficacy Work on the OxPhos Inhibitor IACS-010759, Targeting Metabolic Vulnerabilities in Cancer*
Emilia Di Francesco, MD Anderson Cancer Center

Convener: **Suzanne Tomlinson**, Gulf Coast Consortia

4:45 *CPRIT Update and Company Funding Opportunities*
Cindy WalkerPeach, CPRIT

5:15 **Core Facility Networking Showcase**

- Center for Drug Discovery
- Combinatorial Drug Discovery
- PK/PD and Formulation
- Advanced Microscopy
- High-throughput Flow Cytometry Core Facility
- Therapeutic Monoclonal Antibody
- Accelerating Cancer Therapeutics
- Targeted Therapeutics Drug Discovery Program
- Center for Innovative Drug Discovery

Day 2 Tuesday, May 11

9:00 Welcome **Phil Jones**, MD Anderson Cancer Center

Session 5: Breakthrough Medicines Originating from Academic Drug Discovery

Convener: **Phil Jones**, MD Anderson Cancer Center

9:00 *Towards the Rational Design and Development of Targeted Protein Degraders*
Lyn Jones, Dana-Farber Cancer Institute

9:30 *Drugging Protein-protein Interactions: Discovery and Optimization of WDR5 Binders*
Rima Alawar, Ontario Institute for Cancer Research

10:00 *Drugging RAS: Meeting the Challenge*
Alex Waterson, Vanderbilt University

10:30 Break

Session 6: Biotech Start-ups

Convener: **Sarah Hein**, Texas Medical Center, TMCx

10:45 *TMC Accelerator for Cancer Therapeutics: Supporting the Texas Biotech Ecosystem*
Sarah Hein, TMCx

ACT Users:
11:05 **Raimund Ober**, Astero Alta

11:15 **Andrew Koh**, Aumenta

11:25 **Craig Ramirez**, Tezcat

11:35 *Tvardi Therapeutics: Tvardi Therapeutics: The Next Generation of Texas Companies Advancing Through Clinical Trials for Cancer and Fibrosis of Texas*
Imran Alibhai, Tvardi Therapeutics

11:55 Poster Session-Even numbered posters

12:35 Lunch/networking break

Convener: **Phil Jones**, MD Anderson Cancer Center

1:45 **Keynote Address:**
East to West: A Career in Cancer Care and Drug Discovery
Tom Lynch, Fred Hutch

Session 7: Cell Therapies
Convener: **Stan Watowich**, University of Texas Medical Branch

2:30 *CAR-modified Virus-specific T-cells for the Treatment of Malignancies: Taking Advantage of the TCR*
Cliona Rooney, Baylor College of Medicine

3:00 *MDACC's Approach to Optimizing the Academic/Industrial Interface in Cell Therapy*
Jason Bock, MD Anderson Cancer Center

Session 8: Future Directions and Opportunities: Technologies, Trends, Etc.
Convener: **Stan Watowich**, University of Texas Medical Branch

3:30 **Keynote Address:**
Academic Entrepreneurs, New Technologies, and Building a Biotechnology Ecosystem: A Personal History
Harvey Lodish, Massachusetts Institute of Technology

4:15 Closing Remarks
Suzanne Tomlinson, Gulf Coast Consortia
Stan Watowich, UT Medical Branch at Galveston

Rima Al-awar, PhD
Head, Therapeutic Innovation and Drug Discovery

Drugging Protein-protein Interactions: Discovery and Optimization of WDR5 Binders

Dr. Rima Al-awar earned a PhD in synthetic organic chemistry from North Carolina State University and completed a postdoctoral fellowship at the University of North Carolina at Chapel Hill prior to joining Eli Lilly and Company in 1995.

In 2002, while still at Eli Lilly, Dr. Al-awar was promoted to Head in Discovery Chemistry Research and Technologies and later served as Head in Route Selection in Chemical Product Research and Development. In July 2008 she joined the Ontario Institute for Cancer Research (OICR) as Director and Senior Principal Investigator. Dr. Al-awar also serves as an Associate Professor in the Department of Pharmacology and Toxicology at the University of Toronto.

Since joining OICR, Dr. Al-awar's focus has been to build a drug discovery program that can efficiently translate the most promising ideas coming from Ontario's academic community into therapeutic benefits to cancer patients by identifying hits and lead molecules and optimizing them to potential drug candidates.

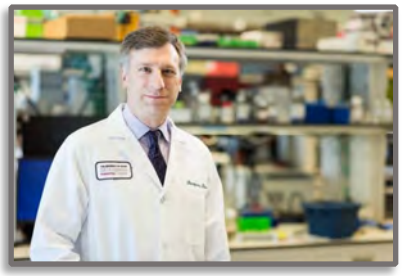
She has built a team comprised of researchers whose collective expertise spans the entire drug discovery process from target identification and validation to clinical candidate selection. The group has extensive experience within pharma and biotech environments and combines the biology, ADME/PK, analytical, medicinal and computational chemistry expertise with the state of the art infrastructure necessary to successfully advance drug discovery projects.

In collaboration with FACIT, the commercialization partner of OICR, her team has now spun out two companies (Novera and Propellon) and partnered two assets with pharmaceutical companies.

Imran Alibhai, PhD
Chief Executive Officer

Tvardi Therapeutics: Tvardi Therapeutics: The Next Generation of Texas Companies Advancing Through Clinical Trials for Cancer and Fibrosis of Texas Companies Advancing Through Clinical Trials for Cancer and Fibrosis

Prior to becoming CEO of Tvardi, Imran Alibhai, Ph.D. was a Senior Vice President and Managing Director of DNAtrix, a clinical stage biotech company developing oncolytic viruses for cancer. Previously, Dr. Alibhai was an investment banker in PJ Solomon's Healthcare Advisory Group, focused on M&A transactions in science-based markets including biopharmaceuticals, medical devices/diagnostics and life science tools. Formerly, he was the Senior Director at Alexandria Venture Investments, where he was responsible for investments in emerging companies and funds in the healthcare sector. He also directed investments for PIPE's and long/short positions in MPM Capital's BioEquities hedge fund. Dr. Alibhai began his career in early stage venture capital at the Accelerator Corp., where he was involved in every facet of company/project formation and management. He currently serves on the Scientific Advisory Board for NASA's Translational Research Institute for Space Health. Dr. Alibhai holds a Ph.D. in Molecular Neuroscience from the University of Texas Southwestern Medical School and a BS in Biology from Duke University.



Jason Bock, PhD
Head of Biologics Product Development
MDACC's Approach to Optimizing the Academic/Industrial Interface in Cell Therapy

Jason Bock, Ph.D., has spent the last 20 years in small, medium and large biotech and biopharma companies developing biologic therapeutics. He has brought 15 novel drugs through preclinical development into clinical studies, as well as driven three biologics through the clinic to commercialization globally.

In 2019, Dr. Bock joined MD Anderson Cancer Center as the Head of Biologics Product Development in the Therapeutics Discovery and Development Division. Biologics Development is a unique group of clinicians, researchers and drug development experts working together to develop the next generation of cell therapies. Dr. Bock is leading the group to work closely with MD Anderson clinicians and researchers to rapidly advance products to patients and clinical proof-of-concept in addition to engaging in strategic industry collaborations to co-develop innovative products and maximize the expansive MD Anderson ecosystem.

Michael A. Davies, MD, PhD
Professor and Chair, Department of Melanoma Medical Oncology
Professor, Departments of Translational Molecular Pathology and Systems Biology

The Role of Oxidative Phosphorylation (OXPHOS) in Metastasis and Therapeutic Resistance in Melanoma

Dr. Davies obtained his Bachelors of Arts from the University of Texas at Austin. He completed the requirements of the combined MD/PhD Program at the University Of Texas Health Science Center Of Houston. He did his Residency in Internal Medicine at the Massachusetts General Hospital, and his Fellowship in Medical Oncology at the University of Texas MD Anderson Cancer Center. Dr. Davies is Professor and Chairman in the Department of Melanoma Medical Oncology at the University of Texas MD Anderson Cancer Center, with joint appointments in the Departments of Systems Biology, Translational Molecular Pathology, and Genomic Medicine. Dr. Davies is a physician-scientist whose research utilizes integrated approaches to study the regulation and clinical significance of oncogenic signaling networks in cancer, particularly in therapeutic resistance and in the molecular pathogenesis of brain metastases. Dr. Davies has been the principal investigator of both individual and team science peer-reviewed grants from several organizations, including the National Cancer Institute (NCI), the US Department of Defense (DoD), the American Society of Clinical Oncology (ASCO), the American Cancer Society (ACS), the Melanoma Research Alliance, and the Melanoma Research Foundation. He has served as the principal investigator of several clinical trials for patients with metastatic melanoma, and has authored or co-authored >180 original research manuscripts in peer-reviewed journals including Cell, Cancer Cell, Science, Cancer Discovery, Lancet Oncology, Cancer Research, JAMA Oncology, and Clinical Cancer Research. Dr. Davies is a member of the American Society for Clinical Investigation and the Executive Committee of the Society for Melanoma Research.

Emilia Di Francesco, PhD
Associate Director,
Medicinal Chemistry

Preclinical and Clinical PK/PD/Efficacy Work on the OxPhos Inhibitor IACS-010759, Targeting Metabolic Vulnerabilities in Cancer

Emilia Di Francesco is the Associate Director of Medicinal Chemistry at the Institute of Applied Cancer Science (IACS) at MD Anderson Cancer Center. She is an experienced medicinal chemist with 20 years' experience in Pharma and Biotech-like organizations. Prior to IACS, she worked across several research sites of Merck Research Laboratories and led medicinal chemistry projects in several different therapeutic areas, including antiviral and oncology. She has contributed to the discovery of two approved medicines, Raltegravir, the first in class HIV integrase inhibitor, and Grazoprevir, a second-generation HCV protease inhibitor. Together with the team at IACS, her research focuses on the discovery and development of novel small molecule cancer therapeutics, with a focus in the areas of cancer metabolism, epigenetics and DNA damage response. The first of these molecules to reach the clinic is IACS-10759, a novel inhibitor of Complex I in the mitochondrial electron chain which is currently in Phase I for treatment of OxPhos dependent tumors.

Di Francesco received her Master's Degree in Organic Chemistry from the University of Rome La Sapienza and her PhD from the University of Cambridge, UK, under the supervision of Prof. Ian Paterson.

Sarah Hein, PhD
Entrepreneur in Residence

TMC Accelerator for Cancer Therapeutics: Supporting the Texas Biotech Ecosystem

Sarah is an Entrepreneur in Residence for the Texas Medical Center Accelerator for Cancer Therapeutics (TMC ACT). Formerly, she was a cofounder and the Vice President of Operations at Courier Therapeutics, a cancer immunotherapy startup. She was also Director of Research at Resonant Therapeutics, an antibody therapeutics platform technology company. She began at Mercury Fund as a Venture Fellow directly after graduating with her PhD from Baylor College of Medicine. She was a core member for Enventure during her graduate training, and launched the Foundations workshop series to provide in-depth entrepreneurship education to the TMC community.

James Inglese, PhD
Principal Investigator
Assay and Screening Strategies for Chemical Probe and Drug Discovery

Dr. James Inglese, Ph.D. directs the Laboratory of Assay Development and Screening Technology (ADST) within the National Center for Advancing Translational Sciences (NCATS) and is an Adjunct Investigator within the National Human Genome Research Institute (NHGRI). His laboratory explores strategies aimed at enabling the identification of novel chemical matter targeting the discovery of chemical probes and therapeutic leads for the study and treatment of rare and neglected disease. The NCATS post-doctoral training program he has established is recognized by disease foundations as an effective collaborative bridge to NCATS expertise and resources enabling a champion-driven effort in early-stage translation. Prior to the formation of NCATS he co-founded the NIH Chemical Genomics Center (NCGC) acting as its Deputy Director. Dr. Inglese received his Ph.D. in Organic Chemistry from the Pennsylvania State University and completed post-doctoral training in the laboratory of future 2012 Nobel Laureate Prof. Robert J. Lefkowitz at Duke University Medical Center. Before coming to the NIH, Dr. Inglese led research teams at the combinatorial chemistry Princeton-based biotech Pharmacopeia and Merck Research Laboratories. Over the past several decades, Dr. Inglese has contributed to over 180 publications and patents; his efforts on the early drug discovery process have resulted in innovative assay formats, reporter systems, and high throughput screening paradigms. Dr. Inglese serves on the scientific advisory boards of several NIH-funded chemistry and screening centers, disease foundations, and international chemical biology consortia.

Head Assay Development & Screening Technology (ADST)
National Center for Advancing Translational Sciences (NCATS), NIH

Sara Jane Johnson
Graduate Student

Microscopy-Based Identification of RNA Foci Modulators in Myotonic Dystrophy Type 1

Sara Johnson is a Ph.D. candidate in the Molecular and Cellular Biology graduate program at Baylor College of Medicine in Houston, Texas. She graduated summa cum laude from Florida Gulf Coast University with a Bachelor of Science in biology with a microbiology concentration.

For her graduate studies, she joined the laboratory of Dr. Thomas Cooper to investigate the metabolism and composition of toxic CUG expanded RNA foci, the pathogenic origin of Myotonic Dystrophy Type 1 (DM1). As DM1 has no disease-modifying therapies, Sara's work has implemented small-molecule screening measuring fluorescent in situ hybridization (FISH)-labeled RNA foci in DM1 myoblasts. This work has identified seven compounds that impact RNA foci formation in DM1 myoblasts, either through reduction of foci counts or enhancement of foci fluorescent intensity.

Lyn Jones, PhD
Director of the Center for Protein Degradation

Towards the Rational Design and Development of Targeted Protein Degraders

Lyn Jones completed PhD studies in synthetic organic chemistry at the University of Nottingham, before starting his post-doctorate research at The Scripps Research Institute, California in the area of chemical biology. He joined Pfizer (Sandwich, UK) initially as a medicinal chemistry team leader, and eventually became Director of Chemical Biology and Lead Discovery Technologies. He transferred to Pfizer Cambridge, MA to become Head of Rare Disease Chemistry and Head of Chemical Biology, before joining Jnana Therapeutics as Vice President of Chemistry and Chemical Biology. He is currently Director of the Center for Protein Degradation at the Dana-Farber Cancer Institute.

He is an author of over 100 publications in chemistry and chemical biology, and is a fellow of the American Association for the Advancement of Science, the Royal Society of Chemistry and the Royal Society of Biology. His interests include protein homeostasis, chemoproteomics and chemogenomics, therapeutic target identification and validation, molecular pharmacology, protein labeling chemical biology and covalent drug discovery.

John Joslin, PhD
Director
Assay Development and High-Throughput Screening

The Evolution of a Fully Automated High Throughput Flow Cytometry Screening System to Enable Drug Discovery

Dr. Joslin received his Ph.D. in cancer biology at the University of Chicago, modeling therapy-related leukemia in mice. He showed that functional loss of Egr1 is an initiating event in the pathogenesis of AML/MDS. Dr. Joslin conducted his postdoctoral training at GNF under the mentorship of Michael Cooke in the Department of Immunology, where he characterized phenotypic differences in various immune cells across 30 different inbred strains of mice. These differences were then correlated with haplotype differences to identify candidate genes underlying the phenotypes. Through this work Dr. Joslin established himself as a leader in phenotypic assay development. After joining the assay development and high-throughput screening group (AD/HTS), his early efforts focused on the development of a fully automated flow cytometry screening system. This was an industry first and is still regarded as the leading platform for automated flow cytometry. Dr. Joslin continues to innovate in high-throughput screening, and is currently leading the AD/HTS group at GNF. Present efforts are focused on highly multiplexed gene expression, 3D biology, and microfluidics.

Andrew Y. Koh, M.D.
Associate Professor
Pediatrics and Microbiology
ACT User

Dr. Andrew Y. Koh is an Associate Professor of Pediatrics and Microbiology at the University of Texas Southwestern Medical Center; Director of Pediatric Hematopoietic Stem Cell Transplantation Program at Children's Medical Center Dallas; and Co-founder of Aumenta. Clinically trained in Pediatric Hematology/Oncology and Infectious Diseases and scientifically trained in microbiology and immunology, Dr. Koh leads an a research program funded by the NIH (NIAID and NCI), industry (Merck, Novartis) and foundations that focuses on understanding how the gut microbiome modulates the host immune response in cancer and stem cell transplant patients – specifically focusing on bacterial/fungal infections originating from the gut, graft-versus-host disease, and host anti-tumor response. Dr. Koh has published his gut microbiome research in high-impact journals such as Nature Medicine, PLoS Pathogens, Nature and Biology of Blood and Marrow Transplantation. Technology developed in the Koh Lab serves as the foundation for Aumenta's novel approach of using gut microbiota to enhance the host's immune response to cancer immunotherapy.

Elisabetta Leo, PhD
Principal Scientist

Development of AZD5305, The Next Generation PARP1 Selective Inhibitor and Trapper

Since she joined AstraZeneca in 2016, Dr Elisabetta Leo has served as the preclinical lead for olaparib (Lynparza) and headed the drug discovery campaing that led to AZD5305, the next generation PARPi currently in Phase 1.

Dr Leo trained as medicinal chemist at the University of Padova, Italy and achieved a PhD in Molecular Pharmacology in 2003, with a study on topoisomerases as targets of drugs at the University of London, UK.

With a postdoctoral position in the University College London (UK), she studied the role of proteins involved in the early stages of DNA replication and their application in translational science. She then moved to Centre for Cancer Research, NCI, NIH, Bethesda, MD, where she focussed on DNA Damage Response (DDR) pathways. Her work shed light to the molecular mechanism of action of novel antiproliferative drugs as well as on the discovery and validation of SLFN11 as novel biomarker for anticancer therapies based on DNA damaging agents.

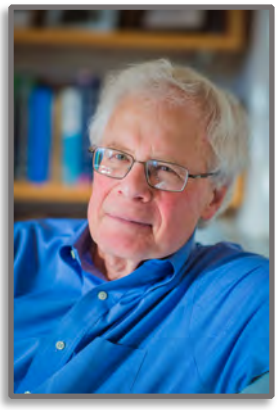
In 2012 Dr Leo joined the Institute for Applied Cancer Sciences at the MD Anderson Cancer Center (Houston, TX); there, she worked with multidisciplinary teams to discover and develop effective anticancer agents targeting epigenetics and the DDR pathways, as well as to evaluate novel strategies for patient stratification in the clinic.

Craig W. Lindsley, PhD
William K. Warren, Jr. Chair in Medicine
Professor, Pharmacology, Biochemistry, and Chemistry

Discovery and Development of GPCR Allosteric Ligands: From Concept to Clinic

Craig W. Lindsley, Ph.D. is the Director of the Warren Center for Neuroscience Drug Discovery (WCNDD), University Professor (also Professor of Pharmacology, Chemistry & Biochemistry) and Editor-in-Cheif of the Journal of Medicinal Chemistry. Craig graduated in 1992 from California State University, Chico with a B.S. in Chemistry, received his Ph.D. degree in Chemistry from the University of California, Santa Barbara (Lipshutz), in 1996, and pursued postdoctoral studies at Harvard University (Shair). In 2001, Craig accepted a position at Merck & Co where he pioneered, in positions of increasing responsibility, the development of allosteric ligands for Akt, mGlu5 and M1, providing critical proof-of-concept compounds that validated the mechanism of allosteric modulation and clinical candidates. In 2006, Craig accepted an Associate Professor position in Pharmacology and Chemistry at Vanderbilt University, and promoted to Full Professor in 2009. In that same year, Craig became the founding Editor-in-Chief of ACS Chemical Neuroscience and was also awarded the ASPET-Astellas Award for Translational Pharmacology. In 2012, he was awarded an endowed chair, the William K. Warren, Jr. Chair in Medicine. The following year, Craig was awarded the Portoghese Lectureship from the ACS MEDI division and the Journal of Medicinal Chemistry for impact in the field of medicinal chemistry, and in 2014, received the John J. Abel Award in Pharmacology from ASPET. More recently, Craig was inducted as an AAAS Fellow, awarded the Pharmacia-ASPET Award in Experimental Therapeutics and named a Thomson Reuters Highly Cited Researcher (2015, 2016 and 2017 (Now Clarivate)) as well as a Thomson Reuters World's Most Influential Scientific Minds (2016). In 2018, Craig was honored as the the 22nd Smissman Memorial Lecturer (KU Department of Medicinal Chemistry) and the 2018 Sato Memorial International Award. Together with Jeff Conn, Craig has pioneered the concept of GPCR allosteric modulation, developing key proof of concept compounds and clinical candidates. Craig holds over 104 issued US patents and has published over 530 manuscripts and another 200 published patent applications. As co-founder and Director of the WCNDD, Craig has raised over $290 million in licensing and research support from NIH, Foundations and companies. In 2016, and without an industry partner, Craig oversaw IND-enabling studies of a novel M1 PAM that was awarded an open IND, and the Phase I trial (SAD) was completed at Vanderbilt prior to licensing to Acadia.

Vanderbilt University



Harvey Lodish, PhD
Professor
Biology and Professor of Bioengineering

Academic Entrepreneurs, New Technologies, and Building a Biotechnology Ecosystem: A Personal History

A leader in the field of molecular and cellular biology, Dr. Harvey F. Lodish has isolated and cloned numerous surface membrane proteins that play a role in blood development, cell signaling, glucose transport, and lipid metabolism. He earned his PhD at the Rockefeller University in 1966. A Founding Member of the Whitehead Institute, Dr. Lodish joined the MIT faculty in 1968 and has been a professor of biology since 1976 and professor of biological engineering since 1999. Dr. Lodish is also the lead author of the widely used textbook Molecular Cell Biology. The book has been translated into 14 languages and the ninth edition appeared in January, 2021. He is a Member of the National Academy of Sciences, a Fellow of the American Association for the Advancement of Science, the American Academy of Arts and Sciences, and the American Academy of Microbiology, and an Associate (Foreign) Member of the European Molecular Biology Organization.

He received the 2010 Mentoring Award from the American Society of Hematology, the 2016 American Society for Cell Biology WICB Sandra K. Masur Senior Leadership Mentoring Award, the 2016 Pioneer Award from the Diamond Blackfan Anemia Foundation, and the Metcalf Lifetime Achievement Award from the International Society for Experimental Hematology in 2020.

Dr. Lodish is a member of the Board of Trustees of Children's Hospital, Boston, where he was Chair of the Research Committee of the Board of Trustees. From 2007 - 2014 he was Founding Chair of the Scientific Advisory Board of the Massachusetts Life Sciences Center, the group charged with oversight of the state's 10- year $1 billion investment in the life sciences. Dr. Lodish was a founder and scientific advisory board member of many biotechnology companies including Genzyme, Millennium and Rubius.

Thomas J. Lynch Jr., MD
President & Director
Raisbeck Endowed Chair

Dr. Thomas J. Lynch Jr. is a world-renowned scientist, highly respected oncologist and successful NCI-designated comprehensive cancer center leader. As the President and Director of Fred Hutchinson Cancer Research Institute, and holder of the Raisbeck Endowed Chair, Dr. Lynch brings more than three decades of experience at highly regarded U.S. cancer centers. He has expertise in solid tumor research, precision medicine and discoveries in fundamental biology. Before joining Fred Hutch, Dr. Lynch held leadership roles as CSO at Bristol-Myers Squibb, CEO of Massachusetts General Physicians Organization, director of Yale Cancer Center, physician-in-chief at Yale's Smilow Cancer Hospital, as well as chief of hematology-oncology at Massachusetts General Hospital and professor of medicine at Harvard Medical School. Dr. Lynch is a member of the American Association for Cancer Research, the American Society of Clinical Oncology, and the International Association for the Study of Lung Cancer.

Raimund Ober, PhD
Professor
Biomedical Engineering and Molecular & Cellular Medicine
ACT User

Raimund Ober is a co-founder, with E. Sally Ward, of Astero Alta Inc. The company was founded to commercialize new approaches for the design of antibody-drug conjugates that were developed in their laboratories at UT Southwestern Medical Center and Texas A&M University. This university-based research had been supported by a CPRIT research grant.

Raimund Ober obtained his Ph.D. from Cambridge University, UK, where he also held a research fellowship at Girton College. In 1990 he joined the University of Texas at Dallas, where he rose through the ranks to Full Professor. From 1996 he also held an adjunct position at UT Southwestern Medical Center. In 2014 he took on a position as Professor in the Department of Biomedical Engineering and the Department of Molecular and Cellular Medicine at Texas A&M University. From 2018, he also holds a position in the Centre for Cancer Immunology at the University of Southampton, UK.

His research interests are in the development of microscopy and imaging techniques, in particular for the development of biotherapeutics. He jointly manages a research laboratory with Prof. E. Sally Ward who specializes in protein engineering for drug development. A particular focus of their research group is the exploitation of the cellular transport pathways of proteins within cells for the design of biologics.

Reid Powell, PhD
Assistant Professor

Implementing Deep Neural Network Technologies Across Experimental Scales

Dr. Powell has a diverse research background with expertise in cell and molecular biology, bio-informatics, and lab automation. He is currently an Assistant Professor in the Gulf Coast Consortia's Combinatorial Drug Discovery Program at Texas A&M Institute of Bioscience and Technology, where he also received his PhD. Throughout his career, he has developed a wide array of image based and biochemical high throughput screening platforms, which have been used to develop and repurpose drugs across multiple disease contexts including cancer, pathogenic infections, and neurologic disorders. He also has an interest in the development of methods used to contextualize high throughput screening data using integrative approaches that combine genomics, transcriptomics, chemical, and pharmacologic data sources.

Craig Ramirez, PhD
Co-Counder & CSO
ACT User

Dr. Ramirez is a cancer cell biologist at heart. He was previously a post-doctoral researcher in the Developmental Therapeutics Lab at UT Austin Dell Medical School. Dr. Ramirez obtained his doctorate in cancer metabolism at NYU School of Medicine under the leadership of Dr. Dafna Bar-Sagi, a world-renowned expert in pancreatic and Ras-driven cancers. During his time in the lab, Dr. Ramirez uncovered many novel potential therapeutic targets and MOAs, several of which represent the backbone of TEZCAT's technology platform. In addition to research, Dr. Ramirez has been engaged in various entrepreneurship and executive programs, including Entrepreneurship Lab (Bio and Health Tech NYC) and the Texas Medical Center Accelerator for Cancer Therapeutics, to further develop the core skill sets and relationships for successful biotech ventures.

Cliona M Rooney, PhD
Professor
Pediatrics, Molecular Virology and Microbiology, and Pathology

CAR-modified Virus-specific T-cells for the Treatment of Malignancies: Taking Advantage of the TCR

Dr. Rooney is a Professor in the Departments of Pediatrics, Molecular Virology and Microbiology, and Pathology and Director of Translational Research Laboratories of the Center for Cell and Gene Therapy at Baylor College of Medicine. Her training in virology and immunology led to the demonstration that adoptively-transferred virus-specific T cells (VSTs) could prevent and cure viral-associated malignancies in humans following hematopoietic stem cell transplantation (HSCT). She extended this successful strategy to develop and clinically test a range of post-transplant viral infections and diseases as well as virus-associated malignancies, such as EBV+ lymphoma and HPV-associated malignancies, that occur outside of the HSCT setting.
She has a particular interest in strategies that render T cells genetically resistant to inhibition by the tumor microenvironment, such as a dominant-negative TGF-beta receptor and a constitutively active IL-7 receptor. To add a safety switch for genetically enhanced T-cells, she developed an inducible caspase 9-based suicide gene that is inducible by dimerization and has proved successful in clinical trials. To overcome the lack of in vivo proliferation of T-cells expressing chimeric antigen receptors (CARs) for tumor antigens, she evaluated the use of virus-specific T cells (VSTs) as hosts, so that CAR-VST activation and expansion can be induced by endogenous viruses, viral vaccines or oncolytic viruses.

Vlad C. Sandulache, MD, PhD
Assistant Professor
Otolaryngology – Head and Neck Surgery

High-throughput Imaging and a Multi-omics Approach Shed Light on Cisplatin Resistance

Dr. Sandulache obtained his MD and PhD from the University of Pittsburgh. He completed his residency in Otolaryngology – Head and Neck Surgery at Baylor College of Medicine, followed by a fellowship in Head and Neck Surgical Oncology at the University of Texas MD Anderson Cancer Center. He currently works at Baylor College of Medicine a surgeon scientist focused on advanced stage head and neck cancer. Dr. Sandulache helped define some of the metabolic features of head and neck squamous cell carcinoma (HNSCC) and the relationship between common oncogenic events and metabolic reprogramming in HNSCC. His laboratory is focused on understanding how metabolic reprogramming contributes to the development of chemotherapy resistance through adaptation to oxidative stress.

Haifa Shen, MD, PhD
Professor
Nanomedicine

Overcoming Transport Barriers in Cancer Treatment

Haifa Shen received his B. Med. degree in China and his Ph. D. degree at University of Texas/ M D Anderson Cancer Center. He spent 8 years in the biopharmaceutic industry after post-doctoral training at the National Cancer Institute, and came back to academia in 2010. He is currently Professor of Nanomedicine in Houston Methodist Academic Institute and Leader of the Innovative Therapeutics Program in Houston Methodist Cancer Center. His research interests and expertise are on delivery of therapeutic agents to the target tissues, with particular emphasis on overcoming drug transport barriers. He performs multidisciplinary research and works closely with biologists, chemists, engineers, drug formulation scientists, material scientists and clinicians. He currently serves as the principal investigator of one U54 center grant and two R01 grants from the National Cancer Institute.

Larry A. Sklar, PhD
Distinguished Professor Emeritus/founding director University of New Mexico Center Molecular Discovery

High Throughput Flow Cytometry for Drug Discovery and Repurposing

Larry A. Sklar (PhD, Physical Chemistry, Stanford), Distinguished Professor Emeritus/founding director University of New Mexico Center Molecular Discovery, has >450 publications and patents in biotechnology, drug discovery and repurposing. With Bruce Edwards and Fritz Kuckuck he invented the HyperCyt high throughput flow cytometry platform and co-founded the Albuquerque startup company IntelliCyt. His inventions contributed to several startup companies and clinical trials. IntelliCyt's acquisition by Sartorius represented a commercialization landmark for UNM. Recent leadership roles included UNM Chemical Biology Consortium Center for the NExT Program; NIH's Illuminating the Druggable Genome; Drug Repurposing Network for the UNM Clinical and Translational Science Center; Cancer Therapeutics Program/Budke and Anderson Chair in Cancer Drug Discovery for UNM Comprehensive Cancer Center, and board member Cures Within Reach. Awards include: at UNM, 53rd Annual Research Lecturer, Innovation Fellow (2011), Presidential Award of Distinction (2016); and National Academy of Inventors (2020).

Abstract: We introduced high throughput flow cytometry (HyperCyt) as a technology for drug discovery, patenting applications of the technology. While the academic mission has traditionally involved research and education it now spans technological innovation, discovery, translation and commercialization. The high throughput flow cytometry platform for drug discovery has been associated with multi-target screening for both cellular and molecular targets such as efflux transporters, integrins, GPCRs, and GTPases, as well as protein-protein, and DNA-protein interactions. These have been accompanied by repurposing screens in association with the NIH Molecular Libraries Program, the UNM Comprehensive Cancer Center, and the UNM Clinical and Translational Science Center through the Drug Repurposing Network. Kinetic measurements in flow cytometry have provided insight into small molecule mechanism of action. Experimental and computational methods have led to the identification of small molecules as first in class chemical probes, leads for drug discovery, and repurposed drugs. These molecules and technologies have been described in several hundred publications and more than 40 patents, and have contributed to several

clinical trials and start-up companies. We have conducted discovery studies in neurological, cardiovascular and infectious diseases as well as cancer that show promising results. We envision future repurposing contributions to personalized medicine. The technology is now commercially available worldwide, with additional applications in antibody discovery and immuno-oncology.

Nakia Spencer, MS
Associate Scientist IV

IACS-6274, a Potent and Selective Inhibitor of Glutaminase (GLS1) Being Developed for KEAP1/NFE2L2 Mutant NSCLC and ASNS-low Ovarian Cancer Patients

Nakia Spencer joined The TRACTION platform at M.D. Anderson Cancer Center in 2012. In her current role, she has contributed to the advancement of a diverse portfolio of translational biology and drug discovery and development programs. Her research efforts focus on defining novel translational strategies through comprehensive mechanistic biology. In partnership with the Institute for Applied Cancer Science within the Therapeutics Discovery Division, Nakia has contributed to the advancement of multiple programs through discovery and development, including advancement of IACS-6274 into Phase 1 clinical trials in patients with unmet medical need.

Prior to joining MD Anderson, Nakia was a research associate in cellular immunology at the Ochsner Clinic Foundation where she studied the role of macrophages in B cell lymphomagenesis and the role of lymphoma microenvironment in mediating drug resistance. Nakia completed her graduate thesis work in the Laboratory for Equine and Comparative Orthopedic Research at Louisiana State University (LSU) School of Veterinary Medicine and received her Master's Degree in Biology from Southern University and A&M College. Nakia's research has been published in multiple journals and she is a co-inventor on multiple patents and patent applications related to her research. She is an active member of the American Association of Cancer Research (AACR) and an In-patient Advocate and New Volunteer Trainer at MD Anderson Cancer Center.

Fabio Stossi, PhD
Associate Professor
Molecular and Cellular Biology
Single Cell Analysis of Estrogen Receptor Actions

Dr Fabio Stossi, a native of Milan, Italy, completed his studies at Universita' degli Studi di Milano, in Pharmaceutical Chemistry and Technology, and in Endocrinology and Metabolism. He then moved to the US as a postdoc in Dr. Benita S. Katzenellenbogen's laboratory at University of Illinois at Urbana-Champaign. He joined Dr. Michael A. Mancini's group in the Department of Molecular and Cellular Biology, Baylor College of Medicine, as an Assistant Professor. He is currently Associate Professor, Technical Director of the Integrated Microscopy Core and group leader for imaging in the GCC Center for Advanced Microscopy and Image Informatics. His interests are imaging and analysis of single cell gene transcription and assay development in environmental toxicology.

Cindy R. WalkerPeach, PhD
Chief Product Development Officer
CPRIT Update and Company Funding Opportunities

Dr. WalkerPeach leads CPRIT Product Development Research which critically evaluates and invests in Texas-based companies with promising novel cancer-focused products and services (drugs, diagnostics, medical devices and other non-traditional oncology applications) that will benefit cancer patients and society.

Prior to joining CPRIT, she served as Program Director for the National Science Foundation (NSF) Innovation Corps (I-Corps) program in Alexandria, VA (Washington, DC area). NSF I-Corps prepared research scientists to extend their focus beyond the university laboratory and accelerated the economic and societal benefits of research projects with commercialization potential. She joined NSF from the University of Texas at Austin where she was a Director at the Austin Technology Incubator, having served as lead advisor for healthcare-focused life sciences startups. She was responsible for evaluating new business ventures, managing a portfolio of bioscience startups and providing business mentoring to technology-focused faculty and entrepreneurs.

Prior to government service, Dr. WalkerPeach had more than 20 years experience in the biotechnology sector as a member of several life science company management teams. Dr. WalkerPeach completed a BS in Chemistry and holds a PhD in Molecular Biology.

Alexandra Walsh, PhD
Assistant Professor,
Biomedical Engineering

Optical Metabolic Imaging to Quantify Heterogeneity in Anti-Cancer Drug Response

Dr. Walsh completed her Ph.D. at Vanderbilt University where she developed an autofluorescence lifetime-based assay for determining the optimal cancer treatment strategy for individual patients. As a post-doc at the Air Force Research Lab, Dr. Walsh used optical techniques to investigate infrared-light activation and inhibition of action potential propagation in neurons. Currently, Dr. Walsh is an Assistant Professor in the Biomedical Engineering Department at Texas A&M University, where her lab group develops tools to detect and quantify cellular heterogeneity, identifies functional, label-free biomarkers of disease progression and drug response, and integrates biomarkers and microscopy tools for improved health outcomes.

Alex G. Waterson, PhD
Research Associate Professor
Pharmacology and Chemistry
Drugging RAS: Meeting the Challenge

Alex G. Waterson, Ph.D. is currently a Research Associate Professor of Pharmacology and Chemistry at Vanderbilt University in Nashville, Tennessee, USA. Classically trained as a synthetic organic chemist, he completed his Ph.D. studies with Professor Al Padwa at Emory University, and conducted postdoctoral research at Colorado State University in the group of the late Professor Albert I. Meyers. Alex joined a medicinal chemistry team at GlaxoSmithKline in the Research Triangle Park area of North Carolina in 2001, contributing to the discovery of covalently modifying ErbB inhibitors and the B-RAF drug Dabrafenib, among other projects.

Upon transitioning to Vanderbilt in 2008, he helped establish the National Cancer Institute-funded Vanderbilt Center for Cancer Drug Discovery, which he now directs, and joined Professor Fesik's fragment-based discovery team. He has led primarily oncology drug discovery projects including those aimed at direct and indirect inhibition of K-RAS, as well as protease, epigenetic, and cancer metabolism targets. As Associate Director of Medicinal Chemistry for the Vanderbilt Institute of Chemical Biology, he continues to impact small-molecule discovery projects across the University and its associated medical center.

Dr. Waterson has co-authored 55 peer-reviewed manuscripts, and is a named co-inventor on 26 published patent applications. He is an active member of the Chemistry in Cancer Research Workgroup of the American Association for Cancer Research, and is currently an Editorial Advisory Board member for ACS Medicinal Chemistry Letters.

Vanderbilt University

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A Novel Role of ADGRF1 (GPR110) in Promoting Cellular Quiescence and Chemoresistance in Human Epidermal Growth Factor Receptor 2-positive Breast Cancer

Abdulkareem NM[1], Bhat R[1,2], Qin L[3], Vasaikar S[3], Gopinathan A[2], Sarmistha Nanda S[3], Thangavel H[2], Zhang B[3,5], De Angelis C[3,4], Schiff R[3,6,7], Trivedi MV[1,2,3,7]

1. Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX 77204, USA
2. Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston, TX 77204, USA
3. Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX 77030, USA
4. Department of Clinical Medicine and Surgery, University of Naples, Federico II, 80131 Naples, Italy
5. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA
6. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA
7. Department of Medicine, Baylor College of Medicine, Houston, TX 77030, USA

Purpose: Adhesion G protein coupled receptors (aGPCRs) remains least explored GPCRs family for their role in health and disease. The aGPCR, ADGRF1 (GPR110), is overexpressed and predicts poor survival in various cancer types. However, ADGRF1's coupling to G proteins and downstream pathways remain unknown in cancer. We have reported that ADGRF1 is overexpressed in tumorigenic population and in anti-HER2 therapy-resistant derivatives of various HER2+ breast cancer (BC) cell line models. Further, we have reported that ADGRF1 knockdown reduces tumorigenesis and metastasis in HER2+ BC cells. In this study, we evaluated the effects of ADGRF1 overexpression (OE) on tumorigenesis, signaling pathways, and outcomes in HER2+ BC.

Methods: Stable clones of 2 HER2+ BC cell lines, BT474 and SKBR3, were generated to overexpress ADGRF1 construct in doxycycline-inducible manner. The effect of ADGRF1 on tumorigenesis was evaluated in vitro using soft agar assays, mammosphere assay, and Aldefluor assay. The effect on the HER pathway was evaluated by assessing expression of phospho- and total-HER1 and -HER2 and potency of anti-HER2 drugs. G protein coupling of ADGRF1 was determined by co-immunoprecipitation of Gαs and Gαq and detecting second messengers, cAMP and IP1. Downstream pathways of ADGRF1 were explored by RNAseq and Reverse Phase Protein Arrays (RPPA) and functional assays. ADGRF1 expression was assessed in BC patients from The Cancer Genome Atlas. ADGRF1 OE effects on survival was evaluated using the Molecular Taxonomy of Breast Cancer International Consortium dataset.

Results: ADGRF1 OE enhanced the number of colonies and mammospheres and increased Aldefluor+ tumorigenic cell population *in vitro* and promoted the tumor growth *in vivo*, suggesting a role in tumorigenesis. ADGRF1 OE did not alter the expression total- or phospho-HER1/-HER2 proteins or efficacy of anti-HER2 drugs. Overexpressed ADGRF1 co-immunoprecipitated with Gαs and Gαq proteins and increased cAMP and IP1. However, inhibition of only the Gαs pathway by SQ22536 reversed the pro-tumorigenic effects of ADGRF1 OE, suggesting pro-tumorigenic effects of the ADGRF1-Gαs pathway. Integration of RNA-Seq and RPPA data showed that ADGRF1 OE inhibited cell cycle. We detected G0/1 arrest by cell cycle analysis with ADGRF1 OE. The protein level of the proliferation marker Ki67 was reduced upon ADGRF1 OE. Also, ADGRF1 OE led to a 10-fold reduction in potency of docetaxel, a chemotherapy drug used in HER2+ BC. ADGRF1 was overexpressed and amplified in basal and HER2 compared to luminal A and B BCs. High ADGRF1 expression predicted poor survival in HER2 subtype, but not in basal BC.

Conclusions: ADGRF1 represents a novel drug target in HER2+ BC, warranting discovery of novel ADGRF1 antagonists.

Support or Funding Information

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Unbiased High Throughput Screening To Identify Novel Combination Therapies For RAS-Mutated Colorectal Cancer

Fan Fan F[1], Park YS[4], Powell R[4], Roszik J[3], Stephan C[4], Ellis LM[1,2], Bhattacharya R[1]

1. Department of Colon & Rectal Surgery, The University of Texas MD Anderson Cancer Center
2. Department of Molecular & Cellular Oncology, The University of Texas MD Anderson Cancer Center
3. Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center,
4. Combinatorial Drug Discovery Program, The Texas A&M Health Science Center

Corresponding Author: Rajat Bhattacharya, Department of Colon and Rectal Surgery, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, Texas.
Email: rbhattacharya@mdanderson.org

Background: Metastatic colorectal cancer (mCRC) is the second leading cause of cancer death in the US. The response rate to current systemic therapies is ~50% and most patients die within 2.5 years from diagnosis of metastasis. Also, only ~5% of patients with mCRC benefit from immunotherapies. 50% of patients with mCRC harbor mutations in RAS (KRAS, NRAS). However, except recent advances for the KRAS G12C mutant, targeting other RAS mutations are challenging. Thus, efforts have been concentrated on targeting MEK, a downstream mediator of RAS. Targeting MEK with single agent therapy is also ineffective in patients with mCRC. Hence, identifying combination strategies that can improve efficacy of MEK inhibitors will benefit a large group of patients with RAS mutated mCRC.

Objectives: We aimed to perform unbiased high throughput screening (HTS) to identify drugs that, when combined to MEK inhibitors, would enhance their efficacy.

Methods: We performed unbiased HTS with CRC cells grown in 3D cell culture using the MEK inhibitor trametinib as a backbone, and two different compound libraries composed of drugs either approved by the FDA or in clinical trials; 1) the NCI oncology set V, and, 2) a custom clinical compound set. The Bliss model of synergy was used to identify multiple compounds that were synergistic with trametinib. Validation of synergy for different drug combinations are being performed *in vitro* and *in vivo*.

Results: Our initial unbiased HTS studies using HCT116 3D cultures identified multiple inhibitors including DNA damaging agents and inhibitors of mTOR, SRC, mitosis and HER family as top candidates that were synergistic with trametinib. These combinations were validated in other KRAS mutated CRC 3D cultures. The combination of trametinib and the SRC inhibitor dasatinib was further validated by colony formation assays. Western blot analyses of markers for apoptosis and various cell proliferation markers demonstrated that targeting SRC with MEK inhibits cell proliferation and increases cell death in multiple CRC cell lines as compared to single agents. *In vivo* studies in mice using RAS mutated CRC PDXs and cell lines with these drugs at *clinically relevant doses,* however, failed to demonstrate significant tumor growth inhibition or tumor regression.

Conclusions: Our unbiased HTS have identified multiple drug combinations that may enhance the efficacy of targeting MEK in RAS mutated CRC. One such combination, trametinib with dasatinib, although promising *in vitro*, failed to demonstrate enhanced efficacy in carefully conducted *in vivo* preclinical studies. These studies highlight the need for performing careful *in vivo* preclinical studies as a prerequisite to translate *in vitro* findings into clinical studies. Further, our studies emphasize the importance for performing *in vivo* studies using drug doses that reflect clinically achievable doses in humans to identify therapies, either single or in combination, that may finally benefit our patients.

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Targeting PI3K Isoforms to Improve Effectiveness of T-cell Mediated Immunotherapy

Bohat R, Liang X, Xu C, Egan NA, Hou J, Peng W

Center for Nuclear Receptor and Cell Signaling, University of Houston, Houston, TX.
Department of Biology and Biochemistry, University of Houston, Houston, TX.

Corresponding author: Ritu Bohat, Center for Nuclear Receptor and Cell Signaling, University of Houston.
Email: rbohat@central.uh.edu

T-cell-based immunotherapies have achieved encouraging response rates in various types of cancer. However, many cancer patients still fail to response to immunotherapy due to the development of immunosuppression. The immunosuppression mechanisms in nonresponding patients are not fully understood. Oncogenic activation of tumor intrinsic phosphatidylinositol-3-kinase (PI3K) pathway has been shown to play an important role in regulating the immunosuppression hence highlights targeting the PI3K pathway to overcome immunosuppression. However, the PI3K pathway is a major intracellular pathway and plays an indispensable role in T-cells function. Therefore, a different strategy is needed to target tumor intrinsic PI3K pathway without immunosuppression.

In this study, we are utilizing PI3K isoform-specific inhibitors to target tumor-specific pathways. Previous studies have suggested the activation of the PI3K pathway depends on different PI3K isoforms. This offers a unique window to target tumor-specific PI3K pathway without affecting tumor-reactive T-cells. Our preliminary results have shown that combination treatment with GSK2636771 (a PI3Kβ inhibitor) and anti-PD1 had improved T-cell infiltration and suppressed tumor growth in a PTEN-loss tumor, which fails to respond to immunotherapy. However, this combination treatment applies only to 20-30% of patients with PTEN loss. Therefore, we aim to find other PI3K isoforms that can be combined with immunotherapy in PTEN present tumors.

We first want to characterize the role of PI3K isoforms in tumor cells and T-cells that will help to identify the combination to target tumor but not the T-cells. In our initial efforts, *in vitro* Genetic and pharmaceutical inhibition of PI3Kα and PI3Kβ in tumor cells downregulated pAKT expression level and cell proliferation. On the other hand, in T-cells genetic inhibition of PI3Kδ isoform and pharmaceutical inhibition of PI3Kδ and PI3Kγ showed a significant reduction in pAKT expression level and their proliferation. These data suggest activation of the PI3K pathway in tumor-reactive T-cells depend primarily on PI3Kδ or PI3Kγ isoforms, while tumor cells rely primarily on PI3Kα or PI3Kβ isoforms. *in vivo* synergic mouse model showed PI3Kα inhibitor with anti-PD1 combination had the greatest suppression in tumor growth and better survival, followed by PI3Kβ inhibitor combination, displaying the synergistic effect of the combination therapy.

Future studies include determining the underlying mechanism that improves immunotherapy efficacy with PI3K isoform-specific inhibitor. This will be done by characterizing the immune profile using flow cytometry analysis and define PI3K pathway changes using RPPA and RNAseq in PI3K inhibited T- cells and tumor cells.

Incorporating HT-SAXS into Drug Discovery Pipelines

Brosey CA[1], Shen R[1], Burnett K[2], Hura G[2,3], Moiani D[1], Jones DE[4], Tainer JA[1,5]

1. Department of Molecular & Cellular Oncology, M.D. Anderson Cancer Center
2. Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory
3. Department of Chemistry & Biochemistry, University of California Santa Cruz
4. Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences
5. Molecular Biology Consortium, Lawrence Berkeley National Laboratory

Corresponding author: Chris A. Brosey & John A. Tainer, Molecular & Cellular Oncology, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit #0108, Houston, TX, CABrosey@mdanderson.org.

High-throughput (HT) methods for discovering single-target protein and nucleic acid ligands are well established and routinely utilized for drug discovery. Many critical biological outcomes, however, are mediated by multi-component assemblies and dynamic macromolecular architectures. HT approaches to assess and fine-tune ligand impact on such functional, dynamic systems remain underdeveloped. Small-angle X-ray scattering (SAXS) provides an opportunity to monitor changes to biomolecular architectures and assemblies under native solution environments, enabling chemical screening and selection for specific architectural states. Here, we have incorporated HT-SAXS into a classic fragment screening pipeline and use this approach to identify chemical allosteric effectors targeting functional architectures of mitochondrial Apoptosis-Inducing Factor (AIF). AIF allosterically switches from monomer to dimer, following NADH-driven reduction of its FAD co-factor and formation of a $FADH^-/NAD^+$ charge-transfer complex within its active site. Dissociation of NAD^+ and subsequent FAD reoxidation returns AIF to a monomeric state. This dimer-to-monomer exchange is proposed to regulate AIF's functional transition from supporting mitochondrial import of respiratory complex factors to facilitating PARP-1-dependent cell death (parthanatos). X-ray scattering from AIF monomers and dimers is well distinguished, providing a robust model system for SAXS analysis.

Using a customized 2500-fragment library, we identified and verified initial fragment leads capable of binding AIF with differential scanning fluorimetry (DSF) and microscale thermopheresis (MST). Analysis of time-resolved HT-SAXS data collected on AIF/fragment samples triaged these leads into three chemotypes – fragments which maintain monomeric AIF or fragments which moderately or robustly induce AIF dimerization upon X-ray induced FAD photoreduction. AIF crystal structures of these representative chemotypes reveal how 'dimer-permissive' fragments configure AIF's active site to allosterically induce dimerization and provide critical SAR for affinity optimization. These results demonstrate how integrating HT-SAXS into fragment screening protocols can focus and customize ligand development toward relevant macromolecular architectures, assembly, and allostery.

This work was supported by the National Institutes of Health R35 CA220430 and P30 GM124169 and the Cancer Prevention and Research Institute of Texas RR140052.

Identification of miR-27a*-combinatorial Therapies that Suppress the Growth of Head and Neck Squamous Cell Carcinoma

Chari NS[1], Powell RT[2], Nguyen N[2], Sobieski M[2], Stephan C[2], Lai SY[1,3,4]*

1. Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
2. Center for Translational Cancer Research, Texas A & M University, Houston, TX, USA.
3. Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
4. Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
   *Corresponding Author: Stephen Y Lai MD, PhD, FACS, Department of Head and Neck Surgery, Unit 1445, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA. Email: sylai@mdanderson.org

Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Cisplatin has been the standard-of-care chemotherapy for treating advanced HNSCC, but can cause significant toxicities and has contributed to a mere ~50% overall five-year survival rate in advanced HNSCC. With almost 300,000 deaths worldwide, there is a clear ***unmet need*** to improve treatment options for patients with HNSCC. Overexpression of epidermal growth factor receptor (EGFR) in 40-70% of HNSCC is associated with decreased survival. However, current efforts targeting EGFR have resulted in marginal clinical benefits. This suggests that other pathways may lead to acquired or baseline resistance of HNSCC to EGFR-targeted agents. We had previously identified miR-27a* (miR-27a-5p) as an inhibitor of EGFR, AKT1, mTOR, and ΔNp63α, oncogenes that are dysregulated in HNSCC and contribute to tumor progression. miR-27a* expression is repressed in HNSCC tumors. Restoring expression of miR-27a* in HNSCC cells inhibits tumor growth in both *in vitro* and *in vivo* models. **The objective of this study** was to identify molecular compounds that augment the ability of miR-27a* to restrict HNSCC growth.

Methods: We used the HNSCC cell line, UM-SCC-22A engineered to conditionally express miR-27a* (22A-miR-27a*) in high throughput screens (HTS) with two well-annotated libraries, an NCI oncology set of approved compounds and, a custom clinical library consisting of compounds in clinical trials or approved by the FDA. Following the unbiased HTS studies, a Bliss model of synergy was used to identify compounds that showed synergistic/additive effects with miR-27a*. These compounds were further validated by examining the effects on cell growth using live-cell imaging.

Results: Our unbiased HTS identified several compounds that augmented the ability of miR-27a* to restrict cell growth. Inhibitors of HDAC and mTOR in combination with miR-27a* induction in the 22A-miR-27a* cells strongly repressed cell growth. Live-cell imaging of selected compounds confirmed these findings.

Conclusion: Our results have identified several potential miR-27a*-combinatorial compounds. Specifically, we found two potential classes of compounds, inhibitors of histone deacetylase and mTOR that augment the ability of miR-27a* to restrict HNSCC growth. Further HTS with libraries comprising a focused collection of agents targeting components of the above identified networks are ongoing.

Funding source: Brandon C. Gromada Head and Neck Cancer Foundation.

The Role of Bacteria Beta Glucuronidase Activity in Irinotecan-Induced Diarrhea

Chemotherapy-induced diarrhea is a common side effect but is an understudied area in cancer management. This problem is significant with irinotecan (CPT-11), a prodrug of SN-38 used in treating metastatic colon cancer as well as lung and pancreatic cancers. It is reported that more than 80% of patients treated with irinotecan experienced diarrhea, with up to 40% experiencing severe (grade 3 and 4) diarrhea. Different anti-diarrhea medications (such as loperamide, octreotide) have been recommended, but diarrhea is still a major concern as many patients do not respond well to these treatments. Metabolism of irinotecan shows that after being administered through intravenous infusion, irinotecan is activated to SN-38 by carboxylesterase and then detoxified to SN-38 glucuronide (SN-38G) by UDP-glucuronosyltransferase in the liver. Irinotecan and its metabolites are secreted into the intestine through biliary excretion, where SN-38G can be hydrolyzed back to SN-38 by *beta*-glucuronidase (*β*-GUS) produced by the intestinal bacteria. Accumulation of SN-38 in the intestinal tract then causes intestinal mucosal injury, resulting in delayed-onset diarrhea. Therefore, the purpose of this study is to determine the role of intestinal bacterial *β*-GUS in irinotecan-induced diarrhea.

At the end of this study we were able to show that SN-38G hydrolysis by bacterial *β*-GUS can be altered and prove that inhibition of the activity of bacterial *β*-GUS using an herbal formula Xiao-Chai-Hu-Tang (XCHT) attenuated irinotecan-induced diarrhea. The toxicity results showed that rats given XCHT treatment showed only grade 1 diarrhea for up to 9 days after CPT-11 injection and rats without XCHT treatment showed severe diarrhea (grade 3 and 4) by day 5 after CPT-11 injection. XCHT attenuated the diarrhea by reducing the amount of GI microflora available to deconjugate SN-38G to SN-38.

Therefore, bacterial *β*-GUS enzyme is the major culprit in the development of irinotecan-induced diarrhea and its manipulation can result in the alleviation of irinotecan-induced diarrhea. This knowledge can be of help in the management of chemotherapy induced diarrhea by alleviating this dose limiting toxicity.

Key words: Diarrhea, Irinotecan, beta-glucuronidase, XCHT

Controlled Release of Osteogenic Factors from a Novel Spinal Fusion Implant

Carcamo-Bahena Y[1], Cabrera FJ[2], Smith ZW[1], Wang DK[1], di Trani N[1], Terracciano R[1], Weiner BK[3], Grattoni A[1,4,5], Filgueira CS[1,6*]

1. Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA
2. Department of Regenerative and Biomimetic Medicine, Houston Methodist Research Institute, Houston, Texas
3. Department of Orthopedic Surgery, Houston Methodist Hospital, Houston, Texas
4. Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX, USA
5. Department of Surgery, Houston Methodist Research Institute, Houston, TX, USA
6. Department of Cardiovascular Surgery, Houston Methodist Research Institute, Houston, TX, USA

*Corresponding author: Dr. Carly S. Filgueira, Department of Nanomedicine and Department of Cardiovascular Surgery, Houston Methodist Research Institute, 6670 Bertner Avenue, Houston, TX 77030, USA, Email: csfilgueira@houstonmethodist.org

Objectives: Spinal fusion is a common surgical procedure used to treat fractures and to heal or "fuse" multiple bone fragments together to create one solid bone. We present a novel, nanotechnology-based spinal implant capable of sustained and constant release of osteogenic growth peptide (OGP). OGP is already found in the human body circulating in the blood, and it is known to play an important role in bone growth and the production of blood cells and platelets. When administered to animals, systemic treatment with OGP (a small, 14 amino acid peptide) has been shown to promote new bone formation. Unfortunately, however, this requires extensive, frequent dosing and can result in off-target effects commonly associated with signaling pathway crosstalk as a result of poorly controlled drug delivery. Our objective is to minimizes unwanted systemic side-effects by providing local, sustained release near the defect site (spine).

Methods: To determine the appropriate nanochannel size for sustained release, in vitro release studies of OGP from different nanochannel sized membranes (3.5 nm, 5 nm, and 20 nm) were first conducted by loading custom release cuvettes with OGP into a top reservoir separated by a nanochannel membrane, such that the peptide was released into a sink reservoir below. The absorbance of the sink solution was measured daily for over two months to determine the nanochannel size most appropriate for in vivo studies. We evaluate our nanofluidic device in skeletally mature New Zealand White rabbits through use of imaging modalities (x-ray, DynaCT) and histology. The implants consist of medical grade polyether ether ketone (PEEK) devices housing two silicon nanofluidic membranes for the sustained and constant release of OGP. We tested their tolerability and efficacy to stimulate local bone growth in New Zealand White Rabbits. The devices, which can be used to treat osteopenia, can be implanted without external fixation. Use of PEEK ensures some flexibility and radiolucency.

Results: In vitro release studies were performed with the microfabricated silicon nanochannel membranes to determine the optimal nanochannel size (3.5 nm) to achieve sustained, constant release. OGP release was relatively linear over the two-month period, and at day 60, cumulative release profiles targeted ~30 ug of peptide. Vehicle releasing (PBS:PBS), mixed (PBS:OGP), or treatment releasing (OGP:OGP) devices were implanted on both sides of the spine in rabbits (n=12) for two months to assess the promotion new bone formation in vivo. Signs of osteogenesis were evaluated via X-ray, cone beam CT, and histology.

Conclusions: Statistics indicate the continuing need for research in the healing and regeneration of bone. Whole body therapies are expensive, time-consuming, and evoke substantial side effects because they do not target a specific defect site. Local drug delivery creates availability of the drug specifically where needed and therapeutic efficacy. We believe OGP can be used therapeutically through local, sustained delivery from our implant positioned near a defect site for bone tissue engineering application. Overall, we demonstrate that our nanofluidic platform for constant OGP delivery can result in local ossification near

the device delivery site. The outcome this research will lead to accelerating fracture healing and bone defect repair.

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Enhancing Intracellular Accumulation and Target Engagement of PROTACs with Reversible Covalent Chemistry

Guo W-H[1,†], Qi X[1,†], Yu X[1], Yang Liu Y[2], Chung C-I[3], Bai F[4], Lin X [5], Lu D[1], Wang L[1], Chen J[1], Su LH[1], Nomie KJ[2], Li F[6,7], Wang MC[8,9,10], Shu X[3], Onuchic JN,[4] Woyach JA[11], Wang ML[2], Wang J[1,7,12*]

1. Department of Pharmacology and Chemical Biology, Baylor College of Medicine, Houston TX 77030, USA.
2. Department of Lymphoma/Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA.
3. Department of Pharmaceutical Chemistry, University of California−San Francisco, San Francisco, CA 94158, USA.
4. Center for Theoretical Biological Physics, Rice University, Houston, TX 77005, USA.
5. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
6. Department of Pathology and Immunology, Baylor College of Medicine, Houston TX 77030, USA.
7. Center for Drug Discovery, Baylor College of Medicine, Houston TX 77030, USA.
8. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston TX 77030, USA.
9. Huffington Center on Aging, Baylor College of Medicine, Houston, TX 77030, USA.
10. Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA.
11. Department of Internal Medicine, Division of Hematology, The Ohio State University, Columbus, OH 43210.
12. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston TX 77030, USA.

† Contributed equally

* Corresponding author; E-mail: wangj@bcm.edu

Current efforts in the proteolysis targeting chimera (PROTAC) field mostly focus on choosing the appropriate E3 ligase for a certain targeted protein, improving the binding affinities towards the target protein and the E3 ligase, and optimizing the PROTAC linker. However, it is well known that due to the large sizes of PROTAC molecules, their cellular uptake level remains an issue, posing a challenge to translate PROTACs into therapeutics. Driven by our fundamental investigation to compare how different warhead chemistry, reversible noncovalent (RNC), reversible covalent (RC), and irreversible covalent (IRC) binders, may affect the degradation of a model protein Bruton's Tyrosine Kinase (BTK), we serendipitously discovered that cyano-acrylamide-based reversible covalent chemistry can significantly enhance the intracellular concentration and target engagement of the PROTAC. Building on this discovery, we developed RC-1 as the first reversible covalent BTK PROTAC, which has high target occupancy and is effective as both an inhibitor and a degrader. Molecular dynamics calculations and phase-separation based ternary complex assays support that RC-1 forms a stable ternary complex with BTK and Cereblon (CRBN). Additionally, RC-1 compares favorably with other reported BTK degraders in cell viability and target engagement assays and has a reasonable plasma half-life for *in vivo* applications. Importantly, this reversible covalent strategy can be generalized and applied to improve other PROTACs. This work can not only help to develop optimal BTK degraders for clinical applications but also provide a new strategy to improve PROTAC efficacy.

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Using a High-Throughput Approach to Identify Effective Agents for the Treatment of Anaplastic Thyroid Carcinoma

Henderson YC[1], Mohamed ASR[1,2,3], Maniakas A[1,4], Chen Y[1], Powell RT[5], Peng S[6], Williams MD[7], Zafereo ME[1], Cabanillas ME[8], Hofmann M-C[8], Wang RJ[1], Johnson FM[3,6], Stephan CC[5], Sandulache V[9], Lai SY[1,2]
1. Department of Head and Neck surgery, The University of Texas MD Anderson Cancer Center
2. Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center
3. MD Anderson Cancer Center UT Health Graduate School of Biomedical Sciences
4. Division of Oto-rhino-laryngology-Head and Neck Surgery, Hôpital Maisonneuve-Rosemont, Université de Montréal
5. IBT High Throughput Screening Core, Texas A&M Health Science Center
6. Department of Thoracic, Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center
7. Department of Pathology, The University of Texas MD Anderson Cancer Center
8. Depart of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center
9. Department of Otolaryngology - Head and Neck Surgery, Baylor College of Medicine
Corresponding author: Dr. Stephen Lai, Department of Head and Neck surgery, The University of Texas MD Anderson Cancer Center, Houston, 1515 Holcombe Blvd, Unit 1445, Houston, TX 77030, sylai@mdanderson.org

Background: Thyroid cancer is the most common endocrine malignancy and its incidence continues to rise in both men and women. Estimated new thyroid cancer cases in women is 32,130 in 2021 and 7th on the list of all cancer cases in women. Papillary (PTC) and follicular thyroid carcinomas are well-differentiated tumors and represent the most common thyroid cancer subtypes with good overall prognosis and response to treatment. However, a subset of these well-differentiated tumors progress to more aggressive poorly differentiated (PDTC) and anaplastic thyroid cancer (ATC). Despite the use of aggressive multimodality treatment, most anaplastic thyroid carcinoma (ATC) patients die within a year of diagnosis. Although the combination of BRAF and MEK inhibitors has recently been approved by FDA for use in *BRAF*-mutated ATC, they remain effective in a minority of patients who are likely to develop drug resistance. There remains a critical clinical need for effective systemic agents for ATC with a reasonable toxicity profile to allow for rapid translational development.
Methods & Methods: Twelve human thyroid cancer cell lines including 7 ATC, 1 PDTC, and 4 PTC with comprehensive genomic characterization were used in a high-throughput screening (HTS) of 257 compounds to select agents with maximal growth inhibition. Cell proliferation, colony formation, orthotopic thyroid models, and patient-derived xenograft models (PDX) were used to validate the selected agents. Combination analysis was performed.
Results: Seventeen compounds were effective and combination between different groups of drugs revealed more inhibitory options. Docetaxel and LBH-589 were selected for additional *in vitro* and *in vivo* analysis as they have been previously approved by the FDA for other cancers. Significant tumor growth inhibition (TGI) was detected in all tested models treated with LBH-589. Docetaxel demonstrated significant TGI only in the context of mutant *TP53*.
Conclusions: HTS identified classes of systemic agents which demonstrate preferential effectiveness against aggressive thyroid cancers, particularly those with mutant *TP53*. Combination analysis provided more options for drug treatments between different groups of potential drugs for future study. Preclinical validation in both orthotopic and PDX models, which are accurate *in vivo* models mimicking tumor microenvironment, may support initiation of early phase clinical trials in non-*BRAF* mutated or refractory to BRAF/MEK inhibition ATC.
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Utilizing Chemical and Functional Genomics to Interrogate Integrator Subunit 11: A Candidate Cancer CYCLOPS Gene

Huang K-L[1], Park YS[2], Powell RT[2], Thomas N[1], Elrod ND[1], Ping J[1], Albrecht TR[1], Stephan C[2], Guenther MG[3], Ward MC[1], Wagner EJ[1]

1.Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, TX, 77555, USA. 2.Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M College of Medicine, Houston, TX 77030, USA. 3. Syros Pharmaceuticals, Cambridge, MA 02140, USA. Corresponding author: EJ Wagner, ejwagner@utmb.edu and MC Ward, miward@utmb.edu

Loss of heterozygosity (LOH) of tumor suppressor genes (TSG) is a driving event that typically disrupts cell cycle control resulting in aggressive cell proliferation. In many cancers, TSG loss through chromosome deletion causes concomitant loss of nearby genes. Recent work investigating the importance of these adjacent genes has led to a subset of them to be classified as 'CYCLOPS' (Copy number alterations Yielding Cancer Liabilities Owing to Partial Loss) genes. CYCLOPS genes are thought to encode proteins important to maintain cell homeostasis, therefore tumor cells will heavily rely on the remaining copy of CYCLOPS genes to sustain dysregulated proliferation. As normal cells possess two copies of CYCLOPS genes, a unique dependency within tumors provides a potential vulnerability if inhibitors of CYCLOPs-encoded proteins can be identified. A well-studied tumor suppressor gene, CHD5, is lost in multiple cancer types through a heterogenous group of chromosome deletions of the 1p36 region collectively termed 'Δ1p36'. Surprisingly, the gene encoding Integrator subunit 11 (IntS11) is within 1p36. By analyzing an extensive collection of shRNA screens conducted in a large panel of cancer cell lines, we found a striking observation that the viability of cancer cells is much more sensitive to reduction in IntS11 expression if one copy of the IntS11 gene is lost. This specific dependency is a property of CYCLOPS genes. IntS11 encodes an RNA endonuclease that is the catalytic subunit of the Integrator Complex. Integrator is highly conserved in metazoans and regulates RNA polymerases II (RNAPII) function at thousands of genes. Our laboratory has recently defined gene targets of Integrator in Drosophila but human genes subject to Integrator regulation have not been well-defined. It is important to identify human promoters genome-wide that are regulated by Integrator to fully understand its role in maintaining cellular physiology. Three specific aims are generated based on these preliminary observations.

Specific Aim 1. Define gene targets of IntS11 in human cells using functional genomics. We will utilize RNA-seq to identify genes with differential expression after IntS11 depletion. We will also employ ChIP-seq using IntS11 antibodies to identify promoters occupied by the Integrator Complex. Utilizing functional genomics (Ward lab), I will analyze these datasets to define direct and functional targets of IntS11.

Specific Aim 2. Demonstrate that IntS11 is a CYCLOPS gene. we have identified GBM cell lines that either possess Δ1p36 (and are $IntS11^{+/-}$) or lack Δ1p36 (and are $IntS11^{+/+}$). We will utilize these two cell lines to compare the impact of partial depletion of IntS11 using shRNA on the growth kinetics, viability, and tumorigenicity in vitro (Wagner lab).

Specific Aim 3. Identify chemical inhibitors of IntS11 using chemical genomics. We will leverage our fluorescence-based reporter cell line that reflects cellular IntS11 function to screen for chemical inhibitors of Integrator. We collaborated with the CPRIT Chemical Genomics Core at TAMU to screen of ~2000 compounds identified few compounds that affect IntS11 function. We will treat cells with these compounds, conduct RNA-seq, and utilize functional genomics to determine if our putative IntS11 inhibitor compounds generate transcriptional changes similar to IntS11 depletion (Ward lab). The compounds found to inhibit IntS11 will be further tested to determine whether they are specifically toxic to Δ1p36 GBM cell lines.

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Drugging the 'Undruggable' Steroid Receptor Coactivators

Lu D[1], Chen J[1], Javed S[1], Qi X[a], Bijou I[1], Qin L[2], Yu Y[2], Jain P[2], Lonard DM[2], O'Malley BW[2], Wang J[1].
1. Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030
2. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030
Corresponding author: Jin Wang, Department of Pharmacology, Baylor College of Medicine, Houston, TX,
E-mail:wangj@bcm.edu

We developed a small molecule inhibitor of steroid receptor coactivator-3 (SRC-3) as a novel targeted cancer therapy. The Holy Grail of drug discovery is to render small molecules the power of biologics to regulate protein-protein interactions. SRC-3 is a large, non-structured nuclear protein which regulates many signaling pathways that are essential for tumor formation. Through high throughput screening and medicinal chemistry optimization, we identified SI-2 that can direct interact with SRC-3 and selectively reduce its transcriptional activities and protein concentrations, leading to selective induction of breast cancer cell death with a low nM IC_{50} value, and inhibition of primary tumor growth in a breast cancer mouse model. This work will not only potentially improve breast cancer treatment through the development of a 'first-in-class' drug that targets oncogenic coactivators, but also encourage other researchers to develop strategies to target protein-protein interactions that are designated as 'important but undruggable' targets in the future.

SRC-3 is an oncogenic coactivator of nuclear hormone receptors and other transcription factors. SRC-3 was found to be amplified and overexpressed in various cancer. SRC-3 has been linked to various aspects of cancer formation and progression. Therefore, targeting SRC-3 with specific inhibitors holds future promise for cancer therapy.
We developed a cell-based functional assay for high throughput screening. Using the high throughput screening for the compound library, we got some hit compounds. Then based on the further validation assay, PK study and medicinal chemistry optimization, we got the lead compound SI-2, which has more potent activity and better PK study. At last, we chose it for in vivo study.

The structure of SI-2 is shown here. As shown in the table, SI-2 is a drug-like molecule. Its physical property meets all of the criteria for Lipinski rules, Veber rules and Oprea rules.
Then we measured the PK profiles of SI-2. Overall, SI-2 has an acceptable PK properties. With oral administration, we could still detect SI-2 at a 24-h time point.

To study the mechanism of action of SI-2, we performed a series of validation assay. Firstly, we test the effects of SI-2 on the SRC-3 transcriptional activity with the luciferase reporter assay. In this assay, as shown in this figure, SI-2 significantly reduced the luciferase reporter activity of pBIND-SRC-3 fusion proteins, but not affect the activity of pBIND protein. This result suggested SI-2 could selectively inhibit the transcriptional activity of SRC-3. Western blotting results showed that SI-2 decreases SRC-3 protein level in a dose dependent manner. But it did not affect the CARM-1 level, which is a transcriptional factor that binds to SRC3. Moreover, SI-2 can also significantly inhibit SRC-3 protein levels in other breast cancer cell lines. Then we used a fluorescence assay to test if SI-2 could directly physical interact with SRC-3. In this assay, if compound could bind to SRC-3 protein, the fluorescence of protein would be quenched. And the result showed it was.
We then test the cytotoxicity of SI-2 on cells. The results showed that SI-2 could inhibit cancer cell with IC50 value of 3.4 nM. In contrary, we did not observe any toxicity in liver cells. Similar activities were also observed in many other cell lines, including MCF-7, BT-474.

Some research has uncovered that down-regulation of SRC-3 could decrease cell motility and metastasis, therefore, we also test the effect of SI-2 on cancel cell motility. We found that SI-2 could significantly reduce the motility of cancer cells. And at this test concentration, SI-2 is not toxic for cell lines, indicating the decrease of cell motility is not because of the decrease of cell numbers.

Safety assessment is very important in drug development. Among them, cardiotoxicity is number one concerned. Therefore, the assessment of hERG inhibition is necessary in the drug development. Fortunately, the test result showed that SI-2 does not affect hERG activity, indicating no cardiotoxicity. Moreover, we also test the in vivo toxicity. We treated mice with 2 mg/kg, twice per day, after 5 weeks, the body weights of mice did not change. No obvious damages were observed in the major organs, including hear, liver, spleen, kidney, lung and stomach.

At last, we test the anti-tumor efficacy of SI-2 in vivo. We could see that SI-2 treatment could significantly inhibit tumor growth. Moreover, immunohistochemistry experiment showed the SRC-3 levels in SI-2 treated tumor tissues were significantly lower than the PBS control group. K67 positive is a cell proliferation marker, was also dramatically decreased in SI-2 treated tumor tissues. Based on these data, we could conclude that SI-2 can significantly inhibit tumor growth through inhibiting SRC-3 in vivo.

In conclusion, we discovered that SI-2 specifically and significantly down-regulates SRC-3 expression levels. It exhibited potent anti-tumor activity in vitro and in vivo. Moreover, SI-2 has a good safety profile and does not induce acute and chronic toxicity. Therefore, we believe that SI-2 is worth for further development to provide a “first-in-class” drug that targets oncogenic coactivators and significantly improve breast cancer treatment.

Harnessing Mechanistic Insights to Target the Oncogenic Functions of hnRNP K

Malaney P[1], Chan LE[1], Link T[2], Cho EJ[3], Cerda J[1], Aitken MJL[1], Zhang X[1], Sobieski M[4], Powell R[4], Nguyen N[4], Stephan C[4], Dalby K[3], Post SM[1]

1. Department of Leukemia, MD Anderson Cancer Center, Houston, TX
2. Department of Molecular and Cellular Oncology, MD Anderson Cancer Center, Houston, TX
3. Targeted Therapeutic Drug Discovery and Development Program, College of Pharmacy, University of Texas at Austin, TX
4. Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University, Houston, TX

Corresponding Author: Sean M. Post, Department of Leukemia, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX. Email: spost@mdanderson.org

Objectives: The identification of therapeutically actionable genetic lesions in hematological malignancies has transformed patient care. However, there remains a large number of individuals that either do not possess these targetable alterations or that relapse by various mechanisms following therapeutic intervention. Therefore, it is imperative to identify novel alterations that drive heme malignancies and lead to treatment resistance.

Methods: Patient samples and transgenic mouse models were used to assess the role of hnRNP K in oncogenesis. RNA-sequencing, RNA immunoprecipitation sequencing (RIP-Seq), mass spectrometry and polysome profiling assays were performed to understand the mechanistic basis for the oncogenicity of hnRNP K. A fluorescence anisotropy assay was developed to determine hnRNP K/RNA binding in vitro. The assay was then used to screen 80,000 small molecule compounds to identify 50 candidates that disrupt hnRNP K/RNA binding. Thermofluor assays, surface plasmon resonance and cell-proliferation assays were used to narrow down to 9 lead compounds.

Results: We identified that the RNA binding protein, hnRNP K, is a novel driver of hematologic malignancies. hnRNP K overexpression contributed to dismal clinical outcomes in patients with diffuse large B-cell lymphoma and B-cell specific overexpression of hnRNP K in mice resulted in a highly penetrant lymphoma phenotype. Mechanistic studies revealed that the oncogenicity of hnRNP K stems, in part, from its ability to post-transcriptionally and translationally regulate the levels of the Myc oncogene. The elevated c-Myc levels consequently rendered hnRNP K overexpressing tumors sensitive to BRD4 inhibition, demonstrating that pathways downstream of hnRNP K are amenable to therapeutic intervention. Mechanistic studies revealed that hnRNP K has critical oncogenic functions outside of the MYC pathway. Consequently, potential therapies that effectively target hnRNP K overexpression will require a direct inhibitor of its RNA binding function. Using our fluorescence anisotropy assay, we screened 80,000 small molecule compounds and identified 50 candidate compounds that disrupted hnRNP K/RNA binding at concentrations from 0.1-40 μM. We then performed cell based assays, thermal shift assays and SPR studies to identify 9 lead compounds that directly bind to hnRNP K and cause cell death in an hnRNP K-dependent manner. Further characterization and structural refinement of these lead compounds is ongoing.

Conclusions: We establish that hnRNP K is a bonafide oncogene that drives lymphomagenesis via its RNA-binding activities. Our data demonstrates that hnRNP K is a valid candidate for therapeutic intervention and that select "lead compounds" disrupt hnRNP K's ability to interact with its target oncogenic transcripts.

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Chemical Genetic Exploration of Clostridium difficile Toxin Metabolism, Toward Defining Anti-virulent Drug Targets

Marreddy RK[1], Olaitan AO[1], Sobieski M[2], Bowling J[3], Stephen C[2], Lee RE[3] & Hurdle JG[1]

[1]Centre for Inflammatory and Infectious Diseases, Institute for Bioscience and Technology, Texas A&M, Houston, TX, USA
[2]Centre for Translational Cancer Research, Institute for Bioscience and Technology, Texas A&M, Houston, TX, USA
[3]Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA

Corresponding author: Julian G. Hurdle, Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M Health Science Center, 2121 West Holcombe Blvd., Houston, Texas 77030, USA, E-mail: jhurdle@tamu.edu

Abstract:
Clostridioides difficile infection (CDI) is the leading cause of hospital-acquired diarrhea, resulting from antibiotic-induced dysbiosis. CDI pathogenesis relies on the biosynthesis of the toxins TcdA and TcdB. While vancomycin is the main recommended treatment, it is not narrow-spectrum and further disrupts the microbiota during therapy. This is thought to contribute to recurrent disease in >20% of patients. Herein, we addressed the urgent need for narrow-spectrum anti-virulent inhibitors that reduce onset of recurrent CDI, by blocking toxin biosynthesis.

Screening of a rationally curated phytochemical library identified a molecule (**TSI-1**) that inhibited toxin production with inhibitory concentration (IC_{50}) of ~16 μM. Interestingly, **TSI-1** did not inhibit the growth of other gut bacterial species (MIC >100 μM), suggesting it was narrow-spectrum. To understand the mode of action of **TSI-1**, we performed click-chemistry proteomics, targeted metabolomics and chemical mutagenesis with ethyl methanesulfonate (EMS). Targeted proteomics identified a key enzyme in purine metabolism, as the molecular target. Consistent with this observation, metabolomics revealed **TSI-1** caused intracellular accumulation of adenosine, but depletion of ATP and GTP. Metabolic bypass experiments, in cells exposed to **TSI-1**, showed toxin production was reinstated by supplementation with purines.**TSI-1** target interaction was confirmed biophysically by Isothermal Titration Calorimetry (ITC) and in biochemical enzyme assays. Genome analysis of five EMS mutants that were refractory to **TSI-1** revealed mutation of CodY, a global transcriptional regulator. Toxin biosynthesis in a CodY-deletion mutant was not inhibited by **TSI-1**, indicating the molecule acted through CodY activation. The discovery of **TSI-1** and accompanying mechanistic studies reveal new pathways that regulate toxin biosynthesis in *C. difficile*, which can be exploited for narrow-spectrum inhibitors.

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Discovery and Optimization of Novel GPR52 Agonists That Reduce Psychostimulant Behavior

Murphy R[1,2], Felsing DE[1,2], Wang P[2], Zhou J[2], Allen JA[1,2]

Center for Addiction Research[1], Department of Pharmacology and Toxicology[2], University of Texas Medical Branch, Galveston, TX

Corresponding author: John A. Allen, Ph.D., Department of Pharmacology and Toxicology, 301 University Blvd., University of Texas Medical Branch, Galveston, TX; joaallen@utmb.edu

GPR52 is a class-A orphan G protein-coupled receptor that is selectively expressed in the striatum, and regulates various brain functions through activation of Gs/adenylyl cyclase/cAMP signaling pathways. GPR52 has been identified as a promising drug target for neurological and psychiatric disorders including schizophrenia, substance use disorders, as well as Huntington's disease. We recently synthesized and pharmacologically evaluated a series of novel GPR52 indoline-carboxamide based agonists (Wang, Felsing et al J. Med. Chem. 2020 Nov 25;63(22):13951-13972). Several potent (EC50: ~100 nM) and efficacious GPR52 agonists were identified, and *in vivo* proof-of-concept investigations revealed that 3 mg/kg of the lead compound PW0787 (**12c)** displayed antipsychotic-like activity by significantly inhibiting amphetamine-induced hyperlocomotor behavior in mice. Here, we further report our efforts to optimize our molecules for addiction-focused drug discovery in a systematic structure-activity relationship study of pharmacophore features crucial for GPR52 activation. Notably, in a HEK293 cell-based GPR52 cAMP assay, we determined the two lower aromatic moieties of the agonist lead were amenable to further medicinal chemistry with substituents shown to modulate both agonist potency and efficacy. Whereas compound modifications to the carboxamide and heterocyclic linkers were largely detrimental to agonist potency. Surprisingly, when the nitrogen containing ring of the indoline system was broken into more flexible variants, this further increased agonist potency (EC50: ~40 nM) and efficacy, while retaining target selectivity, plasma exposure and serum concentration. Collectively, our findings have resulted in several agonists with optimized potency and efficacy, with PW0787 being an orally bioavailable, brain penetrant GPR52 agonist. This work also provides valuable pharmacological tools for investigating the physiological and therapeutic potential of GPR52 activation.

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Determination of OJT008 as a Potential lead Inhibitor of Mycobacterium tuberculosis

Onyenaka C[1], Kaur M[1], John SF[2], Liang D[1], Olaleye OA

1. College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX.
2. University of Saint Thomas, Houston. Texas

Corresponding author: Omonike A. Olaleye, College of Pharmacy and Health Sciences, Texas Southern University, 3100 Cleburne street, Houston, Texas, Omonike.olaleye@tsu.edu

Objective: According to the 2019 World Health Organization (WHO) report, *Mycobacterium tuberculosis* (*Mtb*), causative organism for Tuberculosis (TB), is classified as one of the top ten causes of death globally and ranks above HIV/AIDS as one of the leading causes of infectious diseases. The emergence of extensively, multi-, and total drug resistant strains of *Mtb* has made TB therapeutic management a challenge. As a result, there is an urgent need to discover novel antimycobacterials to combat the emergence of *Mtb* drug resistant strains. Our studies have focused on overexpression, purification, and characterization of methionine aminopeptidase (MetAP) from *Mtb*. MetAP is a universally conserved metalloprotease required for the catalysis of N-terminal methionine excision (NME), an essential biological process required for post- translational modification, translocation, protein stability and biologic maturity. We hypothesized that inhibition of MetAP activity will lead to the inhibition of growth and survival of Mtb. In this study, we successfully induced, overexpressed, and purified recombinant N-terminal poly-histidine tagged MetAP1c protein from *Mtb*. We identified several inhibitors of MetAP1c using the colorimetric assay and characterized OJT008, one of the lead compounds.

Methods: MtMetAP1c was cloned using pET28a vector. The recombinant plasmid was used to transform *E. coli* cells BL2. The target protein, MtMEtAP1c in the cells were over-expressed by adding 1mM final concentration of Isopropyl β-D-1-thiogalactopyranoside (IPTG) and further incubated at 2,4, and 8 hrs. The cells were harvested and sonicated. Using the Immobilized Metal Affinity Chromatography technique, we purified MtMetAP1c. The eluted protein was quantified using the Bradford assay. We determined the in vitro enzyme activity of MtMetAP1c by colorimetric assay using a chromogenic substrate. OJT008 was dissolved in Dimethyl sulfoxide (DMSO) and screened against the purified MetAP1c at a concentration range of 100μM to 300nM. The half maximal inhibitory concentration (IC_{50}) of the pharmacophore was determined with a colorimetric assay.

Results: The induction of the expressed recombinant MtMetAP1c was optimized at 8 hours with a final concentration of 1mM IPTG, this was confirmed using gel electrophoresis.
The average yield for MetAP1c was 3.63mg/L of *E. coli* culture. The IC_{50} and minimum inhibitory concentration value of OJT008 against MetAP1c was in the micromolar range and < 5μg/mL, respectively.

Conclusion: The discovery of a novel Pharmacophore targeting *Mtb* could facilitate the discovery of new therapeutics for drug resistant and drug sensitive and TB infection.

Novel PINK-1 Stabilizing Compounds Identified in C. elegans Model Selectively Eradicate Human Leukemic Cells

Pei J[1], Panina SB[1], Baran N[2], Konopleva M[2], Kirienko NV[1*]

1. Department of BioSciences, Rice University, Houston, TX
2. Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX

*** Correspondence:** Natalia V. Kirienko, Department of BioSciences, Rice University, Houston, TX, kirienko@rice.edu

Background: Acute myeloid leukemia (AML) is a classification for a heterogeneous group of aggressive hematological malignancies that are characterized by the proliferation of undifferentiated or partially differentiated myelogenous blast cells. From the 1970s to 2000, the treatment of acute myeloid leukemia (AML) remained essentially stagnant. In addition, the survival of AML patients is still poor, due to frequent relapses in AML caused by chemotherapy resistance. Novel and more effective treatments are clearly necessary. Mitophagy is a conserved metabolic process that plays a central role in eliminating dysfunctional or damaged mitochondria, and is crucial for cellular responses to physiological stresses and quality control. The PINK1 (serine/threonine PTEN-induced putative kinase 1)/Parkin pathway is the main pathway of mitophagy. Briefly, mitochondrial depolarization stabilizes PINK1 at the outer mitochondrial membrane with subsequent isolation of damaged mitochondria and degradation by autophagic machinery. There is an emerging interest in manipulating mitophagy to improve cancer therapy.

Results: In this study, we identified 6 PINK-1 stabilizing compounds (PS compounds) that exhibit cytotoxicity against AML cells *in vitro* from results of our primary screen in *C. elegans* model. Approximately 50 analogs were tested in a small-scale SAR study. 6 top hits were selected based on following criteria: LD50 in healthy PBMCs (peripheral blood mononuclear cells) is ≥10 LD50 in AML cells. 5/6 top PS leads demonstrated selective killing in multiple AML cell lines with a divers genetic background, and ALL (acute lymphoblastic leukemia) and CML (chronic myelogeneous leukemia) cell lines. Mechanistically, all hit compounds reduced ATP level and selectively impaired basal and ATP-linked mitochondrial respiration in leukemic cells. Compounds of PS127 family significantly up-regulated ROS generation in AML cells, activated Nrf1 expression, and induced ferroptotic/necroptotic cell death. PS30B compound moderately reduced mitochondrial mass and membrane potential in AML cells, but not in healthy blood cells. Lastly, PS molecules were effective against primary AML cells *in vitro* (LD50 < 20 μM) and exhibited synergetic effects when combined with chemotherapeutics of diverse mechanism of action (IACS-010759, doxorubicin, 6-mercaptopurine). Taken together, mitophagy activation is of great potential for future leukemia treatment development.

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High-throughput Drug Screening Identifies Specific Drug Classes That Enhance Cell Death in NOTCH1-mutant HNSCC

Shah PA[1], Mazumdar T[1], Powell RT[2], Shen L[3], Wang J[3], Stephen CC[2], Frederick MJ[4], Johnson FM[1,5]

1. Department of Thoracic/Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX
2. Center for Translational Cancer Research, Institute of Biosciences and Technology Texas A&M College of Medicine, Houston, TX
3. Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX.
4. Bobby R. Alford Department of Otolaryngology, Baylor College of Medicine, Houston, TX
5. The University of Texas Graduate School of Biomedical Sciences, Houston, TX.

Corresponding author: Dr. Faye Johnson, Department of Thoracic, Head and Neck Medical Oncology, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX, E-mail: fmjohns@mdanderson.org

Head and neck squamous carcinoma (HNSCC) is common and lethal. It is driven predominantly by mutations in tumor suppressor genes making it challenging to devise biomarker-based targeted therapy. In order to address this unmet clinical need, our group recently demonstrated the therapeutic vulnerability of *NOTCH1*-mutant HNSCC to phosphoinositide-3 kinase (PI3K) pathway inhibition, in part mediated by PDK1.

Modest clinical responses and acquired resistance are leading causes of failure for targeted therapies that are otherwise well tolerated. In order to address this challenge and identify drugs that could work in combination with PI3K inhibitors against *NOTCH1*-mutant HNSCC, we performed a high throughput screen of 5768 drugs (0-1μM) with diverse targets. We used the IN Cell Analyzer 6000 platform to determine actual cell numbers before and after drug treatment of *NOTCH1*-mutant HNSCC cells (HN31, UMSCC22A, PCI-15B). We selected potential candidates based on 2 efficacy metrics [area under the curve growth rate index (AUC_GRI) $\leq$ 0.9 and area over the curve lethal dose (AOC_LD) > 0] that were calculated using the normalized growth rate inhibition curve, in order to avoid the confounding effect of the rate of cell division. We further excluded chemotherapy, PI3K pathway inhibitors, and non-specific drugs. When several candidates targeted the same pathway, we chose the most specific drugs to use in the combination screen.

We then combined the resulting 74 drugs with PI3K inhibitors, bimiralisib (0-1μM) or copanlisib (FDA approved, 0-100nM), for 72 h. Synergistic effects from these combinations were assessed using Bliss, HAS, Zip, and Loewe models. PDK1 inhibitor SNS-510 (250nM) in combination with copanlisib (50nM) was synergistic and resulted in a 40% increase in apoptosis compared to single agents as seen by TUNEL assay. Likewise, low concentrations of inhibitors targeting EGFR/HER2 pathway (afatinib, sapitinib, poziotinib), MEK pathway (Trametinib), Src (Ecf506), HDAC (Quisinost, Panobinostat), and PLK1 (BI2536, volasertib) were both effective and additive to synergistic with PI3K inhibitors. Notably, the concentrations of these inhibitors are target-specific and clinically achievable. These combinations will be validated *in vitro*. The most promising combinations will be tested *in vivo*.

We have identified six major drug classes that not only maximize the killing of *NOTCH1*-mutant HNSCC, but may also prevent resistance at clinically relevant concentrations. The identified pathways may give us insight into mechanisms of resistance. If validated, these combinations may lead to the first biomarker-specific, targeted therapy for HNSCC.

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Automated Fluorescence Recovery After Photobleaching (FRAP) Analysis Pipeline and Its Application to Measure the Mobility and Residency Changes in Estrogen Receptor-a for Endocrine Disrupting Chemicals (EDCs)

Singh PK[1], Bolt MJ[1], Obkirchner CE[1], Powell RE[1], Mancini MG[2], Stossi F[1,2], Davies P[1], Mancini MA[1,2].

1. Center for Advance Microscopy and Image Informatics, Texas A&M Institute of Biosciences and Technology, Houston, TX 77030, USA
2. Departments of Molecular and Cellular Biology, and Pharmacology and Chemical Biology, Baylor College of Medicine, Houston, TX 77030, USA.

Corresponding author: Pankaj Singh, Center for Advance Microscopy and Image Informatics, Texas A&M Institute of Biosciences and Technology, 2121 W Holcombe Blvd, Houston, TX

Email: psingh@tamu.edu

Fluorescence recovery after photobleaching (FRAP) is a method to study molecule mobility in living cells or tissues. In FRAP, a specific area of a cell or tissue is photobleached by intense laser light, removing fluorescence from that area. Fluorescence in the bleached area will slowly recover as bleached fluorophores move out and healthy fluorophores from adjacent areas move in. We have developed an automated method to quantify mobility and recovery time. It corrects for non-FRAP induced photobleaching. To find the FRAPped regions, we take advantage of the fact that the last pre-bleached (PreB) image and the first post-bleached (PB) image would be the same except for the bleached regions (FRAPed regions). We use Gaussian filtering to blur the images and subtract the blurred PB image from the blurred PreB image. To obtain the FRAPed regions, we use thresholding and other post-processing steps on the subtracted image. We have applied this tool to measure the measure mobility and residency of ERα across a host of Endocrine disrupting chemicals (EDCs) set by the Environmental Protection Agency (EPA). From the data obtained using FRAP analysis, we demonstrate a new class of compounds, inverse agonists that cause higher levels of receptor immobilization without increasing the degradation of ERα.

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The Disposition and Bioavailability of Raloxifene in F344 Rat at Different Age

Du T[1#], Sun R[2#], Zheng Z[2], Etim I[1], Hu M[2]*, Gao S[1]*

1. Department of Pharmaceutical Science, Texas Southern University
2. Department of Pharmacological and Pharmaceutical Sciences, The University of Houston

* To whom correspondence should be addressed
Dr. Song Gao, Department of Pharmaceutical Science, Texas Southern University, 3100 Cleburne St, Houston, TX, E-mail: song.gao@tsu.edu
Dr. Ming Hu, Department of Pharmacological and Pharmaceutical Sciences, The University of Houston, 4901 Calhoun Street, Houston, Texas, Email: mhu@uh.edu
# These authors contributed equally

Abstract:
Raloxifene, a market drug used for the treatment of hormone related diseases in patients at different ages, undergoes extensive glucuronidation in the GI tract and the liver. However, age-dependent disposition and oral bioavailability of raloxifene were under-investigated. In this paper, raloxifene glucuronidation was characterized using S9 fractions prepared from different intestinal segments and the liver of F344 rats at 4, 11, and 28 weeks. PK studies were conducted to determine raloxifene oral bioavailability at different ages. The results showed the ratio of the two major metabolites raloxifene-6-glucuronide and raloxifene-4'-glucuronide were different at different ages in different regions ranging from 2.1 to 4.9 folds in ileum, jejunum, liver, and duodenum and from 14.5 to 50 folds in the colon. The clearances in duodenum at 4-week for both Ral-6-G and Ral-4'-G were significantly lower than those at the other two ages. Additionally, the total CLint was gradually decreased from duodenum to the colon at 11- and 28-week. PK studies showed that oral bioavailability of raloxifene is age dependent. The absolute oral bioavailability of total and free form or raloxifene were significantly lower at 4-week. For iv bolus, raloxifene half-life was longer at 11-week compared to that at 4-week. These findings suggested that raloxifene metabolism in the duodenum was significantly slower at young age in rats, which decreased oral bioavailability of raloxifene. At 11-week, enterohepatic recycling efficiency was higher than that of 4-week. Raloxifene dose at different age should be carefully considered in clinical trials.

Key Words: Raloxifene, glucuronidation, age, intestine, liver, PK

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High-Throughput and High-Content Screening for Mitophagy Activators Identified Therapeutic Compounds for Caenorhabditis elegans Neurodegenerative Models

Tjahjono E*[1], Pei J*[1], Revtovich AV[1], Liu T[1], Hancu M[1], Swadi A[1], Kirienko NV[1]

1. Department of BioSciences, Rice University

Corresponding author: Natalia V. Kirienko, Department of BioSciences, Rice University, 6100 Main Street MS-140, Houston, TX, E-mail: kirienko@rice.edu

As the 'powerhouses' of the cell, mitochondria play a significant role in keeping up with energy (ATP) demands for cellular metabolism. Other indispensable cellular functions, including calcium balance maintenance, metabolites production, and apoptosis, are also facilitated by mitochondria. Removal of dysfunctional mitochondria, known as mitophagy, is therefore essential for mitochondrial homeostasis and cell viability. Accumulation of defective mitochondria and impaired mitophagy have been widely implicated in many neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's disease (PD). Indeed, the mutations of *PINK1* (PTEN-Induced Kinase 1) and *parkin* (an E3 ubiquitin ligase), both are important regulators of mitophagy, are the most common cause of recessive PD. Activation of mitophagy via pharmacological treatments is thus a feasible approach for combating neurodegeneration.

C. elegans have been regularly employed to model and study neurodegenerative diseases. The presence of homologs, the conservation of molecular functions, and the availability of genetic and molecular tools for this model organism are tremendously valuable for aging research. Numerous transgenic *C. elegans* neurodegenerative models have also been constructed and characterized. In this effort, we screened ~45,000 small molecules for the ability to activate mitophagy. Screen was conducted by monitoring PINK1 stabilization, a key event in mitophagy activation, in a worm strain carrying a *Ppink-1*::PINK-1::GFP reporter. We obtained eight hits (termed 'PS' for PINK1-stabilizing compounds) upon elimination of toxic and autofluorescent compounds.

To understand the effects of the PS compounds on mitochondria and mitophagy induction, we utilized multiple *C. elegans* strains and fluorescent dyes to monitor various mitochondrial parameters, including mitochondrial network, ATP production, mitochondrial mass, and reactive oxygen species formation. Notably, treatment with several compounds (PS30, PS34, PS127, and PS143) showed strong mitophagy induction, indicated by increased mitochondrial fragmentation and lysosome formation, with lower ATP production.

Finally, we tested whether PS compounds supplementation provide beneficial effect by utilizing *C. elegans* neurodegenerative diseases models that express either a human beta-amyloid (A-beta-1-42) or polyglutamine (Q82::YFP). We found that treatment with two of the compounds, PS83 and PS106, reduced pathologies, i.e., delayed paralysis and reduced aggregates formation. This rescue was confirmed to be dependent on mitophagy, as animals with PINK-1 deficiency showed similar pathology as the worms treated with vehicle control. This work presents potential application in providing neuroprotection.

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The Paracrine Action of Mutant P53 In Breast Cancer

Williams-Villalobo A*; Zhang Y*#; Liu B#; Xiong SB#; Chau G#; Lozano G#

* Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Texas Southern University

Department of Genetics, The UT MD Anderson Cancer Center

Tumor suppressor *p53* mutations have been identified in breast cancer associated fibroblasts, which correlates with a poor prognosis in patients. However, the effects of fibroblastic mutant p53 on breast cancer remains unclear. Our goal is to investigate whether and how p53R17H mutant in stromal fibroblasts impacts breast cancer. We hypothesize that fibroblastic p53R172H accelerates mouse mammary tumor development via paracrine signaling(s) that alter biological pathways in the tumor. *A* cohort of *MMTV-HER2; Fsp-Cre; p53*$^{wm\text{-}R172H/+}$ (*HFP*) female mice was established, which develops human-epidermal-growth-factor-receptor 2 (HER2) positive mammary carcinomas and contains p53R172H specifically in the fibroblasts. A cohort of *MMTV-HER2; Fsp-Cre* (*HF*) females was also established, where *p53* remains intact in the stromal fibroblasts of HER2 tumors. Tumor development was monitored. RNAs from the *HFP* and *HF* mammary tumors as well as normal mammary glands were subject to sequencing. The raw RNA-sequencing readouts were mapped to the mouse mm10 assembly reference genome and analyzed with DESeq2. The pathway analysis was performed with Ingenuity Pathway Analysis. Secretome analysis was also performed using the Vertebrate Secretome Database. We found that the *HFP* female mice exhibited a significantly shorter median tumor free survival than that of the *HF* females. Extensive transcriptomic changes were observed between the *HFP* and *HF* tumors. Several biological pathways that are known to facilitate tumor development were enriched in the *HFP* tumors. A list of secretory factors, which either up- or down-regulated in the mammary glands containing mutant p53, has also been determined. The RNA-sequencing data and secretome analysis are under further investigation to reveal the mutant p53-driven paracrine signaling(s) that are potentially responsible for triggering the pro-tumor pathways. Our results show that fibroblastic mutant p53 promotes mammary tumor development, potentially through a paracrine mechanism that alters gene expression profiles in the tumor. This study will broaden our knowledge of the p53 functions in cancer and may lay a groundwork for further mechanistic and therapeutic studies in the future.

RIPK1 PROTAC Demonstrates Great Potentials in Overcoming the Resistance to Immune Checkpoint Blockade Therapy

Xin Yu X[1], Lu D[1]

1. Department of Pharmacology and Chemistry Biology, Baylor College of Medicine

Corresponding author: Jin Wang, Department of Pharmacology and Chemical Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, E-mail: wangj@bcm.edu

The emergence of immune checkpoint blockade (ICB), first demonstrated in melanoma, has revolutionized cancer treatments. However, despite the tremendous success of cancer immunotherapies, the majority of patients fail to respond to ICBs, highlighting the need for more effective and novel approaches. Receptor interacting protein kinase 1 (RIPK1) has emerged as the core component of the inflammatory response activated downstream of several immune receptors, where it paradoxically functions as a scaffold to protect the cell from death or instead as an active kinase to promote the killing of the cell. Genetic deletion of RIPK1 can sensitize tumors to immunotherapy and strategies to alter the stability of RIPK1 in tumors provide therapeutic avenues to enhance tumor immunity. Therefore, we hypothesize that pharmacologically degradation of RIPK1 by Proteolysis Targeting Chimera (PROTAC) can synergize with ICB to boost immunotherapy. We have developed a potent RIPK1 PROTAC LD4172 and demonstrated its pharmacological effects both *in vitro* and *in vivo*. LD4172 potently degrades RIPK1 in a panel of human cancer cell lines, including melanoma, hematological and breast cancer, with DC_{50} (concentration of PROTAC required to achieve 50% degradation of target protein) in the range of 5-40 nM. Different from RIPK1 kinase inhibitor, in mouse melanoma B16F10 cells, LD4172 can potentiate TNFα-initiated apoptosis by significantly inhibiting NF-κB signaling activities, which cannot be observed in cells treated with TNFα plus RIPK1 kinase inhibitor. Furthermore, we established a syngeneic B16F10 mouse model to test whether LD4172 can combine with ICB to overcome the tumor immune resistance. We found that consistent with published RIPK1 knockout data, our RIPK1 PROTAC can degrade RIPK1 in tumors, turning the “cold” B16F10 melanoma tumor into “hot” tumor, with enhanced responses to ICB. Taken together, in addition to its well-known kinase activity, the kinase-independent function of RIPK1 makes it an oncogenic driver in various cancers, suggesting the requirement of novel therapies that can abolish its kinase-irrelevant activity, which is perfectly satisfied by our RIPK1 PROTAC.

High-throughput Morphological Screening of Mesenchymal Mammary Tumor Organoids to Identify Drugs that Reverse Epithelial-Mesenchymal Transition

Zhao N[1#], Powell RT[2#], Yuan X[1], Bae G[2], Roarty KP[1], Stossi F[1,3], Strempfl M[4], Johnson HL[3], Mani SA [5], Jones P[6], Stephan CC[2], Rosen JM[1]

1. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA.
2. Center for Translational Cancer Research, Texas A&M Health Science Center, Institute of Biosciences and Technology, Houston, Texas, USA.
3. Integrated Microscopy Core, Baylor College of Medicine, Houston, Texas, USA.
4. Graz University of Technology, NAWI Graz, Graz, Styria, Austria.
5. Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
6. Institute of Applied Cancer Science (IACS), University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
[#] These authors contributed equally to this work.

Corresponding author: Jeffrey M. Rosen, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA. jrosen@bcm.edu

The epithelial-mesenchymal transition (EMT) has been implicated in conferring stem cell properties and therapeutic resistance to cancer cells. Therefore, identification of drugs that can reprogram EMT may provide new therapeutic strategies. Here, we found that cells derived from claudin-low mammary tumors, a mesenchymal subtype of triple-negative breast cancer, exhibit a distinctive organoid structure with extended "spikes" in 3D matrices. Upon a miR-200 induced mesenchymal-epithelial transition (MET), the organoids switch to a smoother round morphology. Based on these observations, we developed a high-throughput morphological screening method with accompanying analytical pipelines that leverage deep neural networks and nearest neighborhood embedding to screen for EMT-reversing drugs. Through screening of a targeted epigenetic drug library, we identified multiple class I HDAC inhibitors and Bromodomain inhibitors that reverse EMT. These data support the use of high-throughput morphological screening of mesenchymal mammary tumor organoids as a novel platform to identify drugs that reverse EMT.

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