4th Annual Gulf Coast Consortia Innovative Drug Discovery and Development Conference

Leveraging Big Data for Transformative Healthcare

May 9-10, 2023 **Houston, Texas**

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 research consortia gather interested faculty around research foci within the quantitative biomedical sciences and currently include Innovative Drug Discovery and Development, Antimicrobial Resistance, Cellular and Molecular Biophysics, Immunology, Mental Health Research, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, and Translational Pain Research. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Pharmacological Sciences, Precision Environmental Health Sciences, and Antimicrobial Resistance. 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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Gulf Coast Consortia - Research

Agenda

May 9, 2023

<u>Day 1</u> **Big Picture**

7:45-8:15 Registration and breakfast

8:15-8:20 **Welcome:** Suzanne Tomlinson, Gulf Coast Consortia

8:20-9:00 Keynote Presentation
The Study of Human Diversity: The Key to Understanding of Human Disease
Kári Stefánsson
deCODE Genetics, Iceland

9:00-9:40 Keynote Presentation
Generative AI for Drug Discovery and Development
James Zou
Stanford Univ.

Session 1: **Pharma and State of the Field**
Convenors: Jason Cross, MD Anderson Cancer Center
Phil Jones, MD Anderson Cancer Center

9:40-10:05 *The Reality of AI in Drug Discovery*
Bissan Al-Lazikani
MD Anderson Cancer Center

10:05-10:30 *In Vitro-In Vivo Correlations and Human PK/Dose Predictions: What are the Hurdles?*
Leslie Benet
Univ. of California San Francisco

10:30-10:55 *Can Humans Learn from Machine Learning in Drug Discovery?*
Tudor Oprea
Univ. of New Mexico School of Medicine

10:55-11:10 **Break**

Session 2: **Leveraging Preclinical Databases for Drug Discovery**
Convenors: Pete Davies and Cliff Stephan, Institute of Bioscience and Technology, Texas A&M Univ.

11:10-11:35 *Knowledge-primed AI to Power the Design of Rational Combination Therapies*
Maxwell Sherman
Serinus

11:35-12:00 *Pre-Clinical Oncology Studies using Patient-Derived Xenografts*
Janine Low-Marchelli
The Jackson Laboratory

Agenda

12:00-12:25 *Establishing Pre-clinical Databases for Drug Repositioning*
Reid Powell
Institute of Bioscience and Technology, Texas A&M Univ.

12:25-1:30 Lunch

Session 3: Diversity in Healthcare Datasets
Convenor: Suzanne Tomlinson, Gulf Coast Consortia

1:30-2:10 Keynote Presentation
The All of Us Research Program Researcher Workbench
Sheri Schully
NIH All of US

2:10-2:35 *Values and Barriers to Diversity in Healthcare Datasets*
Veronica Ajewole
Texas Southern University

2:35-3:00 *What We Don't Know CAN Hurt Us: Representation in Healthcare Datasets*
Rayne Rouce
Texas Children's Hospital/Baylor College of Medicine

3:00-3:25 Panel Discussion

3:25-3:40 Break

Session 4: Leveraging Clinical and Real-world Data
Convenor: Stan Watowich, Univ. Texas Medical Branch

3:40-4:05 *Unlocking the Potential of Real-World Data in Oncology Research and Development*
Vasu Chandrasekaran
Ontada

4:05-4:30 *Challenges and Opportunities of Real-World Evidence Data for Therapeutics and Drug Development*
Georgiy Golovko
Univ. of Texas Medical Branch

4:30-4:55 *From Real-world Data to Real-world Evidence: An Upstream Bespoke EHR Strategy*
Guo-Qiang Zhang
Univ. of Texas Health Science Center Houston

4:55-5:15 Panel Discussion

5:15-6:15 Posters and Networking Reception (event hall)

Agenda

May 10, 2023

Day 2 **Disease-Specific Data-Driven Drug Development**

8:00-8:30 Registration and breakfast

Session 5: **Session 5 Neurotherapeutics**
Convenor: Jim Ray, MD Anderson Cancer Center

8:30-9:00 *AI Rodeo: Riding the Wave of Large Language Models in Drug Discovery*
Brett Abrahams
Heppinn Biosciences

9:00-9:30 *Breaking Down Barriers in Neuroscience Drug Discovery Through an AI-powered, Human-Centric Approach*
Virginie Buggia-Prevot
Valo Health

9:30-9:45 Panel Discussion

9:45-10:05 **Networking Break**

Session 6 RNA Therapeutics
Convener: John Cooke, Houston Methodist Research Institute

10:05-10:30 *Systematic Bioinformatics Studies for the Development of the Targeted Therapeutics (Targeting Diverse RNA Level Mechanisms and Gene Fusions)*
Pora Kim
Univ. of Texas Health Science Center, Houston

10:30-10:55 *Mapping and Programming RNA-protein Interactions to Build RNA Therapeutics*
Kristopher Brannan
Houston Methodist Research Institute

10:55-11:20 *Targeting RNA Splicing in Cancer and the Immune System*
Trey Westbrook
Baylor College of Medicine

11:20-12:50 Lunch and Cores
11:20 Lunch
11:50 Core Showcase-Core and Core representatives can be found here

Session 6: **Cardiovascular**
Convenor: John Cooke, Houston Methodist Research Institute

12:50-1:15 *Big Data Infrastructure Transforming the Future of CV Health Research: Happening As We Speak*
Khurram Nasir
Houston Methodist Research Institute

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1:15-1:40 *Beyond Genes: Understanding Cardiovascular Health through Exposomics*
Sadeer Al-Kindi
Case Western Reserve Univ. School of Medicine

Session 7: Infectious Disease & Microbiome
Convenors: Zhiqiang An, Univ. of Texas Health Science Center Houston

1:40-2:05 *Metagenomic Enzymes: A Window into Evolution for Next-Generation Pharmaceuticals*
Peter McCaffrey
Pragma Biosciences

2:05-2:30 *Targeting the Microbiome and Other Factors to Promote Health and End Cancer*
Jenn Wargo
MD Anderson Cancer Center

2:30-3:00 *Audience Brainstorming: Needed Initiatives to More Rapidly Advance Healthcare Research*
Suzanne Tomlinson
Gulf Coast Consortia
Stan Watowich
Univ. Texas Medical Branch
Peter Davies
Institute of Bioscience and Technology, Texas A&M Univ.

3:00 **Closing remarks**
Stan Watowich
Univ. Texas Medical Branch

Presenters
(in alphabetical order)

Brett Abrahams, PhD
President
Heppinn Bioscience
AI Rodeo: Riding the Wave of Large Language Models in Drug Discovery

Dr. Abrahams is a neuroscientist, geneticist, and drug hunter with experience developing therapeutics and building startups. He is the founder and President of Heppinn Biosciences, a consultancy practice launched to support venture investors, foundations, biotech companies, and academic founders diligence opportunities, develop drugs, and start biotech companies. He's also an Advisory Board Member for Autism Speaks, CureShank, FAST, and Accelerator Life Science Partners.

Prior to launching Heppinn, Dr. Abrahams served as Executive Vice President of Research and Development of Magnolia Neurosciences. The company was founded to develop novel therapeutics for neurodegenerative disorders and built out around investment from Arch, Pfizer Ventures, Eli Lilly, and others. Dr. Abrahams joined Magnolia from Ovid Therapeutics, leaving as Senior Director and Head of Pre-Clinical Biology. While there, he helped to move multiple autism and epilepsy-related assets into the clinic and was part of the team that showed clinical benefit of Gaboxadol (OV101) in Angelman Syndrome and Soticlestat (TAK935) in Dravet Syndrome.

Prior to this, Dr. Abrahams was full-time faculty with an independent laboratory at the Albert Einstein College of Medicine, where he retains an adjunct appointment. His lab applied genomic strategies to identify novel disease genes and then studied the molecular, cellular, and behavioral consequences of identified variants in disease models and patients. He was also closely involved in the development of the Simons Foundation's SFARI Gene, an autism-focused knowledge base for researchers and clinicians. His research, published in numerous high impact journals including Cell, New England Journal of Medicine, Nature, and Science Translational Medicine, has been cited more than 10,000 times.

Veronica B. Ajewole, PharmD, BCOP
Associate Professor, Pharmacy Practice
Texas Southern University
Clinical Pharmacist Specialist, Oncology
Houston Methodist Hospital

Values and Barriers to Diversity in Healthcare Datasets

Dr. Ajewole serves as Associate Professor in the Department of Pharmacy Practice at Texas Southern University (TSU) and as a Clinical Pharmacist and an Adjunct Assistant Professor of Oncology at Houston Methodist Hospital. She received her PharmD from TSU and completed her oncology pharmacy residency at Houston Methodist Hospital. Dr. Ajewole is a Board-Certified Oncology Pharmacist with clinical practice in oral chemotherapy Houston Methodist Hospital Cancer Center.

Dr. Ajewole and her team received a Susan G. Komen community grant to establish a Breast Cancer Screening and Prevention Center at TSU. She is the Director of the recently funded National Institutes of Health-Research Centers in Minority Institutions Center for Biomedical and Minority Health Research—Community Engagement Core and the Principal Investigator of a Centers for Medicare & Medicaid Services-funded grant on prostate cancer in African American men and a Cancer Prevention and Research Institute of Texas Funded grant on breast cancer prevention program for ethnic minority women. She is also the founder of Community Resource Solutions Group: an organization committed to promoting Health Equity, Research, and Training. Learn more at www.communityrsg.com

When Dr. Ajewole is not serving her patients, students, community, or church, she is serving and enjoying quality time with her husband and four wonderful children.

Bissan Al-Lazikani, PhD
Professor, Genomic Medicine
Director, Therapeutics Data Science
MD Anderson Cancer Center

Bissan Al-Lazikani, PhD, is professor of Genomic Medicine and director of Therapeutics Data Science. She joined MD Anderson in 2021 and is a founding member of our Institute for Data Science in Oncology. She is a data scientist and drug discoverer with experience in academia and industry. She pioneered integrating multidisciplinary data and AI in drug discovery. She is the creator of canSAR.ai, the world's largest public cancer drug discovery platform, now hosted at MD Anderson, and used by researchers worldwide. Before MD Anderson, she was Chair of Data Science and Drug Discovery at the ICR, UK. There, she led AI-based approaches for objective and systematic evaluation of therapeutic targets for cancer which led to several drugs in clinical and preclinical development. She studied in the United Kingdom where she earned a doctorate in computational biology from Cambridge University and a master's degree in computer science from Imperial College.

Leslie Benet, PhD
Professor
Bioengineering & Therapeutic Sciences
Schools of Pharmacy & Medicine
University of California San Francisco

In Vitro-In Vivo Correlations and Human PK/Dose Predictions: What Are the Hurdles?

Dr. Benet, Professor and former Chairman (1978-1998), Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, University of California, San Francisco, received his A.B. (English), B.S. (Pharmacy), M.S. from the University of Michigan and Ph.D. from the University of California. He has received nine honorary doctorates: Uppsala University, Sweden (Pharm.D., 1987), Leiden University, The Netherlands (Ph.D., 1995), University of Illinois at Chicago (D.Sc., 1997), Philadelphia College of Pharmacy and Science (D.Sc., 1997), Long Island University (D.Sc., 1999), University of Athens, Greece (Ph.D., 2005), Catholic University of Leuven, Belgium (Ph.D., 2010), University of Michigan (D.Sc., 2011) and University of Lisbon, Portugal (Ph.D., 2016). His research interests, more than 615 publications, 7 books and 12 patents are in the areas of pharmacokinetics, biopharmaceutics, drug delivery and pharmacodynamics. Dr. Benet is listed by Clarivate Analytics as one of the most highly cited pharmacologists worldwide with his published peer-reviewed papers cited over 32,000 times, while Google Scholar credits him with over 50,000 citations. His most recent work has addressed the deficiencies of in vitro-in vivo extrapolation (IVIVE) and different approaches to advancing our understanding and prediction of clearance processes. Dr. Benet was a Founder/Editor of the JOURNAL OF PHARMACOKINETICS AND BIOPHARMACEUTICS (1973-98) and Associate Editor of PHARMACOLOGY AND THERAPEUTICS (1995-2000). He presently serves a member of the Editorial boards of PHARMACOLOGY and AAPS JOURNAL. He is a Fellow of the American Association for the Advancement of Science (AAAS), the American Association of Pharmaceutical Scientists (AAPS), the American College of Clinical Pharmacology (ACCP) and the Controlled Release Society (CRS). In 1982, Dr. Benet received the APhA Academy of Pharmaceutical Sciences (APS) Research Achievement Award in Pharmaceutics and the University of Michigan, College of Pharmacy, Distinguished Alumnus Award. In 1985, he served as President of APS. During 1986, Dr. Benet was a Founder and first President of the AAPS. In 1987, Dr. Benet was elected to membership in the National Academy of Medicine (NAM) of the U.S. National Academy of Sciences. In 1988, he received the ACCP Distinguished Service Award and in 1989, the AAPS Distinguished Pharmaceutical Scientist Award. In 1990, he was the recipient of the Rho Chi Lecture Award, and the UCSF Long Teaching Award, while in 1991, he received the Volwiler Research Achievement Award of the American Association of Colleges of Pharmacy

(AACP). In 1993-94, he served as AACP President. In 1995, he received the Rawls-Palmer Progress in Medicine Award of the American Society for Clinical Pharmacology and Therapeutics (ASCPT); in 1996, the AAPS Distinguished Service Award; and in 2000 the APhA Takeru Higuchi Research Prize and the AAPS Wurster Research Award in Pharmaceutics. In 2001 he was awarded the Høst-Madsen Medal of the International Pharmaceutical Federation (FIP) and the UCSF Outstanding Faculty Mentorship Award (repeated in 2016). In 2004, he was awarded the Pharmaceutical Sciences World Congress Research Achievement Award and the CRS Career Achievement in Oral Drug Delivery Award. In 2007 he was selected as the UCSF Distinguished Clinical Research Lecturer; in 2010, the ASCPT Hunter Memorial Award in Therapeutics; and in 2011 the ACCP Distinguished Investigator award. In 2012, the September issue of PHARMACEUTICAL RESEARCH was dedicated in his honor and he was made an honorary member of FIP. In 2013, he was awarded the APhA Ebert Prize, the AAPS Journal Outstanding Manuscript award and the September issue of JOURNAL OF PHARMACEUTICAL SCIENCES was dedicated in his honor. In 2015 he received the ISSX North American Scientific Achievement award; in 2016 the Remington Honor Medal from the APhA, the highest honor in American Pharmacy; and in 2017 a full day symposium in his honor entitled “The Cutting Edge in Pharmaceutical Sciences—50 Years of Progress Celebrating Les Benet’s 80th Birthday” was held in conjunction with the Pharmaceutical Sciences World Congress in Stockholm, Sweden. In 2023 he received the Lifetime Achievement Award of the Frankfurt Foundation for the Quality of Medicines. Dr. Benet formerly served as Chair of the Pharmacology Study Section and the Pharmacological Sciences Review Committee for the NIH, the FDA CBER Peer Review Committee, the FDA Expert Panel on Individual Bioequivalence, the Board of Pharmaceutical Sciences of the FIP, the Organizing Committee for the FIP Millennial World Congress of Pharmaceutical Sciences, the Congressionally mandated NAM/National Research Council (NRC) Committee on Accelerating the Research, Development and Acquisition of Medical Countermeasures Against Biological Warfare Agents and the FIP Foundation for Education and Research and as a member of the FDA Science Board, the FDA Generic Drugs Advisory Committee and the NRC Biodefense Standing Committee. He presently serves as Chair of the Board of Directors of Medicines360, a nonprofit pharmaceutical company, emphasizing products for women’s health.

Kristopher W. Brannan, PhD
Assistant Professor, Center for RNA Therapeutics, Department of Cardiovascular Sciences
Houston Methodist Research Institute

Mapping and Programming RNA-protein Interactions to Build RNA Therapeutics

Dr. Kristopher Brannan is an Assistant Professor and CPRIT Scholar at the Center for RNA Therapeutics in the Department of Cardiovascular Sciences at Houston Methodist Research Institute. Dr. Brannan holds a BA in Molecular Biology and Biochemistry from the University of Colorado Boulder, and a Ph.D in Molecular Biology from the University of Colorado School of Medicine. Dr. Brannan's research integrates technology-development and systems approaches to study how RNA-protein interactions mechanistically regulate gene expression, and how disruption of these interactions drives cancer and neurological disease. Dr. Brannan is developing tools to probe RNA-binding and RNA-translation landscapes, to programmably target and modify RNA, and to build and test RNA-based medicine.

Virginie Buggia-Prevot, PhD
Senior Director of Drug Discovery
Valo Health

Breaking Down Barriers in Neuroscience Drug Discovery Through an AI-Powered, Human-Centric Approach

Dr. Virginie Buggia-Prevot is a neurobiologist/cell biologist with over 15 years of experience in academia and the biotechnology sector. She is currently Senior Director of Drug Discovery at Valo Health, a technology company using human-centric data and artificial intelligence (AI) powered computation to transform the drug discovery and development process, and has been at the company for over 2.5 years. In her role she leads Neurology Discovery, developing human-centric computational approaches to discover new targets for neurodegenerative diseases. Prior to Valo, Virginie led novel target discovery and validation for Alzheimer's disease at the Neurodegeneration Consortium, MD Anderson, an academic collaboration of leaders in the field of neurodegeneration from the Baylor College of Medicine, Massachusetts Institute of Technology, Icahn School of Medicine at Mount Sinai, New York University and more. The mission of the Consortium is to better understand the underlying biology of Alzheimer's disease and translate the knowledge into therapeutic interventions that can effectively alleviate symptoms by delaying, reversing and/or eliminating the pathology of the disease and other neurological diseases. Her work on a neuroprotective small molecule program contributed to the launch of Magnolia Neurosciences, a company focused on the development of a new class of neuroprotective medicines. A new strategic research agreement was formed with Denali Therapeutics from data generated by Virginie and her team.

Dr. Buggia-Prevot received her PhD in cell and molecular biology from the University of Nice Sophia-Antipolis and completed her post-doctoral training at the University of Chicago Department of Neurobiology. In 2020, she was named to In Vivo's List of Rising Leaders in the life sciences, one of 30 individuals across the biopharma, medtech and health technology sectors.

Vasu Chandrasekaran, PhD
Vice President
Real-World Data & Analytics
Ontada (McKesson)

Unlocking the Potential of Real-World Data in Oncology Research and Development

Dr. Chandrasekaran has over 15 years of experience in the pharmaceutical and healthcare industry at the intersection of data science, real-world data and drug discovery/development.

He joined Ontada in 2021 and has responsibility for the development, delivery and growth of real-world data products and data science services to biopharma and life sciences customers seeking to leverage real-world data across the discovery, development and commercialization lifecycle.

Prior to joining Ontada, he spent 5 years at Merck & Co. in the Center for Observational and Real-World Evidence (CORE) where he led a team of data scientists responsible for data science capabilities and strategy. Before Merck, Dr. Chandrasekaran held positions of increasing responsibilities at UMass Medical School, Cubist and Novartis.

Dr. Chandrasekaran obtained his Ph.D. in structural bioinformatics from the University of Georgia and completed postdoctoral fellowships at UNC-Chapel Hill and MIT prior to joining the pharmaceutical industry. He has published in several peer-reviewed journals and presented at scientific and professional meetings, including the American Chemical Society, Keystone Symposia, Machine Learning in Healthcare, American Medical Informatics Association (AMIA) and Observational Health Data Sciences and Informatics (OHSDI).

George Golovko, PhD
Assistant Professor, Pharmacology
Director. Microbiome Sequencing and Analysis Core, UTMB
Data Science Lead, West African Center for Emergent Infectious Diseases
Challenges and Opportunities of Real-World Evidence Data for Therapeutics and Drug Development

Dr. George Golovko is an Assistant Professor in the Department of Pharmacology and Toxicology at the University of Texas Medical Branch. Dr. Golovko serves as director of the Microbiome Sequencing and Analysis Core (UTMB) and Lead Data Scientist at the West African Center for Emerging Infectious Diseases. He is an expert in bioinformatics with decades of academic and industrial research experience in pathogen detection, metagenomics, transcriptomics analysis, and clinical data science.

Dr. Golovko's academic research centers on developing decision-support tools for translational and clinical applications. As a lead member of the UTMB Institute for Translational Sciences, Dr. Golovko engages in the development of biomedical infrastructure, research activities, and education focused on Big Data multi-omics projects at UTMB. As the data science lead at the West African Center for Emerging Infectious Disease, Dr. Golovko's lab addresses global data challenges, overseeing data acquisition, harmonization, and quality control for clinical and animal samples to monitor the spread of infectious diseases in West Africa.

Pora Kim, PhD
Assistant Professor
Bioinformatics
Univ. of Texas Health Science Center
Houston

Systematic Bioinformatics Studies for the Development of the Targeted Therapeutics (Targeting Diverse RNA Level Mechanisms and Gene Fusions)

Dr. Kim is an Assistant Professor in Bioinformatics at the School of Biomedical Informatics of The University of Texas Health Science Center at Houston. Dr. Kim's research expertise lies in computational biology for precision medicine based on accurate cellular mechanisms with bioinformatics and genomic data science approaches. She was awarded the Outstanding Investigator Award in 2020 by NIGMS with a study aiming to infer the origin and functional aspects of new genes using bioinformatics and deep learning approaches. In this awarded project, she and her lab members are studying the origin of new genes and downstream effects, and genomic features through multiple Bioinformatics tools and deep learning methods. Recently, they predicted the 3D structures of new proteins, which were translated from new genes (fusion genes), performed the virtual screening of the potentially interacting small molecules, functional annotation of fusion proteins, and build a protein version FusionGDB (FusionPDB). Her group will report multiple integrative studies of fusion proteins per important gene groups such as kinase, transcription factor, and transmembrane proteins to provide new knowledge for the therapeutic target candidates in human fusion genes. In this talk, she will present her previous and ongoing studies on developing therapeutic targets in RNA-level mechanisms and gene fusions.

Janine Low-Marchelli, PhD
Manager, Technical Information Services,
The Jackson Laboratory
Pre-Clinical Oncology Studies using Patient-Derived Xenografts

Dr. Janine Low-Marchelli earned a Ph.D. in Biomedical Sciences from the University of California, San Diego (UCSD) studying angiogenesis in breast cancer metastasis in 2011. Dr. Low-Marchelli ontinued on at UCSD as a postdoctoral fellow targeting leukemic stem cells with novel small molecule inhibitors. During her postdoctoral training, Dr. Low-Marchelli was awarded a K12 IRACDA fellowship, which provides training in Scientific Teaching, in which teaching is approached with the same rigor as science. Dr. Low-Marchelli joined The Jackson Laboratory in 2014 as a Technical Information Scientist, where she was able to apply both her bench and education experience to support researchers with best-practices in human disease modeling. Dr. Low-Marchelli has managed the Technical Information Services team since 2020.

Peter McCaffrey, MD
Clinical Pathologist and Co-Founder and Chief Technology Officer
Pragma Biosciences

Metagenomic Enzymes: A Window into Evolution for Next-Generation Pharmaceuticals

Peter McCaffrey, MD is a Clinical Pathologist and Co-Founder and Chief Technology Officer at Pragma Biosciences. Peter leads the integration of Pragma's wet lab and dry lab efforts in bridging computational enzyme discovery with high-throughout synthetic biology. Following significant advances in both artificial intelligence and laboratory automation, Pragma Biosciences is scaling intelligent discovery with iterative biomanufacturing in the pursuit of novel enzymes and their novel chemistries. Peter completed his medical training at Johns Hopkins and Massachusetts General Hospital and his Graduate Training in Artificial Intelligence at Stanford.

Khurram Nasir, MD, MPH, Msc
Chief of Cardiovascular Disease Prevention
Houston Methodist Research Institute
Big Data Infrastructure Transforming the Future of CV Health Research: Happening As We Speak

Khurram Nasir MD MPH Msc is the Chief of Cardiovascular Disease Prevention and Wellness as well as serves as the Chief Division of Health Equity & Disparities Research and Co-Director for Center for Outcomes Research at Houston Methodist. He is also the inaugural Director for the newly founded Center for Cardiovascular Computational Health & Precision Medicine (C3-PH). He is Professor of Medicine at Weill Cornell Medical College and Professor of Cardiology at Houston Methodist Academic Institute.

Dr. Nasir received his MD from Pakistan, followed by Master's degree in public health at John Hopkins University. Dr. Nasir completed his internal medicine residency at Boston Medical Center and cardiology fellowship at Yale University. He also received postdoctoral research training at the division of cardiology at Johns Hopkins Hospital and NIH T-32 fellowship at Massachusetts General Hospital, Harvard University. In 2017, he earned a Master's degree in Health Economics and Policy Management from London School of Economics & Political Science.

His clinical and research interests in lies in role of precision medicine, health system & big data initiatives. He has published more than 850 high impact articles published in top academic journals with h-index>100. He is currently Associate Editor for the journal "Circulation: Cardiovascular Quality and Outcomes". He has served on the board of Directors for Society of Cardiac CT (SCCT) & American Society of Preventive Cardiology (ASPC). In recognition of his contributions, he was recognized with 1) the "Johns Hopkins Distinguished Alumni Award" in 2013, which honors alumni who have typified the Johns Hopkins tradition of excellence and brought credit to the University by their personal accomplishment, professional achievement, or humanitarian service and , 2) "Arthur S. Agatston Cardiovascular Disease Prevention Award" in 2020 that recognizes individuals whose pioneering efforts have saved lives from the leading killer throughout the world, coronary artery disease.

Tudor I Oprea, MD, PhD
Chief Scientific Officer
Expert Systems Inc

Can Humans Learn from Machine Learning in Drug Discovery?

Tudor I. Oprea is a digital drug hunter with three decades of experience in knowledge management applied to target and drug discovery. He co-developed ChemGPS, the “lead-like approach”, systems chemical biology and a knowledge-based classification for human proteins. He co-discovered the first GPER agonist (now orphan drug designated) and GPER antagonist, and several GLUT transporter inhibitors. His machine learning models include cheminformatics and drug discovery, disease and target biology. His team maintains DrugCentral and Pharos, part of an NIH Common Fund project. He co-authored over 320 publications, 11 US patents, and edited 2 books on informatics in drug discovery.

Reid Powell, PhD
Assistant Professor
Combinatorial Drug Discovery Program and High Throughput Flow Cytometry Program
Texas A&M Institute of Biosciences and Technology

Establishing Pre-clinical Databases for Drug Repositioning

Dr. Powell is an Assistant Professor in the Gulf Coast Consortia's Combinatorial Drug Discovery Program and High Throughput Flow Cytometry Program at Texas A&M Institute of Bioscience and Technology. He has a diverse set of research interests with expertise in cell and molecular biology, bioinformatics, and lab automation. This has been exemplified throughout his academic carrier where he initially received a Bachelor of Science (BS) in Biochemistry with a minor in Biology from Texas Tech University. He later went on to receive his PhD in Medical Science from Texas A&M Health Science Center, where he also completed his post-Doctoral research in the Drug Discovery area. Throughout his career, he has developed a wide array of biochemical, image-based, and flow-based high throughput screening platforms with accompanying analytical methods. This has included multiple fully automated image analysis routines as well as methods to contextualize high throughput screening data using integrative approaches that combine genomics, transcriptomics, chemical, and pharmacologic data sources. He has supported multiple early-stage drug developed and drug repurposing campaigns, which have been performed across multiple disease contexts including cancer, pathogenic infections, and neurologic disorders.

Research Areas

High throughput screening, High throughput/content microscopy, Bioinformatics, integrative analysis, deep Learning, Machine Learning, drug discovery, pharmacogenomics/transcriptomics

Rayne Rouce, MD, BS
Center for Cell and Gene Therapy (CAGT)
Texas Children's and Baylor College of Medicine

What We Don't Know CAN Hurt Us: Representation in Healthcare Datasets

Dr. Rayne Rouce is a pediatric oncologist and physician scientist whose research and clinical interests focus on refractory hematologic malignancies, specifically how to harness the immune system to recognize and attack cancers. She has spent the past 9 years in the translational research laboratories of the Center for Cell and Gene Therapy (CAGT) at Texas Children's and Baylor College of Medicine, leading a translational and clinical research program creating novel early phase cell and gene therapy products and translating them to first-in-human immunotherapy trials. She has significant experience in every aspect of translation and clinical trial development, from study conception (specifically chimeric antigen receptor and virus-specific modified T cells for leukemia and lymphoma) to preclinical laboratory-based validation and ultimately clinical practice. She has additional experience in the regulatory hurdles associated with these trials, such as submission of protocols and INDs to the IRB and other regulatory agencies, as well as the requirements of clinical trial conduct. She is especially passionate about addressing barriers to access to novel cancer therapies including CAR T-cells and other cellular immunotherapies and is working within society groups to identify and address these barriers. Dr. Rouce also leads the Task Force for Promoting Diversity in Clinical Trials within the Dan L Duncan Comprehensive Cancer Center and has gained notoriety in her work to enhance diversity in clinical trials leading her to present on this important topic to Cancer Centers, patient advocacy groups, and scientific organizations around the country. She leads the DEI initiatives for ASH, ASGCT and ASTCT, thus integrating her passion for science and advocacy, and is active in national working groups inclusive of multiple stakeholders with a shared goal of enhancing access specifically to cancer cell therapy trials. She also serves as the Director of Community Outreach and Engagement within Baylor College of Medicine's Office of Institutional Diversity, Equity and Inclusion and the Associate Director of Community Outreach and Engagement for DLDCCC. In these roles, she leads numerous institutional, national and community initiatives within biomedical science. These programs provide a clear pathway for underrepresented minorities to excel in STEM careers as well as clear approaches to community engagement in every aspect of the bench-to-bedside scientific process.

Sheri Schully, PhD
All of Us Research Program
Deputy Chief Medical and Scientific Officer
National Institutes of Health
The All of Us Research Program Researcher Workbench

Sheri Schully, Ph.D., is the deputy chief medical and scientific officer and the lead for ancillary studies in the All of Us Research Program at the National Institutes of Health (NIH). Through her leadership, she is establishing ancillary studies as a core and scalable capability of the program that will expand the cohort and deliver new phenotypic, lifestyle, environmental, and biological data to the All of Us Researcher Workbench. Dr. Schully has been involved with shaping the program and setting the scientific vision and strategy since its inception.

Before taking this role, Dr. Schully was a team lead and senior advisor for disease prevention in the Office of Disease Prevention (ODP). There she led the effort to systematically monitor NIH investments in prevention research and assess the progress of that research. She also served as the team lead for the Knowledge Integration Team, as well as a program officer in the Epidemiology and Genomics Research Program at the National Cancer Institute (NCI). She came to NIH as an NCI-designated Presidential Management Fellow in 2005.

Dr. Schully's research interests include genomics, personalized medicine, and the integration of genetic and genomic information into clinical and public health practices. Her work has been published in numerous high-impact scientific journals. She earned both a Ph.D. in biological sciences with a concentration in population genetics and a B.S. in zoology with a minor in chemistry from Louisiana State University.

Keynote Presenter

Max Sherman, PhD
Co-founder and CTO
Serinus Biosciences, Inc.
Knowledge-primed AI to Power the Design of Rational Combination Therapies

Max Sherman is the co-founder and CTO of Serinus Biosciences, Inc., a biotechnology startup building an integrated computational and experimental platform to design safe and effective combination therapies for cancer patients. Fueled by his background in medicine and mathematics, Max's research focuses on uniting deep-learning and statistics to build approaches customized for the unique characteristic of biomedical data. The goal of his work is to extract actionable insights from next-generation genomics data that can inform therapeutic development and patient care.

Max has a PhD in Computer Science from MIT where he studied under computational biology pioneer Bonnie Berger and statistical geneticist Po-Ru Loh. Prior to his PhD, he studied statistics at the University of Cambridge and applied mathematics and biology at Brown University and worked as a statistician at Harvard Medical School.

Max's research has appeared in high-impact journals including Science, Cell, Nature Biotechnology, Nature Genetics, and Nature Neuroscience. His work has been recognized with awards from the American Society of Human Genetics, European Association of Cancer Research, and the National Institutes of Health.

Kari Stefansson, MD, PhD
Founder and CEO
deCODE Genetics
The Study of Human Diversity: The Key to Understanding of Human Disease

Kari Stefansson MD, PhD is a founder and CEO of the Icelandic biotechnology company deCODE genetics. He pioneered the use of population genetics in the study of human diversity. The population approach he advanced in Iceland has served as a model for large scale genome projects around the world. Before founding deCODE in 1996 Dr. Stefansson was a professor of neurology, neuropathology and neuroscience at Harvard Medical School. Dr. Stefansson is an International Member of the US National Academy of Sciences, he is a member of the European Molecularbiology Organization (EMBO) and he is the recipient of the American Society of Human Genetics (ASHG) William Allan Award, the European Society of Human Genetics Award, the Anders Jahre Award, the Federation of European Biomedical Societies Sir Hans Krebs Medal, the European Heart Association Gold Medal, the World Glaucoma Association Award, the American Alzheimer's Association's Inge Grundke-Iqbal Award, the Wallace H. Coulter Distinguished Award, International KFJ Award from Rigshospitalet in Denmark, and the Jakobus Award. Chosen by Time magazine as one of the 100 most influential men of the year for 2007 (Time100 list for 2007), chosen 2007 by Newsweek as one of the 10 most important biologists of the 21 century, Chosen by BusinessWeek as one of the stars of Europe in 2000 at the forefront of change, on the Reuter´s/Thompson´s list of the world´s 10 most cited scientists of 2010, Clarivate Analytics 2016, 2017 and 2018 Highly Cited Researcher in molecular biology and genetics, Clarivate Analytics 2019 and 2020 Highly Cited Researcher Cross-Field.

Keynote Presenter

Jennifer Wargo, MD, MMSc
Professor
Surgical Oncology and Genomic Medicine
University of Texas MD Anderson Cancer Center

Targeting the Microbiome and Other Factors to Promote Health and End Cancer

Jen Wargo's career commitment has always been to advance the understanding and treatment of disease through science. After completing her medical degree, she entered surgical residency training at the Massachusetts General Hospital where she became interested in the biology and treatment of cancer. During her training, she completed two fellowships in surgical oncology with a focus on cancer immunotherapy.

She was recruited to MGH in 2008 to join the faculty in the Division of Surgical Oncology and established a translational research laboratory focusing on better understanding response and resistance to treatment for melanoma, pancreatic cancer, and other cancers. During that time, her laboratory demonstrated that treatment with molecularly targeted therapy could sensitize tumor cells to treatment with immunotherapy, providing the rationale for combined targeted therapy and immunotherapy combinations. She also began studies on the tumor microbiome in pancreatic and other cancers while at Harvard.

Jen was recruited to the University of Texas MD Anderson Cancer Center in 2013 to help lead the Melanoma Moonshot efforts - and also continued important translational research work on targeted therapy, immunotherapy, and the impact of the gut and tumor microbiome in cancer. Jen is currently a Professor of Surgical Oncology and Genomic Medicine, and the leader of the Platform for Innovative Microbiome and Translational Research (PRIME-TR) at MD Anderson. Importantly, Jen is deeply invested in working with investigators across the institution and across the world to find better ways to treat, intercept, and ultimately prevent cancer.

Thomas "Trey" Westbrook, PhD
Executive Director
Therapeutic Innovation Center (THINC)
Welch Chair in Chemistry, and a Professor of Molecular & Human Genetics and Biochemistry & Molecular Biology
Baylor College of Medicine

Targeting RNA Splicing in Cancer and the Immune System

Thomas "Trey" Westbrook Ph.D. is Executive Director of the Therapeutic Innovation Center (THINC), Welch Chair in Chemistry, and a Professor in the Departments of Molecular & Human Genetics and Biochemistry & Molecular Biology at Baylor College of Medicine. His research seeks to unravel how the genetic drivers of cancer create new cancer vulnerabilities that can be exploited therapeutically. Nearly two decades ago, Dr. Westbrook contributed to the development of the first barcoding approaches for genetic screens ("functional genomics") in human cells (Westbrook, Cell 2005), an approach that underpins most RNAi and CRISPR screening strategies in mammalian systems today. Since this time, his team has focused on using synthetic lethality, chemical biology, and other approaches to identify and credential new dependencies of cancer. For instance, his team contributed the first synthetic lethal screens for MYC-driven cancers (Kessler, Science 2012) and has spent the past decade studying how common oncogenes like MYC drive unanticipated cancer vulnerabilities in RNA processing and metabolism. His team actively collaborates with academic, patient advocacy, and pharma/biotech partners to translate these vulnerabilities into patient benefit.

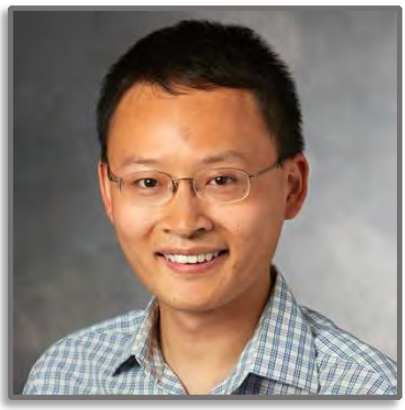
Dr. Westbrook has served as a faculty member at Baylor College of Medicine since 2007 and is an Era of Hope Scholar in Breast Cancer Research, McNair Scholar in Cancer Research, and Scholar of The V Foundation for Cancer Research. Dr. Westbrook's innovations have been widely recognized by the community in journals including Science, Nature, and Cell, and he has received numerous awards for his contributions to science and commitment to cancer patients.

Guo-Qiang Zhang, MS, PhD
Professor and Distinguished Chair , Digital Innovation VP & Chief Data Scientist, Office of Data Science (ODS) Co-Director, Texas Institute for Restorative Neurotechnologies (TIRN)
University of Texas Health Science Center at Houston

From Real-world Data to Real-world Evidence: An Upstream Bespoke EHR Strategy

Dr. Zhang is Vice President and Chief Data Scientist for UTHealth. He is a Professor in the Department of Neurology, at McGovern Medical School and Co-Director, Texas Institute for Restorative Neurotechnology. Prior to joining UTHealth, he was a Professor of Internal Medicine and Computer Science at the University of Kentucky, where he also served as the university's inaugural Director for the Institute for Biomedical Informatics, and Associate Director for the Center for Clinical and Translational Science. His longest career stretch has been spent at Case Western Reserve University, where his role included Division Chief of Medical Informatics, Co-Director of Biomedical Research Information Management Core of the Case Western CTSA, and Associate Director for Case Comprehensive Cancer Center.

Dr. Zhang received his Ph.D. from the University of Cambridge. His earlier research interests included theoretical computer science and the semantics of programming languages. In the last decade, his research has revolved around Human-Data Interaction (HDITM), achieved through the development of innovative software and web-based applications spanning the biomedical data lifecycle. Software tools include query interface for clinical research, data management software for clinical trials and biomedical research and tools for multi-site data integration. He led the development of data infrastructures and manages data resources, following the vision of NIH Data Commons, for the National Sleep Research Resource and for the Center for Sudden Unexpected Death in Epilepsy Research, the largest and most comprehensive, well-annotated clinical data sets in the two disease areas. He also has a track record of research in biomedical metadata including ontologies and terminology systems, to bring them to bear on HDI. Dr. Zhang effectively brings cutting-edge computer science and informatics methodology to addressing biomedical data/big data challenges through the translation of theory, algorithms, methods, and best practices to functional and usable tools impacting the clinical research data lifecycle.



James Zou, PhD
Assistant Professor
Biomedical Data Science, Computer Science and Electrical Engineering
Stanford Univ.

Generative AI for Drug Discovery and Development

James Zou is an assistant professor of Biomedical Data Science, CS and EE at Stanford University. He develops machine learning/AI for precision medicine, from drug and biomarker discovery to clinical trial design. He has received a Sloan Fellowship, an NSF CAREER Award, two Chan-Zuckerberg Investigator Awards, a Top Ten Clinical Achievement Award, several best paper awards, and faculty awards from Google, Amazon, Tencent and Adobe. He is also the faculty director of Stanford AI for Health.

Keynote Presenter

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Poster 1

A Single-cell, Multi-parametric High Content Assay to Quantify and Classify Endocrine Disrupting Chemicals in Environmental Contaminants that Affect ERβ Functions

Abbott D[1], Bolt MJ[1,2], Szafran AT[1], Mancini MG[1], Gorelick D [1,3], Stossi F[1], Mancini MA[1,2]
1. Baylor College of Medicine
2. Texas A&M Institute for Bioscience and Technology
3. Center for Precision Environmental Health

Here, we present an inducible biosensor model for ERβ, GFP-ERβ:PRL-HeLa, a single-cell-based high throughput (HT) *in vitro* assay that allows direct visualization of GFP-tagged ERβ binding to ER-specific DNA responsive elements, ERβ-induced chromatin remodeling, and monitor transcriptional alterations via mRNA FISH for the prolactin (PRL)-dsRED2 reporter gene. The model was used to accurately (Z-Prime 0.58-0.8) differentiate ERβ-selective ligands from ERα ligands when treated with a panel of selective agonists and antagonists. Next, we tested an EPA-provided set of 45 estrogenic reference chemicals with known ERα and some ERβ *in vivo* activity. Following a 2-hr exposure and HT imaging with a high throughput spinning disk confocal and custom software platform, we demonstrate the ability to distinguish between agonists and antagonists, identifying 32 ERβ-specific ligands, 17 of which previously had been characterized as inactive by a published model, emphasizing the sensitivity (EC50 values up to -4.5 log) of the model. We then used an orthogonal transgenic zebrafish (ZF) model[1] to cross validate ERβ and ERα selective activities at the organism level. The ZF model has previously been used to identify water samples with estrogenic activity and compounds with ER subtype selectivity. Using this environmentally relevant ZF assay, these compounds were confirmed to have ERβ activity, validating the GFP-ERβ:PRL-HeLa assays screening potential. These data demonstrate the value of sensitive multiplex mechanistic data gathered by the GFP-ERβ:PRL-HeLa (receptor levels/localization, DNA binding, chromatin modeling and transcriptional output) and the orthogonal zebrafish model to rapidly identify environmentally relevant ERβ EDCs and improve upon currently available technologies.

References

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Poster 2

Assessing the Pharmacological Mechanism of OJT009: A Novel Inhibitor of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Purpose

The ongoing pandemic of coronavirus disease (COVID-19) caused by the highly infectious pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents a global public health challenge. The emergence of deadly SARS-CoV-2 variants with mutations on the viral genes has made it more imperative to discover therapeutics that target the host receptors for COVID-19 treatment. Therefore, our research has targeted the critical host entry receptor for SARS-CoV-2 entry into the human cells, Angiotensin-converting enzyme-2 (ACE2). Through a high-throughput screen we identified OJT009 as a novel inhibitor of SARS-CoV-2. Herein, our purpose is to identify the primary mechanism of action of OJT009 against SARS-CoV-2

Objective(s)

The Interaction between ACE2 and the RBD region of Spike protein is the most crucial step in the viral life cycle. Therefore, the ACE2- RBD interaction has remained a key target for therapeutics in COVID19 treatment. Although ACE2 facilitates viral entry, it provides defense against acute lung injury through its physiological function of converting Ang 2 to Ang 1-7 thereby counterbalancing the effects of ACE 1 in the Renin-Angiotensin pathway, Therefore the ACE2/Ang 1-7 pathway must be carefully manipulated without disrupting the balance of the renin angiotensin system. We investigated the effect of OT009 on ACE2-RBD interaction as well as its impact on the renin angiotensin pathway. To assess the impact of OJT009 on the key interaction between recombinant human ACE2 (rhACE2) and the RBD of the S protein of SARS-CoV-2, we utilized a COVID-19 Spike-ACE2 Binding Assay. To investigate the impact of OJT009 on renin angiotensin pathway we first tested the effect of OJT009 on the exopeptidase activity of ACE2 using an ACE2 activity assay. Furthermore, we tested the effect of OJT009 on ACE1 exopeptidase activity and we also examined the impact on OJT009 on expression levels of both ACE2 and ACE1.

Result(s)

We evaluated the effects of OJT009 on SARS-CoV-2 infection-induced CPE *invitro.* OJT009 inhibited SARS-CoV-2 infection-induced CPE in vitro with a 50% Inhibitory Concentration value at about 21.7µm. A unique dose-response curve was observed when the effect of OJT009 on the binding affinity of rhACE2 and RBD of SARS-CoV-2 S protein was evaluated using an adapted *in vitro* enzyme-linked immunosorbent assay (ELISA). The concentrations tested ranged from 100 nM to 100 µM. OJT009 inhibited the binding of SARS-CoV-2's S (RBD) protein to rhACE2 receptor at lower concentrations ranging from 100 nM to 10 µM; but enhanced the interaction at higher concentrations from 50 µM. Hence, the bell-shaped model generated two IC_{50} values. OJT009 inhibited ACE 2 activity *in vitro* at high concentrations (>100µM) but does not affect the activity at low concentrations (50µM). The expression levels of both ACE2 and ACE1 remained unchanged after treatment with OJT009 in cell culture. Further investigations through additional computational modelling studies validated our invitro findings that OJT009 might inhibit viral attachment and entry.

Conclusion(s)

The unconventional dose-response curve observed, could suggest additional binding site(s) and/or target(s), such as other sites on rhACE2 or the Spike (RBD) protein. To further understand the impact of OJT009 on the RAAS pathway we investigated its role on the exopeptidase activity and expression of both ACE1 and ACE2. At high concentrations (above 50µM), OJT009 inhibits the ACE2 exopeptidase activity but enhances the binding of ACE2 with RBD. While at low concentration, it does not affect ACE2 activity but disrupts the interaction of ACE2 and RBD. Although OJT009 inhibits the activity of ACE1 at concentrations above 50µm it does not affect the expression of either ACE1 or ACE2.

Based on our findings, OJT009 represents a promising drug class that could be further evaluated as a lead series in developing chemotherapeutics for COVID-19 treatment.

Poster 3

Gene-Specific Primers Identify and Differentially Quantify Microorganisms DNA Contents in a Competitive Environment of Fermented Food

Adeyemo SM[1,2], Morgem M[1], Eltayab H[1], Johnson T[1], Sodipe A[1], Jackson DA[1], Olufemi SE[1]

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Abstract

Background: Fermentation is an age-long method of preserving food. As a result, the shelf life of such food is also extended, the flavor, organoleptic and nutritional attributes are improved, spoilage microorganisms are eliminated, and the foods are further bio-preserved. A group of organisms known as lactic acid bacteria (LAB) is responsible for food bio-preservation. LAB are probiotics and produce lactate after fermentation. In addition, they produce organic acids, polyols, exopolysaccharides, and antimicrobial compounds, which protect cellular functions, benefit organisms' health and detoxify Mycotoxins. They also prevent Aspergillus species from growing in the same food during the first few hours of fermentation. Aspergillus sp are filamentous, cosmopolitan, and ubiquitous fungi found in nature. They have a significant worldwide economic impact on food products and cause a major health challenge in humans and animals; producing aflatoxin, if ingested, causes different health problems.

Goal and Hypothesis: Several morphological, biochemical, microscopic, and molecular methods are often employed to identify and isolate LAB and *Aspergillus species* from fermented corn/grains and cassava used as food. Still, these conventional identification methods have limitations and lack quantification of DNA content. Therefore, this study aims to show that gene-specific primers combined with semi- or quantitative PCR (qPCR) can detect and differentially quantify *Lactobacillus* and Mycotoxigenic fungi in a competitive environment of fermented foods. To achieve the goal of this innovative study, we develop an aim. **Aim:** Gene-specific primers combined with semi-qPCR or qPCR will identify and quantify the DNA content of Lactobacillus and Mycotoxigenic fungi in a competitive environment of fermented foods. Overall the DNA content provides the estimated parameter of the microorganisms in a competitive food environment.

Methods: Typical African diet samples, comprised of *garri* and *ogi,* were purchased from local markets and analyzed for the presence of LAB and *Aspergillus sp*. The diet samples were inoculated into De Mann Rogosa and Sharpe broth and incubated for 48h at 30^{o}C under anaerobic conditions. Gene-specific primers were synthesized (Sigma-Genosys, Woodland, TX) and combined with semi-qPCR to determine the DNA content of microorganisms in a competitive food environment.

Results: The gene-specific method identified and differentially quantified DNA contents of six different LAB species, *Lactobacillus plantarum*, *L. fermentum*, *L. acidophilus*, *L. brevis*, and *L. pentosus*, including four different *Aspergillus species*, *Aspergillus niger*, *A. nidulans*, *A. fumigatus*, and *A. flavus* in a competitive food environment.

Conclusion: Even though PCR and qPCR methods are well known and yet to be attempted to identify and quantify microorganisms based on their DNA contents in a fermented competitive food environment. Therefore, the approach described in the study has an advantage over the current methods used for identifying and quantifying microorganisms in a competitive food environment. Furthermore, it is a versatile tool that is easy to employ and identify and differentially quantify DNA contents of microorganisms that are beneficial and detrimental (e.g., LAB and fungi), and growing simultaneously in a competitive environment with limited nutrients.

Poster 4

Cryo-EM Structure of Human Long-Chain Acyl-CoA Dehydrogenase and Basis for Substrate Fatty Acyl Chain Specificity

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Background
Long-chain acyl-CoA dehydrogenase (LCAD) is one of the mitochondrial acyl-coenzyme A dehydrogenases (ACADs) that catalyze the first enzymatic step in fatty acid β-oxidation (FAO). LCAD catalyzes the conversion of acyl-coenzyme A to trans enoyl-coenzyme A, specifically for straight chain fatty acids with 12-18 carbons with preference for palmitoyl-CoA (Ikeda et al., 1985). LCAD has been identified as a key dependency in Glioblastoma (Puca et al., 2021). Commonly occurring point mutations that disrupt LCAD enzyme function have also been implicated as a risk factor in infant death syndrome, so it plays an important role in human disease (Goetzman et al., 2014). Crystal structures of short-chain, medium-chain, very long-chain acyl-CoA dehydrogenase (SCAD, MCAD, VLCAD) were determined previously, but not for LCAD. Here we report first structure of LCAD by cryo-EM single particle analysis.

Goals: Obtain the high-resolution structure of LCAD by cryo-EM single particle analysis and map the binding pocket with other ACADs to identify substrate specificity.

Methods: Purified recombinant human LCAD protein (1 mg/ml) was used to prepare cryo-EM specimen using Vitrobot mark IV. Cryo-EM datasets were collected at a 300 kV Titan Krios microscope (ThermoFisher Scientific) equipped with a Falcon 4 detector. Single particle image analysis was carried out using Relion 4 software. To build the structures of the LCAD, an Alphafold2 model was model docked into the cryo-EM electron density map and further refined using Phenix and Coot.

Results: Single-particle analysis yielded a density map of LCAD at 3 Å. From this density map, we built an atomic model, that is a homotetramer and two protomers resolved with the FAD cofactors. We have also solved the crystal structure of the close homologue Human MCAD protein with hexanoyl-CoA and octanoyl-CoA bound in the active site. Comparing the substrate binding pocket of LCAD with substrate bound MCAD and other ACADs identified crucial residues differences that allow up to 18 carbon long chain substrates to be accommodated into LCAD but not to MCAD, explaining the substrate specificity for these enzymes.

Conclusions: First structural evidence for substrate specificity of Long-chain acyl-CoA dehydrogenase (LCAD) by cryo-EM single particle analysis.

Acknowledgements: Some of this work was performed at the NCCAT and the Simons Electron Microscopy Center located at the New York Structural Biology Center, supported by the NIH U24 GM129539, and by grants from the Simons Foundation SF349247 and NY State Assembly.

Poster 53

Boceprevir is an HCV NSP3 inhibitor that was explored as a repurposed drug for COVID-19. It inhibits the SARSCoV-2 main protease (MPro) and contains an α-ketoamide warhead, a P 1 β-cyclobutylalanyl moiety, a P2 dimethyl cyclopropyl proline, a P3 tert-butyl glycine, and a P4 N-terminal tert-butyl carbamate. By introducing modifications at all four positions, we synthesized 20 boceprevir-based MPro inhibitors including PF-07321332 and characterized their MPro inhibition potency in test tubes (in vitro) and 293T cells (in cellulo). Crystal structures of MPro bound with 10 inhibitors and cytotoxicity and antiviral potency of 4 inhibitors were characterized as well. Replacing the P1 site with a β-(S-2-oxopyrrolidin-3-yl)-alanyl (Opal) residue and the warhead with an aldehyde leads to high in vitro potency. The original moieties at P2, P3 and the P4 N-terminal cap positions in boceprevir are better than other tested chemical moieties for high in vitro potency. In crystal structures, all inhibitors form a covalent adduct with the MPro active site cysteine. The P1 Opal residue, P2 dimethyl cyclopropyl proline and P4 N-terminal tert-butyl carbamate make strong hydrophobic interactions with MPro, explaining high in vitro potency of inhibitors that contain these moieties. A unique observation was made with an inhibitor that contains a P4 N-terminal isovaleramide. In its MPro complex structure, the P4 N-terminal isovaleramide is tucked deep in a small pocket of MPro that originally recognizes a P4 alanine side chain in a substrate. Although all inhibitors show high in vitro potency, they have drastically different cellular potency to inhibit ectopically expressed MPro in human 293T cells. In general, inhibitors with a P4 N-terminal carbamide or amide have low cellulo potency. This trend is reversed when the P4 N-terminal cap is changed to a carbamate. The installation of a P3 O-tert-butyl-threonine improves cellulo potency. Three molecules that contain a P4 N-terminal carbamate were advanced to cytotoxicity tests on 293T cells and antiviral potency tests on three SARS-CoV-2 variants. They all have relatively low cytotoxicity and high antiviral potency with EC50 values around 1 μM. A control compound with a nitrile warhead and a P4 N-terminal amide has undetectable antiviral potency. Based on all observations, we conclude that a P4 N-terminal carbamate in a boceprevir derivative is key for high antiviral potency against SARS-CoV-2.

Poster 5

A Peptoid Interleukin-15 Receptor Antagonist Suppresses Inflammation and Arthritis in Mice

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Background: Rheumatoid arthritis (RA) is an autoimmune disease that most commonly affects the joints as well as the cardiovascular or respiratory systems. IL-15 is a cytokine that stimulates the generation of memory CD8 T cells and natural killer (NK) cells and has been placed at the apex of the proinflammatory cytokine cascade. IL-15 is constitutively up-regulated in synoviocytes of RA patients and is a potent T-cell attractant, and therefore, may play a major role in the pathogenesis of RA.

Hypothesis/Goals: To discover a novel peptoid antagonist that targets the interleukin-15 (IL-15) receptor and to evaluate its therapeutic efficacy in the treatment of inflammation and arthritis.

Methods: A new compound (IFRA3, interleukin-15 receptor antagonist 3) was discovered using a unique on-bead two-color combinatorial cell screening of a peptoid library. The direct interaction of IFRA3 with IL-15 receptor was confirmed by *in vitro* pull-down and thermal shift assays. To determine the effects of IFRA3Q1 (a tetramer of IFRA3) on the function of IL-15, CTLL-2 cells (whose growth depends on IL-15 activity) were used to assess IL-15 activity. The efficacy of IFRA3 in treating inflammation and arthritis was evaluated in carrageenan-induced inflammation mouse model and collagen-induced arthritis mouse models.

Results: IFRA3Q1 inhibited the function of IL-15 in a dose dependent manner. In the inflammation induced mouse models a decreased swelling of the mice footpad and reduced levels of pro-inflammatory cytokines in the IFRA3Q1-treated group. Furthermore, IFRA3Q1 reduced arthritis symptoms in collagen-induced arthritis in DBA/1J mice and showed a reduced levels of NK cells, NKT cells, memory $CD8^+$ T cells and naïve T cells in the spleens of treated mice. In addition, these mice also exhibited a reduced the levels of inflammatory cytokines, TNF-α, INF-γ and IL-6 in the plasma.

Conclusion: By binding to and inhibiting the function of IL-15 receptor, IFRA3Q1 exhibited significant anti-arthritis activity. Our findings suggest that IFRA3Q1 represents a new paradigm for arthritis therapy by targeting IL-15 signaling.

Acknowledgements: This work was supported by the Innovative Research Award from The Rheumatology Research Foundation (B Guo, DG Udugamasooriya, and C Mohan) and the University of Houston Drug Discovery Institute Research Seed Grant (B Guo, DG Udugamasooriya, and C Mohan).

Poster 6

FGF13 Ligands Represent Promising Scaffolds for the Development of New Anti-Pain Medications

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Background: Pain is a multifactorial symptom that is directly and indirectly associated with a wide array of acute and chronic clinical conditions. Despite the wide variety of pathologies associated with pain, the most used FDA approved drug classes for pain are opioids, which are Schedule 2 controlled substances with major abuse liabilities. Thus, a paradigm shift toward medications based on druggable targets restricted to pain mechanisms is needed. From the central to the peripheral nervous system, there are nine voltage-gated Na+ channel (Nav) isoforms (Nav1.1-1.9) heterogeneously expressed throughout the human body some of which mediate peripheral pain signaling. With respect to a unique class of intracellular fibroblast growth factors (FGF11-14), advancements in our knowledge have highlighted this group as intrinsic modulators of Nav channels in the context of pain signaling. Under pain-related conditions, firing in peripheral dorsal root ganglia (DRG) sensory neurons is increased upon activation of Nav1.7 through enhanced protein-protein interactions with FGF13. Genetic knockout of FGF13 has potent and selective anti-nociceptive effects in preclinical pain models, while a genetically inherited mutation that reduces FGF13 protein expression has been identified in patients with enhanced tolerance to pain. Thus, by interrogating the biologically relevant pair of FGF13 and the Nav1.7 channel, the potential to create a new drug class based on protein-protein interactions that specifically target pain relevant pathways could advance non-opiate pain management.

Hypothesis/Goals: Given the literature surrounding the FGF13/Nav1.7 interaction and its relevance in the context of pain, it reasonably stands that disrupting or modifying this specific interaction by the means of small molecules could provide a therapeutic strategy to develop new first-in-class drugs for pain management.

Methods: Homology modeling, virtual screening, split-luciferase complementation assay (LCA).

Results: Analysis of the FGF13 crystal structure revealed four potential cavities at the FGF13 surface one of which, predicted to be at the FGF13/Nav1.7 interface, was chosen for a 50 million compound virtual screening (VS). As a result of the VS, 127 small molecules were selected and prioritized based on synthetic derivation and optimization potentials leading to a list of 20 compounds. At a single concentration of 50 μM, these top 20 hits were screened using the split-luciferase complementation assay (LCA) in which the FGF13/Nav1.7 protein complex was reconstituted in cells by the means of transient transfection. In the LCA, 5 out of the 20 prioritized hits showed statistically significant activities compared to relative controls and are currently being further evaluated for functional activity against Nav1.7-mediated currents.

Conclusions: FGF13 ligands represent novel scaffolds suitable for future drug optimization and mechanistic studies.

Acknowledgments: We acknowledge our funding sources as follows: NIH R01MH124351 (FL), NIH 1R01MH132226 (FL), U18DA052504 (FL), Technology Commercialization Program (FL).

Poster 7

Development of an in vitro Assay Screening Funnel to Enable the Discovery of Potent PRMT1 Inhibitors

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Background

With limited therapeutic options and poor overall 5-year survival rates, pancreatic cancer is estimated to become the second leading cause of cancer deaths by 2030. We identified protein arginine N-methyltransferase 1 (PRMT1) as a novel genetic vulnerability in pancreatic ductal adenocarcinoma (PDAC) through an *in vivo* loss-of-function screen. PRMT1 catalyzes the formation of asymmetric dimethylarginine (ADMA) and is the predominant Type I enzyme in the PRMT family. The potential benefits from inhibiting PRMT1 in a clinically challenging context led us to embark upon a drug discovery effort focused on the identification of novel small molecule inhibitors of PRMT1 activity.

Goal

Our goal was to develop a robust screening funnel that included *in vitro* biochemical and cellular automated high throughput assays. This was essential to support medicinal chemistry efforts in the design and synthesis of a proprietary series of potent PRMT Type I inhibitors.

Methods

The enzymatic activity of recombinant full-length human PRMT1 was measured using the LANCE TR-FRET assay. A cellular target engagement assay in RKO cells was developed and optimized for the detection of ADMA. Long-term phenotypic assays were also established using PDAC models DOHH2, PATC53 and CFPAC1.

Results

IACS-53152 inhibited enzymatic activity of the full-length PRMT1 recombinant protein. Target engagement was observed in RKO cells, with the reduction of ADMA. We also observed reduced viability of DOHH2 and PATC53 cells.

Conclusions

In conclusion, our efforts led to the discovery of the preclinical development compound IACS-53152, a nanomolar Type I PRMT inhibitor in enzymatic, target engagement and phenotypic assays.

Acknowledgements

UT MD Anderson Moon Shots Program; Sheikh Ahmed Center for Pancreatic Cancer Research; Pancreatic Cancer Action Network; Harrington Discovery Institute.

Poster 8

Targeting the Nav1.6:GSK3β Protein:Protein Interaction Complex to Mitigate Hippocampal Hyperexcitability in Neuropsychiatric Disorders

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Background: A vast array of neuropsychiatric disorders are facilitated by aberrant hippocampal network activity. The hippocampus plays a critical role in learning, memory formation, social cognition, and emotional processing. A multitude of recent studies indicate a causative relationship between hippocampal hyperexcitability and memory impairment, cognitive deficits, and epileptiform activity. Voltage-gated Na^+ channels (Nav channels) have critical regulatory roles in synaptic function and neuronal firing. Of the three Nav channel isoforms expressed in the adult human brain, (Nav 1.1, 1.2, and 1.6), Nav1.6 is the most densely expressed and plays a critical role in action potential initiation due to its subcellular localization at the axon initial segment. Thus, targeting the Nav1.6 macromolecular complex represent a promising strategy for modulation of neuronal excitability. Recent studies from our laboratory have revealed that glycogen synthase kinase 3β (GSK3β) binds the Nav1.6 C-terminal tail and phosphorylates the T1938 residue of its C-terminal domain, indicating that GSK3β regulates the Nav1.6 channel via a dual-function scaffolding and phosphorylation mechanism. Functionally, genetic silencing of GSK3β suppresses Nav1.6-encoded currents, while overexpression produces opposing phenotypes. This evidence suggests that the Nav1.6:GSK3β PPI interface represents a promising target for alleviation of aberrant hippocampal hyperexcitability in neuropsychiatric disorders.

Hypothesis/Goals: The goals of this study are to identify the critical residues conferring Nav1.6:GSK3β complex formation, optimize a chemical probe identified to inhibit the PPI complex, and evaluate this compound for functional modulation of Nav1.6-mediated hyperexcitability and other disease-related phenotypes in hippocampal neurons. We hypothesize that pathological hippocampal hyperexcitability can be diminished through small-molecule modulation of the Nav1.6:GSK3β PPI complex.

Methods: Split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), whole-cell patch clamp electrophysiology, ex-vivo whole-cell voltage clamp recordings

Results: We have identified a chemical probe that exhibits inhibition of Nav1.6:GSK3β complex assembly in the LCA and appreciable binding to both proteins using SPR. A mutagenesis screen of GSK3β and the Nav1.6 CTD has revealed putative regions of the PPI interface that we predict are critical for conferring the functional effects of our representative ligand. Initial studies have revealed that our compound decreases Nav1.6 channel activity *in vitro* in a manner reminiscent of genetic silencing GSK3β and reduces pathological hyperexcitability *ex-vivo* in 3x-Tg-AD mice.

Conclusions: The Nav1.6:GSK3β PPI complex is a key regulatory element contributing to hippocampal excitability, and further elucidation of the molecular determinants of this interaction will guide optimization of our representative ligand for modulation of the Nav1.6:GSK3β complex.

Acknowledgements: We acknowledge our funding sources as follows: NIH Grant R01MH12435101 (FL), R01ES031823 (FL); U18DA052504 (FL); Technology Commercialization Program, HAMBP training grant no. T32GM008280 (NMD), NIH Grant no. T32AG0679 (TB), PhRMA Foundation (PAW).

Poster 54

AKR1C3 Inhibitors Induce Allosteric Changes in Ligand Binding Interactions - Implications for Androgen Receptor Coactivation

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Prostate cancer is a common cancer affecting men in the United States, and despite the effectiveness of androgen deprivation therapy in treating it, castration-resistant prostate cancer (CRPC) often develops, leading to poor outcomes and limited treatment options. Therefore, there is a need for novel drug targets to inhibit the androgen signaling axis. A promising target is Aldoketoreductase 1C3 (AKR1C3), which is overexpressed in CRPC tumors and activates the androgen receptor. AKR1C3 activates the androgen receptor both through the enzymatic catalysis of potent androgens as well as coactivation via binding directly to the androgen receptor. The compound GTx-560 has been shown to inhibit both enzymatic production of potent androgens and direct coactivation of the receptor, but its mechanism of action is not well understood. Our hypothesis is that GTx-560 induces allosteric changes in the structure of AKR1C3, rendering the protein coactivation-incompetent in a distinct manner from other enzymatic inhibitors such as indomethacin which do not inhibit coactivation. To test this hypothesis, we used solution NMR to observe potential allosteric effects of ligand binding. Our results show that GTx-560 and indomethacin eliminate the presence of slow exchange in HNCO spectra, suggesting conformational selection upon ligand binding. Chemical shift perturbations (CSPs) in HNCO spectra due to ligand binding also reveal many large CSPs distant from the binding site, indicating the ability of these ligands to act as allosteric modulators of AKR1C3 structure. Preliminary residual dipolar coupling data show that the uninhibited solution state ensemble of AKR1C3 is dynamic, further suggesting that ligand binding may be selecting distinct conformations sampled by AKR1C3.

Supported by a Cancer Therapeutics Training Program fellowship (CTTP), (CPRIT Grant No. RP210043).

Poster 9

Evolution of SARS-CoV-2 Main Protease Inhibitors Utilizing X-ray Crystallography

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Background: As an essential enzyme of SARS-CoV-2, the COVID-19 pathogen, main protease (M^{Pro}) is a viable target to develop antivirals for the treatment of COVID-19.

Hypothesis/Goals: We were uniquely positioned as a lab to transition into discovering small molecule inhibitors for M^{pro} of SARS-CoV-2 at the onset of the pandemic.

Methods: By varying chemical compositions at both P2 and P3 positions and the N-terminal protection group, we synthesized 18 tripeptidyl M^{Pro} inhibitors that contained an aldehyde warhead and β-(S-2-oxopyrrolidin-3-yl)-alaninal (Opal) at the P1 position. Boceprevir was another inhibitor that showed potency against the SARS-CoV-2 M^{Pro} and contains an α-ketoamide warhead, a P1 β-cyclobutylalanyl moiety, a P2 dimethylcyclopropylproline, a P3 *tert*-butylglycine, and a P4 *N*-terminal *tert*-butylcarbamide. By introducing modifications at all four positions, we synthesized 20 boceprevir-based M^{Pro} inhibitors. X-ray crystal structures of M^{Pro} bound with all inhibitors were determined.

Results: In all structures of the Opal based inhibitors, the M^{Pro} active site cysteine interacts covalently with the aldehyde warhead of the bound inhibitor to form a hemithioacetal. Systematic characterizations of these inhibitors were conducted, showing that all the electron density around residues 45-50 is relatively weaker compared to the other parts of M^{pro}, indicating that the M49-containing loop is flexible after the inhibitor is bound. During crystallographic analyses of M^{Pro} crystals that were exposed to the air, a uniquely Y-shaped, S–O–N–O–S-bridged post-translational cross-link that connects three residues C22, C44, and K61 at their side chains was frequently observed.

Conclusions: Replacing the P1 site with an Opal residue and the warhead with an aldehyde leads to high *in vitro* potency. As a novel covalent modification, this cross-link serves potentially as a redox switch to regulate the catalytic activity of M^{Pro}, a demonstrated drug target of COVID-19. The formation of this linkage leads to a much more open active site that can potentially be targeted for the development of novel SARS-CoV-2 antivirals.

Acknowledgements: This work was supported by the Welch Foundation (Grants A-1715 and A-2089), the National Institutes of Health (Grants R21AI164088, R21EB032983, R35GM143047, R35GM145351, P41GM128577, and R01GM138863), and the Texas A&M X Grants Mechanism.

Poster 55

Development and In Vivo Evaluation of Potent and Selective Inhibitors of CDK11, A Novel Transcription Associated Cyclin Dependent Kinase

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Background: Cyclin dependent kinases (CDKs) are a family of serine/threonine kinases whose activity requires the formation of heterodimer complexes with specific cyclin partners. CDK family members are roughly categorized as either cell cycle-associated or transcription-associated, although they are involved in a multitude of cellular functions. While dual CDK4/6 inhibitors have gained FDA approval and a number of inhibitors that target additional family members are in clinical trials, interrogation of CDK11 target biology has been limited by a lack of pharmacological tools. To date, no potent and selective inhibitors of CDK11 that are suitable for in vivo studies have been reported.

Goals: Develop potent and selective tools for evaluating the pharmacological inhibition of CDK11 *in vivo*

Methods: Given the lack of known CDK11 inhibitors at the onset of the program, we screened a focused library of hinge-binder directed compounds to identify starting points for development. Two structurally distinct micromolar hits were selected for optimization, and with the support of CDK11 homology models, were developed into selective *in vivo* tool compounds IACS-054647 and IACS-074527.

Results: Both compounds exhibit low nanomolar activities in biochemical and cellular target engagement assays and are potent in a PATC53 phenotypic assay. Additionally, they demonstrate excellent selectivity across the CDK family (>100x over CDK9, the closest family off-target) and the broader kinome (>200x over a panel of 340 wild- type kinases). IACS-054647 exhibits a favorable *in vitro* and *in vivo* pharmacokinetic profile, suitable for oral dosing in mouse models. Exposures of IACS-074527 are limited with oral dosing; however, high metabolic stability allows for sufficient exposures to be achieved with intraperitoneal dosing.

Conclusions: Pharmacological inhibition of CDK11 induced a potent anti-proliferative response across a panel of PDAC cell lines, revealing a disconnect between genetic ablation of the protein versus pharmacologic CDK11 inhibition. Furthermore, minimal tumor growth inhibition was observed in mouse efficacy studies due to dose limiting toxicities resulting from systemic exposure. Taken together, the data suggests that while there is a dependency of a subset of tumor cells on CDK11, systemic inhibition in a whole organism is not tolerated, thereby limiting the therapeutic potential.

Poster 11

Evaluation of SARS-CoV-2 Main Protease Inhibitors Using a Novel Cell-Based Assay

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Abstract

As an essential enzyme of SARS-CoV-2, main protease (M^{Pro}) triggers acute toxicity to its human cell host, an effect that can be alleviated by an M^{Pro} inhibitor. Using this toxicity alleviation, we developed an effective method that allows a bulk analysis of the cellular potency of M^{Pro} inhibitors. This novel assay is advantageous over an antiviral assay in providing precise cellular M^{Pro} inhibition information to assess an M^{Pro} inhibitor. We used this assay to analyze 30 known M^{Pro} inhibitors. Contrary to their strong antiviral effects and up to 10 μM, 11a, calpain inhibitor II, calpain XII, ebselen, bepridil, chloroquine, and hydroxychloroquine showed relatively weak to undetectable cellular M^{Pro} inhibition potency implicating their roles in interfering with key steps other than just the M^{Pro} catalysis in the SARS-CoV-2 life cycle. Our results also revealed that MPI5, MPI6, MPI7, and MPI8 have high cellular and antiviral potency. As the one with the highest cellular and antiviral potency among all tested compounds, MPI8 has a remarkable cellular M^{Pro} inhibition IC_{50} value of 31 nM that matches closely to its strong antiviral effect with an EC_{50} value of 30 nM. Therefore, we cautiously suggest exploring MPI8 further for COVID-19 preclinical tests.

Funding source: Welch Foundation, National Institute of Allergy and Infectious Disease, The Texas A&M University President's Excellence Fund, TAMU COS Strategic Transformative Research Program, Texas A&M X grants

Poster 12

EnGens: A Computational Framework for Generation and Analysis of Representative Protein Conformational Ensembles

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Background

Proteins are dynamic macromolecules that execute vital functions in the cells. The structure of a protein determines its function, but this structure is not static. Instead, proteins change their conformation to achieve various functions. Understanding the conformational landscapes of proteins is essential to understand their mechanism of action. A conformational ensemble is a set of representative protein conformations that summarizes such complex landscapes and provides better insights into protein function than a single conformation. Recent advances in computational methods are leading to an increased number of available structural datasets which span conformational landscapes and enable building representative conformational ensembles. Generating a representative ensemble from such datasets is not an easy task and while many methods have been developed to tackle it, there is no unified framework for such analysis.

Goals

In this work we propose and develop a pipeline that we call EnGens. EnGens collects different methods into a unified framework for generating and analyzing protein conformational ensembles from datasets of protein structures. In our work we: (1) provide an overview of existing methods and tools for protein structural ensemble generation and analysis; (2) unify the existing approaches in an open-source Python package, a portable Docker image and provide interactive visualizations within a Jupyter Notebook pipeline; (3) test our pipeline on a few canonical examples found in the literature.

Methods

EnGens pipeline consists of four consecutive workflows with a goal of extracting a representative ensemble from a large dataset of protein structures. First, the features are extracted for each conformation in the dataset. Second, the dimensionality of the extracted featurization is reduced. Third, conformations are clustered into groups of similar conformations. Finally, a single representative conformation is extracted from each cluster, forming a diverse representative ensemble. The whole analysis is visualized, and the analysis of the clusters is available as part of the final workflow.

Results

EnGens pipeline is tested on structural datasets for three different molecular systems: a large PI3K protein complex, a peptide ligand Compstatin and a small drug Nelfinavir. For each of the systems EnGens was able to recover diverse ensembles that coincided with previously reported results.

Conclusions

EnGens is a novel tool for the end-to-end processing of large protein structural datasets with the aim of generating and analyzing representative protein conformational ensembles. EnGens ensembles can be useful for many downstream tasks related to drug discovery such as molecular docking and drug-target interaction prediction. Additionally, EnGens can serve as a platform for further algorithmic development. Overall, we see the EnGens pipeline becoming part of many new efforts to utilize the structural data generated by novel structure prediction tools.

Acknowledgements
A.C. and L.K. have been supported in part by National Institutes of Health NIH [U01CA258512]. Other support included: University of Edinburgh and Medical Research Council [MC UU 00009/2 to D.D.]; Computational Cancer Biology Training Program fellowship [RP170593 to M.M.R.]; The Brazilian National Council for Scientific and Technological Development [CNPq no. 440412/2022-6 to G.Z]; University of Houston Funds and Rice University Funds.

Poster 13

Developing an Animal Model to Evaluate Mycophenolic Acid-induced Diarrhea

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Abstract: Purpose: Gastrointestinal side effect is a serious concern of mycophenolic acid (MPA), an active metabolite of the prodrug mycophenolic mofetil (MMF) that is used as an immunosuppressive agent in clinical settings. The purpose of this study is to optimize animal models to mimic the symptom MPA-induced diarrhea (MID) in humans for future antidiarrhea efficacy screening. **Method**: F344 rats were used to develop the model, fecal conditions and body weigh were evaluated every day after administration of MMF, histological exam was conducted to evaluate the intestinal tissue damages. The exposure of MMF and its metabolites in rats' tissues were evaluated using LC-MS/MS method. **Results**: The result show that the MID was dose-depend in rats, diarrhea occurred from day 4, and all the rats became severe diarrhea on day 8 and day 9, then recovered on day 11, only 50% of rats showed MID with a lower dose of MMF, and no rats survived with 100 mg/kg of MMF; significant differences in MMF-induced diarrhea severity were observed with the female rats as they experienced greater GI side effects than the male rats. The PK profile of MPA and MPAG, the concentration of MPA and MPAG on day 6 was higher than on day 3. Additionally, the tissue exposure was 4-fold higher in the intestine in female than that in male. **Conclusion**. A rat model for MID was successfully developed with appropriate diarrhea grade, body weight loss and tissue damage.

Poster 14

A Genetically Encoded Phage Display Technique Targeting Bromodomain Protein 9 (BRD9) for Discovery of Peptide Inhibitors

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Background: Acetylation is the most dynamic protein translational modification often associated with increased DNA accessibility and transcription. These acetylated histones recruit transcription and remodeling factors, and their deregulation could result in aberrant expression of survival and growth-promoting genes. Recognition of acetylated lysine is principally mediated by bromodomains (BRDs). Recent studies have shown that BRD9 is preferentially used by cancers that harbor SMARCB1 abnormalities such as malignant rhabdoid tumors and sarcomas. BRD9 is an essential component of the SWI/SNF chromatin remodeling complex, and a critical target required in acute myeloid leukemia. As the biological function of BRD9 in tumorigenesis becomes clear, bromodomain of BRD9 has become a new hot target for effective tumor treatment method.

Hypothesis: BRD9 has a different architecture than other bromodomains. Due to larger hydrophobic cavity of BRD9, it can recognize longer propionyl and butyryl marks on lysine. Thus, N^{ε}-butyryl-lysine (BuK) can selectively bind to BRD9. Our group is specialized in the amber suppression-based noncanonical amino acid (ncAA) mutagenesis technique. Herein (Fig 1), we propose to extend this technique using phage-displayed ncAA-containing peptide libraries for the identification of high-affinity and highly selective BRD9 inhibitors.

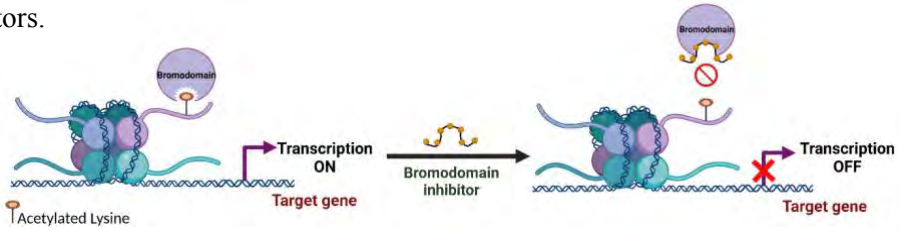

Fig 1. *Schematic representation of bromodomain 9 peptide inhibitor turning off transcription process.*

Methods: Phage display is a technique for rapid screening of potential ligands. It is facilitated through the creation of a genetic fusion between a randomized peptide sequence and pIII, a phage coat protein. This direct link between genotype and phenotype allows for peptide screening. We utilized Phage-assisted, Active site Directed Ligand Evolution (PADLE) approach to target BRD9. To identify the binders, we choose 7mer phagemid library which generates $1.5x10^{10}$ randomized possible peptides displayed on PIII of bacteriophages. The peptides screened were tested for binding using Bio-Layer Interferometry and inhibition by Alpha Screen assay. Based on Structure Activity Relationship studies second-generation focused selection was done to screen for more potent peptides.

Results: Studies resulted in identification of BRD9 binders with increased specificity and varying affinities. The estimated IC_{50} for peptide *EBuKAYPVC* was 0.74 µM and K_d was determined to be 0.53 µM. Focused selection peptide *QBuKRYPVC* inhibits protein with IC_{50} 0.54 µM and K_d value 0.104 µM.

Conclusion: Selected peptides successfully bind and inhibit BRD9, and we aim to further optimize its cellular target engagement and on-target effects in inhibiting leukemia cell growth and suppressing the expression of BRD9 target genes.

Acknowledgement: : This work was supported by the Welch Foundation grant A-1715, National Institutes of Health awards R01CA161158 and R35GM145351, Texas A&M X-Grants mechanism, Texas A&M Chancellor EDGES Fellowship and Cancer Prevention and Research Institute of Texas grant RP170797.

Poster 15

Modulation of Sodium Channel Inactivation and Medium Spiny Neuron Excitability by Small Molecule Targeting the FGF14:Na_v1.6 Complex

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Background: Voltage-gated Na^+ (Na_v) channels are determinants of action potential (AP) firing. Given this function, compounds targeting the Na_v channel have been employed to treat conditions caused by perturbed neuronal excitability. Most compounds, however, target regions of the Na_v channel that are highly conserved across isoforms (Na_v1.1-Na_v1.9). Resultantly, these compounds lack isoform selectivity, giving rise to off-target side effects.

Hypothesis/Goals: As a strategy to achieve isoform selectivity, we sought to target the Na_v channel auxiliary protein fibroblast growth factor 14 (FGF14), which interacts with the C-terminal domains (CTD) of Na_v channels and confers differential regulation of Na_v channel isoforms.

Methods: The split-luciferase complementation assay (LCA); surface plasmon resonance; whole-cell patch-clamp electrophysiology; *in vivo* single unit recordings; reward cue task.

Results: Focusing on the complex formed between FGF14 and Na_v1.6 on account of its regulation of the excitability of medium spiny neurons (MSN) of the nucleus accumbens (NAc), which is a central hub of reward circuitry, we tested the effects of ~45,000 compounds on FGF14:Na_v1.6 complex assembly. This screening identified 1028, which selectively modulated Na_v1.6 channel inactivation. These effects were demonstrated to occur through 1028's modulation of the interaction between $FGF14^{R117}$ and the $Na_v1.6^{D1846:R1866}$ salt bridge and dependent upon an intact interaction between the III-IV linker and CTD of the Na_v1.6 channel. Through these effects on Na_v1.6 channel inactivation, 1028 potentiated the excitability of MSNs of the NAc. These electrophysiological changes were shown to correlate with alterations in behaviors associated with motivation.

Conclusions: Collectively, these results provide evidence for a mechanism of small molecule-mediated regulation of Na_v channel inactivation that can affect behaviors associated with motivation by increasing excitability of neurons in the NAc.

Acknowledgements: NIH Grant R01MH095995 (FL), R01MH12435101 (FL), NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01DA038446 (JZ), P30DA028821 (JZ), CPRIT RP150578 (CS), and HAMBP training grant no. T32GM008280 (NMD).

Poster 58

Remodeling DNA Methylation Landscapes to Prolong CAR T-cell Activity to Overcome Cancer Relapse

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Background: Although chimeric antigen receptor (CAR) T-cell based immunotherapy has achieved promising outcomes in patients with lymphoma and leukemia over the past five years, antigen escape and gradual reduction of T cell activity led to cancer relapse in nearly 50% of patients after initial treatment.

Hypothesis: Recent studies uncovered the critical role of DNA methylation in boosting CAR T activity, hence pointing to the possibility of overcoming cancer relapse through precise control over the DNA methylation regulatory pathways in therapeutic T cells.

Methods: Novel classes of DNMT enzyme inhibitors and TET protein engineering technologies are used to remodel the DNA methylation landscapes in CAR T-cells. In vitro co-culture of CD19 expressed Raji lymphoma cells and CAR T-cells and in vivo injection of CAR T-cells into tumor bearing mice systems are applied to test the CAR T-cell activities after remodeling their DNA methylation landscapes.

Results: We identified two classes of small molecules which might have inhibitory effects on DNMT1 enzyme. In vitro enzymatic activity assay indicated that several candidate compounds had strong DNMT1 inhibition. The inhibitory effect of these candidates was further tested in a co-culture system composed of CD19-positive Raji lymphoma cells and engineered CAR T-cells. Luciferase assay showed that some candidates could significantly increase luciferase level and enhance CAR T-cell activity. To modulate TET activity, we developed more than 15 nanobodies against TET2. Immunofluorescence staining and immunoprecipitation experiments showed that two nanobodies showed strong colocalization with TET2, suggesting that they can recognize TET2 efficiently. In parallel, the nanobodies against TET2 were fused with various proteasomal degradation systems to modulate intracellular TET2 protein levels and will be used in the following experiments to modulate TET2 activity in CAR T-cells.

Conclusions: We identified candidates that can specifically inhibit DNMT1 activity and boost CAR T-cell activity in vitro and produced TET2-specific nanobodies, the latter of which can be used to modulate TET2 activity. The CAR T-cell modulating activities of both DNMT1 inhibitors and TET2 nanobodies will be tested in mouse models of CD19-positive tumor, thereby establishing the preclinical rationale of manipulating the DNA methylation landscapes to benefit CAR T-cell therapy.

Acknowledgement: Shaohai Fang is the Cancer Therapeutics Training Program fellow (supported by CPRIT Grant No: RP210043).

Poster 16

APE-Gen2.0: Conformational Ensemble Generation of Peptides Bound to MHC Receptors

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Background: The recognition of peptides bound to Major Histocompability Complexes (MHC) receptors by T-cell Receptors (TCRs) is a determinant to trigger the adaptive immune response. The exact interaction features that drive the immune response when TCRs bind are still unknown. However, studies have suggested that the geometry of the joint peptide-MHC (pMHC) structure plays an important role in T-cell recognition. As such, knowing/predicting the pMHC structure can be vital for therapeutics, such as peptide vaccine development, or T-cell based immunotherapy.
Hypothesis/Goals: In an effort to contribute to this field we previously developed APE-Gen, a tool for generating conformational ensembles of pMHC complexes by sequence alone. However, predicting the fine-grained structural effects that certain peptide modifications (such as single-point mutations or post-translational modifications) will have on the pMHC complex is still a very difficult task. There is a need for accurate modeling of pMHC complexes that exhibit these modifications, as well as other non-canonical geometrical cases, as there is evidence that they are excellent targets for cancer therapy.
Methods: To this end, we have developed APE-Gen2.0, a tool that improves upon its predecessor. Some of the improvements include: (i) the ability to model peptides that have different types of post-translational modifications such as phosphorylation, nitration and citrullination; (ii) a new and improved anchor identification routine in order to identify and model peptides that exhibit a non-canonical anchor conformation; (iii) a web server that provides a platform for easy and accessible pMHC modeling.
Results: APE-Gen2.0 is able to model most pMHC structures in public databases with near-native accuracy (< 1 Å). It outperforms its predecessor, as well as other state-of-the-art pMHC modeling tools in the literature. Most importantly though, APE-Gen2.0 is able to correctly identify and model non-canonical cases in regards to both geometry and biochemical properties that previously could not be modeled.
Conclusions: We developed APE-Gen2.0, a tool that expands the pMHC modeling repertoire, by including peptides that are important for cancer treatments and the field of immunoinformatics in general.
By using APE-Gen2.0 to generate pMHC structures, we hope to expand and improve processes that were previously dependent solely on sequence data, such as affinity and immunogenicity prediction/assessment, and are crucial for therapeutics. Lastly, APE-Gen2.0 will be provided as a web server that provides a platform for easy and accessible pMHC modeling, which will be freely available at https://apegen.kavrakilab.org, in order to facilitate the use of pMHC to downstream tasks by the community.
Acknowledgements: R Fasoulis and LE Kavraki are supported in part by NIH U01CA258512 and by Rice University funds. DA Antunes and MM Rigo are supported by a Computational Cancer Biology Training Program fellowship (CPRIT Grant No. RP170593). DA Antunes is also supported in part by University of Houston funds.

Poster 17

Design, Synthesis and Evaluation of PKMYT1 Inhibitors for Cancer Treatment

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Background:

In Eukaryotic cell cycles, the WEE kinase family plays a pivotal role to maintain genomic stability. PKMYT1 inhibits CDK1 at the Thr14 and Tyr15 residues, interrupting the cell cycle at the G2 phase to repair damaged DNA. In contrast, WEE1 phosphorylates CDK1 at the Tyr15 residue blocking the cell cycle at the G1/S or G2/M transition. Interestingly, in over 50% of cancer cases, p53 is highly mutated, compromising the G1 checkpoint. As a result, cancer cells rely massively on the G2 checkpoint to repair their damaged DNA. In addition, PKMYT1 is overexpressed in several types of solid tumors including brain cancer, which makes it an attractive therapeutic target.

Hypothesis/Goals:

PKMYT1 is more important for cancer cells than normal cells, making it a promising target for cancer treatment. However, due to the high structural similarity between PKMYT1 and WEE1, the design of selective PKMYT1 inhibitors is very challenging. Therefore, the goal of this project is to design and synthesize selective PKMYT1 inhibitors and to establish a structure-activity relationship in order to facilitate the development of selective, potent, and brain permeable PKMYT1 inhibitors.

Methods:

In this study, computational docking was used to design and synthesize selective PKMYT1 inhibitors. NanoBRET cell-based assays were established for PKMYT1 and WEE1 in order to determine inhibitors Ki and selectivity.

Results:

We have identified selective PKMYT1 inhibitors and established a structure-activity relationship. This initial study provides insight to develop more potent and brain permeable PKMYT1 inhibitors.

Conclusion:

Due to its overexpression in several solid tumors and its critical role in the G2 checkpoint, the understudied PKMYT1 is an essential target for cancer treatment. Therefore, the initial development of selective PKMYT1 inhibitors and the establishment of a structure-activity relationship are crucial steps to designing effective brain permeable PKMYT1 inhibitors for cancer therapy.

Acknowledgments: This project is supported in part by a Cancer Therapeutics Training Program fellowship (CPRIT Grant No. RP210043 to R.G.), NCI SPORE (P50-CA186784 to M.J.E.) SPORE Developmental Research Project to J.W.

Poster 18

Development and Clinical Evaluation of CD5.CAR T-cells in Patients with T-cell Malignancies

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Background: Patients with treatment-refractory or relapsed (r/r) T-cell lymphoma (TCL) and T-cell acute lymphoblastic leukemia (T-ALL) have few therapeutic options and poor prognosis, with 3-year survival not exceeding 20% in aggressive subtypes. Chimeric antigen receptor (CAR) T-cells are very effective in patients with B-cell malignancies but extending its potency to T-cell cancers is difficult due to high risk of CAR-T self-elimination and ablation of normal T-cells expressing the target antigen. We developed a CD5-targeting CAR that, when expressed on T-cells, induces rapid and complete degradation of CD5 thus protecting CAR T-cells from self-targeting (Mamonkin et al., *Blood 2015, CIR 2018*). CD5.CAR T-cells can be expanded in GMP and produce robust cytotoxicity in preclinical models of T-cell malignancies.

Study goals: We aimed to determine whether CD5.CAR T-cells will be safe and will produce clinical benefits in patients with r/r T-cell malignancies. We also aimed to understand the impact of modifications in the cGMP manufacturing process of CD5.CAR T-cells on their anti-tumor activity in patients.

Methods: We conducted a Phase I clinical trial of CD5.CAR T-cells in patients with r/r T-ALL and TCL (NCT03081910) at Baylor College of Medicine. CD5.CAR T-cells were manufactured in BCM cGMP facility from patients or prior stem cell donors. A subset of patients received CD5.CAR T-cell products manufactured with optimized protocols utilizing selected tyrosine kinase inhibitors (TKI) to enrich for highly potent, minimally-differentiated CAR T-cell subsets based on preclinical studies at BCM.

Results: We treated 24 adult and pediatric patients with T-cell malignancies on three dose levels. Infusions were well tolerated, with no Grade 3+ cytokine release syndrome or neurotoxicity observed. Autologous CD5.CAR T-cells produced clinical responses in 4/9 (44%) patients with r/r TCL, including 3 complete remissions (CR) enabling allogeneic stem cell transplant. Two of these patients remain alive 4 years later. Initial responses in patients with T-ALL were rare (1/8 CR, 13%) but were improved in the subsequent cohort who received CD5.CAR T-cells manufactured with TKI on highest dose levels (4/6 MRD-negative CR, 67%). T-cell aplasia was avoided by transient or permanent downregulation of CD5 on normal T-cells.

Conclusion: CD5.CAR T-cells are safe in patients with r/r T-ALL and TCL and produce robust anti-tumor activity. Responses in patients with T-ALL were improved by incorporating TKI into cGMP manufacturing, but these improved processes have not yet been tested with TCL patients. The CD5.CAR T-cell technology, now MB-105, has been licensed to March Biosciences to further close the manufacturing process and prepare it for commercial scale in collaboration with the Cell Therapy Manufacturing Center (CTMC). March Biosciences will advance MB-105 to the next clinical stage of development to validate the safety and efficacy of the improved product in T-cell lymphoma via a company-sponsored Investigational New Drug (IND) application in Q2'2024.

Acknowledgments: CD5.CAR T-cell development and clinical trials were funded by NIH NCI SPORE in Lymphoma and ASH Scholar Award (to M.M.). We also acknowledge the efforts of the March Biosciences team in the continued development of this project.

Poster 19

The Synergistic Antitumor Effect of Irinotecan and Flavonoids on Human Colon Cancer Xenograft Mice

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Abstract:

Background:
Camptothecin (CPT)-11 (irinotecan) is one of the first-line therapeutic agents in the treatment of metastatic colorectal cancer, but its efficacy and safety can be compromised because of its severe side effects, such as gastrointestinal injury/inflammation and severe diarrhea. CPT-11 is a prodrug that is converted to the active cytotoxic molecule SN38 by the action of liver carboxylesterases. SN-38 is subsequently conjugated primarily by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite (SN-38G). However, SN-38G is susceptible to β-linkage cleavage in the intestinal tract, producing free SN-38 which can be reabsorbed. Previous studies reported that natural flavonoids such as wogonin and chrysin have anticancer and anti-diarrheal activities.
Hypothesis/Goals:
We hypothesize that the co-administration of wogonin/chrysin mix with irinotecan, might improve the anti-tumor efficacy without compromising the safety. This study aims to investigate the efficacy and safety of irinotecan when co-administered with flavonoids in human colon cancer xenograft model.
Methods:
Xenograft model has been established using human colorectal adenocarcinoma HT-29 cell line. When the tumor volume was around 500-600 mm^3, the flavonoids mix (wogonin/chrysin) was administered by oral gavage at 100mg/kg per day for three days and then co-administered with CPT-11, intraperitoneally, at two different doses i.e., 50mg/kg and 75mg/kg per day for seven consecutive days. Mice that received CPT-11 monotherapy served as control.
Results:
Our study demonstrated that the tumor volume decreased 36 % with the treatment of 50mg/kg/day CPT-11 plus 100mg/kg/day wogonin/chrysin and 57 % with 75mg/kg/day CPT-11 plus 100mg/kg/day wogonin/chrysin compared to conventional irinotecan treated animals with no major impact on body weights. Interestingly, female mice showed a 2-fold decrease in tumor volume compared to monotherapy group and is statistically significant with $p<0.05$. This confirms the previous reports that females mount a more robust cellular and humoral response, resulting in greater anti-tumor efficacy.
Conclusions:
Taken together, our data show that CPT-11 and flavonoids (wogonin and chrysin) exhibit a gender-specific synergistic anti-tumor effect and can be safely administered together for metastatic colon cancer treatment. Therefore, this combination therapy could be a promising approach in anti-tumor chemotherapy for better clinical outcomes.
Acknowledgements:
NIGMS grant award 1SC2GM135111-01, NIMHD grant award U54MD007605, CPRIT grant RP180748 and RCMI – CBMHR.

Poster 20

Proteomic-based Interactome of Nav1.6 Reveals Sex-specific Biosignatures of Resilience and Vulnerability

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Background: Resilience and vulnerability to neuropsychiatric disorders are linked to molecular changes underlying excitability that are still poorly understood. In previous studies, we identified the voltage-gated Na^+ channel Nav1.6 as a mediator of neuroplasticity induced by environmentally enriched (EC) or isolated (IC) conditions which are used as models for resilience and vulnerability. Protein-protein interactions play a key role in regulating Nav1.6 channel function and whether the relative composition of the Nav1.6 interactome in EC/IC models is different is not known. This study aimed at characterizing the interactome of Nav1.6 in EC/IC models to search for proteomic-based biosignatures of maladaptive plasticity underlying resilience and vulnerability to neuropsychiatric disorders.

Hypothesis/Goals: The hypothesis tested in this study was that the Nav1.6 protein-protein interaction network is differentially regulated by EC/IC in the striatum and hippocampal regions.

Methods: Immunoprecipitation, Western blotting, LC/MS/MS.

Results: To determine the impact of the EC/IC behavioral paradigm on the composition of the Nav1.6 channel macromolecular complex, we housed rats in environmentally enriched (EC) and isolated conditions (IC) for 30 days. The Nav1.6 immunoprecipitated fractions of EC and IC rats revealed 165 and 63 protein interactors of Nav1.6 differentially expressed in the striatum and hippocampus respectively. PANTHER protein class analysis revealed that most of the differentially expressed proteins in the striatum and hippocampus are involved with RNA metabolism (19%) and translation (25%) respectively. Classification by biological process revealed 31% of proteins in the striatum and 34% of proteins in the hippocampus are involved in cellular processes. Also, 32% and 31% of proteins in the striatum and hippocampus respectively are involved in binding while 23%(striatum) and 25%(hippocampus) have catalytic activity.

Conclusion: The results of this study offer valuable information for identifying new molecular targets suitable for the development of novel neurotherapeutics.

Acknowledgments: We acknowledge our funding sources as follows: NIH R01MH124351 (FL), NIH 1R01MH132226 (FL), and U18DA052504 (FL).

Poster 21

Development of Selective ENL Inhibitors and PROTACS for Acute Myeloid Leukemia Therapy

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Abstract: Acute myeloid leukemia (AML) is the most diagnosed and the deadliest subtype of leukemia. It is characterized by uncontrolled proliferation of abnormal myeloblasts that lacking the ability to further differentiate into normal blood cells and usually linked to gene mutations or chromosomal rearrangements in myeloblasts. Recently genetic loss-of-function studies have demonstrated that a human YEATS domain-containing protein named eleven-nineteen-leukaemia (ENL) functions as a transcriptional coactivator and is essential for the proliferation of AML and acute lymphoblastic leukemia that harbour oncogenic multiple lineage leukemia (MLL) rearrangements. Our previous studies have demonstrated a series of ENL inhibitors (**1**, **7**-**15** and **24**) displaying significant and specific inhibitory effects targeting ENL YEAST domain. In this study, we developed a NanoBRET system which allows the analysis of cellular permeability, potency, selectivity, and stability of synthesized ENL inhibitors. Followed by *in vitro* metabolic stability and cell growth inhibition studies, we identified a potent and specific ENL YEATS domain inhibitor **13** with both high *in vitro* metabolic stability and strong anti-proliferation ability on MLL-fusion leukemia cell lines. Cumulatively, this study established **13** as a promising inhibitor to disrupt the pathogenic functions of ENL for acute myeloid leukemia treatment.

Acknowledgement

The project was supported in part by National Institutes of Health (Grants NIH-R21CA267512 and R35GM145351) and Welch Foundation (Grant A-1715). X. Guo was supported by a postdoctoral fellowship from Cancer Prevention and Research Institute of Texas (Grant RP210043).

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Poster 22

A Glu-Gly-Cit Tripeptide Linker for Maximizing the Therapeutic Index of Antibody–Drug Conjugates

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Abstract

Valine-citrulline (VCit) is an industrial-standard protease-cleavable linker commonly used in antibody-drug conjugates (ADCs) for cancer therapy. However, its in vivo linker instability can cause various clinical adverse effects including neutropenia and hepatotoxicity. In phase II and III studies of Adcetris (VCit-based ADC), neutropenia and hepatotoxicity were observed in 16%–22% and 7% of patients respectively, leading to dose delay or treatment discontinuation. Here, we report that a glycine-based tripeptide linker sequence, glutamic acid-glycine-citrulline (EGCit), has the potential to solve these clinical issues without compromising the ability of traceless drug release and ADC therapeutic efficacy.[1] We demonstrate that our EGCit ADC resists human neutrophil protease-mediated degradation and spares differentiating human neutrophils. Notably, our anti-HER2 ADC shows almost no sign of blood and liver toxicity in healthy mice at 80 mg kg^{-1}. Our EGCit ADCs also exert greater antitumor efficacy in multiple xenograft tumor models compared to the FDA-approved ADCs including Kadcyla® and Enhertu®. Because of the linker's simplicity, desirable physicochemical properties, and independence from conjugation modality and payload type, the EGCit linker is transferable to a wide range of ADC designs and other drug delivery agents. We believe that the EGCit linker technology will help expand the repertoire of effective, safe targeted drug delivery systems. This may provide clinicians and patients with cancer with access to otherwise unrealistic treatment options such as high-dose ADC therapy.

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Acknowledgments

This work was partly supported by the National Institute of General Medical Sciences (R35GM138264 to K. Tsuchikama), the Department of Defense Breast Cancer Research Program (W81XWH-19-1-0598, to K. Tsuchikama), the Cancer Prevention and Research Institute of Texas (RP150551 and RP190561, to Z. An), the Welch Foundation (AU-0042-20030616, to Z. An), and the Japan Society for the Promotion of Science (postdoctoral fellowship to Y. Anami). We gratefully acknowledge the following researchers for providing the cell lines used in this study: U87ΔEGFR from Prof. Balveen Kaur (UTHealth) and KPL-4 from Dr. Junichi Kurebayashi (Kawasaki Medical School). We thank Prof. Momoko Yoshimoto (UTHealth) for providing scientific advice on studies using human HSPCs, and Dr. Yoshihiro Otani and Dr. Kyotaro Ohno (Okayama University) for providing a clinical opinion for MRI and liver tissue analysis.

Poster 23

Structure-based Drug Design Reveals Druggable Pockets within the FGF12/Nav1.2 Complex

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Background: Voltage-gated sodium channels (Nav) are transmembrane proteins responsible for the generation and propagation of the action potential. There are nine pore-forming α subunits of the Nav channel (Nav1.1-Nav1.9) and genetically inherited mutations in these subunits can lead to channelopathies affecting the nervous system. A major barrier in developing therapeutics against Nav channels is the high degree of primary sequence conservation between the nine isoforms. Protein-protein interaction (PPI) interfaces formed by the Nav α subunit C-terminal domain (CTD) and the intracellular fibroblast growth factors (iFGFs; FGF11-14) are unique and specific for each iFGF/Nav pair. Thus, gaining a better understanding of the thermodynamic properties of iFGFs/Nav complexes could guide the design novel Nav isoform-specific compounds. Here, we have studied the FGF12/Nav1.2 channel complex, a key regulator of neuronal excitability in the hippocampus and cortex, using homology modeling to characterize critical PPI-hot spots. Particular attention was given to hot spots that correspond to genetically inherited single-point mutations of both the Nav1.2 channel and FGF12 and that have been previously associated with brain disorders.

Hypothesis/Goals: The goals of this study were to: 1. build an FGF12/Nav1.2 channel homology and AlphaFold2 (AF2) models; 2. characterize the role of single-point mutations at the PPI interaction interface; 3. assign hot spots associated with human disease; 4. conduct initial structural-activity relationship studies toward the synthesis of selective FGF12/Nav 1.2 channel modulators.

Methods: *In silico* drug discovery approaches such as homology modeling, molecular docking, and binding site analysis available through Glide of the Schrödinger platform were used. The impact of targeted PPI hot spots on FGF12/Nav1.2 complex formation will be determined experimentally.

Results: We built a homology model based on available crystallographic and AF2 structures and used them for prediction of the FGF12/Nav1.2 PPI interface, evaluation of hot spots and selection of candidate residues for mutagenesis and experimental validation. We found critical hydrogen bonds and salt bridges formed between the Nav1.2 CTD and FGF12 that could guide future structure-based drug design.

Conclusions: These studies provided invaluable information on the importance of the FGF12/Nav1.2 channel complex in health and disease that could guide new efforts in neurotherapeutics development.

Acknowledgements: We acknowledge our funding sources as follows: NIH R01MH124351 (FL), NIH 1R01MH132226 (FL), U18DA052504 (FL); Technology Commercialization Program (FL); T32AG067952 (TJB).

Poster 24

FIS103, a Novel SULT1A1-dependent Prodrug, Demonstrates Potent Antitumor Activity in Renal Cell Carcinoma Models.

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Background:
Improved characterization of the etiology and biology of cancer cells have led to the development of targeted chemotherapeutics, which rely on alterations of particular molecules in cancer cells to exert therapeutic effects. As heterogeneity is an important characteristic of cancer, precision medicine has become a core strategy in treating cancer patients. One approach for targeted therapeutics is the delivery of prodrugs designed to be activated by tumor-associated enzymes (TAEs). SULT1A1, a sulfotransferase enzyme, is overexpressed in about 5-15% of cancer patients including breast, prostate, and renal cell carcinoma (RCC); however, it is either not expressed or expressed at low levels in most normal tissue. We have identified a new compound, FIS103, which is a small molecule anti-cancer prodrug that is activated by SULT1A1. This class of compounds, N-benzyl indole carbinols (N-BICs), cause rapid cell death by inducing widespread non-specific covalent alkylation of proteins in the cancer cell.

Hypothesis/Goals:
FIS103 can provide an effective therapeutic option for the treatment of SULT1A1 overexpressing cancers (e.g., RCC) in a subset of patients who fail standard chemotherapy – either as a standalone therapy, or in rationale combination with other drug options.

Methods:
Correlations between SULT1A1 overexpression in cancer types and expected patient prognoses is determined via bioinformatics analyses. FIS103 dependency on SULT1A1 expression for cytotoxicity is demonstrated with multiple cell lines (SULT1A1-positive and -negative RCC and breast cancer cell lines) *in vitro*. *In silico* modeling reveals FIS103-SULT1A1 predicted docking interaction. Preclinical proof-of-concept of FIS103 efficacy *in vivo* is shown in mouse xenograft models injected with A498 (RCC) cells.

Results:
Bioinformatic analyses revealed that SULT1A1 overexpression in tumors, including RCC, is correlated with worse patient prognosis. FIS103, an N-BIC analog, has potent antitumor activity in SULT1A1 high-expressing RCC cell lines (A498 and Caki-1) and has no effect in the SULT1A1 low-expressing cells (786-O and ACHN), indicating low toxicity in the absence of SULT1A1. *In silico* modeling validated the SULT1A1-FIS103 interaction. Furthermore, FIS103 demonstrates potent SULT1A1-dependent antitumor activity in NU/J mouse xenografts injected with A498 (RCC) cells. Remarkably, the tumors in mice became non-detectable 14 days post-FIS103 treatment and remained absent through the study conclusion (41 days).

Conclusions:
Since only a subset of patients will express this enzyme, it is critical to identify those who are more likely to respond. Thus, a companion diagnostic to screen for patients whose tumors overexpress SULT1A1 is essential to the success of this approach. Collectively, targeted therapeutics aims to ultimately move patient care away from broadly toxic, non-specific chemotherapies, and towards more rationally designed treatments. Therefore, the development of FIS103 has the potential to improve survival as well as quality of life of RCC patients and may have application in other SULT1A1 expressing cancers.

Acknowledgements:
Rincon Bio was contracted to conduct the mouse xenograft experiments. *In silico* FIS103 docking modeling was conducted by the Texas Medical Center's Accelerator for Cancer Therapeutics.

Poster 25

Pharmacokinetic Characterization of a Novel COVID-19 Inhibitor OJT010 in a Rat Model

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Purpose: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiological agent responsible for the COVID-19 global pandemic. We identified a novel compound, OJT010, as an inhibitor of the host receptor ACE2 that is required for SARS-CoV-2 viral entry into the host cell. **Methods:** Single dose crossover pharmacokinetic studies were conducted in jugular vein cannulated adult male Sprague Dawley rats to evaluate oral bioavailability of OJT010. Multiple dose steady-state pharmacokinetic studies were further conducted in the rats to evaluate the drug accumulations in the lungs. Serial timed blood samples were collected before and after dose administration. Plasma concentrations of OJT010 were measured using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Pharmacokinetic parameters were obtained using WinNonlin program. **Results:** Following a 50 mg/kg IV administration, OJT010 showed a bi-exponential disposition with a mean terminal half-life of 7.3 hours. Mean maximum plasma concentration (C_{max}) was 2560 ng/mL at 25 min after a single 250 mg/kg oral dosing. Absolute oral bioavailability of OJT010 was 15.5%. In another set of study, C_{max} was 2176, 827 and 1118 ng/mL following a single oral 250 mg/kg, a daily oral 250 mg/kg for 5 days, and a daily oral 400 mg/kg for 5 days administration, respectively. A dose-normalized Turkey post hoc analysis showed significant decrease in C_{max} between the single versus multiple 250 mg/kg oral dosing (P=0.05). The observed decrease of drug exposure following multiple administration was probably due to self-induced hepatic drug metabolism. Interestingly, we observed significant accumulation of the drug in the lungs with an average ratio of C_{lung}/C_{plasma} at 17.7. Further studies are ongoing to explore mechanisms of the unique tissue drug disposition and metabolism using PBPK modeling. **Conclusion:** Pharmacokinetics of OJT010 were characterized using rat as an animal model. The drug appears to accumulate in the lungs.

This work is supported in part by a NIMHD U54MD007605 grant at Texas Southern University

Poster 26

EGFR Regulation of LGR5 Expression and Strategies to Enhance Efficacy of Antibody-Drug Conjugates Targeting Colorectal Cancer Stem Cells

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Background: Cancer stem cells (CSCs) are a sub-population of therapy-resistant cells within a tumor with infinite replicative potential that can also differentiate to drive tumor initiation and promote relapse. Targeting CSC populations has emerged as a promising anti-cancer therapeutic strategy. Antibody-drug conjugates (ADCs), which utilize monoclonal antibody-mediated specificity to hone cytotoxic payloads to cancer cells, are one therapeutic modality being employed to target CSCs. Our lab has targeted leucine-rich repeat containing G protein-coupled receptor 5 (LGR5), which is highly expressed in colorectal cancer (CRC) and is a well-defined biomarker of normal stem cells and CSCs. Our anti-LGR5 ADCs have demonstrated in vitro and in vivo efficacy in gastrointestinal cancer models. However, tumor relapse following anti-LGR5 ADC treatment is a major obstacle, in part due to intrinsic plasticity wherein residual cancer cells interconvert to an LGR5- state during treatment and back to LGR5+ state after treatment. Interestingly, FDA-approved EGFR-targeted therapy has been shown to increase LGR5 *mRNA* expression levels in patient-derived models of CRC and treatment with EGFR signaling inhibitor, gefitinib, increased the number of Lgr5+ cells in normal mouse intestine. The thrust of this work, then, is to evaluate combination therapies targeting both LGR5 and EGFR to eliminate CSCs.

Hypothesis: We hypothesize that EGFR-targeted therapies can increase LGR5 expression levels in CSCs to enhance the efficacy of anti-LGR5 ADCs to improve CRC treatment.

Methods: $KRAS^{mut}$ DLD-1, LoVo, LS180, and SW620 and $KRAS^{WT}$ LIM1215 CRC cells were treated with a panel of EGFR and HER2 inhibitors including cetuximab, nimotuzimab, gefitinib, lapatinib and trastuzumab and MEK1/2 inhibitor trametinib. EGF was used to test the effect of EGFR activation. LGR5 expression was measured via Western Blot. EGFR-directed siRNA was used to determine the effect of EGFR knockdown (KD) on LGR5 expression. CRC cell lines were treated with anti-LGR5 ADC in the presence or absence of cetuximab for 4 days and viability was measured using CellTiter-Glo assay.

Results: EGFR inhibitors upregulated LGR5 expression in CRC cells in a time-dependent manner irrespective of KRAS mutation status. LGR5 levels were unchanged in $EGFR^{neg}/HER2^{low}$ SW620 cells. EGFR KD or treatment with EGF or trametinib substantially decreased LGR5 levels. Additionally, ADC combination treatment with EGFR-targeted therapy enhanced anti-LGR5 ADC potency in the LoVo cells.

Conclusions: This preliminary data shows EGFR-targeted therapy is able to upregulate LGR5 expression and enhance anti-LGR5 ADC potency in CRC cell lines. In vivo studies examining the effects of combination treatment on tumor growth and relapse will help to assess the overall efficacy versus monotherapy. Future studies to determine the mechanism of EGFR regulation of LGR5 expression will be performed. Taken together, our results support the growing body of evidence that combining ADCs with other systemic therapies may be more effective in treating cancer.

Acknowledgements: This work is supported by NCI R01 CA226894 and R21 CA270716 to K.S.C. and The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences.

Poster 27

Inhibitory Mechanism of Ojt009 and Ojt0010 as Potent Blockers of Molecular Interaction Between Sars-Cov-2 Spike Protein and Human Angiotensin-Converting Enzyme-2

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Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects the host through interaction of its spike protein (S(RBD)) with human angiotensin-converting enzyme 2 (rhACE-2). Thus, disruption of this molecular interaction will lead to reduction in viral infectivity.

Hypothesis/Goals

This study aimed to analyze the inhibitory potentials of two mucolytic drugs; OJT009 and OJT0010, to serve as potent blockers of these interactions and alters the binding affinity between the proteins.

Methods

To achieve the aim of this study, a combinatory technique of both *in vitro* and computational methods were employed.

Result

The *in vitro* data showed OJT0010 displayed the highest inhibition of S(RBD)-rhACE2 protein interaction at lower micromolar concentrations (100nM to 10μM); compared to higher concentrations of OJT009 from 50 μM. Interestingly, we found that OJT009 inhibited the binding of S(RBD) protein to rhACE2 receptor at lower concentrations (100 nM to 10 μM). Computational data revealed that the binding of the two drugs at the S(RBD)-rhACE-2 site does not alter the binding affinity and interaction between the proteins. However, the binding of OJT0010 (-56.931 Kcal/mol) and OJT009 (-46.354 Kcal/mol) at the exopeptidase site of rhACE-2, significantly reduced the binding affinities between the proteins compared to the unbound, S(RBD)-rhACE2 complex (-64.856 Kcal/mol). The result further showed the two drugs have good affinity at the hACE-2 site, inferring they might be potent inhibitors of rhACE-2.

Conclusions

Residue interaction networks analysis further revealed the binding of the drugs resulted in loss of interactions between the proteins. This study suggests the binding of the two drugs at the exopeptidase site reduces the binding affinity of the proteins, and consequently might inhibit viral entry.

Acknowledgments

This project is supported from RCMI grant number 5U54MD007605-28 from NIMHD/NIH and CTTP

Grant number RP210043

Poster 28

Targeting Treatment Resistant Colorectal Cancer Using Novel Epiregulin Antibody-Drug Conjugates

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Background: Colorectal cancer (CRC) is the second leading cause of cancer related deaths in the U.S. Despite increases in incidents over the past decade, successful treatment of metastasized CRC has been limited at best. One of the primary methods of treatment is targeting Epidermal Growth Factor Receptor (EGFR) using monoclonal antibody therapy. While this treatment can be effective against patient tumors with high expression of EGFR ligands such as Epiregulin (EREG), patients with oncogenic protein Kras mutations are completely unresponsive to this treatment. Recent clinical and translation research have shown that Kras mutant tumors upregulate downstream MAP Kinase signaling to overexpress EREG through an autocrine mediated EGFR signaling loop. EREG's high expression in CRC tumors, including those with Kras mutation , and its low- to undetectable expression in normal tissue, make EREG a promising therapeutic target.

Hypothesis: Therefore, I hypothesize that EREG can mediate CRC tumor growth and metastasis and an EREG-targeted antibody-drug conjugate (ADC) can act as a guided missile to deliver cytotoxic drugs to EREG-expressing tumors for targeting treatment resistant CRC.

Methods: To develop a novel EREG ADC, I cloned an EREG monoclonal antibody (mAb) construct based on patented variable regions of a humanized EREG mAb and tested for binding affinity, the ability to internalize, and to block exogenous and endogenous EREG activity. Using the EREG mAb as well as a non-targeting IgG1 control antibody (cmAb) we performed radiolabeling with Zr89 to trace antibody tumor uptake in vivo using a CRC cell line xenograft model. We then conjugated the EREG mAb and cmAb to potent cytotoxic agents using either a cleavable , or non-cleavable peptide linker. Our EREG ADCs were evaluated in vitro against a wide panel of CRC cell lines of various *KRAS*, *BRAF*, and *PI3CKA* statuses and various EREG expression. Drug efficacy was determined using cytotoxicity assays to measure percent cell survival given varying doses of the cAb and EREG mAb as well as control ADC (cADC) or EREG ADC.

Results: Our EREG antibody showed high binding affinity, internalization and lysosomal colocalization in vitro and tumor specificity in vivo. While the EREG mAb alone does not produce significant cytotoxicity, conjugated to a chemical payload, the EREG ADC has a much higher efficacy at lower concentrations in causing cytotoxicity compared to cADC. Ongoing studies include large-scale ADC production with the lead anti-EREG ADC and cADC for cytotoxicity profiling in patient derived organoid culture and safety and therapeutic efficacy studies in vivo.

Conclusions: By targeting EREG, we can develop an ADC capable of acting as a potent clinical drug for targeting cellular plasticity and eliminate colorectal cancer resistance and recurrence.

Acknowledgements: Funded by the Gulf Coast Consortia Training Interdisciplinary Pharmacology Scientists (TIPS) program National Institutes of Health grant T32 GM139801 to The University of Texas Health Science Center at Houston.

Poster 29

Synthetic Oxidized Carbon Enzymes (Nanozyme) As a Platform for Treatment of Secondary Injury from Intracerebral Hemorrhage

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Abstract: Background: Intracerebral hemorrhage (ICH) is the deadliest form of stroke, affecting approximately 50,000 new patients per year in the US. It occurs when a blood vessel in the brain ruptures, releasing toxic blood products such as hemin and iron. Unfortunately, there is currently no effective treatment for ICH, and the prognosis is poor. **Hypothesis**: Our team has developed a new class of therapeutics, oxidized carbon nanozymes (OCNs) to address this urgent medical need. OCNs are synthesized from carbon rich sources, such as medicinal activated charcoal, and undergo harsh acid treatment to transform them into 3-5 nanometer discs with transformed chemical properties. OCNs act as synthetic enzymes, enhancing a variety of biological reactions including localizing to mitochondria to support essential oxidative phosphorylation, catalyzing the quenching of superoxide free radical, and enhancing the metabolism of hydrogen sulfide to protective polysulfides. **Validation Results:** Preclinical validation studies in both in vitro and in vivo models have shown that OCNs are nontoxic, can pass through the blood-brain barrier, and reduce key toxic effects of hemoglobin breakdown products in neurons, brain endothelial cells and following injection of hemolyzed blood into the cerebrum. Given their flexible chemistry, modifications to OCNs to target different conditions can be readily performed. In the case of ICH, we have linked OCNs with Deferoxamine, an already approved FDA drug, to target the iron toxicity caused by the leakage of blood components during hemorrhage. Deferoxamine-linked OCNs mimic enzyme-like protect against damaging effects of hemin (oxidative injury, senescence) and iron (Fenton reaction and ferroptosis). **Future Milestones**: Federal Funding of $6 million has been obtained to establish the mechanisms of action and pre-clinical efficacy in several models. Many pre-clinical milestones are completed, including scalable synthesis and in-vitro and early in-vivo efficacy. Gerenox, Inc. has been founded to seek non-dilutive funding to complete efficacy and toxicity studies. A student led start-up team, Neurnano, has been formed to seek dilutive funding for ICH indication, and awarded 3rd place in the 2023 Rice Business Plan Qualifying competition. The next steps involve scaling up OCN production under GLP and GMP regulations, pre-clinical efficacy and toxicity in 2 species, complete ADME (absorption, distribution metabolism and excretion) IND-enabling studies, and form strategic alliances for this exciting new, pleiotropic platform. **Acknowledgements:** Discovery and development validation studies funded by Department of Defense and National Institutes of Health (R01NS094535; TAK, PJD, AL), the Welch Foundation Grant BE-0048 (TAK) and Texas A&M Presidential Clinical Research Partnership Awards (TAK and AV).

Poster 30

An Azapeptide Platform in Conjunction with Covalent Warheads to Uncover High-Potency Inhibitors for SARS-Cov-2 Main Protease

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Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent for the COVID-19 pandemic. The SARS-CoV-2 (SC2) genome encodes for 10 open reading frames (ORFs), of which ORF1ab is the largest one that encodes two polyproteins pp1ab and pp1a. The two polyproteins are cleaved by two proteases, namely main protease (Mpro), and papain-like protease (PLpro) to yield 16 non-structural proteins (nsps). Mpro operates at 11 cleavage sites on the large polyprotein pp1ab to process 12 out of the total 16 nsps, which play a critical role in viral replication and pathogenesis. Therefore, inhibition of this protease is sufficient in preventing the virus from replicating in infected cells which makes it a potential drug target.

Goals: The function of Mpro relies on three active site pockets to recognize P1, P2, and P4 amino acid residues in a substrate and a catalytic cysteine residue for catalysis. Scrutinizing the substrate preference of SC2Mpro and inspired by the previous works on the development of inhibitors against SC1Mpro, the Liu lab has developed reversible covalent inhibitors to target SC2Mpro. By bringing about structural manipulation to the amino acid residues in the inhibitor design to improve cell membrane permeability and metabolic stability, we have generated a cohort of peptidomimetic inhibitors that target SC2Mpro.

Methods: In a different approach, by converting the P1 Cα atom in an M^{Pro} substrate to nitrogen, we showed that a large variety of azapeptide inhibitors with covalent warheads targeting the M^{Pro} catalytic cysteine could be easily synthesized (**Figure 1A**).

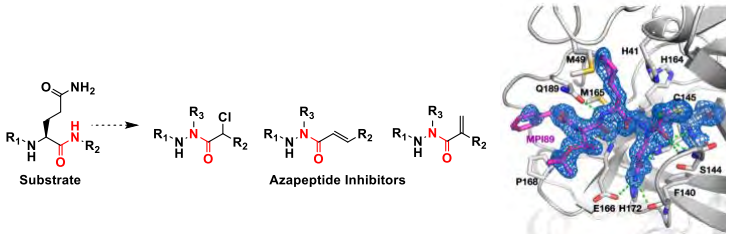

Figure 1. (**A**) A graphic representation depicting the transformation of a substrate into an azapeptide with different warhead groups. (**B**) Crystal structure of Mpro bound to MPI89.

Results: Through the characterization of the developed inhibitors, we identified several highly potent M^{Pro} inhibitors. MPI89 that contained an aza-2,2-dichloroacetyl warhead, displayed a 10 nM EC_{50} value in inhibiting SARS-CoV-2 from infecting $ACE2^{+}$ A549 cells and a selectivity index of 2,000. The crystallography analyses of M^{Pro} bound with 6 inhibitors, including MPI89, revealed that inhibitors used their covalent warheads to covalently engage the catalytic cysteine and the aza-amide carbonyl oxygen to bind to the oxyanion hole (**Figure 1B**).

Conclusions: MPI89 represents one of the most potent M^{Pro} inhibitors developed so far, suggesting that further exploration of the azapeptide platform and the aza-2,2-dichloroacetyl warhead is needed for the development of potent inhibitors for the SAR-CoV-2 M^{Pro} as therapeutics for COVID-19.

Acknowledgements: This work was supported by the Welch Foundation (grant A-1715), National Institutes of Health (grants R35GM145351 to W.R.L., R21AI164088 to S.X., and R21EB032983 to W.R.L.), Texas A&M X Grants, and Texas A&M EDGES Fellowship Program.

Poster 31

Discovery and Preclinical Development of IACS-52825, A Potent and Selective DLK Inhibitor for the Treatment of Chemotherapy-Induced Peripheral Neuropathy

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Background: Chemotherapy-Induced Peripheral Neuropathy (CIPN) is a major unmet medical need in which cancer patients on chemotherapy develop pain and/or loss of sensation in their extremities. Currently there is no effective treatment for prevention or reversal of CIPN.

Hypothesis: While the exact mechanisms underlying CIPN are debated, it is thought that the active axon degeneration program, which involves activation of the dual leucine zipper kinase (DLK, or MAP3K12), is engaged following chemotherapy and contributes to CIPN.

Methods: Starting from a literature compound designed for a non-CIPN indication, we developed our own DLK inhibitor program. A DLK screening funnel was used to select the inhibitors with best in vitro potencies and in vivo properties. In vivo mouse CNS target engagement of the compounds was measured by reduction of p-c-Jun/c-Jun levels. Neuroprotective efficacy at preventing cisplatin-induced mechanical allodynia was measured with the Von Frey test. We optimized potency of the series using structure-based drug design through displacement of a water and introduction of multiple multipolar interactions, and then fine-tuned metabolic stability with a strategically placed fluorine.

Results: We have developed a series of inhibitors of DLK protect against axonal degeneration of sensory neurons and prevent or reverse the effects of cisplatin in mouse models of CIPN. In this talk we will present the discovery and preclinical development of IACS-52825, a potent and selective DLK inhibitor containing a novel bicyclo[1.1.1]pentylimidazole core.

Conclusions: IACS-52825 showed strong efficacy in mice reversing cisplatin-induced allodynia, and generally excellent properties supporting development as a preclinical candidate.

Acknowledgements: Magnolia NeuroSciences, Magnolia Tejas Corp.

Poster 59

Generation of Highly Selective Monoclonal Antibodies Inhibiting Matrix Metalloproteinases by Periplasmic Genetic Selection

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Background: Matrix metalloproteinases (MMPs) play pivotal roles in controlling a wide variety of physiological and pathological procedures, and thus abnormal MMPs expression leads to many disorders. Among them, MMP-9/-14 are promising pharmacological targets and their inhibitors have great potential as valuable drugs in multiple pathologies including neuropathic pain and cancer. Some MMPs are antitumorigenic in certain cancer environment, and thus target-specific inhibition is highly required. However, selective inhibition of target MMP without cross-reactivity toward other MMPs is a main challenge for inhibition therapy because catalytic domains of MMPs share homologous protein folds and catalytic mechanisms with highly conserved catalytic Zn^{2+} and associated motifs, sequential and conformational diversity. In addition, single MMP has both detrimental and beneficial effects through the cleavage of different substrates. Especially, cleavage of syndecan-1 by MMP-14 stimulates tumor cell migration, invasion, and metastasis, therefore its inhibition provides a promising strategy for cancer treatment. In contrast, MMP-14 also cleavages monocyte chemotactic protein 3 (MCP3), which results in dampening inflammatory responses, and thus degradation of MCP3 by MMP-14 is beneficial and should not be inhibited. Consequently, there is a requirement to develop substrate-specific inhibitors blocking the cleavage of syndecan-1 by MMP-14 but not MCP3.

Hypothesis/Goals: Existing monoclonal antibodies (mAbs) selection/screening methods are mainly based on binding affinities but not inhibitory function, therefore few or none of the selected binders have required target/substrate specific inhibition. This study aims to generate MMP-9/-14 inhibitory mAbs with high potency, stability and target/substrate-specific. Objectives: 1) Developing a function-based selection method; 2) Generation of target/substrate-specific mAbs by using novel function-based selection; 3) Characterization of isolated mAbs.

Methods, Results: By combining convex paratope antibody library and function-based periplasmic genetic selections, MMP-9/-14 inhibitory mAbs were successfully isolated. In the case of MMP-9 inhibitory mAbs, binding affinity and proteolytic stability measurements indicated that isolated mAbs bound to MMP-9 with nano molar range binding affinity and high proteolytic stability. In inhibition assays, isolated mAbs effectively inhibited MMP-9 proteolytic activity from cleaving MMP-9 peptide substrate and physiological/macromolecular substrates including collagen type IV, gelatin and interleukin-1β (IL-1β). Importantly, isolated mAbs were highly selective toward MMP-9 over (cd)MMP-2/-12/-14/-24. As regards MMP-14 inhibitory mAbs, isolated mAbs showed nanomolar affinity and potency toward cdMMP-14 with high selectivity over (cd)MMP-2/-12/-14/-24. More importantly, isolated mAbs inhibited the cleavage of syndecan-1 by MMP-14 not MCP3.

Conclusions: Technological developments within this study allow us to discover target/substrate-specific mAbs inhibiting MMP-9/-14 of biomedical importance, which otherwise is not able to be achieved by current selection/screening technologies. We expect that in addition to therapeutic application, new MMP-9/-14 inhibitory mAbs can be applied for diagnostic and research tools to identify functionality and role of MMP-9/-14 in normal and pathological conditions as well as extensive pharmaceutical testing will represent future.

Acknowledgements
This study was supported by NIH R35GM141089 and CTTP traineeship from CPRIT Grant No. RP210043.

Poster 32

The Effect of Traumatic Brain Injury on Pharmacokinetics and Biodistribution of Riluzole and Etoricoxib in Long-Evan Rats

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Background: Traumatic brain injury (TBI) is a brain dysfunction resulting from violent blow of sport injury or car accident to the head. Currently, no FDA approved therapeutics are available to treat TBI. Riluzole has shown to be safe and promisingly efficacious for neuro-recovery in the treatment of cervical spinal cord injury. Etoricoxib inhibits the inducible cyclo-oxygenase-2 (COX-2) pathway to reduce inflammation induced by TBI.

Goals: The aim of this study was to investigate the effects of TBI on the pharmacokinetics (PK) and biodistributions of riluzole and etoricoxib as a single or combination regimen.

Methods: The TBI model of Long-Evans (L.E.) rats was generated using the "Marmarou" weight drop injury approach. Riluzole and etoricoxib concentrations in plasma, brain, and spinal cord were quantified by using validated liquid chromatography tandem mass spectroscopic (LC-MS/MS) assay. A PK 2-compartmental model analysis was performed using Phoenix WinNonlin®. Drug exposures were calculated from PK profiles by the area under the concentration curves from 0 to 24 hours ($AUC_{0\text{-}24hr}$).

Results:

1. For riluzole, TBI resulted in lower plasma concentration at 15 min post dose ($p<0.05$). The PK compartmental analysis revealed that the peripheral volumes of distribution (V_2) was decreased, with 1.85 L/kg in healthy group and 0.83 L/kg.
   The biodistributions of riluzole to brain and spinal cord at 24 hr post dose were higher with TBI (B/P=0.9 and S/P=1.1) than the healthy group (0.5 and 0.4, respectively).
2. For etoricoxib, TBI impacted the PK in combination group. The plasma concentration was lower at 15 min post dose with TBI. The V_2 was reduced from 1.09 L/kg to 0.67 L/kg. The B/P and S/P in TBI group were reduced (0.1 and 0.09, respectively) compared to the healthy group (0.2 and 0.9, respectively).
3. In the etoricoxib single agent group, the etoricoxib plasma concentration was significantly lower with TBI at 24 hours post injury ($p<0.05$). The clearance of etoricoxib was 0.48 L/kg*hr, faster in TBI group than that in the healthy group, 0.35 L/kg*hr.
   In the healthy rats, the combination treatment reduced riluzole $AUC_{0\text{-}24hr}$, approximately 57% lower than that in single agent group. In TBI rats, the exposures of etoricoxib were reduced in both combination and single agent groups. However, no statistical significance was established because of the large variabilities.

Conclusions: TBI changed PK profiles of the drugs, with decreased V_2 of riluzole and etoricoxib in combination therapy, and reduced etoricoxib plasma concentrations in single agent group. The drug exposure and biodistribution were also affected by TBI, with more riluzole in brain and spinal cord and less in plasma. However, TBI reduced etoricoxib biodistribution in plasma, brain, and spinal cord.

Acknowledgements: This work was a proud collaboration with late Dr. Robert G Grossman (Methodist) and late Dr. Raymond Grill (UMMC). Funding source was from Institute for Drug Education and Research (IDER), College of Pharmacy, University of Houston.

Poster 33

Prediction Of Synthetic Lethal Relationships Using Siamese Neural Networks

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Background Synthetic lethality occurs when the perturbation of two genes individually is well tolerated but their combined perturbation causes a loss of cellular fitness or cell death. Therefore, synthetic lethality may be exploited to develop anticancer therapeutics. However, experimental discovery of synthetic lethal relationships is costly and time-consuming. Computational discovery of novel and robust synthetic lethal relationships will accelerate therapeutic drug discovery.

Goals Develop a computational model to predict synthetic lethal relationships using publicly available molecular data.

Methods Towards this goal, we designed a deep learning algorithm following the Siamese neural network architecture. Siamese neural networks are specifically designed to handle pairs of input samples and learn a similarity function between them, where the two subnetworks process each input sample separately before combining the extracted features for classification. Here, we adapt the network to take as input the DepMap Chronos dependency scores and TCGA copy number profiles of a pair of genes. These input features represent the concepts of codependency and mutual exclusivity, which previous computational approaches have leveraged to nominate potential synthetic lethal relationships. Using SynLethDB, a database for synthetic lethal interactions, gene pairs are labeled as synthetic lethal and used as positive pairs in the model. Random gene pairs are used as negative pairs in the model. The trained model will predict the probability of any two genes having a synthetic lethal relationship.

Results To evaluate our model we used experimentally validated synthetic lethal pairs derived from publicly available combinatorial knockout screens. Our model predicted significantly higher probabilities for gene pairs with experimentally validated synthetic lethal interactions compared to gene pairs without.

Conclusions In conclusion, our study demonstrates the feasibility of using deep learning to predict synthetic lethal relationships using publicly available data sets. Our model shows promising results in identifying experimentally validated synthetic lethal pairs and has the potential to accelerate the development of anticancer therapeutics.

Acknowledgements We appreciated the help from Heyuan Li, Jošt Vrabič Koren, Min Thiha Myo, Nick Neill.

Poster 34

Nanoemulsion Formulation Development and Characterization of PC257, a Novel Anti-CRPC Agent

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Background

In 2022 the National Cancer Institute reports that prostate cancer accounted for 14 percent of overall new cancer occurrences with a 5.7% death rate. Early detection provides high survival and remission rates; however, there are limited options for castration resistant prostate cancer (CRPC). Current treatment strategies for CRPC exploit the dependence of AR for hormone activation, but the available therapies are ineffective in FKBP52 cochaperone, which is a promising therapeutic target for the disruption of multiple mechanisms in prostate cancer. PC257 was developed as an inhibitor of novel targets such as FKBP52 by Dr. Marc Cox's group at UT-El Paso.

Goal

In this study, we developed a non-hemolytic nanoemulsion formulation of PC257 for parenteral administration. A nanoemulsion would increase the drug's bioavailability while efficiently delivering the drug to the targeted site.

Methods

A nanoemulsion formulation of PC257 was prepared using a low energy stirring/heat inversion method to combine an oil phase and water phase for the final nanoemulsion. PC257 is added at various formulation phases to achieve optimal drug concentrations. The nanoemulsion was characterized for particle size, zeta potential, encapsulation efficiency, and drug loading.

Results

Zeta Sizer data for unfiltered and filtered emulsions both have mean PDI of 0.289 +/- 0.034, Z-Average of 210.33 +/- 25.53 d.nm, and Zeta potential of -20.02 +/- 5.27 mV. NanoDrop UV-Vis shows an absorbance of the compound between 280 to 300 nm range. Preliminary UPLC data shows a concentration of 190.95 ug/mL with 10% drug loaded. Further development of this nanoemulsion is undergoing in our lab.

Conclusions

By developing non-haemolytic nanocarriers, poorly soluble anti-cancer drugs are more effective in targeting tumor sites. These routes of administration address improved bioavailability, multi-drug resistance and provide less invasive treatments

Acknowledgements

National Institute on Minority Health and Health Disparities of the National Institutes of Health (NIH) under award number 2 U54 MD007605-27A1.; Dr. Marc Cox & Group at University of Texas El Paso; Dr. Dong Liang, Co-Advisor & Group, TSU COPHS; RCMI – Research Centers in Minority Institutions

Poster 35

Delivering Nanoparticles as Theranostic Agents in the Heart

Abstract: The use of gold nanoparticles in the biomedical field has a wide and diverse range of applications. Given their low cytotoxicity, optical properties, and high biocompatibility, they are highly valued for applications that include biosensing, bioimaging, and drug delivery. Furthermore, the high atomic weight of gold makes them visible with X-ray imaging. These properties make gold nanoparticles as well as core-shell gold nanoshells advantageous as both therapeutic and diagnostic agents. However, when administered as a solution in the body, the particles rapidly dissipate and do not localize to a target area. Specifically, when trying to deliver nanoparticles to the heart, a highly vascularized organ, administering them as a solution quickly results in their systemic distribution, leading to possible off-target effects and hampered efficacy. We leverage a hydrogel formulation to administer gold nanoshells into the heart, so that they remain at the target site and do not undergo rapid coronary washout. In cardiac tissue engineering, integration of metal nanoparticles, like gold nanoparticles, within a scaffold could be advantageous to help guide delivery and duration of therapy due to their high contrast properties. In this work, we exploited computed tomography (CT) imaging to identify hydrogels containing nanoparticles in the heart in a porcine model. The technique is based on differences in signal attenuation, whereby strong signal is attributed to the nanoparticle-gel (4 mg [Au]/ml, ~400 HU) and weaker signal (~80 HU) is attributed to the background cardiac tissue. A threshold of 150 HU is used to segment particles inside a 3D cardiac reconstruction of a porcine heart using 3DSlicer, a free and open-source software for visualization and processing of 3D images, to identify and quantify nanoparticle-gel volume. Notably, we found that the particles remain in a semi-solid agglomerate inside the cardiac tissue over several hours. We also identified the signal attenuation differences of between gold and calcium, the cross-linking agent used to create the hydrogel. This is important as calcium is also present in the heart. Elemental analysis confirmed the presence of gold and was used to quantify gold content for each injection spot. The particles were visible in the gel in both ex vivo and in vivo porcine hearts and could be tracked after in vivo administration without adverse effects in an acute study.

Understanding the Paradoxical Behavior of Haloperidol-Induced PKB/Akt Regulation of Cell Proliferation and Apoptosis

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Abstract

Background: Haloperidol is a typical antipsychotic drug; it is widely used for people with various psychiatric conditions such as schizophrenia, bipolar disorder, major depressive disorder, and dementia. Despite its effectiveness in controlling delusions, hallucinations, agitation, and other disruptive behavioral symptoms in these psychiatric conditions, it has adverse side effects such as extrapyramidal manifestation and endocrinologic and metabolic changes. Initial evidence shows that Haloperidol has antitumor properties in several cancer types by inhibiting cell proliferation and inducing apoptosis. However, other reports indicate that Haloperidol promotes cell proliferation in other cells. Protein Kinase B (PKB/Akt) promotes proliferation, survival, metabolism, and tumorigenesis by phosphorylating the downstream target proteins. Therefore, understanding PBK/Akt paradoxical behavior in cellular environments is paramount when using Haloperidol to treat cancer and psychiatric conditions.

Aim and Hypothesis: Most Haloperidol studies focused on cancer cells, not normal cells. However, a study showed that Haloperidol protects lung endothelial cells from injury. Nevertheless, the role of Haloperidol on the immortalized non-tumorigenic epithelial cell line, human bronchial epithelium BEAS-2B, remains unknown. In addition, the paradoxical behavior of PKB/Akt induced by Haloperidol is puzzling. Therefore, this study aims to decipher whether the derail of microRNAs (miRNAs) plays a role in PKB/Akt paradoxical behavior. We hypothesized that overexpression of miRNA (i.e., hsa-Let-7c-5p) targets PKB/Akt mRNA) would reduce PKB/Akt-induced expression by Haloperidol in BEAS-2B cells, reducing cell proliferation and promoting apoptosis. Furthermore, the study aims to show that up- or down-regulation miRNAs are suitable biomarkers for determining the paradoxical behavior of PBK/Akt in different cellular environments.

Methods: BEAS-2B cells treated with 3.5 μM of Haloperidol for 24 and 48 hours were also transfected with hsa-Let-7c-5p DNA construct. Controls were included. After each interval, the cells were examined under an inverted microscope; protein and RNA were isolated; cDNA was synthesized; semi-qPCR and Western blot were performed. The LI-COR Image Studio Software (LI-COR, Lincoln, NE) was used for visualization and quantification.

Results: Haloperidol increases BEAS-2B cell proliferation without hsa-Let-7c-5p expression, suggesting it promotes cell cycle progression. Conversely, overexpression of miRNA, hsa-Let-7c-5p, inhibits Haloperidol-induced cell proliferation and PKB/Akt, including the downstream genes regulated by PKB/Akt.

Conclusion: PBK/Akt plays a role in the proliferation of BEAS-2B cells. However, Haloperidol increases the proliferation of BEAS-2B cells and PKB/Akt. Overexpression of hsa-Let-7c-5p inhibits Haloperidol-induced of PKB/Akt, the downstream genes, and cell proliferation. This study demonstrates that miRNAs are suitable biomarkers for solving the issues of genes with paradoxical behaviors involving cell proliferation and apoptosis.

Acknowledgments Funds from the TSU Seed Grant, COSET and COSET-SURP, the Department of Biology, and the Department of Environmental Toxicology and Interdisciplinary Sciences support this study.

Poster 37

Targeting a Novel Cryptic Pocket in ATG4B Disrupts its Functional Activity

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Background: During conditions of cellular stress, cells utilize a survival mechanism, autophagy, to degrade intracellular material, recycle metabolites and meet their nutrient requirements. Autophagy is upregulated in cancer cells and helps ameliorate intrinsic and extrinsic stresses, thereby promoting tumor survival and progression. Consequently, disrupting the autophagy pathway in cancer cells dysregulates cellular homeostasis and promotes deleterious effects such as nutrient deprivation, energy deficit, and hypoxic stress. The Autophagy Related 4B (ATG4B) cysteine peptidase that plays a key role in the formation of the autophagosome, a critical step for autophagy, has emerged as a prominent drug target in this context. Active site inhibitors against ATG4B are known, but the current chemical classes have not progressed to the clinic.

Goals: This study aimed to identify new chemical starting points for an ATG4B drug discovery program.

Methods: We executed fragment screens using differential scanning fluorimetry and nuclear magnetic resonance spectroscopy to identify inhibitors against ATG4B. We followed this with X-ray crystallography to identify the binding site and binding mode of these compounds. The functional impact of the hits was assessed in an ATG4B enzymatic assay using GATE16 as the substrate. Direct binding of the hits to ATG4B was further evaluated using microscale thermophoresis and surface plasmon resonance. Finally, we used computational molecular dynamics simulations to understand the functional effect of compound binding on ATG4B.

Results: From our fragment screens, we identified 144 fragment compounds that bound to ATG4B. Subsequent X-ray crystallographic studies with the hits then revealed binding of 5 of these compounds to a previously unreported cryptic pocket in ATG4B. Follow-up studies enabled the identification of **Compound 6** that showed an IC50 of 20μM in the ATG4B enzymatic assay. Enzymatic and biophysical characterization of the pocket suggests that fragment binding in this pocket can disrupt the protein-protein interaction between ATG4B and its substrate. Further, MD simulations revealed significant changes in the structure and dynamics of ATG4B upon binding to **Compound 6**. We propose a mechanism where the C-terminal disordered loop docking on the surface of the protein and increased fluctuations in residues 259-261 is essential for ATG4B-ATG8 interactions. Fragments binding in the cryptic pocket disrupt the intrinsic dynamics of ATG4B, consequently destabilizing the ATG4B-ATG8 interactions, resulting in decreased ATG4B activity.

Conclusions: We discovered a novel cryptic pocket in ATG4B through fragment screening and subsequent X-ray crystallographic analysis. Compound binding weakly disrupted the functional activity of ATG4B, which is predicted to originate from an allosteric change in the structure and dynamics of residues in both the catalytic site and the binding interface between ATG4B and its substrate protein, ATG8. We hypothesize that as these compounds are more fully elaborated, they will show a biological impact via a decrease in autophagosome formation.

Acknowledgements

This work was supported by Vescor LLC, and internal funds from The University of Texas MD Anderson Cancer Center

Poster 57

Targeting DPF2, a Repressive Histone-acyl Reader in Colorectal Cancer

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Abstract

Histone modifications are dynamic and versatile post-translational modifications that are intimately connected to human development and disease pathogenesis, including cancer etiology [1]. As histone 'marks' are potentially reversible, they represent promising therapeutic targets. Anticancer drugs such as Vidaza™, Tazemetostat™, Vorinostat™ and JQ1 provided proof-of- concept for 'drugging' the epigenome, and combinatorial approaches are in the pipeline [2], including drug/dietary agent combinations [3]. In a recent multi-omics study on the antitumor efficacy of dietary spinach (SPI) in an Apc mutant rat model of familial polyposis, linoleate and butanoate metabolites 13(S)-HODE and 2-hydroxybuyrate (HB) targeted histone deacetylase (HDAC) activity and IFN-γ signaling [4]. Subsequent analyses revealed distinctive histone K-acylation changes, particularly H3K14Cr, H3K14But, and H4K5But, increased in adenomatous polyps obtained after 30-day SPI intake (SPI30d). Double PHD finger (DPF) and YEATS domains have been reported as preferential 'readers' for distinct histone lysine acylation, with a preference for Cr>Bu>Pr>Ac at specific histone locations [5]. Among these acyl readers, DPF2 is an accessory component of the BAF-family chromatin remodeler with a repressive role in myeloid differentiation [6]. We observed high DPF2 expression linked to lower survival rates in CRC and other human cancers, and DPF2 was overexpressed in human CRC cell lines compared to normal colonic epithelial cells. DPF2 also was highly expressed in adenomatous lesions from the polyposis in rat colon (Pirc) model, compared with adjacent normal-looking colonic tissues. In Pirc colon tumors, SPI30d decreased DPF2 expression and increased H3K14Cr, H3K14But and H4K5But, and this was corroborated by 2-HB treatment in human colon cancer cell lines. Knockdown of DPF2 increased histone acyl marks and inhibited cell growth. We conclude that DPF2 is a repressive histone K-acylation reader that might be targeted for interception of CRC and adenoma stages.

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Poster 38

Development and Validation of an LC-MS/MS Method for the Quantification of Hydrogen Sulfide in Isolated Tissues from Porcine Eyes

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Background: Hydrogen sulfide (H_2S) is a gaseous neurotransmitter that is produced in several mammalian tissues including the eye[1]. Most of the current methods available to measure H_2S in biological samples such as the methylene blue assay and amperometry are associated with errors due to the lack of precision[2]. Based on its simple molecular structure, H_2S can be derivatized with monobromobimane to produce sulfide dibimane (Sdb).

Hypothesis/Goals: This study was aimed at developing and validating a sensitive and robust LC-MS/MS method to measure changes in H_2S levels in tissues from isolated porcine eyes.

Methods: Isolated porcine iris-ciliary bodies (ICB) were cut into quadrants of approximately 50 mg and homogenized using a 1:3 volume of homogenizing buffer. Sdb was synthesized from samples as well as sodium hydrosulfide using a previously described methodology[3,4]. For the LC-MS/MS analysis, a reverse phase Waters Acquity BEH C18 column (1.7 µm, 2.1 x 100 mm) and an injection volume of 2 µL was used. H_2S quantification was performed using multiple-ion reaction monitoring (MRM) in positive mode, with the transitions of m/z 415.0 → m/z 223.0 for Sdb and m/z 353.0 → m/z 101.0 for internal standard (griseofulvin). The calibration curve of Sdb ranged from 5-2500 ng/ml. Accuracy and Precision were evaluated using intra- and inter-day coefficient of variation (CV). Extraction recovery and matrix effect were performed by comparing the responses before and after protein precipitation and spiked post-extraction and neat solvent samples, respectively. Stability of Sdb was determined under different conditions. This method was applied to measure H_2S levels in isolated tissues from porcine ICB previously treated with lipopolysaccharide (LPS, 5 – 100 ng/ml), a pro-inflammatory agent.

Results: The method displayed linearity over a range of concentrations for Sdb (5 – 2500 ng/ml). Intraday and inter-day CV ranged from 2.04% to 5.94% and 5.30% to 6.86% respectively. Extraction recoveries for Sdb were 98.7%, 113%, and 92.9 % at low, middle, and high QC, respectively. Matrix effect was 5.6%, 10.4%, and 6.4% respectively at low, middle, and high QC, respectively. Sdb showed stability in porcine ICB with mean recoveries up to 4h at room temperature (93.3%, 98.3%, 98.6%), after three freeze-thaw cycles (92.6%, 88.3%, 94.3%), and at -80 ^{o}C for up to 14 days (90.1%, 88.2%, 92.2%) respectively at low, middle, and high QC. The level of basal H_2S was significantly decreased (3.89 ng/mg ± 0.30 ng/mg tissue) in the presence of 100 ng/ml LPS compared to blank (5.55 ng/mg ± 0.20 ng/mg tissue) (n=10). The method was able to measure changes in tissue H_2S concentration in the presence of varying concentrations of LPS.

Conclusions: To our knowledge, this is the first study conducted to quantify H_2S in ocular samples by liquid chromatography-tandem mass spectrometry. We have developed and validated a sensitive LC-MS/MS method to quantify H_2S in ocular samples. Advantages of this method include small tissue sample size and measurement of changes in H_2S levels in the presence of lipopolysaccharide.

Acknowledgments: This study was funded in part by NIH Grant Numbers: IRI5EY032274 and G12MD007605; CPRIT grant number RP 180748.

Poster 39

Assessing Population Pharmacokinetics of Riluzole in Acute Spinal Cord Injury through Covariate Analysis

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Background
Riluzole in Acute Spinal Cord Injury Study (RISCIS) Phase II/III trial (NCT01597518) was conducted to determine the efficacy and safety of riluzole in patients with Spinal Cord Injury (SCI). Our previous PK study showed that there are great inter-subject variabilities in patients with riluzole treatment.
Hypothesis/Goals
To better understand these heterogeneous patients in acute SCIs, population pharmacokinetics through covariate analysis was performed.
Methods
Patient data were obtained from RISCIS, a multi-center, randomized, placebo-controlled, double-blinded, phase 2/3 clinical trial. With patients' plasma samples, the concentrations of riluzole were quantified using a validated LC-MS/MS assay with an LLOQ of 0.5 ng/ml. PK analysis was performed using Monolix version 2023 R1, with the use of a one-compartment structural model. Covariate analysis on age, sex, actual body weight, BMI, total motor score, and AIS category at admission were explored.
Results
In the basic one-compartmental popPK model, interindividual variability in Ka, C, and CL was 21.1%, 22.53%, and 31.7%, respectively. Riluzole clearance in acute SCI was independent of age, actual body weight, BMI, total motor score, and AIS category at admission, but sex was the most important patient covariate in clearance.
Conclusions
Sex serves as the primary covariate in explaining inter-subject variability among patients undergoing riluzole treatment for acute SCI.
Acknowledgements
US ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY, Contract No. W81XWH-16-C-0031, IDER (Institute for Drug Education and Research, Medicine of the Future)

Poster 56

Development of a Screening Platform to Evaluate the Therapeutic Potential of CDK11 in Pancreatic Ductal Adenocarcinoma (PDAC) Models

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Background
Cyclin-dependent kinases (CDKs) are master regulators of cell division and cell-cycle progression. CDK11, a relatively understudied member of the family, has pleiotropic roles in transcriptional regulation, splicing, and other critical cellular processes. CDK11 recently emerged as an attractive drug discovery target via our patient-derived xenograft functional genomics discovery platform, Patient-based *In vivo* Lethality to Optimize Treatment (PILOT). Preliminary data suggests not only differential sensitivities, but also differential molecular perturbations in responders compared to non-responders, providing an opportunity for a targeted therapy in PDAC patients. Thus, a drug discovery program was initiated for CDK11.

Goals
The aim of these drug discovery efforts was to identify first-in-class chemical matter with potent inhibition of CDK11 and high selectivity over closely related family members that could be used to develop a therapeutic tool for underserved patient populations. To support the rational progression of chemical matter targeting CDK11, a screening funnel was developed.

Methods
At project inception there were no known commercial sources of active CDK11 bound to cyclin, nor was there any literature precedence for a *bona fide* substrate of CDK11. Thus, no 'off-the-shelf' assays were available to conduct a high-throughput screening campaign. Through extensive testing of custom made CDK11 and cyclin protein reagents, an active, high quality CDK11/CyclinD3 complex was selected for our internal screening funnel. During development of the first known catalytic activity assay for CDK11/CyclinD3 a novel substrate was discovered. Kinetic characterization was conducted on our custom probe compound, thereby facilitating development of a high-throughput binding assay that correlates with inhibition of kinase activity.

Results
Two curated small molecule libraries (10,320 compounds) were screened via our internally developed binding assay. 593 hits representing 30 unique scaffolds were identified from the screen which showed >60% inhibition at 50 uM and >0.30 ligand efficiency. After scaffold clustering, removal of PAINS compounds, virtual docking in homology models, and follow up in full dose-response, that was narrowed down to 72 compounds to progress into the hit-to-lead stage.

Conclusions
Here we briefly describe screening efforts as well as development of our *in vitro* assay infrastructure consisting of a CDK11/CyclinD3 binding assay, catalytic activity assays for CDK2/CyclinA2, CDK9/CyclinT1, and CDK11/CyclinD3, an intracellular target engagement assay, and a 120-hour anti-proliferation phenotypic assay. Through rational drug design, this led to the development of small molecule inhibitors with single-digit nanomolar potency against CDK11 and 200-fold selectivity over other CDK family members.

Poster 41

Preclinical Development and Characterization of GT-14, a Novel Giα_i2 Inhibitor for Prostate Cancer

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Background

Giα_i2 (heterotrimeric G-protein subunit alphai2) protein plays a critical role in cell migration and invasion of prostate cancer cells. Using a structure-based approach, several small molecule inhibitors were synthesized by Dr. Adegboyega Oyelere at George Tech and characterized by Dr. Shafiq Khan at Clark Atlanta University. Those compounds could specifically prevent the activation of the Gαi2 subunit. One of the lead molecules is GT-14 which showed significant inhibition of the migratory behavior of the PC3 and DU145 prostate cancer cell lines at a concentration of 10 μM. We further conducted the preclinical development of GT-14 at TSU.

Methods

Physicochemical properties (pKa, log P and solubility) of GT-14 were determined and a cosolvent formulation was developed. *In vitro* metabolic study was performed rat liver microsomes with 1mM of GT-14 to identify Phase I and II metabolites. A pharmacokinetic study of GT 14 with intravenous dosing (5 mg/kg in cosolvent formulation) was performed in male SD rats. PK parameters were derived using Phoenix WinNonlin.

Results

GT-14 is a weakly basic compound with very poor water solubility and high lipophilicity (log P> 3). It has a pKa of 3.46 and 10.53. It is freely soluble in DMSO and DMA and shows high solubility in organic solvents. A cosolvent formulation consisting of PEG 300: PEG 400: Propylene glycol in a ratio of 3:2:2 was developed for Intravenous administration and solubility of GT-14 is 8mg/ml. The cosolvent formulation was well tolerated by rats. The concentration- time profile of GT-14 fits a 2-compartment model. GT-14 shows a distribution and elimination half-life of approx. 12 min and 4 hours, respectively. *In vitro* metabolic study showed GT-14 undergoes only Phase I metabolism.

Conclusions

The Physicochemical and pharmacokinetic parameters of GT-14 have been characterized that can used or future formulation development.

Acknowledgements

National Institute on Minority Health and Health Disparities of the National Institutes of Health (NIH) under award number 2 U54 MD007605-27A1.

Poster 42

Impact of Host Physiological and Pathological Conditions on the Activity of Gut Microbial Beta-glucuronidases Towards Hydrolysis of Flavonoids: Baicalin, Wogonoside and Lutoelin

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Purpose: The purpose of this paper is to determine the impact of host physiological and pathological conditions on the activity of gut microbial beta-glucuronidases towards hydrolysis of flavonoid-glucuronides.

Methods: Fecal S9 fractions were prepared using feces collected from different types of rats at different ages with different genders. Baicalin, wogonoside, and luteolin-glucuronide were used as the substrates. A waters acquity performance liquid chromatography (UPLC) system was used to quantify the metabolite baicalein to analyze the rate of the reaction of the enzymes. The rates were compared by obtaining enzymes through S9 fractions to confirm microbiota ability to hydrolyze the glucuronide and release of the parent compound, baicalein. Fecal S9 prepared from The Fischer 344 (F344) rats at three different ages (i.e., 5, 9, and 16 weeks) and different inflammatory conditions treated with Docusate Sodium (DSS) or anti-inflammatory agent herbal mixture Xiao-Chai-Hu Tang (XCHT). Additionally, fecal S9 from genetically modified pirc rats, which spontaneously have inflammation in the colon, was also tested.

Results: The results depicted that age had an impact on hydrolysis of the compound baicalin into its parent compound and this method was best suited to determine the rate of hydrolysis. The $p<0.05$ making the results statistically significant. The wild type enzymes had a clear increase in Km and Vmax. While PRIC enzymes and enzymes treated with DSS and XCHT had a clear difference in rates, but the Km and Vmax did not increase significantly.

Conclusion: The data shows that microbial GUS activity was higher at elder age. Fecal S9 from Pirc rats has lower activity and anti-inflammatory agent XCHT can increase microbial GUS activity.

Acknowledgements: Dr. Gao, Dr.Ting and Imoh

Funding Source: NIH grant funding for a Center for Biomedical and Minority Health Research

Poster 43

Peptide-based Probes Reveal a Scaffolding Function of GSK3β in the Nav1.6 Channel Complex

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Background: Signaling pathways regulating ion channel macromolecular complexes play a crucial role in fine-tuning neuronal activity and remodeling synapses. Yet, the molecular underpinnings of activity-dependent signaling mechanisms regulating these molecular complexes are still poorly understood. In recent studies conducted in the nucleus accumbens (NAc), we have shown that vulnerability to depression-like and stress-induced disorders induces a form of maladaptive plasticity consisting of hyperexcitability of medium spiny neurons (MSNs). Vulnerability in these cells is mediated by the increased interaction between glycogen synthase kinase 3β (GSK3β) and the C-terminal domain (CTD) of the voltage-gated Na+ channel Nav1.6. A decoy peptide mimicking the Nav1.6 segment interacting with the kinase or *in vivo* genetic silencing of GSK3β was found to be sufficient to prevent MSN maladaptive plasticity.

Hypothesis/Goals: We hypothesized that inhibition of the GSK3β/Nav1.6 protein-protein interaction complex can counteract maladaptive plasticity of MSNs. Our goal was to develop chemical probes capable of modulating the scaffolding function of GSK3β in the Nav1.6 channel complex.

Methods: Molecular docking of rationally designed peptide-derived small molecules, split-luciferase complementation (LCA), surface plasmon resonance (SPR), whole-cell patch-clamp electrophysiology in heterologous cells and *ex-vivo* acute slice preparation, and viral vector-based gene silencing for target validation studies.

Results: We identified ZL141, a peptide-derived small molecule that targets specifically and selectively the GSK3β/Nav1.6 complex with no predicted interference with the kinase enzymatic activity. ZL141 was found to significantly inhibit GSK3β/Nav1.6 complex formation using the LCA and to bind to GSK3β using SPR. In addition, whole-cell patch-clamp recordings in HEK293 cells stably expressing Nav1.6 showed that ZL141 regulates peak current density, voltage-dependent activation, and steady-state inactivation curves as well as long-term inactivation of Nav1.6 in a GSK3β-dependent manner. Studies employing GSK3β *in vivo* genetic silencing and *ex vivo* slice recordings of MSN in the NAc are in progress.

Conclusions: We expect ZL141 and other small molecules targeting the GSK3β/Nav1.6 channel complex to inhibit maladaptive firing of MSNs ultimately reducing susceptibility to depression-like and stress-induced disorders. These studies lay the groundwork for the development of novel neurotherapeutics based on modulation of the scaffolding role of GSK3β in the Nav1.6 macromolecular complex.

Funding: We acknowledge our funding sources as follows: NIH Grant NIH R01MH124351 (FL), R01MH132226 (F.L.), R01 MH111107 (F.L., J.Z.), R01 DA038446 (JZ), U18DA052504 (FL), and HAMBP training grant no. T32GM008280 (NMD).

Poster 44

Targeting Cancer Stem Cell Plasticity to Overcome Colorectal Cancer Resistance and Relapse

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Background

Colorectal cancer (CRC) relapse can be attributed to cancer stem cells (CSC), an immortal cell population thought to potentiate metastatic progression by exploiting its self-renewability and differentiation capacity. Owing to these mystifying properties, CSCs are an attractive drug target. In the past, our group and others have attempted targeting CSCs using antibody-drug conjugates (ADCs) against the Leucine-rich repeat-containing G protein-coupled Receptor 5 (LGR5), a well-recognized CSC marker that is frequently upregulated in CRC tumors. Our previous attempts at eradicating CRC by targeting $LGR5^+$ CSCs with an LGR5-directed ADC have resulted in incomplete tumor regression or relapse. Follow-up studies suggest that CRC cells evade this therapeutic insult by converting into a quiescent $LGR5^-$ state. Further, the $LGR5^-$ drug-resistant cells use the MET-STAT3 signaling cascade to bolster their invasive and metastatic potential.

Hypothesis

Plasticity and drug intolerance in $LGR5^+$ CSCs are regulated by the IQGAP1 mediated MET-STAT3 pathway and can be overcome through concurrent MET and LGR5 inhibition.

Methods

I will evaluate the mechanistic relationship between MET-STAT3 and LGR5 on CRC plasticity through MET ablation. I will investigate the effect of the feedback loop between MET-STAT3 and LGR5 on downstream signaling via immunoprecipitation and pulldown assays. Two anti-MET monoclonal antibodies (mAbs) binding different MET structural domains will be cloned, evaluated *in vitro* for internalization, and subsequently conjugated to cytotoxic drug payloads. Efficacy of MET-and LGR5-ADCs alone or in combination will be evaluated *ex vivo* and *in vivo*.

Results

Both anti-MET mAbs clones are promising candidates following evaluations for specificity, and internalization. My preliminary data also suggests the two candidates demonstrate high cell-killing efficacy using a secondary ADC conjugated with Pyrrolobenzodiazepine (PBD).

Conclusions

My proposed dual-targeted therapeutic modality will act as a guided missile that delivers cytotoxic agents to the heterogenous CRC tumors. I believe that elimination of $LGR5^+$ CSCs and their $LGR5^-$ counterparts will be the linchpin of tumor eradication in CRC patients.

Acknowledgments

This work is supported by funding from the National Institutes of Health (NCI R01 CA226894) to Carmon KS and a fellowship of the Gulf Coast Consortia, on the Training Interdisciplinary Pharmacology Scientists program (T32 GM139801) to Jacob J.

Poster 45

Designing an Immunofluorescence-Based Biomarker Assay for Detecting Rb and Phospho-Rb Expression in HR+/HER2- Breast Cancer

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Background: Cyclin-dependent kinases 4/6 inhibitors (CDK4/6i) have emerged as a major advance in treating hormone receptor-positive/human epidermal growth factor receptor-2-negative breast cancer (HR+/HER2- BC), which accounts for over 60% of all breast tumors. However, response rate to CDK4/6i combined with endocrine therapy (ET) is only 50% or less, with *de novo* resistance seen in 15-30% in patients with HR+/HER2- metastatic BC. Therefore, predictive biomarkers that can help select patients who are likely to derive clinical benefit from adding CDK4/6i to ET are urgently needed to spare non-responders from toxicities and cost.

Goals: The long-term objective of our study is to design and develop a predictive biomarker assay that can be used on circulating tumor cells for evaluating tumor response to CDK4/6i [palbociclib, ribociclib, and abemaciclib]. In this research, we aimed to select internal controls that can be used for the immunofluorescence (IF) assay to evaluate the expression of Rb and phospho-Rb as potential predictive biomarkers for CDK4/6i therapy.

Methods: MCF7 and T47D parental (P) cells were treated with various concentrations of abemaciclib and palbociclib to determine their effects on cell growth by automated cell counting using Ensight® Multimode Plate Reader and on Rb and phospho-Rb expression by western blotting (WB) and IF using Leica SP8 STED confocal microscope. We also utilized derivatives of MCF7 and T47D cells resistant to estrogen deprivation (EDR) and/or Palbociclib (PalboR).

Results: Abemaciclib and palbociclib treatment resulted in a concentration-dependent decrease in cell growth of MCF7 P (IC_{50}: 37.4 and 254.7 nM, respectively) and T47D P (IC_{50}: 2.7 and 59.1 nM, respectively) cells. Abemaciclib also reduced the expression phospho/total-Rb in a concentration-dependent manner by both WB and IF. The IC_{50} for abemaciclib was 19 nM in MCF7 P and 24 nM in T47D P cells when detected by WB. Similarly, there was a concentration-dependent decrease in the phospho/total-Rb with abemaciclib treatment as detected by IF. Expression of both total Rb and phospho-Rb was undetectable in the P/PalboR T47D cells compared to T47D P cells by both WB and IF. Therefore, this cell line can be used as a control for lack of Rb expression. EDR/PalboR derivative of MCF7 had ~50% reduction in Rb and ~70% reduction in phospho-Rb by IF, supporting its use as a control for reduced Rb expression.

Conclusion: In summary, we have identified cell line models that can be used as internal controls for detecting the expression of total and phospho-Rb as predictive biomarkers of CDK4/6i treatment response in HR+/HER2- MBC patients. Ongoing performance validation studies are underway, including automated quantitation using digital whole slide fluorescent imaging to optimize the throughput and dynamic range of the assay.

Acknowledgements: This research was conducted by the funding from the Cancer Prevention & Research Institute of Texas (CPRIT, RP210148) and the Grants to Enhance & Advance Research program (GEAR, I0506932) from UH.

Poster 46

Characterizing Interferon-Stimulated Genes as Targets of Interest Against SARS-CoV-1 and -2 Infection in Pathologically Relevant Human Lung Epithelial Cells

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Background: Severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) and -2 are beta-coronaviruses (β-CoVs) that have caused significant morbidity and mortality worldwide. Improved understanding of the host innate immune responses to β-CoVs would allow us to identify potential targets for the development of effective and novel medical countermeasures.

Hypothesis/Goals: We hypothesize that SARS-CoV-1 and -2 induce similar and dynamic innate immune gene profiles and pathways in pathologically relevant human lung bronchial epithelial cells (Calu-3/2B4), enabling selection of interferon-stimulated genes (ISGs) as potential targets for host-directed antivirals. Our goals are to examine the innate immune responses triggered upon SARS-CoV-1 and -2 infection over time in Calu-3/2B4 cells and characterize certain ISGs for further examination through systems biology and experimental approaches.

Methods: We analyzed RNA from virally infected Calu-3/2B4 cells through RNA sequencing, and genes with an expression value of more or less than a log2fold change of ± 1.5 and a statistically significant p_{adj}-value of < 0.05 were chosen. We then performed QIAGEN's Ingenuity Pathway Analysis and functional enrichment analysis through Cytoscape App ClueGO and Gene Analytics™. Selected ISGs expression were validated through RT-qPCR.

Results: We selected four ISGs for further examination: bone marrow stromal cell antigen 2 (BST2), Z-DNA binding protein 1 (ZBP1), interferon induced transmembrane protein 1 (IFITM1), and C-X-C motif chemokine ligand 11 (CXCL11). The ISGs of interest are related to negative regulation of the viral life cycle and the response to type I interferon, and they are predicted to defend the host against both β-CoVs infection. The highest levels of expression occur at 48 hpi, and SARS-CoV-2 infection generally elicits a stronger response compared to SARS-CoV-1.

Conclusions: Antiviral signaling and the innate immune response are significantly induced following SARS-CoV-1 and SARS-CoV-2 infection. In addition, we have identified BST2, ZBP1, IFITM1, and CXCL11 as potential targets for host-directed therapeutics. Our next steps are to continue characterizing the innate immune response against β-CoVs infection *in vitro*.

Acknowledgements: We would like to thank Dr. Steven Widen and the Next Generation Sequencing Core for performing RNASeq and Dr. Kamil Khanipov and the Microbiome Sequencing and Analysis Core for performing Ingenuity Pathway Analysis.

Poster 47

Nanozymes: A New Class of Synthetic Therapeutics That Promote Several Critical Biological Reactions to Protect From Tissue Injury and Mitochondrial Dysfunction

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Background:

Nanozymes are a recently conceptualized class of synthetic nanosized therapeutics that emulate natural enzymes. While their affinities for biological reactions they promote are typically less than native enzymes, nanozyme advantages include enhanced stability and flexible chemistry. Their mechanisms of action typically involve redox mediation (e.g., electron transfer) rather than facilitating conformational changes or other common enzyme actions that catalyze reactions. We and others have reported that acid oxidation of carbon rich sources generates nanozymes with cellular protective properties in disorders including brain ischemia and inflammation. These studies mostly focused on the protective role of their superoxide dismutase (SOD) mimetic nanozyme actions. Unlike native SOD, harshly oxidized carbon nanoparticles (OCNPs) developed by our laboratories facilitate dismutation without transition metals and achieve dismutation rates within an order of magnitude of native SOD (Samuel et al, PNAS 2015). Mechanistic explanations for their catalytic activity include the role of quinone moieties generated through acid oxidation, resulting in a strong intrinsic radical that participates in electron transfer reactions. We recently reported that particle size and broad redox potential are important parameters for *in-vitro* and *in-vivo* cellular protection; the former contributes to rapid cellular uptake and the latter encompasses multiple biologically relevant oxidation and reduction reactions (McHugh et al, Advanced Materials, 2023). Given their broad redox potential, we explored other nanozyme actions.

Hypothesis:

In addition to high-capacity SOD mimetic activity, we hypothesize that OCNPs improve mitochondria functions, accelerate NAD+ production from NADH, and promote endogenous antioxidant response Nrf2/Keap1 with its sulfide transferase-like activity.

Results:

Our poly(ethylene glycol) (PEG)-modified, OCNPs mediate the oxidation of NADH and reduction of cytochrome C, actions that support mitochondrial function in genetic conditions and acquired injuries (Derry et al, Nanoscale 2019). Adding to this range of nanozyme actions, we recently discovered these particles oxidize hydrogen sulfide to polysulfides and thiosulfates, products that enhance endogenous antioxidant protectants through mechanisms including Nrf2/Keap1 pathway. Here, we show that our OCNPs protect from H_2S toxicity, an action of growing interest in allergic and inflammatory conditions and genetic disorders, e.g., Down Syndrome.

Conclusion:

We anticipate the range of nanozyme actions that OCNPs mediate to expand suggesting promising therapeutic applications given mechanisms of injury common to many disorders.

Acknowledgements:

Discovery and development validation studies funded by Department of Defense
and National Institutes of Health (R01NS094535; TAK, PJD, AL), the Welch Foundation Grant BE-0048 (TAK) and Texas A&M Presidential Clinical Research Partnership Awards (TAK and AV).

Poster 48

The Identification of a Bisulfite Adduct of NI7, and its Application for NI7 Quantification

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Background NI7, is a potent inhibitor of NADPH oxidases (NOX) which has recently been identified as a novel agent targeting to triple-negative breast cancer. NI7 is unstable in bio-matrix, and it completely disappeared in mouse whole blood samples in 10 days stored under -70°C. In the present study, a stabilized form of NI7, NI7 bisulfite adduct, was identified, and it was successfully applied for NI7quantification using LC-MS/MS.
Methods The identification of NI7 and its bisulfite adduct was conducted on an X500B QTOF mass spectrometer (SCIEX, Framingham, MA, USA). TOF-MS and MS/MS data were monitored using the Information-dependent-acquisition (IDA) in negative mode by SCIEX OS software 1.6.1. NI7 quantification via monitoring its bisulfite adduct was conducted on a 6500+ Triple Quad LC-MS/MS System (AB SCIEX LLC, CA, USA) coupled with a Synergi Fusion-RP column (50 x 2 mm, 4 μm, 80 Å, Phenomenex Inc.). The measurement of the analyte and the internal standard warfarin were employed to detect the MRM transition at m/z 340.1 to 127.0 for the analyte and at m/z 307.1 to 160.9 for the IS using negative multiple reaction monitoring (MRM) mode Data were acquired by Analyst software 1.6.3.
Results NI7 bisulfite adduct was identified using QTOF MS/MS, and a UPLC-MS/MS method was developed and validated by stabilizing and monitoring NI7 in its bisulfite adduct form. No interference was observed for both analyte and the IS. There was no carryover for both analyte and IS. The linearity range of the calibration curves was between 0.5 to 500 ng/mL with regression correlation coefficients > 0.99. The intra-day and inter-day accuracy (RE%) were from -3.13 to 3.33% and -5.58 to 5.18%, respectively. The intra-day and inter-day precision (CV%) ranged 3.86 ~ 12.34% and 5.28 ~ 12.19%, respectively. These data indicate that the method is accurate and precise for the quantification of NI7 in mouse whole blood. Mean matrix effect and recovery were 105.26 ± 5.84 and 92 ± 7.73 % at QC levels, meaning the matrix effect was negligible and the extraction was thorough in this study. The stability results indicate that no significant degradation occurred under the experimental conditions.
Conclusions A bisulfite adduct of NI7 was identified and a UPLC-MS/MS method for the quantification of NI7 in mouse whole blood samples was developed and validated. The method could be applied to NI7 PK studies in mouse whole blood.
Acknowledgements This study was funded in part by the NIH/NIMHD-Research Centers in Minority Institutions Program (U54MD007605), the Cancer Prevention & Research Institute of Texas (CPRIT) Core Facilities Support Awards (RP180748) to DL and HX; and NCI R01CA251206 to S-YL.
Keywords: NOX inhibitor NI7; Bisulfite adduct; LC-MS/MS; Pharmacokinetic study

Poster 50

Bring the Power of Antibodies to the Bone

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Abstract: Over the past 30 years, antibody-based therapies against cancer have been developed and proved to be successful in the clinic. Despite their clinical success, delivery of these antibodies to the bone niche has proven to be difficult due to relatively low vascularization and the physical barriers of penetration. Inspired by the bone-targeting mechanism of natural biomolecules, we have developed an innovative bone targeting (BonTarg) technology that enables the preparation of antibodies with both antigen and bone specificity. The resulting bone-targeting antibodies exhibit improved *in vivo* therapeutic efficacy in the treatment of breast cancer micrometastasis and in the prevention of secondary metastatic dissemination from the initial bone lesions. This study establishes a new strategy for transitioning antibody-based therapies from antigen-specific to both antigen and tissue-specific, thus providing a promising new avenue for advancing antibody therapy toward clinical translation.

Poster 49

Integrative Bioinformatic Analysis of p53 and Pathway Alterations in Two Different Lung Cancer Subtypes

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Background
Whether p53, either wild type (WT) or mutant, plays cell-specific or uniform role remains controversial.
Goals
In this study, we aim to investigate the role of *p53* the lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), two lung cancers with different cellular origins and frequent *p53*mutation.
Methods
The analysis of *p53* gene status and its association with clinical outcomes in human cancers was performed on cBioPortal platform. The data for the genes that are co-expressed along with p53 were retrieved from cBioPortal, which contains gene name, cytoband, spearman's correlation, *p* value and *q* value (the *p* value adjusted for the False Discovery Rate) for each gene that is correlated with p53 in mRNA expression. The data for genes that have a *q* value less than 0.05 were uploaded into Qiagen's IPA system (www.ingenuity.com) for core analysis to determine canonical pathways in each cancer. Pathway comparison analysis was also performed to generate unsupervised hierarchical clustering heatmap. Additionally, raw counts were downloaded from TCGA database in GDC data portal. The samples were classified based on *p53* status (wild type vs. missense mutation in the DNA binding domain). The DESeq2 R package was used to perform the differentially expressed gene analysis between the two classified groups in LUAD and LUSC and generate the principal component analysis (PCA) plot and MA-plot.
Results
Mutant p53 more strongly correlates with different genomic alteration and protein expression profiles in LUAD than in LUSC. *p53* mutation in LUAD and LUSC is associated with multiple exacerbated clinical outcomes. Although the presence of *p53* mutation does not change the survival of LUAD patients, LUSC patients containing *p53* mutation exhibit surprisingly prolonged survivals. Ingenuity Pathway Analyses with genes co-expressed with WT or mutant p53 in both LUAD and LUSC show that mutant p53 in these two cancers are correlated with different signaling. Additionally, WT p53 in LUAD are largely associated with activation of tumor suppressive pathways and suppression of the tumor promotive ones, a pattern different from what is observed for WT p53 in LUSC. Furthermore, pathway analyses of genes differentially expressed between cancers with mutant and WT p53 for both LUAD and LUSC revealed different pathway fashions for these two cancers.
Conclusions
Our study indicates that both WT and mutant p53 may have cell-specific functions, which needs to be validated with future experimental investigations.
Acknowledgements
Y.Z. has been funded by NIGMS [1SC2GM135111-01], NIH RCMI [U54MD007605] and CPRIT [RP180748].

Poster 51

Development of ADAR1 Inhibitors to Improve Cancer Immunotherapy

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Cancer immunotherapies have had unprecedented success in recent years by harnessing the power of the immune system to recognize and eliminate cancer cells. Recent genetic screens have identified ADAR1 as a promising target for improving immunotherapies. ADAR1 is an enzyme that catalyzes the posttranscriptional conversion of adenosine to inosine in double-stranded RNA. Editing levels of dsRNA are elevated in most tumor types relative to normal tissue, making ADAR1 an attractive target for cancer therapy. Our ultimate goal is to develop safe and effective inhibitors that can improve cancer therapy. Developing ADAR1 inhibitors for cancer therapy faces two main challenges: low throughput assays for ADAR1 activity and lack of structural information for ADAR1 protein. To overcome these challenges, we are exploring alternative methods for drug screening and designing inhibitors, such as developing new assays and using computational methods. We are developing a biochemical assay for ADAR1, as well as an in-cell activity assay, to enable high-throughput drug screening and minimize false positives. These assays will allow us to efficiently identify potential inhibitors of ADAR1 and prioritize hits for further investigation. In addition, we are conducting high-throughput docking studies using the recently resolved ADAR1 cryo-EM structure. By combining wet lab assays and dry lab computational methods, we aim to quickly identify effective hits for ADAR1 inhibition and optimize their chemical structures for improved potency and selectivity. This collaborative approach will allow us to leverage the strengths of both wet and dry laboratory work, and ultimately accelerate the discovery of safe and effective ADAR1 inhibitors for cancer therapy.

Acknowledgments: We are grateful for the generous support from Cancer Prevention and Research Institute of Texas funded Cancer Therapeutics Training Program CPRIT Grant No: RP210043

Poster 52

Identification of StagX1, an Isoquinolinone Compound, as a Potential Therapeutic Agent for Ewing Sarcoma Targeting Carboxylesterase 1 (CES1)

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Ewing sarcoma (EWS) is a highly aggressive tumor and the second most common bone or soft tissue tumor in children and young adults. It is characterized by chromosomal translocations involving a member of the FET gene family and an ETS transcription factor, such as *EWSR1–FLI1* (occurring in ~85% of cases), as well as *EWSR1-ERG* and other less frequent fusions (~10-15% of cases). The fusion proteins resulting from these translocations are tumor-specific transcription factors that reprogram the transcriptome and epigenetics of the genome.

Using high-throughput screening, we identified a compound named StagX1 that inhibits the growth of Ewing sarcoma (EWS) cell lines containing carboxylesterase 1 (CES1). Mechanistic studies have shown that StagX1 triggers apoptosis in sensitive EWS cell lines by activating caspases and cleaving Parp, but has no effect on the cell cycle. However, StagX1 is a carboxyl ester with limited stability in liver microsomes and mouse plasma, and it gets hydrolyzed to form an acid product (StagX1-acid). Although StagX1-acid is stable, it cannot inhibit cell growth, likely because of its hydrophilic nature that prevents it from entering cells.

Inhibitors of CESs prevented StagX1 from being converted to StagX1-acid, thereby eliminating the effect of StagX1 on cell growth inhibition. RNA-seq and immunoblotting assays indicated that StagX1-sensitive cell lines had significant overexpression of *CES1*, suggesting that CESs might be responsible for the hydrolysis of StagX1. *In vitro* enzymatic assays confirmed that CES1 is the enzyme responsible for hydrolyzing StagX1. StagX1 was stable in tissue culture medium and was gradually converted to StagX1-acid when added to cells. Both StagX1 and StagX1-acid were found inside cells within 15 minutes, suggesting that StagX1 is a prodrug that is hydrolyzed into StagX1-acid by CES1 inside cells.

Currently, the effect of StagX1-acid on cell growth is being investigated.

This study was funded by the CPRIT Grant # RP190002 and Kate Amato Foundation