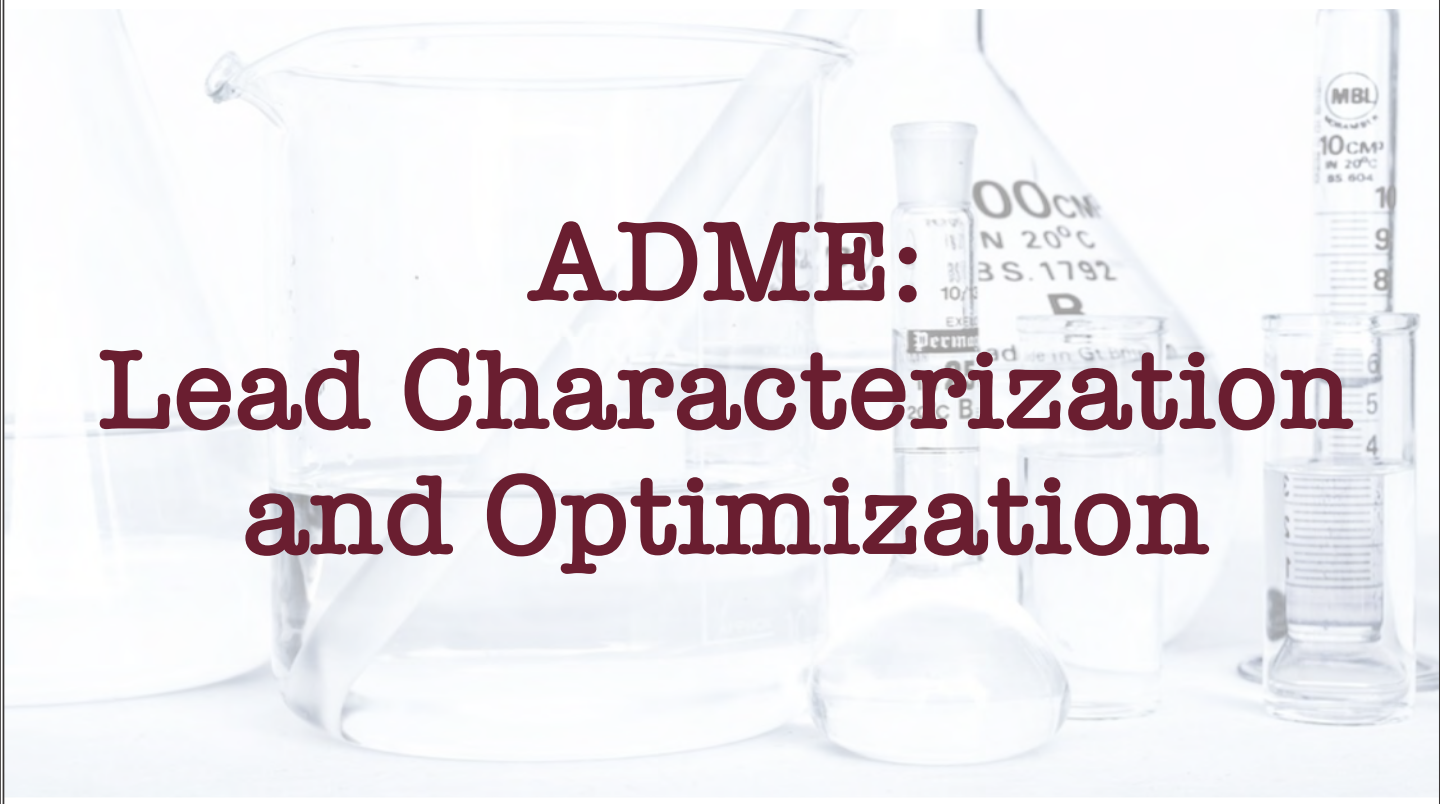
October 29, 2019

BioScience Research
Collaborative
Auditorium
6500 Main St.



ADME:
Lead Characterization and Optimization

CPRIT

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

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

Gulfcoastconsortia.org

Conference Organizing Committee:

Diana Chow, University of Houston
Dong Liang, Texas Southern University
Omonike Olaleye, Texas Southern University
Suzanne Tomlinson, Gulf Coast Consortia
Huan Xie, Texas Southern University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8:30 Breakfast and Poster Setup

9:00 Welcome and Introduction to GCC Center for Comprehensive PK/PD and Formulation (CCPF)
Suzanne Tomlinson, Gulf Coast Consortia for Quantitative Biomedical Sciences
Omonike Olaleye, Texas Southern University
Dong Liang, Texas Southern University College of Pharmacy and Health Sciences

Convener: **Diana Chow**, University of Houston

9:10 **Keynote:** *You Have an Active Compound; Where Do You Go from Here in Drug Development*
Leslie Benet, University of California San Francisco

9:50 *Clinical Drug Development*
S. W. Johnny Lau

10:25 Break

Session 1: Drug Metabolism and PK/PD and Formulation 101
Convener: **Omonike Olaleye**, Texas Southern University

10:45 *Preclinical Pharmacokinetic Studies*
Dong Liang, Texas Southern University

11:10 *Preclinical Pharmacokinetic and Pharmacodynamic (PK/PD) Correlation and Allometric Scaling*
Diana Chow, University of Houston

11:35 *Preclinical Drug Characterization and Formulation Development*
Huan Xie, Texas Southern University

12:00 Lunch and Poster Session
1:00 Poster Session (Poster presenters stand at posters)

Session 2: Progress and Potential: CCPF Users
Convener: **Huan Xie**, Texas Southern University

2:00 *Developing a Novel Mucoadhesive Patch for Drug Delivery and Chemoablation of Oral Premalignant Lesions*
Robert Tsai, Institute of Biosciences and Technology, Texas A&M Health Science Center

2:10 *A Hindsight Reflection on the Clinical Development of Opaxio (paclitaxel poliglumex): Lessons Learned*
Chun Li, PhD, MD Anderson Cancer Center

2:20 *Discovery and Development of Novel Mechanism-of-Action Oral Drugs to Reverse Obesity-linked Type 2 Diabetes*
Stan Watowich, Ridgeline Therapeutics, University of Texas Medical Branch

2:30 *Small Molecule Targeting of Androgen Receptor-associated Cochaperones for the Treatment of Prostate Cancer*
Marc Cox, UT El Paso

2:40 Break

Session 3: IND-Enabling Studies: Regulatory Considerations, Current State-of-the-Art and New Opportunities
Convener: Dong Liang, Texas Southern University

3:00 *In Vitro Transfer Tests De-Risk Decisions to IND*
Fady Ibrahim, Pfizer

3:20 *Pre-clinical Pharmacokinetics, Tissue Distribution and Physicochemical Studies of CLBQ14, a Novel Methionine Peptidase Inhibitor for Infectious Diseases*
Oscar Ekpenyong, Merck

3:40 *Quantitative Pharmacology in Support of Design, Selection, & Development of Biologic Modalities*
Mohammad Tabrizifard, Ascendis Pharma

4:00 *Formulation Considerations in Developing Large Molecule Drugs*
Cinzia Stella, Genentech

4:20 *Early In Vitro and In Vivo Toxicology Studies to Push Your Drug into Development*
Jeff Larson, Iterion

4:40 Reception

Leslie Z. Benet, PhD
Professor
Bioengineering & Therapeutic Sciences

You Have an Active Compound; Where Do You Go From Here in Drug Development?

Dr. Benet, Professor and former Chairman (1978-1998) of Bioengineering and Therapeutic Sciences, University of California San Francisco (UCSF), received his AB, BS and MS from the University of Michigan, and PhD from UCSF. He has received nine honorary doctorates, five from Europe and four from the US. Dr. Benet served as President of the Academy of Pharmaceutical Sciences (1985) and in 1986 was a founder and first President of the American Association of Pharmaceutical Scientists. In 1987 he was elected to membership in the National Academy of Medicine of the US National Academy of Sciences. In 1993-4 he served as President of the American Association of Colleges of Pharmacy and from 1996-2000 as Chair of the International Pharmaceutical Federation (FIP) Board of Pharmaceutical Sciences. Among his many more recent honors were dedication of the September 2012 issue of Pharmaceutical Research, the 2013 APhA Ebert Prize, dedication of the September 2013 issue of Journal of Pharmaceutical Sciences, selection for the 2013 AAPS Journal Manuscript Award, ISSX North American Achievement Award in 2015, and the Remington Honor Medal of APhA in 2016, the highest award in American Pharmacy. Dr. Benet has published over 590 scientific articles and book chapters, holds 12 patents and served as editor of 7 books. He has been listed by Clarivate Analytics among the most highly cited pharmacologists worldwide since 2001 with his peer reviewed publications being referenced in the peer reviewed literature on more than 28,000 occasions. This year, he founded his 5th start-up biopharmaceutical company, Squal Pharma Inc. (www.squalpharma.com).

Abstract: I am presently in the process of starting a new company, my fifth www.squalpharma.com, so the topic is relevant to you as well as me. First, do you have funding? If not, here are the key points to cover in your presentations: a) Problem/Opportunity; b) Solution; c) Data; d) IP; e) Regulatory and Reimbursement; f) Go to Market (Competition); g) Financials/Exit and Partnering; h) Management Team. With funding you must leverage the work done to date to complete an IND. These IND enabling studies include: i)Synthesis, formulation, characterization, and scale-up; j) Stability studies; k)Preclinical verification in animal models; l)Safety/toxicology studies; m) Additional patent filings; n) Clinical development plan; o)Pre-IND meeting and p)File IND with FDA. In my presentation, I will present an overview of these processes.

S.W. Johnny Lau, RPh, PhD

Senior Clinical Pharmacologist

The Science of Clinical Pharmacology in Clinical Drug Development

Johnny Lau is a senior clinical pharmacologist at the Office of Clinical Pharmacology of the Food and Drug Administration. He helps guide drug development through the review of the clinical pharmacology and biopharmaceutics studies for New Drug Applications, Investigational New Drug Applications, Pediatric Study Plans, Proposed Pediatric Study Requests, and study protocols for metabolic, endocrine, bone, reproductive, urologic, pulmonary, and rheumatologic drug products. He was formerly a clinical pharmacokineticist at the Procter & Gamble Pharmaceuticals. He also worked for the Norwich Eaton Pharmaceuticals. as a clinical biopharmaceutics scientist. He received his B.S. (pharmacy) and M.S. (pharmacokinetics) from the University of Washington, and Ph.D. (pharmaceutics) from the University of Houston. He is a diplomate of the American Board of Clinical Pharmacology. He founded and chairs the Geriatric Scientific Interest Group in his current position. He is a registered pharmacist in the state of Texas and Washington.

Abstract: This presentation aims to help attendees understand the basics of clinical drug development. This presentation will discuss the product label-driven clinical drug development, which consists of iterative learn-confirm cycles. One fundamental objective of drug development is to find a dose, or dose range, for a drug candidate that is both efficacious and safe. This presentation will show the application of principles of Clinical Pharmacology in drug development. This presentation will also discuss important regulatory issues in clinical drug development. This presentation will provide ample references for attendees to further learn clinical drug development.

Special Thanks to Dr. Dong Liang, Director, GCC Center for PK/PD and Formulation

Dong Liang, PhD
Professor and Chair
Pharmaceutical and Environmental Health Sciences
Preclinical Pharmacokinetic Studies

Dr. Liang has industrial and academic expertise in pre-clinical and Phase I PK and drug development studies. He has worked on Phase I PK studies from protocol design (clinical study monitoring; bioanalysis; and statistical PK data interpretation) to FDA submissions for over 35 generic drug product approvals. He is co-inventor of 5 U.S. patents in dosage formulation development. He is the Director of CCPF with responsibility for the overall operations of the program, as well as providing support in PK studies in rats.

Abstract: In August 2018, Texas Southern University in collaboration with the University of Houston College of Pharmacy and the Gulf Coast Consortia (GCC) received a CPRIT award to establish GCC Center for Comprehensive Pharmacokinetics and Pharmacodynamics (PK/PD) and Formulation (CCPF). While there is no shortage of brilliant cancer investigators throughout the Texas Medical Center, and exciting cancer discoveries on a regular basis, academic resources for developing those discoveries into actual cancer drugs/therapeutics, has been largely unavailable except through CROs, which are often very expensive and inaccessible to many cancer researchers. Developmental pharmacokinetics/pharmacodynamics (how the body handles a potential drug and how the drug affects the body) and dosage formulation studies are necessary for advancing a potential drug candidate to clinical trials. This presentation aims to introduce CCPF core resources to attendees in the areas of pre-clinical pharmacokinetic studies, from bioanalytical LC-MS/MS method quantifying drug concentrations in biological media to in vitro metabolic and permeation studies, and to in vivo pharmacokinetic studies using rodents as animal models. Pharmacokinetic study design and data interpretation will be discussed.

Diana Shu-Lian Chow, PhD, FNAI
Professor, Pharmaceutics
Director, IDER

Preclinical Pharmacokinetic and Pharmacodynamic (PK/PD) Correlation and Allometric Scaling

Dr. Chow has been faculty for 38+ years at COP-UH, focusing on translational research to move bench-top research to clinical trial and FDA approved product. Dr. Chow is expert in IND enabling preclinical and clinical pharmacokinetics/pharmacodynamics, particularly in the development, modeling and analyses of novel drug formulations and drug-delivery systems. Her research resulted in more than 10 U.S. and international patents, and product of FDA-approved Busulfex®. She is inducted Fellow of the National Academy of Inventors (2016), and recipient of Houston Intellectual Property Law Association "Inventor of the Year Award" (2009). She is author or co-author of more than 250 peer-reviewed journal articles, book chapters, and presentations.

Dr. Chow has mentored 37 doctoral students and 19 postdoctoral fellows, who are all well placed with successful careers in academic, industrial or regulatory settings.

Abstract: The GCC-CCPF has establish the infrastructure to support PK/PD model analyses and simulation at Institute for Drug Education and Research (IDER), College of Pharmacy, University of Houston (COP-UH). The PK/PD model analyses correlate PK characteristics (achieved concentration-time profile, systemic exposure, and clearance kinetics) with PD outcomes (tumor size changes, protein levels of predetermined biomarker(s) in tumor tissues, vital signs, and histology of highly perfused organs at predetermined times) in mouse tumor models from respective studies. The analysis will define key PK parameters that predict PD outcomes. Modeling results will predict rational dose and dose regimen recommendations for efficacy studies and interspecies PK allometric scaling to determine First in Human (FIH) dose for Investigational New Drug (IND) applications, effectively advancing a potential drug candidate to clinical trials.

This presentation aims to introduce the broad capability of PK/PD modeling at IDER, using various PK software, WinNonlin, Phoenix, GastroPlus and Symcyp to develop pre-clinical and clinical PK/PD models. A case of mechanism-based PK model to extrapolate from preclinical study to clinical trial will be discussed.

Huan Xie, PhD
Professor
Pharmaceutics

Preclinical Drug Characterization and Formulation Development

Dr. Huan Xie is Professor of Pharmaceutics at College of Pharmacy and Health Sciences, Texas Southern University. She has a broad background in nano-formulation and targeted drug delivery, with specific expertise in cancer therapy applications. Dr. Xie has 4 years of industry experience contributing to the development of silica/gold nanoshell cancer therapy, now in several clinical trials. She is an inventor on 3 patents related to novel drug formulation and PK study and has many collaborators across Texas. She has been consistently funded by NIH, and now serves as Director of NIH-RCMI Pharmacology Core as well as Co-Director of the CCPF, providing support in traditional and advanced drug formulation and delivery.

Abstract: Preclinical drug development involves studies from the identification of a new chemical entity (NCE) all the way to clinical testing. PK/PD, pre-formulation and formulation development are key steps leading to an Investigational New Drug (IND) application. It is very common that many NCEs are either unstable or unsuitable for conventional routes of drug administration. Therefore, characterizing its physicochemical properties then developing an optimal dosage formulation are critical for further NCE's development. Unfortunately, a major challenge for cancer scientists and clinicians pursuing new cancer therapies is the lack of access to the specialized preclinical drug development resources that are required for IND.

CCPF focuses on preclinical drug development to facilitate rapid advancement of novel cancer drugs to clinical trials. This presentation aims to introduce CCPF core resources to attendees in the areas of pre-formulation characterization and dosage formulation development for potential anti-cancer drugs. Several aspects will be covered: 1) drug solubility, stability and other physicochemical properties evaluations; 2) basic formulation such as co-solvents, cyclodextrins and emulsions; and c) advanced dosage formulations including liposomes, polymer-based nanoparticle formulations, nano-suspension, and gold nanoparticle-based drug delivery systems. Specific research projects will be discussed as examples.

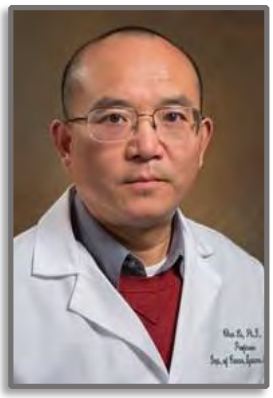
Texas Southern University

Robert Y. Tsai, MD/PhD
Associate Professor
Center for Translational Cancer Research

Developing a Novel Mucoadhesive Patch for Drug Delivery and Chemoablation of Oral Premalignant Lesions

Dr. Tsai received his M.D. from the National Taiwan University in 1988 and Ph.D. in Neuroscience from the Johns Hopkins University School of Medicine in 1996. He finished his Neurology residency training at The National Taiwan University Hospital in 1991 and Post-Doctoral training at National Institutes of Health in 2003. He is currently an Associate Professor at the Texas A&M University Health Science Center, Institute of Biosciences and Technology and has been a faculty member at Texas A&M since 2003. Dr. Tsai's research focus is to discover new mechanisms underlying stem cell self-renewal and to translate this knowledge into medical applications aiming at advancing the management of tissue repair, premature aging, and tumor malignancy. His current research topics include: (1) delineating the molecular and cellular mechanism of nucleostemin that drives the self-renewal of normal and cancerous stem cells, with a special emphasis on how nucleostemin protects the integrity of genome against replication stress; (2) determining the importance of self-renewal programs in liver regeneration, liver cancer progression, and liver aging; and (3) developing new screening and therapeutic devices for cancer prevention, with a special emphasis on oral squamous cell carcinoma prevention and non-alcoholic fatty liver disease-related hepatocellular carcinoma risk assessment. His work is funded by NIH and CPRIT and has led to a US patent and the establishment of a startup company, Post Oak Pharmaceuticals. Dr. Tsai has co-authored 43 peer-reviewed journal articles, 29 of which were published from his lab at Texas A&M.

Abstract: Oral cancer is the 8th most common cancer in male and 15th in female in the US. Its five-year survival rate remains at an abysmal 64% over decades, in contrast to some of the more common cancers, such as breast cancer (90%) and prostate cancer (98%). Furthermore, patients who survive oral cancer also have to live with impaired functions and sometimes disfiguration caused by the surgical or radiation treatment. Pathologically, more than 90% of the oral cancers are oral squamous cell carcinomas (OSCC) that may develop from clinically visible oral premalignant lesions (OPLs), which affect as many as 1.5-4.3% of the world population. Histologically, OPLs are divided into those with low- or high-grade dysplasia. Even though the majority of oral dysplastic lesions remain stationary for years or even undergo spontaneous regression, a significant minority of them (4-11% for low-grade dysplasia and 20-43% for high-grade dysplasia) develop into OSCC with a short period of time. Currently, there is no standard treatment for patients with oral high-grade dysplasia, especially for those with diffuse or multiple lesions. We have discovered a drug combination, consisting of a chemotherapeutic agent (oxaliplatin, OXP) and a chemo-sensitizing agent (mycophenolate, MPS), which have been shown to exert a strong synergistic effect on killing dysplastic oral keratinocytes as well as OSCC cells in culture. The objective of this study is to develop a new chemoablation product based on this synergistically acting novel drug combination and a topically controlled-release device to ablate high-risk OPLs for the sustained prevention of oral cancer. A computer-aided design (CAD) three-dimensional (3D) printing technique was used to formulate a novel mucoadhesive patch for topical delivery of the OXP-MPS combination. Patch formulations were optimized based on the in vitro release profiles of OXP and MPS. The final patch product was tested for its mechanical properties, mucoadhesiveness, and in vivo drug release profile on rat mucosa.



Chun Li, PhD
Professor
Cancer Systems Imaging

A Hindsight Reflection on the Clinical Development of Opaxio (paclitaxel poliglumex): Lessons Learned

Chun Li, Ph.D. is a professor and Director of the John S. Dunn Foundation Chemistry Laboratory for Imaging Sciences in the Department of Cancer Systems Imaging at The University of Texas M. D. Anderson Cancer Center. Dr. Li earned his doctorate in chemistry at Rutgers-The State University of New Jersey. His undergraduate degree was obtained from Peking University, Beijing, China. Research in Dr. Li's laboratory is primarily focused on two areas: 1) Develop targeted imaging probes for noninvasive characterization of molecular events associated with tumor progression and regression. Multiple imaging modalities, including PET, SPETC, MRI and optical imaging are used to acquire complementary data with increased sensitivity and selectivity for early tumor detection, tumor-marker profiling and the monitoring of early treatment responses. 2) Develop novel drug-delivery systems for selective delivery of diagnostic and therapeutic agents to the disease sites. Nanometric drug carriers are designed for selective delivery of anticancer agents to the tumor to maximize their therapeutic efficacy and minimize their toxic side effects to normal tissue. The long-term goal of Dr. Li's laboratory is to apply the "seek and treat" strategy in the development of targeted imaging/ therapeutic (theranostic) agents that will eventually be translated to the clinic to improve the management of cancer through early tumor detection and individualized therapy. A polymer-drug conjugate (PG-TXL) originated from his laboratory has advanced into clinical phase III trials studies. Dr. Li has more than 150 papers published in peer-reviewed journals (Google Scholar H-Index 62), 28 patents (4 of which have been licensed), 1 edited book, and 14 book chapters.

Abstract: Chemotherapy for cancer treatment is limited by the excessive toxicity to normal tissues. The design of chemodrug-loaded nanoformulations provide a unique approach to improve the treatment efficacy while minimizing toxicity. Despite the numerous publications of nanomedicine for the last several decades, however, only a small fraction of the developed nanoformulations have entered clinical trials, with even fewer being approved for clinical application. Poly(L-glutamic acid)-paclitaxel (PG-TXL) belongs to the few formulations that reached phase III clinical trials. Unfortunately, the development of PG-TXL stopped in 2016 due to the inability to show significant improvement over current standard care. This review will provide an overview of the preclinical and clinical evaluations of PG-TXL, and discuss lessons to be learned from this ordeal. The precise identification of suitable patients for clinical trial studies, deep understanding of the mechanisms of action, and an effective academic-industry partnership throughout all phases of drug development are important for the successful bench-to-bedside translation of new nanoformulations.

UT MD Anderson Cancer Center

Stan Watowich, PhD
Associate Professor
Biochemistry & Molecular Biology

Discovery and Development of Novel Mechanism-of-Action Oral Drugs to Reverse Obesity-linked Type 2 Diabetes

Dr. Watowich is Associate Professor in the Department of Biochemistry & Molecular Biology at the University of Texas Medical Branch, Galveston, TX. He is also Founder of Ridgeline Therapeutics, a Houston-based biotechnology startup developing new mechanism-of-action therapies to treat age-related muscle degeneration, obesity-linked Type 2 diabetes, glioblastoma, and muscular dystrophies. He was recent director of the University of Texas (UT) System's state-wide entrepreneurship program developed in partnership with UT McCombs School of Business, and brings this experience to bear on translating research discoveries to clinical practice. He is an accomplished educator, researcher, inventor, entrepreneur, and developer of world-class innovative resources (e.g., the global "DiscoveryingDengueDrugs-Together" project with IBM and the DrugDiscovery@TACC supercomputer-based virtual screening portal). Among his prime interests are the development of effective drug discovery and optimization approaches, with a long-standing focus in the discovery and development of small molecule enzyme inhibitors using innovative combinations of structural, computational, biophysical, cellular, and chemical biology. Dr. Watowich graduated from the Carleton College, received his PhD in Physical Chemistry from the University of Chicago, and was a research fellow at Harvard University before migrating to Texas.

Abstract: There is a critical need for new mechanism-of-action drugs that reduce adiposity (excess adipose tissue), a prime driver of Type 2 diabetes. A novel target to treat adiposity and reverse Type 2 diabetes is nicotinamide-N-methyltransferase (NNMT), a cytosolic enzyme highly expressed in select tissues (e.g., adipose, aged skeletal muscle), where it plays a critical role in cell metabolism, epigenetic gene regulation, and adiposity-induced insulin resistance. We have recently developed first-in-class small molecule NNMT inhibitors. Extensive characterization of the physicochemical, pharmacological, pharmacokinetic (PK), and off-target safety profiles of NNMT inhibitor leads has identified a highly efficacious, orally bioavailable, and safe drug candidate for IND-enabling and clinical studies. By directly targeting the underlying cause of Type 2 diabetes, our novel mechanism-of-action drug has the potential to help the 26 million Americans suffering with obesity-linked diabetes.

Marc Cox, PhD
Professor
Biological Sciences

Small Molecule Targeting of Androgen Receptor-associated Cochaperones for the Treatment of Prostate Cancer

Marc Cox, Ph.D. is Professor in the Department of Biological Sciences, Co-Director of the Toxicology and Cancer Cluster within the Border Biomedical Research Center, Deputy Director of the BUILDing SCHOLARS Center, and Director of the Center for Faculty Leadership and Development at the University of Texas at El Paso (UTEP). Dr. Cox is a molecular endocrinologist with expertise in intracellular receptor signaling pathways. In addition to identifying, characterizing, and therapeutically targeting steroid hormone receptor regulatory proteins for the treatment of prostate cancer, he also has expertise in various model systems, including yeast, that prove useful in large-scale toxicity screens, as well as for high throughput screens for novel drug candidates. Dr. Cox also has expertise in the molecular chaperone-mediated stress response and maintains a wealth of reagents relevant for research in any system and/or disease involving chaperones and the stress response including a wide variety of cancers, neurodegenerative diseases and toxicant-induced cellular stress. As a result Dr. Cox collaborates on a number of projects that are outside of his major research foci. In addition to environmental monitoring and prostate cancer therapeutics, Dr. Cox has published with collaborators in areas as diverse as Alzheimer's Disease, stress and depression, and chronic pain. Dr. Cox was named the 2016 Inventor of the Year by the Intellectual Property Law Section of the State Bar of Texas for his breast and prostate cancer treatments developed at UTEP.

Abstract: The FKBP52 cochaperone is a positive regulator of androgen (AR), glucocorticoid (GR) and progesterone receptor (PR) function and represents an attractive target for the treatment of castration resistant prostate cancer. Towards this end, we previously identified MJC13, which represents a first-in-class drug for targeting the regulation of AR by FKBP52 through binding a putative FKBP52 regulatory surface on AR. While the targeting of the FKBP52 regulatory surface on AR is a promising therapeutic strategy, we propose that the direct targeting of FKBP52 offers a number of advantages over MJC13 that would lead to a more potent and effective drug. Thus, we performed a high-throughput in silico screen of the ZINC database consisting of 20-million lead-like compounds. We identified GMC1 as the initial hit molecule with the most potent inhibition of FKBP52-mediated AR reporter expression. GMC1 effectively blocks AR, GR, and PR activity, blocks endogenous AR-mediated gene expression, and inhibits the proliferation of prostate cancer cell lines. As proof-of-concept we developed a soluble GMC1 co-solvent formulation and demonstrated that GMC1 prevents tumor growth and causes tumor recession in LNCaP and CW22Rv1 xenograft mouse models. These studies are at the forefront of an emerging concept to target novel AR co-regulators for the treatment of prostate cancer.

Fady Ibrahim, PhD
Senior Principal Scientist
Pharmaceutical Sciences

In Vitro Transfer Tests De-risk Decisions to IND

Fady Ibrahim is a Senior Principal Scientist in Pharmaceutical Sciences at Pfizer Worldwide R&D in Groton CT. Fady has a BSc in Clinical Pharmacy and PhD in Pharmaceutical Sciences from the University of Houston, TX. His PhD started to develop his core research interests of formulation, pharmacokinetics and biopharmaceutics. He moved to The University of British Columbia, Vancouver, Canada, as a postdoc to work on developing an oral lipid-based formulation for amphotericin B. Then, he moved to Pfizer in Groton to join the Biopharmaceutics Group and he is currently leading the translational Biopharmaceutics Group. His research interest is understanding oral absorption and the effect of physicochemical properties of drug substance and drug product on performance of the drug molecules. His main areas of expertise are dissolution, formulation, IVIVC oral absorption modeling and simulation.

Abstract: Physicochemical properties of drug molecules dictate the release from the dosage from and the ADME properties of the molecule. For weakly basic compounds, the low pH of gastric media provides optimal conditions for high solubility that is diminished upon progression to intestinal contents. The crash out of solution could be a rate limiting step for absorption of weakly basic compounds and could be mitigated by formulation and/or form change. In this presentation, the application of pH-shift in vitro model to assess the formulation fix to enable plasma exposure will be discussed.

Oscar Ekpenyong, RPh, PhD
Senior Scientist, Pharmacokinetics, Pharmacodynamics and Drug Metabolism

Pre-clinical Pharmacokinetics, Tissue Distribution and Physicochemical Studies of CLBQ14, a Novel Methionine Peptidase Inhibitor for Infectious Diseases

Oscar Ekpenyong, RPh, PhD is a registered pharmacist turned pharmaceutical scientist. He is currently a Senior Scientist in the Pharmacokinetics, Pharmacodynamics and Drug Metabolism department at Merck & Co., Inc. His expertise is in the preclinical development of small molecules, peptide and protein therapeutics and is accomplished in bioanalytical method development and validation, and pharmacokinetic and pharmacodynamics studies. Oscar is proficient in the design, planning and execution of new LC-MS/MS and ligand binding assays for the quantitative analysis of exogenous and endogenous compounds (xenobiotics, biomarkers, peptides and proteins) to support pharmacokinetic, pharmacodynamics, and endogenous metabolomic studies in a variety of preclinical models and challenging matrices. He has demonstrated impact across multidisciplinary discovery research including cardiovascular, renal, metabolic, ophthalmology and immuno-oncology. Prior to joining Merck, Oscar worked as a Pharmacist in retail, hospital and pharma industry settings.

Dr. Ekpenyong earned his Ph.D. in Pharmaceutical Sciences from Texas Southern University, where his doctoral research focused on the pre-clinical studies of small molecules intended for the treatment of tuberculosis and castration-resistant prostate cancer respectively. This involved the development and validation of LC-MS/MS methods for the determination of the new chemical entities in solutions, plasma, urine and tissues; pre-formulation studies and formulation of the molecules for application to pharmacokinetic and pharmacodynamics studies in pre-clinical models. His doctoral research also extended to gold nanoparticles-antibody conjugation for hypoxic tumor targeting and photo-thermal ablation. His patented and peer-reviewed works have been published in esteemed journals and presented at numerous scientific meetings.

Oscar is a member of the American Association of Pharmaceutical Scientists, International Pharmaceutical Federation, American Society of Mass Spectrometry, and Pharmaceutical Society of Nigeria. He holds a State of Texas Pharmacist license and several other professional certifications. Outside of work, Oscar likes to travel, explore different cultures, food, and wine.

Abstract: CLBQ14, a derivative of 8-hydroxyquinoline exerts its chemotherapeutic effect by inhibiting methionine amino-peptidase (MetAP), the enzyme responsible for the post-translational modification of several proteins and polypeptides. Preclinical efficacy studies in Mycobacterium tuberculosis (Mtb) shows that it has great potency against replicating Mtb; an increased potency against aged non-growing Mtb and exhibits great selectivity for the two MetAPs in Mtb over human MetAPs. Early evaluation of the ADME, physicochemical properties, and tissue distribution of CLBQ14 is expedient in its development process. This presentation explores the pre-clinical development of CLBQ14 with a focus on the development of a bioanalytical method for its quantitation in biological samples, and the characterization of its physicochemical properties, pre-clinical pharmacokinetic disposition and tissue distribution. A liquid chromatography tandem-mass spectrometry (LC-MS/MS) method for the quantification of CLBQ14 in solution, and in rat plasma and urine was developed and validated. The solubility and lipophilicity of CLBQ14 were determined by the shake-flask method; the plasma stability and protein binding, microsomal stability and CYP metabolism phenotyping were determined following respective incubation at suitable condition. The pharmacokinetic properties, bioavailability and tissue distribution were studied in adult male Sprague Dawley rats. CLBQ14 has a characteristic physicochemical profile, bio-exponential pharmacokinetic disposition in rats, substantial oral and subcutaneous bioavailability, extensive tissue distribution and its biotransformation is catalyzed by an NADPH-dependent CYP metabolism. These studies are imperative to the development of CLBQ14 as a new chemical entity for infectious diseases.

Merck Research Laboratories

Mohammad Tabrizi, PhD
Senior Director Pharmacology
Quantitative Pharmacology in Support of Design, Selection, & Development of Biologic Modalities

Mohammad Tabrizi (Tabrizifard) Ph.D. is an expert in translational sciences and integrative pharmacology with a key focus on development of targeted modalities, novel biologics and antibody-based therapeutics. He has extensive experience (20+) in research and development with product development experience spanning many therapeutic areas including immunology, oncology, immune-oncology, and inflammatory diseases; his technical expertise includes preclinical pharmacology and safety, preclinical and clinical pharmacokinetics (PK), pharmacodynamics (PD), GLP-compliant bioanalytics, and clinical pharmacology. He has been an author and co-inventor on more than 50 original papers, reviews, scientific books and patents and has been an invited speaker to numerous national and international conferences Additionally, he has served in numerous scientific capacities including as the Guest Editor for AAPS Journal, a member of the Grant review committees for Canada Foundation for Innovation and Terry Fox New Frontiers, a reviewer for AAPS Journal, Drug Discovery Today, and the Clinical Therapeutics. He received his bachelor's degree in Pharmacy from University of Houston (Summa Cum Laude) with honor and his PhD from University at Buffalo, State University of New York (SUNY) with focus on Quantitative and Pharmaceutical Sciences (QPS). He completed a postdoctoral training in pharmacology at University of New York at Buffalo (SUNY) with a focus on therapeutics.

Abstract: Biologics are one of the fastest growing subsets of pharmaceuticals today. With advances in antibody technology, it is now possible to rapidly and effectively generate highly tailored and specific antibody-based therapeutics that interact with a diverse array of soluble or cell-associated antigen targets. Biologics and antibody-based therapeutics are becoming progressively complex. With such complexities in the design of novel constructs, foundational and robust approaches in translation of preclinical data in support of the later stages of drug development are becoming increasingly vital. Understanding of the target biology across species and application of a science-based approach for integration of pharmacology principles are an essential cornerstone for translational efficiency across species. This presentation outlines the application of quantitative approaches for design, selection and development of biologic modalities

Ascendis Pharma, Inc.

Cinzia Stella, PhD
Senior Scientist and Team Leader

Early Development Technical Considerations for Complex and Large Format Biotherapeutics

Cinzia Stella received her PhD in Pharmaceutical Analytical Chemistry from the University of Geneva (Switzerland), after her Master's Degree in Pharmaceutical Sciences from the University of Pavia (Italy). Following a post-doctoral fellowship at Imperial College London (UK) on Metabolomics funded by Unilever, she held a position at the University of Geneva in the School of Pharmacy, where she was responsible for the development and optimization of protein based pharmaceutical formulations in collaborations with industrial partners. She currently is a Sr Scientist and Team leader at Genentech and she is responsible for the analytical development and control strategy of Early Stage programs in the context of CMC development.

Abstract: With the introduction of increasingly complex product formats and the growing demand of biotech companies to reduce the time it takes from DNA to IND, improvements in speed and efficiency are needed more than ever to enable a faster technical development of new therapeutics. Therefore, the limits of standard analytical characterization tools and formulation considerations have become more apparent with next-generation biologics, such as novel scaffolds (e.g. fusion products), bispecific antibodies and protein-polymer conjugates.

In this presentation, we describe some technical considerations and challenges that were encountered during the early development of a protein-polymer conjugate. In order to increase the retention of a therapeutic fragment antibody (Fab) in the eye, a branched polyethylene glycol (PEG) scaffold was coupled to the protein fragment as a means to increase the hydrodynamic radius (Rh). The increased molecular size of the conjugate, compared to the Fab alone, produces a significant longer half-life and requires less frequent intravitreal (ITV) injections, which can greatly benefit the patient.

Here we describe i) the impact of the raw material (PEG) on the quality of the final conjugate; ii) some of the formulation considerations for ocular products with viscosity challenges and iii) the limitations of standard QC control strategies with complex formats, such as branched protein-polymer conjugates.

This work highlights key considerations to be taken into account during the early clinical development of a non-standard format therapeutic product and provide valuable insight into how to overcome some of the challenges that might be associated to next generation therapeutics.

Genentech

Jeff Larson, PhD, DABT
VP of Product Development

Early In Vitro and In Vivo Toxicology Studies to Push Your Drug into Development

Dr. Larson has 25+ years of experience in drug development, primarily in nonclinical research, safety toxicology and pharmacokinetics. Previously, Jeff held the Vice President of Nonclinical Development for both Beta Cat Pharmaceuticals (now Iterion) and Salarius Pharmaceuticals and prior to that was the Vice President of Texas BioAlliance (BioHouston), a nonprofit association leading life science companies in the State of Texas, where he provided a wide array of consulting services on preclinical research and development for a number of leading life science companies. Jeff has held positions of increasing responsibility at NexBio, Inc. and at Tanox, Inc.

Dr. Larson earned a Ph.D. in Toxicology/Pharmacology from Washington State University and is a Diplomate of the American Board of Toxicology.

Abstract: Early characterization of a new chemical entities (NCEs) is important in lead candidate selection and may perhaps uncover potential class effects. Guiding these early studies is an understanding of the target product profile that defines disease, desired route of administration, frequency and desirable drug attributes. At this early stage in development, toxicology studies are limited by compound availability and by data surrounding potential drug product formulation; i.e. solubility, stability, compatibility with excipients). Nonetheless, one can begin to determine the lead candidate from early toxicity screening. To facilitate screening, it is important to know the in vitro activity at the cell of interest and, if available, data surrounding any in vivo efficacy work. It is possible to use in vitro data, coupled with likely volume of distribution estimates and in vitro metabolic characterization, to set dose levels for efficacy studies and from this build a toxicity database. Early in vitro toxicity is primarily designed to identify alerts that would eliminate potential NCEs. The most common alerts to be evaluated are potential mutagenicity and cardiac toxicity. Early off target screening (usually a CEREP panel) is also performed to identify other potential targets. From this in vitro screening, one moves into single dose and multiple dose non-GLP toxicology studies, generally in rats as these are the species for rodents preferred by regulatory agencies. Study designs will be presented. In vivo studies in a non-rodent species will also be discussed and study designs presented. The objectives of early toxicity screening are two-fold; 1) weed out potential candidates before spending money on development and 2) provide data to guide formal GLP toxicology studies.

Iterion Therapeutics

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Poster #16

Pharmacokinetic and Bioavailability Studies of 5-Amino-1-Methyl Quinolinium in Rats

Ololade Awosemo[1], Harshini Neelakantan[2], Jing Ma[1], Lei Wu[3], Diana S-L Chow[3], Stanley Watowich[2], Liang Dong[1]
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Purpose: 5-amino-1-methyl quinolinium is a potent nicotinamide N-methyl transferase (NNMT) inhibitor that has been shown to promote weight and fat loss in high-fat diet-induced obese mice. The objective of this study is to characterize the pharmacokinetic profile and oral bioavailability of the compound using rat as an animal model.

Methods: Cross-over design was used in this study to evaluate the oral bioavailability of 5-amino-1-methyl quinolinium. Jugular vein-cannulated, adult, male Sprague Dawley rats (n=5) received a 10 mg/kg intravenous dose of 5-amino-1-methyl quinolinium (10 mg/mL in normal saline). Serial blood samples (0.2 mL) were collected from each rat before dose and at 2, 5, 15, 30, 60, 90 minutes, and 2, 4, 6, 8, 10 and 24 hours post dose. Following a week of washout period, the rats were fasted overnight and then received a 100 mg/kg oral dose of 5-amino-1-methyl quinolinium (25 mg/mL in water). Serial blood sampling was repeated as described earlier. Urine samples were also collected for 24 hours for both IV and oral groups. Plasma 5-amino-1-methyl quinolinium concentrations were measured by a validated sensitive, specific and reproducible LC-MS/MS method. Briefly, chromatographic separation was achieved using an ACE® Excel™ C18 column (2 µm, 50×2.1 mm) with a binary gradient solvent system comprising of water (A) and acetonitrile (B) containing 0.1% formic acid as the mobile phase. 5-amino-1-methyl quinolinium was extracted from plasma by protein precipitation using acetonitrile. Deuterated 5-amino-1-methyl quinolinium was used as internal standard. The assay was linear in the 5-amino-1-methyl quinolinium concentration range of 10–2500 ng/mL. The extraction recovery was ≥ 90% and there was no matrix effect. Plasma 5-amino-1-methyl quinolinium concentration-time profiles (Figure 1 & 2) were analyzed with Phoenix WinNonlin 8.0 using compartmental pharmacokinetic methods.

Results: 5-amino-1-methyl quinolinium displayed a bi-exponential disposition in rats following intravenous administration of the drug. The mean area under the curve ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), total clearance (CL), distribution half-life ($t_{1/2\alpha}$), terminal elimination half-life ($t_{1/2\beta}$), and apparent volume of distribution (V_d) were 2945.9 hr.ng/mL, 7599.8 ng/mL, 3540.7 mL/hr/kg, 0.23 hr, 2.75 hr and 2939.9 mL/kg respectively for the IV group. After oral administration, maximum plasma concentration was reached within 1.6 hours, followed by a prolonged elimination process. The mean area under the curve ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), absorption rate constant (K_a), terminal elimination half-life ($t_{1/2\beta}$), total clearance (CL) and apparent volume of distribution (V_d) were 12788 hr.ng/mL, 1913.7 ng/mL, 0.8 hr, 7.8 hr, 4315 mL/hr/kg, and 14114 mL/kg respectively. The oral bioavailability of 5-amino-1-methyl quinolinium ranged from 20.91% to 58.40%. The average percentage dose excreted unchanged in the urine within 24 hours post dose was 58.9% and 26.2% for IV and oral groups, respectively, which suggests renal excretion as a major route of elimination and significant first-pass metabolism. The significantly longer terminal elimination half-life observed after oral administration could be due to the drug re-absorption after 8 hours through the large intestine.

Conclusion: 5-amino-1-methyl quinolinium displayed a bi-exponential disposition in rats. The drug was well absorbed after oral administration with a significant first-pass effect. Majority of intravenously administrated drug was eliminated unchanged in the urine.

Poster #15

Method Development and Validation of Liquid Chromatography-Tandem Mass Spectrometry for AC1LPSZG in Rat Plasma and Urine

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Abstract

Purpose: AC1LPSZG is a mammalian target of rapamycin (mTOR) inhibitor that can significantly reduce the viability in lung adenosquamous carcinoma cell line HTB-178 cells at a dose of 4.57 µg/mL. To meet the demand for preclinical pharmacokinetic and toxicological studies, a precise, rapid and robust analytical method is highly desirable with the FDA guidelines.

Method: AC1LPSZG was extracted from 50 µL rat plasma and urine by protein precipitation with acetonitrile (ACN) containing 0.1% formic acid and 100 ng/mL griseofulvin (the internal standard, the IS). The chromatographic separation is achieved on C_{18} analytical UPLC column using a gradient from 20% to 100% ACN containing 0.1% formic acid at a flow rate of 0.5 mL/min. Mass spectrometric detection was carried out using AB Sciex API-4000 Qtrap triple quadrupole mass spectrometer equipped with a TurboIonSpray in positive ion mode. Multiple-reaction monitoring mode (MRM) was employed for the detection by monitoring the transition pairs of m/z 457.10 precursor ion to the m/z 349.00 and m/z 353.27 precursor ion to the m/z 285.10 product ion for the IS.

Results: It was found that calibration curve of the method had an excellent linearity (r_2>0.999) for the analyte concentration ranging from 15 to 5000 ng/mL with acceptable inter and intra-assay, precision, accuracy, and stability. Stable and high extraction recovery was achieved in the ranges of 88.46-102.51% for plasma and 92.74-103.48% for urine with no significant matrix effect. The application of the assay for measurement of AC1LPSZG concentration in plasma and urine is demonstrated by a pharmacokinetic (PK) study conducted in six male Sprague-Dawley (SD) rats with single intravenous administrated 5 mg/Kg dose.

Conclusion: A LC-MS/MS method has been successfully established for the quantification of AC1LPSZG in rat plasma and urine.

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Poster #2

PLEV Analogs Act As Potent Inhibitors Of FGF14:Nav1.6 Complex Assembly

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With the support of a diverse ensemble of auxiliary proteins tightly regulating their function, voltage-gated sodium (Nav) channels serve as the primary molecular determinants of neuronal excitability. Chief among the auxiliary proteins modulating the Nav1.6 channel is fibroblast growth factor 14 (FGF14), a member of the intracellular FGF family that has been demonstrated to directly interact with the intracellular C-terminal tail of Nav1.6 channels. With translational studies increasingly illuminating an associative link between FGF14 dysfunction and a diverse array of neuropsychiatric conditions associated with mood disorders, the development of compounds to modulate the FGF14:Nav1.6 complex formation is hugely consequential in the quest to provide improved treatment options for patients with refractory depression, anxiety, and bipolar disorder. In an effort to develop such compounds, the present investigation sought to test the modulatory effects of a series of 17 analogs derived from the peptide PLEV, a four amino acid sequence previously shown to map onto the FGF14:Nav1.6 complex protein:protein interaction (PPI) interface. As part of an initial screening, the 17 peptidomimetics were reconstituted in DMSO and administered to HEK293 cells stably expressing constructs that allow reconstitution of the FGF14:Nav1.6 complex using split-luciferase complementation assay (LCA). This initial screening revealed that PW0531, PW0543, and PW0564 were potent inhibitors of FGF14:Nav1.6 complex assembly, with percentage luminescence values (normalized relative to 0.5% DMSO) of 11.9±0.9, 14.0±1.1, and 4.7±0.3, respectively (n=6 for each compound). Subsequently, the three most potent inhibitors were tested at varying concentrations (0.5 μM-100 μM; n=4 per concentration) to determine the IC_{50} value of each compound. This latter inquiry revealed that the IC_{50} values for PW0531, PW0543, and PW0564 are 19.8±0.52 μM, 38.4±1.25 μM, and 22.4±0.8 μM, respectively. Collectively considered, these findings suggest that, with further chemical optimization, PW0531 and PW0564 could serve as viable candidates to advance into later stages of preclinical testing as potential protein-protein interaction-based antidepressants or mood stabilizers.

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Poster #18

Development and validation of a new LC-MS/MS method for measuring transmucosal delivery of mycophenolate in vivo

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Purpose: Mycophenolate has shown a strong synergistic effect when used in combination with oxaliplatin on inhibiting the growth of oral premalignant cells. We are developing novel mycophenolate patch formulations as a non-invasive therapeutic device for treating oral premalignant lesions. The purpose of this study is to develop a specific, sensitive and reproducible LC-MS/MS method capable of quantifying the levels of mycophenolate in rat plasma for pharmacokinetic study and in rat tongue for tissue distribution studies.

Method: Patch formulations were applied to the tongue of adult male Sprague Dawley rats for 4 hrs. Tongue tissues were then collected and washed with phosphate buffer. Mycophenolate was extracted from the tongue tissue by removing proteins using acetonitrile precipitation. Mycophenolate separations were carried out on an ACE Excel 2 Super C_{18} column (50 x 2.1 mm, 2 µm) with a mobile phase run in gradient elution of 0.1% FA in water (solvent A) and 0.1% FA in acetonitrile (solvent B) at a flow rate of 0.4 mL/min. Griseofulvin was used as internal standard. LC-MS/MS analysis was carried out on an API 4000 QTRAP LC-MS/MS system with a Turbo Ion Spray ion source. Tandem mass spectrometry was employed under positive electrospray ionization to detect the specific precursor to product ion transitions m/z 321.2 $\rightarrow$ 207.2 for mycophenolate and m/z 353.2 $\rightarrow$ 285.1 for the internal standard. Method validation was performed based on the FDA guideline for Bioanalytical Method Validation. The validity of this assay has been confirmed for measuring mycophenolate in rat plasma for pharmacokinetic studies following intravenous administration of 0.5 mg/kg of mycophenolate sodium, as well as in rat tongues for tissue distribution studies following a 4-hour transmucosal delivery of 357 µg/cm^2 of mycophenolate sodium.

Results: Blank rat plasma or tongue tissue homogenates coupled with griseofulvin, as internal standard, was used for generating standard curves ranging from 0.5 – 1000 ng/mL ($r > 0.9990$) for both plasma and tongue tissue homogenates. Mass detection was performed under positive ionization electrospray. Inter- and intra-day accuracy and precision of the assay were ≤15% in both plasma and tongue tissue homogenates. The matrix effect was non-significant and extraction recovery rates were in the range of 87.99% to 109.69% in plasma and tongue homogenates, respectively. Mycophenolate samples were stable in room temperature and autosampler for 16 hrs in rat plasma and tongue.

Conclusion: We have established a specific, sensitive and reproducible LC-MS/MS method for the quantification of mycophenolate in rat tongue and plasma. The validated method was successfully applied to investigate the tissue distribution of mycophenolate following tongue administration to and pharmacokinetic studies by intravenous administration to the Sprague-Dawley rats.

Poster #18

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Poster #1

Pharmacodynamic Study Services Provided by the Gulf Coast Consortia (GCC) Center for Comprehensive PK/PD & Formulation (CCPF)

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The Gulf Coast Consortia (GCC) Center for Comprehensive PK/PD & Formulation (CCPF), funded by Cancer Prevention & Research Institute of Texas (CPRIT), is a state-of-the-art drug development core facility with experienced faculty from Texas Southern University College of Pharmacy and Health Science (TSU COPHS), University of Houston College of Pharmacy (UHCOP), and the GCC for Quantitative Biomedical Science. It provides pre-clinical drug development services that include a wide variety of *in vitro*, *in situ* and *in vivo* studies in different areas. One focus area is to utilize various *in vitro*/*in vivo* assays to measure the efficacy of candidate anti-cancer drugs. Our facility offers *in vitro* assays to measure how candidate drugs could affect cell proliferation, apoptosis, DNA damage or cell migration/invasion. Specifically, our facility will, upon request, present to investigators results of MTT, Caspase-3, γH2AX, or Boyden chamber assays. Our PD facility also offers evaluation of drug efficacy *in vivo* using xenograft and/or genetic mouse models for a variety of malignant cancers such as that of the breast, pancreas and colon. In addition, if needed, we can follow through to examine or identify biomarkers or proteins of interest in the tumors using immunoblotting, immunohistochemistry and/or immunofluorescence assays.

To highlight our PD services, we will present data exemplifying an efficacy study of a candidate anti-cancer compound (TSU-01) performed at the CCPF. The highly aggressive, triple-negative breast cancer cell line MDA-MB-231 was used to perform MTT assays which revealed extensive cell death caused by 1 or 2 day-treatment of TSU-01 at the concentration of 5μm or higher. Further, Caspase-3 assays indicated that apoptosis did not seem to be the underlying mechanism. However, cells treated with a nonlethal dose of TSU-01 exhibited mild DNA damage, as measured by the γH2AX assay. Further studies on elucidating the mechanism of action of this novel potential anti-cancer compound TSU-01 will include examining potential protein degradation in MDA-MB-231 cells, as well as testing the potential combinatory effect of TSU-01 with other common drugs. Results from these *in vitro* assays will guide performance of i*n vivo* assays.

PD services provided by CCPF will be invaluable for researchers who are interested in testing the efficacy of their candidate anti-cancer drugs but have limited expertise or resources to perform PD studies on their own. Please visit our website (https://www.gcc-ccpf.com) to view the comprehensive list of services offered by the GCC CCPF. The GCC CCPF is supported by CPRIT-Core Facilities Support Awards RP180748.

Poster #19

Flow-Through Cell USP 4 apparatus for *In vitro* release testing of drug loaded PLGA Nanoparticles

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ABSTRACT

PURPOSE
The present work is the first to investigate the feasibility of SOTAX®USP apparatus 4 in studying the *in vitro* sustained release profile of PLGA nanoparticles. A synthetic chemotherapeutic drug AC1LPSZG , a novel mammalian target of rapamycin (mTOR) inhibitor, was chosen as a model poorly water-soluble drug.

METHOD
The polymeric nanoparticles were prepared by "nanoprecipitation" technique using biocompatible polymer Poly(lactide-co-glycolide) (PLGA-50:50) and nonionic surfactant poloxamer P188. The prepared nanoparticles were characterized for particle size, size distribution, zeta potential and drug entrapment effeciency The *in vitro* drug release was determined in phosphate buffer pH 7.4, employing a USP-4 apparatus CE7-smart (SOTAX®) incorporated with Float-A-Lyzer dialysis cells at 300 kDa molecular weight cut–off (MWCO). The flow rate and the temperature of release medium were set at 16 mL/min at 37°C, respectively. Experiments were done in triplicate and data are presented as the mean ± SEM.

RESULTS
In this study, the prepared PLGA nanoparticles were 150±7 nm in size with narrow polydispersity index (PDI) 0.193±0.05. The zeta potential value was -17±4 mV with standard deviation 7.18±1.14 mV. The drug entrapment efficiency was 60±5 %; and 40±6 % drug was released at the end of 7 days.

CONCLUSION
Sotax™ USP 4 apparatus can serve as a tool for determination of *in vitro* drug release from PLGA NPs. This work was the first to investigate the *In vitro* release profile of PLGA NPs using Flow Through Cell USP 4 apparatus.Sensitive LCMS method was used to detect the very low dissolution concentrations after performing liquid-liquid extraction.A drug release profile of AC1LPSZG has been successfully obtained with this method.

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Poster #3

Novel Immune Modulators Enhance *C. elegans* Resistance to Multiple Pathogens

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Traditional treatments for bacterial infection have focused upon directly inhibiting growth of the pathogen. However, an equally important determinant of infection outcome is the host defense response. We previously performed a high-throughput chemical screen to identify small molecules that rescued the nematode *Caenorhabditis elegans* from infection by *Pseudomonas aeruginosa*. Over 20 of the hits stimulated host defense gene expression. During in-depth studies of five such molecules using microarray analysis, bioinformatic clustering, and RNAi knockdown of candidate gene targets, we identified PMK-1/p38 MAPK and SKN-1/Nrf2 as two key pathways modulated by these hits. Interestingly, the molecules studied did not depend on a single pathway for ameliorating *P. aeruginosa* pathogenesis in liquid-based assay, but did rely on the PMK-1/p38 MAPK pathway during infection on agar. A subset of these molecules was also protective against *Enterococcus faecalis* and *Staphylococcus aureus*. In general, the compounds showed little toxicity against mammalian cells or worms, consistent with their identification in a phenotypic, high-content screen. These molecules possess significant potential for use as tools to study innate immune processes.

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Poster #5

Applying Graph Convolutional Neural Networks for Drug Metabolism Prediction

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A pharmaceutical drug is a chemical compound of a specific structure that induces certain biological activity when it enters the human body. Once a chemical compound is ingested, it may interact with enzymes which can alter its structure through metabolic reactions. This process may result in reduced therapeutic action of the drug or even toxicity through the production of harmful metabolites. In order to ensure efficacy and safety, drug design studies must account for the metabolic activity of the drug, which results in a very large number of laboratory experiments. Over the years, various computational tools have been developed in order to assist this process. At the same time, intensive efforts have been made for the development of chemical databases specifically for drug-related data. Such datasets provide information regarding the structure of drugs, as well as the enzymes that interact with them and the metabolites that are formed. Such extensive chemical datasets have enabled the application of machine learning algorithms for predicting drug metabolism. Most of these efforts focus on predicting which atoms in the molecule will participate in the chemical transformation when the molecule is metabolized by a specific family of enzymes. This information gives pharmacologists insights on how to optimize the structure of the drug in order to manipulate its metabolism without compromising its therapeutic action. These existing efforts for drug metabolism prediction, though, have been hindered by the fact that chemical molecules do not have a straightforward numerical representation, which traditional machine learning algorithms require. Instead, chemical molecules are represented as graphs, where the graph nodes correspond to the atoms in the molecule and the graph edges correspond to the bonds formed between atoms. Most of the existing machine learning-based approaches rely on expert knowledge to engineer features that represent either the individual atoms within the molecule or the entire molecule, depending on the prediction task.

In this project, we are exploring Graph Convolutional Neural Networks (GCNNs) for predicting drug metabolism, in order to automatically derive task-specific atom and molecule representations. We divide the drug metabolism prediction problem into two prediction subtasks: First, we are predicting which enzymes are more likely to metabolize a given molecule. Second, we are predicting which atoms are more likely to be involved in an enzymatic reaction. For each prediction task, we are using GCNNs to learn task-specific graph embeddings. A GCNN is a neural network-based architecture that learns vector representations either at a graph or at a node level based on the structure of the graph and the prediction task. We are utilizing the graph level embeddings to predict which enzymes will more likely metabolize a given molecule. We are also learning node level embeddings to predict whether a given atom can be involved in a metabolic transformation by a given enzyme. These embeddings have been obtained by training the GCNN on two separate datasets. For the enzyme prediction task, we have constructed a dataset by collecting information about enzymatic interactions from publicly available datasets. For the prediction of reacting atoms, we are using a standard dataset that has been used in the literature for developing and testing computational methods that predict reacting atoms in metabolizing drugs. Preliminary results show that our method for predicting the atoms involved in metabolic reactions can fairly compete against existing approaches without the need for expert-engineered features. Coupled with the model that predicts which enzymes can metabolize a chemical molecule, we aim to develop a computational tool that provides a more comprehensive study of drug metabolism in order to ensure safety and efficacy of drugs.

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Poster # 4

Metabolic Pathways of c-MET Tyrosine Kinase Inhibitor Tepotinib in Human and Mouse Liver Microsomes

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Tepotinib (TPB) is a highly selective and potent c-Met inhibitor that has shown promising activity in a phase I trial for non-small cell lung cancer (NSCLC). Recent studies indicate that TPB can overcome resistance to epidermal growth factor receptor inhibitors driven by aberrant MET activation, and the Food and Drug Administration has granted a breakthrough therapy designation for tepotinib in patients with metastatic NSCLC harboring *MET*ex14 skipping alterations who have progressed on prior platinum-based chemotherapy. The metabolism of TPB remains largely unknown, but is likely to play a critical role in both safety and efficacy: understanding the metabolism of TPB could improve safety and predict possible adverse effects and drug-drug interactions. In the current study, we investigated the metabolism and bioactivation of TPB in human and mouse liver microsomes (HLM and MLM) using LC-MS-based metabolomic approaches. We identified over 20 TPB metabolites and adducts in liver microsomes. Monohydroxyl-TPB (O+TPB) is the dominant metabolite in both HLM and MLM, and demethyl-TPB is secondary to O+TPB. Using methoxyamine as a trapping agent, we infer that two aldehydes are generated in HLM; one of these was also observed in MLM. No adducts were observed in liver microsomes incubated with TPB in the presence of trapping agents glutathione or potassium cyanide. Using recombinant CYP450 isoenzymes, we showed that CYP3A4 and CYP3A5 are the primary contributors to the formation of both monohydroxyl-TPB and the methoxyamine-trapped TPB aldehydes. This study offers a comprehensive view of TPB metabolism and identifies metabolites that will be helpful to evaluate adverse effects of TPB and possible drug-drug interactions in TPB-treated hepatocytes and model organisms.

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Poster #17

Oral Bioavailability and Pharmacokinetics of OJT007, an Inhibitor of Methionine Aminopeptidase, in Rats.

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Purpose: OJT007 is an inhibitor of Methionine Aminopeptidase 1 (MetAP1) with potent activity against *Leishmania major*, the causative agent of cutaneous leishmaniasis infection. Inhibition of MetAP1 interferes with parasitic survival. This makes OJT007 an attractive therapeutic agent for the development of new targets for the treatment of cutaneous leishmaniasis. The objective of this study is to characterize the pharmacokinetics of OJT007.

Methods: Crossover design was used in this study to evaluate oral bioavailability of OJT007. Three jugular vein-cannulated adult, male, Sprague-Dawley rats received a 5 mg/Kg intravenous dose of OJT007 cosolvent formulation (1 mg/mL). Serial Blood samples (0.2 mL) were collected before dosing, and at the following times after drug administration: 0.033, 0.0833, 0.25, 0.5,1, 1.5, 2, 4, 6, 8, 10, 24 hours. Following a week washout period, the rats were fasted overnight and received a 10 mg/Kg oral dose of OJT007 cosolvent formulation (1 mg/mL). Blood samples were collected before dosing, and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 hours post dose. Urine samples were collected for 24 hours for both intravenous and oral groups. OJT007 concentrations in plasma and urine were quantitated using sensitive, specific and reproducible LC-MS/MS methods. Briefly, OJT007 was extracted from plasma by protein precipitation using acetonitrile. Urine samples were analyzed directly after a 100 times dilution with water. Voriconazole was used as an internal standard. Chromatographic separation was achieved using C_{18} column (50 x 2.1 mm, 1.7 µm), with a binary gradient system comprising of water (A) and acetonitrile (B) containing 0.1% formic acid as the mobile phase. OJT007 was measured using SCIEX QTRAP 4000 mass spectrometer. The OJT007 assay was linear in the concentration range of 5 ng/mL- 1000 ng/mL. The pharmacokinetic parameters were calculated with Phoenix WinNonlin 8.1 using non-compartmental model.

Results: OJT007 displayed a biexponential disposition after intravenous administration. The mean area under the curve ($AUC_{0-\infty}$), systemic clearance (CL), volume of distribution (V_d), and terminal elimination half-life ($T_{1/2}$) were 3.140 h.mg/L, 2.306 L/kg/hr, 4.926 L/Kg and 1.864 hr, respectively. After oral administration, OJT007 had limited absorption with a mean maximum plasma concentration of 92 ng/mL and a mean absorption rate constant of 0.38/hr. $AUC_{0-\infty}$, apparent CL, apparent V_d, and $T_{1/2}$ were 0.504 mg.h/L, 22.55 L/kg/hr, 78.3 L/kg and 2.49 hr, respectively. The oral bioavailability of OJT007 ranged from 5.34% to 10.8%. Low oral bioavailability may be related to the low aqueous solubility of OJT007 (<1 mg/mL). The mean percentage of OJT007 excreted unchanged in urine after 24 hours following intravenous and oral administration was 0.62% and 0.45%, respectively, suggesting OJT007 was extensively metabolized *in vivo.*

Conclusion: OJT007 displayed a biexponential disposition in rats after intravenous administration. It is poorly absorbed following oral administration with limited bioavailability. The drug is extensively metabolized in rats. Further studies are warranted to improve oral bioavailability and investigate the metabolic mechanisms of OJT007.

Poster #6

Acute Treatment with Nicotine-derived Nitrosamine Ketone (NNK) Causes Disruption of Blood Brain Barrier (BBB) and Microglia Activation on Mice

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Cigarette Smoke (CS) continues to be a leading cause for decline of quality of life as well as deaths globally. CS has been shown to have an association with neuroinflammation and several neurological disorders. Mounting evidence indicates a correlation /association between Cigarette Smoke Toxins with studies indicating that smoking can aid in blood brain barrier (BBB) impairment by subsequent inflammatory activity. While there is indication that CS induces neuroinflammation mediated by microglia and BBB breakdown, no focused effort has been taken to understand the role of BBB disruption and subsequent gliovascular responses. Thus, there is a lack of studies on the effects of CS on markers of neurodegeneration, though epidemiological evidence indicates a potential link. To address this, we combine the use of transgenic mouse line (CX3CR1-GFP) with intravital neuroimaging (IN) in an acute intra-nasal delivery model of nicotine-derived nitrosamine ketone (NNK). This study focuses on investigating the patterns of neuroinflammation caused by NNK across the brain using IN. This approach has the potential to reveal a connection between inhaled toxicants, neuronal vulnerability and progression of neurodegeneration through use of a CX3CR1 transgenic mouse line (expressing a GFP+ microglia). The *in vivo* tracking of CS effects provided a real time assessment of BBB and microglia responses to a common aggressor found in cigarette smoke.

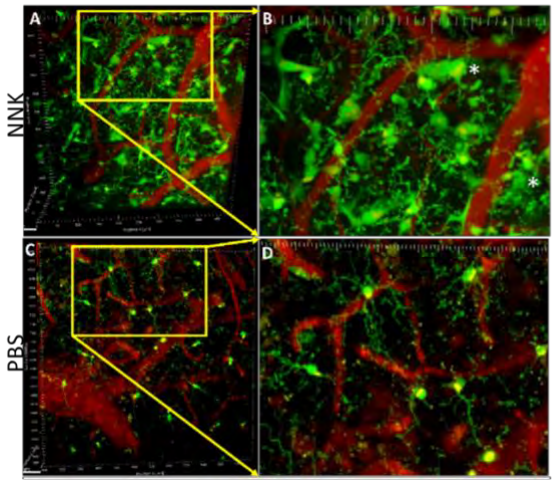

Fig.1: Representative 3-D volumes of NNK treated (A, B) and sham mice (C, D). Qualitative analysis shows amoeboid microglia in NNK treated mice, suggesting microglial activation. Sham mice display more ramified processes, suggesting inactive state. A zoomed in view of microglial bodies can be appreciated in panels B and D. Microglial clusters were also noted in NNK treated mice but not on sham, delineated by asterisks. Objective Nikon 40x, NA 0.8.

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Design and Synthesis of Novel Ispinesib Prodrugs

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Background: Glioblastoma multiforme (GBM) is the most malignant form of brain tumors and remains largely incurable. The median survival rate is 15 months on a standard chemotherapy. In search for a more effective approach, researchers have been focusing on targeting mitotic kinases (CDK, AURK,, PLK) to inhibit the tumor's cell cycle. Kinesin spindle protein (KSP) plays a critical role in mitosis by mediating centrosome separation and bipolar spindle assembly. Inhibition of KSP function results in prolonged mitotic arrest leading to cell death. Bioinformatic analyzes of the GBM genome revealed an overexpression of KSP. Several KSP inhibitors are currently being investigated in clinical trials. However, like many mitotic kinase inhibitors KSP inhibitors lack a therapeutic window due to toxicity against rapidly proliferating non-cancerous cells.

Objective: Given their high potencies and high affinities, we contend that KSP inhibitors can be repurposed for clinical efficacy by adopting the bioactivation themes common to conventional chemotherapeutic pro-drugs such as pH and enzymatic activation. This study focuses on the design, synthesis and *in vitro* biological evaluation of derivatives of the KSP inhibitor ispinesib.

Methods: *Chemistry:* Phosphonium and phsophoramidate derivatives of ispinesib were synthesized via Mitsunobu reaction and Copper-Catalyzed Oxidative Cross-Coupling respectively. Full characterization of the products was achieved by ultra performance liquid chromatography mass spectrometry (UPLC-MS) and nuclear magnetic resonance spectroscopy. *Proliferation assays:* Cell proliferation of glioblastoma cell lines (D423, LN319) was assayed through crystal violet staining. Cells were seeded at 1500 cells/well in 96-well plates, and after 24 h, were treated with dilution series of KSP inhibitors, with each treatment concentration in duplicate wells. After 5 days cells were fixed with 10% formalin and stained with crystal violet. Dye extraction was performed using 10% acetic acid solution, and absorbance was read at 595 nm. The results were plotted using GraphPad Prism 8. *Stability:* The synthesized compounds were examined for their stability in phosphate buffer at pH 3.3, 7.0 and 8.8 at 10 min, 30 min, 60 min, 120 min and 24h using UPLC-MS. Compounds back extracted from the culture media after treatment in cells were also tested.

Results: Phosphine derivatives were stable in pH 3.3 and 7.0 phosphate buffer for 24 h, whereas at pH 8.8 they hydrolyzed into ispinesib and phosphine oxides within 60 min. Phosphate ester were stable in all test pH ranges within 2 h. Ispinesib derivatives inhibited the proliferation of D432 and LN319 with IC50 values in nanomolar range. Ispinesib was recovered from the culture media of cells treated with phosphonium derivatives, whereas intact phosphoramidates derivatives of ispinesib were retrieved.

Conclusion: Novel ispinesib prodrug candidates have been synthesized and showed potencies similar to ispinesib. The rapid degradation of phosphonium derivatives in alkaline pH implies an intracellular release of the parent drug. The prodrug candidates will advance to *in vivo* studies.

Funding sources This study is made possible by the generous support of the Brockman family foundation.

Poster #8

Protein: Protein Interaction-based Peptidomimetics Targeting the Nav1.6 Channel Complex

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The voltage-gated Na+ (Nav) channel Nav1.6 is regulated by accessory proteins such as intracellular fibroblast growth factor 14 (FGF14). Studies have shown that FGF14 binds directly to the Nav1.6 C-tail leading to modulation of the channel's biophysical properties. Thus, short peptides or small molecules interfering with the FGF14:Nav1.6 channel interface might serve as specific inhibitors of Nav1.6 and modulators of neuronal excitability. Recently, we have applied homology modeling to define the FGF14:Nav1.6 interface and designed putative interfering peptides of the FGF14:Nav1.6 channel complex formation. Here, we applied *in silico* docking and a combination of split-luciferase complementation assay (LCA) and patch-clamp electrophysiology to reconstitute the FGF14:Nav1.6 channel complex and test each peptide efficacy in disrupting the complex formation. *In silico* peptide docking predicted FLPK to interact with the previously identified "hot-spots", $FGF14_{Y158}$ and $FGF14_{V160}$, at the FGF14:Nav1.6 channel complex interface, finding that was confirmed by surface plasmon resonance (SPR) studies. In cell LCA demonstrated that FLPK disrupts the FGF14:Nav1.6 channel complex formation and that the effect is abolished by $FGF14_{Y158N/V160N}$ mutations. Whole-cell patch clamp electrophysiology studies showed that FLPK prevents FGF14-dependent regulation of Nav1.6 currents, reversing previously reported changes in peak current density and voltage sensitivity of Nav1.6 that occur in the presence of FGF14. On the basis of these results, we designed a series of peptidomimetics derived from FLPK which are currently under investigation for functional activities toward Nav1.6. In conclusion, our findings identify the FGF14:Nav1.6 interface as an attractive target for future therapeutics against Nav channelopathies.

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Poster #10

Targeting the GSK3 Pathway with Modulators of Voltage-gated Na+ Channels

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Resilience and vulnerability to neuropsychiatric disorders are linked to molecular changes underlying neuronal excitability that are still poorly understood. In recent studies, we have shown that vulnerability to depression is mediated by a form of maladaptive plasticity consisting of increased firing of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), a brain area associated with reward-related behaviors. In these cells we showed that maladaptive firing is mediated by phosphorylation of the intracellular C-tail of the voltage-gated Na^+ channel Nav1.6 by glycogen-synthase kinase 3β (GSK3β) and that a GSK3β-Nav1.6$_{T1936}$ competing peptide reverses maladaptive plasticity of MSNs in animal models of depression-like behaviors. Furthermore, in vivo genetic manipulations demonstrated that GSK3β and Nav1.6 are molecular determinants of MSN excitability and that silencing of GSK3β prevents this form of maladaptive plasticity of MSNs. Building on these results, we are now designing a series of small molecules with improved drug-like properties that target specifically and selectively the GSK3β:Nav1.6 complex. One compound, ZL141, inhibits the formation of the GSK3β:Nav1.6 complex in cells as determined by the luciferase complementation assay and fluorescence spectroscopy, and binds to the Nav1.6 C-terminal tail as assessed by surface plasmon resonance. In addition, whole cell patch-clamp recordings in HEK293 cells stably expressing Nav1.6 showed that ZL141 rescues GSK3β-dependent modulation of Na+ currents and of Nav1.6 steady-state inactivation and long-term inactivation. We expect ZL141 and other small molecule analogues targeting the GSK3β:Nav1.6 channel complex to inhibit maladaptive firing of MSNs ultimately reducing susceptibility to depression and stress disorders. These studies lay groundwork for the development of compounds targeting the GSK3β signaling pathway by targeted modulation of functionally relevant substrates such as Nav channels.

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Poster #10

Identification and Functional Validation of Allosteric Modulators of Nav 1.1 Channels

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Voltage-gated sodium (Nav) channels provide the basis for neuronal excitability in the brain. Of the nine Nav channel isoforms (Nav1.1-Nav1.9), Nav1.1 exhibits cell-specific distribution in fast-spiking parvalbumin (PV) interneurons in the cortical circuit. Reduced firing of these cells is a common feature in neuropsychiatric and neurodegenerative disorders, raising the need to develop selective allosteric modulators targeting Nav1.1. The fibroblast growth factor 14 (FGF14) directly binds to the C-terminal tail of Nav channels resulting in isoform-specific modulation of Na+ currents and channel biophysical properties. These unique structure-function properties of protein:protein interaction (PPI) interfaces between FGF14 and different Nav isoforms could provide a novel target for developing isoform-specific small molecule modulators of Nav channels. Here, we have conducted a ligand-based high-throughput virtual screening against the FGF14:Nav1.1 complex using Autodock. We used a grid box encompassing a portion of the FENYYV sequence (residues 155-160) of FGF14 within a distance of 8Å from the Nav1.1 C-tail; this region is part of a previously identified druggable pocket within the β9 sheet of FGF14. We initially identified 1001 ZINC compounds predicted to bind this interaction site out of 642,759 screened ligands, and these were further narrowed down to 14 hits based on putative binding scores. Finally, we selected ZINC1 and ZINC3 for further studies based on chemical properties, including predicted cLogP. Surface plasmon resonance and whole-cell patch clamp electrophysiology confirmed binding of ZINC3 to FGF14 and functional activity of the compound against Nav1.1-mediated Na+ currents. Interestingly, ZINC3 functional effect on Nav1.1 currents was FGF14 isoform dependent.

The compound suppressed Nav1.1 peak transient currents ($n=14$, $p<0.0031$) and decreased channel availability through regulation of long-term inactivation ($n=14$, $p<0.005$) in the presence of FGF14-1b, while induced a depolarizing shift in the voltage-dependence of activation in the presence of FGF14-1a ($n=6$, $p<0.002$). In conclusion, ZINC3 and other small molecules targeting the FGF14:Nav channel PPI interface could serve as scaffolds to develop Nav channel isoform-specific allosteric modulators with broad applicability for neuropsychiatric and neurodegenerative disorders.

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Poster #11

High-throughput Screen Identifies Novel Mitochondrial-Targeting Small Molecule Drugs with Anti-cancer Properties

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It has long been established that tumorigenesis drives profound metabolic changes as the cell prepares for unrestrained division. In solid tumors, oxidative phosphorylation is often dysregulated and glycolysis shifts into high gear (a condition commonly referred to as Warburg metabolism), partially to compensate for poor vascularization and low oxygen tension in tumor microenvironments. This led to long-lasting consideration of the importance of glycolysis for tumorigenesis, while the importance of oxidative phosphorylation (OXPHOS), and mitochondria in general, was relatively neglected. However, in recent years the paradigm has begun to shift back, and understanding the unique relationships between cancer and mitochondria has gained in importance. For example, a number of cancer subtypes upregulate OXPHOS, in contradiction with the Warburg Hypothesis. These include both blood cancers and solid tumors. Meanwhile, carcinogenic transformation causes substantial mitochondrial stress and damage as cancerous cells leverage them to continue their aberrant division.

Recent results from our lab demonstrated that by inflicting mitochondrial damage (particularly in combination with glycolytic inhibition), we can selectively trigger cancer cell death. To identify novel mitochondria-targeting leads, we screened ~50,000 small molecules to identify hits capable of stabilizing the kinase PINK1. This key regulatory enzyme phosphorylates Parkin2, which is necessary and sufficient for targeting mitochondria for autophagic degradation. Using *Caenorhabditis elegans* as a whole-organism model, we identified 13 compounds that activated mitophagy. Hits were evaluated for their impact on cell lines derived from acute myeloid leukemias (OCI-AML-2 and MOLM-13) and glioblastomas (U251 and U138MG). Several of the molecules demonstrated increased toxicity towards cancer cells specifically, as compared to controls (PBMCs or glial cells, respectively). Interestingly, one of the potential mitocans, PS127, is also a potent inducer of DAF-16 nuclear localization. This characteristic will be investigated in further studies using analogs of the original compound and RNAi knockdown of insulin signaling. Additionally, analogs of identified hits are being screened to identify critical features as well as decrease toxicity to non-cancerous cells. The ability of these compounds to activate mitochondrial stress responses pathways was also assayed.

Poster #12

Mining Protein: Protein Interaction Interfaces For Voltage-Gated Na_+ Channel Drug Discovery

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As key players in cell function, ion channels are important targets for drug discovery and therapeutic development against a wide range of health conditions. Thus, developing assays to reconstitute ion channel macromolecular complexes in physiological conditions and screen for chemical modifiers of protein:protein interactions within these complexes is timely in drug discovery campaigns. For most ion channels, expressing their pore-forming subunit in heterologous mammalian cells has now become a routine procedure. However, reconstituting protein-channel complexes in physiological environments is still challenging, limiting our ability to identify tools and probes based on allosteric mechanisms, which could lead to more targeted and precise modulation of the channel function. Here, we describe our assay development to stably reconstitute the interaction between voltage-gated Na+ (Nav) channel Nav1.6 and its accessory protein, fibroblast growth factor 14 (FGF14) using the split-luciferase complementation assay (LCA), followed by assay miniaturization and optimization in 384-well plates for in cell high-throughput screening (HTS) against protein-channel interactions. Throughout development, we used a small test library of 267 FDA-approved compounds targeting known mediators of cellular signaling to assess assay performance, as well as to demonstrate its utility. Of the 65 hits initially detected, 24 were excluded based on counter-screening and cellular toxicity. Based on target analysis, potency and dose-response relationships, 5 compounds were subsequently repurchased for validation and confirmed as hits. Among those, the tyrosine kinase inhibitor lestaurtinib was highest ranked, exhibiting nanomolar inhibition of FGF14:Nav1.6 assembly. While providing evidence for a robust in-cell HTS platform that can be adapted to search for any channelopathy-associated regulatory proteins, these results lay the potential groundwork for repurposing cancer drugs for neuropsychopharmacology. Overall, the flexibility of this assay enables rapid hypothesis generation in early phase drug discovery campaigns narrowing down targets prior to more labor-intensive in vivo studies.

Funding: CPRIT grant RP150578 (CCS); PhRMA Foundation Fellowship in Pharmacology (PAW); NIH/NIA Grant T32AG051131 (PAW); NIH/NIMH Grant R01MH111107 (FL); NIH/NIMH Grant R01MH095995 (FL); John Sealy Memorial Endowment Funds (FL); UTMB MD/PhD Combined Degree Program (PAW)

Poster #13

High-Throughput Screening Against Protein:Protein Interactions Within the Voltage-Gated Na+ Channel Complex

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Ion channel macromolecular complexes play a critical role in regulating and finely tuning neuronal firing. Minimal disturbances to these tightly controlled and highly specific protein:protein interactions (PPI) can lead to persistent changes in plasticity of the brain circuit. As such, PPI interfaces are potentially ideal targets for neurotherapeutic development, as they could lead to selective and specific drugs with limited side effects. We used the split-luciferase complementation assay (LCA) to reconstitute the voltage-gated Na^+ (Nav) channel 1.6 and its regulatory protein, fibroblast growth factor 14 (FGF14), a molecular complex that mediates action potential firing in medium spiny neurons (MSNs) in the nucleus accumbens, a brain area that controls reward-related behaviors and mood states. Following assay optimization in 384-well plates, we conducted an in-cell HTS against the FGF14:Nav1.6 complex using the LCA. We screened ~50,000 small molecules and rationally-designed drug-like analogues in duplicate, and compound Z-scores were calculated by normalizing luminescence to per plate controls (0.3% DMSO). A fluorescence-based cell viability assay was conducted in parallel, and potentially toxic compounds were excluded. Using cut-offs of $Z \leq -5$ for inhibitors and $Z \geq 3$ for enhancers, we initially identified 960 hits. Of these, 640 compounds failed to achieve significance during validation screening (n=3), and an additional 149 were identified as false positives based on counter-screening against luciferase ($Z \leq -3$ or $Z \geq 3$). The remaining 168 hits were then stratified by structural and chemical properties including predicted permeability (logP), and an initial dose response was conducted for 60 compounds with the greatest potential for blood-brain barrier permeability. We repurchased 24 promising compounds for validation by an expanded 10-point dose response, and hits were then ranked based upon their potency (EC_{50}/IC_{50}) and efficacy. Estimated in-cell IC_{50} of the top 14 inhibitors ranged from 0.95 to 15 μM, while estimated EC_{50} of the top 4 enhancers ranged from 0.65 to 1.21 μM. Cell-free orthogonal screenings including surface plasmon resonance (SPR), protein thermal shift (PTS), and isothermal titration calorimetry (ITC) were subsequently used to assess hit binding affinity for purified FGF14 and Nav1.6 protein, and *in silico* docking was used to predict potential binding sites. Promising hits are now being functionally evaluated as modulators of Nav1.6 currents and neuronal firing from MSNs in the nucleus accumbens. Lead compounds targeting this complex could lay groundwork for developing a new class of anti-depressants or mood stabilizers based on fine-tuning of Nav channels.

Funding: CPRIT grant RP150578 (CCS); PhRMA Foundation Fellowship in Pharmacology (PAW); NIH/NIA Grant T32AG051131 (PAW); NIH/NIMH Grant R01MH111107 (FL); NIH/NIMH Grant R01MH095995 (FL); John Sealy Memorial Endowment Funds (FL); UTMB MD/PhD Combined Degree Program (PAW)

Poster #14

Development of Antivirulence Compounds Targeting Pyoverdine

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Objectives and Methods: *Pseudomonas aeruginosa* exhibits resistance to multiple antibiotics and causes serious infections for people with compromised immune systems. One strategy to treat *P. aeruginosa* infections is to develop inhibitors that act on its virulence factors directly. One key virulence factor is pyoverdine (PVD), which is key for obtaining iron from the pathogen's environment and is also involved in the regulation of other acute virulence determinants. Previously, we carried out a high-throughput phenotypic screen to identify compounds that prevent *P. aeruginosa* virulence by interfering with pyoverdine. Preliminary characterization of one of our hits, a novel small molecule referred to here as PQ3, was identified in the high-throughput screen, and suggested that it prevented pyoverdine function, possibly by directly interacting with pyoverdine. In this study, we apply solution nuclear magnetic resonance (NMR) and molecular modeling to: 1) study the structure-activity relationships (SAR) for PQ3 and several related compounds, 2) identify interactions between PVD and the inhibitors, and 3) develop structure models of PVD complexed with each compound.

Results: PQ3, PQ3a, and PQ3c were shown to reduce the pathogenic effect of PVD and to improve survival of *Caenorhabditis elegans* when challenged with *P. aeruginosa*. This rescue vanishes if a pyoverdine biosynthetic mutant is used in place of wild-type bacteria, suggesting that pyoverdine is the relevant target. **1)** The study demonstrates that the compounds inhibit the innate fluorescence of PVD that results from the chromophore at its core. **2)** Site-specific NMR studies showed the compounds interact with the chromophore and the N-terminal amino acids of pyoverdine, particularly D-Ser1 and the sidechain of Arg2 in PVD. PQ3 causes the most structure perturbations on PVD, while PQ3a exhibits the least effect. **3)** B-factors from molecular dynamics (MD) simulations reveal that the N-terminus of PVD is highly dynamic. The conformational heterogeneity plays an essential role in the conformational selection by ligand. Finally, an ensemble of the representative conformations from the most populated clusters was used for molecular docking. The derived structure models are consistent with our NMR observations and reveal that electrostatic interactions are important for the compound binding.

Conclusions: We discovered a panel of antivirulence compounds which act on PVD by the interaction with the N-terminal chromophore and residues. The molecular modeling provides the structural and dynamic basis for the binding event and can be utilized for lead optimization.

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