NE-ADME

NEW ERA OF ADME & BEYOND

JUNE 16, 2022

Novartis Institute for BioMedical Research
CAMBRIDGE, MA

SPONSORED BY:

BIOIVT
ELEVATING SCIENCE™

SCIEX
XENOTECH
A DIVISION OF SEKISUI

DISCOVERY HYPHA

SHIMADZU
Excellence in Science

EMITIMAGING

cyprotex
AN EVOTEC COMPANY

GVBBS

Medicilon
Medicilon Inc.
Integrated Drug Discovery Services

Organized by: The Boston Society

www.bostonsociety.org

NE-ADME
NEW ERA OF ADME & BEYOND

2022 NE-ADME SPONSORS

PLATINUM SPONSOR

BIOIVT
ELEVATING SCIENCE™

GOLD SPONSORS

SCIEX

XENOTECH
A DIVISION OF SEKISUI

SILVER SPONSORS

cyprotex
AN EVOTEC COMPANY

EMITIMAGING
ABSOLUTELY

GUBBS

DISCOVERY
HYPHA

Medicilon Inc.
Integrated Drug Discovery Services

SHIMADZU
Excellence in Science

The Boston Society

NE-ADME
NEW ERA OF ADME & BEYOND

ORGANIZERS' WELCOME

Welcome to the 2022 NE-ADME Conference.

Our organizers have gathered another excellent group of speakers for the annual NE-ADME conference. The program is arranged to incorporate extensive audience participation and discussion. We encourage attendees to take full advantage of the opportunity to engage in discussion in order to receive the maximum benefit from the NE-ADME experience. Thank you for your participation.

ORGANIZING COMMITTEE

PRESIDING OFFICERS

Conference Chair:
Ruchia Duggal, Merck

COMMITTEE MEMBERS

Steven Louie, Novartis
Dallas Bednarczyk, Novartis
Vinayak Hosagrahara, Nimbus Therapeutics
John S. Janiszewski, NIH/NCATS
Mitesh Patel, Novartis
Chris Rowbottom, Moderna
Jun Zhang, BMS
Ron Xu, ORIC Pharmaceuticals
Hui Zhang, Entos Pharmaceuticals
Maria Fitzgerald, Epizyme

The Boston Society

NE-ADME
NEW ERA OF ADME & BEYOND

NE-ADME 2022 CONFERENCE AGENDA

THURSDAY, JUNE 16

8:00 - 9:00 AM **Registration & Breakfast**

9:00 - 9:10 AM **Conference Opening**
Ruchia Duggal, Merck

SESSION I: Advances in ADME of New Modalities
Chairs: Mitesh Patel, Novartis & Ruchia Duggal, Merck

9:10 - 9:15 AM **Session Introduction**

9:15 - 9:40 AM ***Of Mice and Man: Path to Clinical CRISPR/Cas9 Genome Editing Therapeutic Development***
Yuanxin Xu, Intellia Therapeutics

9:40 - 10:05 AM ***The Knowns and Unknowns of ADME/PK of Targeted Protein Degraders***
Haojing Rong, Kymera Therapeutics

SPONSOR SHOWCASE: XENOTECH A DIVISION OF SEKISUI

10:05 - 10:45 AM **PLENARY:** ***How Understanding ADME Properties of Multispecific Biologics Can Increase Speed to the Clinic***
Brooke Rock, Amgen

10:45 - 11:05 AM Break

SPONSOR SHOWCASE: SCIEX

SESSION II: Novel In Silico Tools for ADME in Drug Discovery
Chairs: Maria Fitzgerald, Epizyme & Vinayak Hosagrahara, Nimbus Therapeutics

11:05 - 11:10 AM **Session Introduction**

11:10 - 11:35 AM ***Populations of Models for Virtual Patient/Cohort Construction in ADME/PK***
James Kozloski, IBM

11:35 - 12:00 PM ***Prediction Accuracy of Volume of Distribution Using In Vitro, In Vivo, PBPK, and QSAR Methods***
Li Di, Pfizer

The Boston Society

12:00 - 12:25 PM ***Mechanistic Modeling of Caco-2 Experiments to Estimate Intrinsic Permeability***
Mailys Mendes, Certara UK

12:25 - 1:25 PM Lunch

1:25 - 1:50 PM **VENDOR PRESENTATION:**
Long-term HEPATOPAC® Cultures: Model Validation for ADME-Tox Applications
Dipen Vyas, BioIVT

BIOIVT ELEVATING SCIENCE™

SPONSOR SHOWCASE: XENOTECH A DIVISION OF SEKISUI

SESSION III: Contributions of Vascular and Lymphatic Compartments to Drug Absorption

Chairs: Dallas Bednarczyk, Novartis & Jun Zhang, BMS

1:50 - 1:55 PM **Session Introduction**

1:55 - 2:35 PM **PLENARY:** ***Vascular and Lymphatic Barrier Function in the Brain and Subcutaneous Compartments***
Roger Kamm, MIT

2:35 - 3:00 PM ***Tools Toward Predicting Oral Lymphatic Adsorption***
Ryan Pelis, Novartis

3:00 - 3:20 PM Break

SPONSOR SHOWCASE: SCIEX

SESSION IV: ADME of COVID Therapeutics and Beyond

Chairs: Steven Louie, Novartis & Chris Rowbottom, Moderna

3:20 - 3:25 PM **Session Introduction**

3:25 - 3:50 PM ***Assessment of Amino Lipid Biodistribution, Metabolism, and Elimination in Rats after LNP IV Administration to Understand In Vivo Fate and Potential for DDI***
Doug Burdette, Moderna

3:50 - 4:15 PM **Panel Discussion:** ***Expedited Drug Discovery Post-COVID***
Brooke Rock, Amgen; Li Di, Pfizer; Doug Burdette, Moderna; Charlie Knutson, Novartis

4:15 - 4:20 PM **Closing Remarks**
Chris Rowbottom, Moderna

4:20 - 5:20 PM **Reception**

ABSTRACTS

SESSION I

The Knowns and Unknowns of ADME/PK of Targeted Protein Degraders

Haojing Rong, Kymera Therapeutics

One of the two big questions for the "druggability" of targeted protein degradation (TPD) platform was "will a degrader have the drug-like ADME/PK properties in human?". Much progress has been made in the last decade as TPDs made their transition to the industry, resulting more than ten TPDs entering clinical development in the past two years (Békés et al, 2022). The talk will share the translation of PK from preclinical species to human for degraders. Characteristics of key underlining process for degrader absorption, distribution, and elimination in vivo will be presented. Opportunities to improve oral bioavailability for degraders will be discussed.

PLENARY

How Understanding ADME Properties of Multispecific Biologics can Increase Speed to the Clinic

Brooke Rock, Amgen

With the increase in the use of biologic drugs, understanding absorption, distribution, metabolism and excretion (ADME) properties is critical to tuning the pharmacokinetic (PK) profiles of next generation multispecific biologics. Knowledge of the fate of a drug is essential for the enhancement of the design processes, elongation of exposure at the desired site of action and achieving efficacy with minimum toxicity. In vivo proteolytic cleavage of biotherapeutics may lead to undesirable in vivo properties, such as rapid clearance, low bioavailability and loss of pharmacodynamic (PD) effect which may affect drug efficacy and/or generate safety concerns through increases in immunogenicity or off-target toxicity. The presentation will focus on ADME assays to de-risk instability of multispecific biologics as well as stream lined processes to decrease the time to clinical testing.

SESSION II

Populations of Models for Virtual Patient/Cohort Construction in ADME/PK

James Kozloski, IBM

Mechanistic models of biological systems provide insights into processes that underly observations at a cellular, organ or patient level. These models require parameter tuning to fit model outputs to data. Furthermore, heterogeneity in biological processes is fundamental at all levels, creating the need for populations of models (PoMs). Pharmacokinetic (PK) models can recreate drug concentration profiles across a virtual patient cohort (VPC) by varying parameters such as rate constants among models. Covariates, such as genetic polymorphisms in CYP, may correlate with distributions of concentration measures and create heterogeneity within sub-cohorts. Often methods for parameter fitting specify only a single model parameter set given some specific covariates. Here we present a generative adversarial network (GAN) that solves a stochastic inverse problem (SIP) formulation of VPC generation as in [1] while enabling stratification of distributions of parameter samples by covariate information. In an extension of the model, termed cr-GAN, we incorporate covariate data as conditioning variables, and the cr-GAN learns the joint distribution of covariates with model parameters. We demonstrate cr-GAN on parameter inference problems using synthetic data with a standard two-compartment PK model. Our method for tackling PoM problems in the PK domain is novel, generic and extensible to other types of mechanistic models used in pharmacometrics and quantitative systems pharmacology. A neural network-based approach to model population generation holds several important advantages, such as the ability to train on large datasets that may

have high-dimensional parameter or feature spaces, or to incorporate prior knowledge of data structure, as in the use of convolutional layers for reducing dimensionality of inputs in imaging problems.

[1] Parikh et al., (2022). Generative adversarial networks for construction of virtual populations of mechanistic models: simulations to study omecamtiv mecarbil action. J Pharmacokinet Pharmacodyn. 49(1):51-64.

Prediction Accuracy of Volume of Distribution Using In Vitro, In Vivo, PBPK and QSAR Methods

Li Di, Pfizer

Steady state volume of distribution (Vss) is one of the most important PK parameters of drug candidates as it impacts not only half-life, but also dose. Many methods are available to predict human Vss, including QSAR models, PBPK approaches, scaling from preclinical species and in vitro methods. However, the top performing methods are not always consistently identified based on literature information, due to different datasets and input parameters used. In this presentation, Vss prediction accuracy will be discussed using multiple methods with three large datasets, including (1) the rat dataset with 56 compounds, (2) the human dataset with 1276 compounds, and (3) the preclinical species dataset of 125 compounds containing Vss data of four species, i.e., rat, dog, monkey and human. The results indicated that the global QSAR models outperformed the PBPK methods in Vss prediction. Tissue fraction unbound (fu,t) method with adipose and muscle predicted Vss accurately. Overall, the high performing methods for human Vss prediction are the global QSAR models, Øie-Tozer and equivalency methods from scaling of preclinical species, as well as PBPK methods with Kp scalar from preclinical species. Prediction accuracy of tissue Kp were also examined. The fu,t method predicted Kp values more accurately than the PBPK methods for adipose, heart and muscle. All the methods overpredicted brain Kp and underpredicted liver Kp, potentially due to efflux (brain) and uptake (liver) transporter effects. Successful Vss prediction involves strategic integration of in silico, in vitro and in vivo approaches.

Mechanistic Modeling of Caco-2 Experiments to Estimate Intrinsic Permeability

Maïlys De Sousa Mendes, Certara

Passive permeability is a key parameter to predict drug absorption and tissue exposure. In vitro derivation of passive permeability can be affected by the structural elements of the assay and the conditions under which it is performed. If an appreciation of the assay's structural components and conditions is not undertaken when deriving permeability estimates, an 'apparent' rather than 'intrinsic' permeability is generated, and employing the 'apparent' permeability for extrapolation to in vivo, may compromise predictive success. Hence, a mechanistic appreciation of the assay can be of benefit when performing IVIVE in permeability-limited in silico models. This presentation will bring forward considerations for key parameters that can impact the 'apparent' permeability, and approaches to model the data to obtain an 'intrinsic' permeability, thus limiting potential bias introduced by differences between the in vitro and in vivo conditions, to facilitate translation through IVIVE-PBPK.

VENDOR PRESENTATION

Long-term HEPATOPAC® Cultures: Model Validation for ADME-Tox Applications

Dipen Vyas, BioIVT

The long-term viability and functionality of HEPATOPAC cultures makes this an ideal system to evaluate compounds with low clearance and low metabolism rates. IVIVE can be improved using HEPATOPAC technology, providing better data for lead selection and optimization decisions and IND submissions. The session will provide an overview of the HEPATOPAC system, a micropatterned hepatocyte-fibroblast co-culture and study designs for metabolic and toxicity studies using this platform.

SESSION III

PLENARY

Vascular and Lymphatic Barrier Function in the Brain and Subcutaneous Compartments

Roger D. Kamm, MIT

Microphysiological models are rapidly being developed to enable in vitro simulation of normal and diseased function of human organs and tissues. In addition, blood and lymphatic vascular function can now be mimicked producing models of transport across various vascular barriers. The latter have proven to be especially relevant to drug delivery via multiple pathways. In this presentation, two models of clinically relevant transport will be described. In one, trans-endothelial exchange across a blood-brain barrier (BBB) model that has similar morphology and function to that found in vivo is described and characterized. This model is then used to study transport of therapeutic molecules or nanoparticles across the BBB in neurological disease (Alzheimer's disease) or cancer (metastatic breast cancer and glioblastoma). In the other, biodistribution of monoclonal antibodies injected into the subcutaneous space is studied. In both models the microvasculatures are created from mixed cell populations introduced in gel solution, that subsequently form into in vivo-like structures through self-assembly and self-organization. Current results as well as applications envisioned in the future will be discussed, highlighting both the capabilities and limitations of these in vitro technologies.

Tools Toward Predicting Oral Lymphatic Adsorption

Ryan Pelis, Novartis

The lymphatic circulatory system is a promising physiologic route for improving pharmacokinetics of compounds that otherwise have poor oral absorption properties. By targeting oral lymphatics first-pass hepatic metabolism is bypassed, and the flow rate of the lymphatic system is magnitudes lower than blood flow, leading to extended pharmacokinetic profiles. Also, formulation strategies can be used to high-jack this pathway. Our understanding of oral lymphatic drug absorption is largely limited to lymph-duct cannulated pre-clinical models, which bears uncertainty of clinical translatability. Presented are first steps in establishing an in vitro human intestinal cellular model, combining information from established in silico and pre-clinical in vivo models, to better understand the impact of drug physicochemical properties and formulation on oral lymphatic drug absorption. This presentation focuses on comparing the use of Caco-2 vs. human primary intestinal cells (duodenal vs. ileal) for this purpose. Our results suggest that primary human intestinal cells are more representative and likely a more appropriate model system for assessing oral lymphatic drug absorption, and translating the information from in vitro-to-in vivo.

SESSION IV

Assessment of Amino Lipid Biodistribution, Metabolism, and Elimination in Rats after IV Administration to Understand In Vivo Fate and Potential for DDI

Doug Burdette, Moderna

Lipid nanoparticle (LNP) encapsulated mRNA therapeutics are complex assemblages of naturally occurring and xenobiotic chemical components. The lipid components that encapsulate the therapeutic RNA drive the distribution and productive mRNA delivery to cells as well as being critical to stabilizing the mRNA and protecting it from in vivo degradation. The work described here assesses the in vivo distribution, metabolic fate, and routes of elimination of a critical, xenobiotic lipid (Lipid 5) after IV administration of [14C]Lipid 5-containing LNPs. The spleen and liver were the main organs of 14C distribution with some exposure in the lung, kidneys, and brown fat. Only background levels were seen in brain or eyes and there was no differential exposure in pigmented vs. non-pigmented rats. Circulating Lipid 5 was BQL within 10 h after dose and a mix of circulating mono-and di-acids collapsed to a single β-oxidized diacidic species after 10 h with overall plasma 14C levels approaching BQL by 24h. Renal (65%) and hepatic (25%) routes of

elimination after extensive metabolism were identified based on quantitative whole-body autoradiography (QWBA) and excreta data. Greater than 90% of the administered 14C was recovered in urine and feces within 168 h after dose. The 14C chemical species recovered were a mix of mono- and diacid metabolites in feces with only diacids in urine. Additionally, the biodistribution of Lipid 5 and mRNA from an LNP of similar composition that contained a different mRNA, showed similar biodistribution to that seen in this study via QWBA. These data support the hypothesis that LNP lipids drive the biodistribution of LNP encapsulated mRNA therapeutics, that traditional biodistribution studies with unlabeled drug product accurately represent the in vivo distribution of these complex biotherapeutics and that Lipid 5 is rapidly metabolized and eliminated by well understood biochemical pathways.

NE-ADME
NEW ERA OF ADME & BEYOND

SPEAKER BIOGRAPHIES

DOUG BURDETTE, PHD, Moderna Dr. Doug Burdette is Head of DMPK and Clinical Pharmacology at Moderna Therapeutics. He received his academic training at the University of Michigan (BS), Michigan State University (MS, PhD), and U. C. Berkeley (postdoctoral research). His 25+ year career in the pharmaceutical/biopharma industry spans early target discovery through post-approval support (global) across a wide range of therapeutic areas and drug modalities. He has actively participated in and consulted for academic/industry/regulatory consortia and professional societies throughout his career. His peer reviewed publications and presentations include topics related to assay development; high throughput screening; enzymology; protein biochemistry; drug discovery, ADME and safety; and DDI.

LI DI, PHD, Pfizer Dr. Li Di has over 25 years of experience in the pharmaceutical industry including Pfizer, Wyeth and Syntex. She is currently a research fellow at Pfizer Worldwide Research and Development, Groton, CT. Her research interests include the areas of drug metabolism, pharmacokinetics, drug-drug interactions, absorption, transporters, and blood–brain barrier. She has over 170 publications including two books and presented over 95 invited lectures. She is a recipient of the Thomas Alva Edison Patent Award, the New Jersey Association for Biomedical Research Outstanding Woman in Science Award, the Wyeth President's Award and Peer Award for Excellence.

ROGER D. KAMM, PHD, MIT Professor Kamm began his career at Northwestern University earning a degree in Mechanical Engineering. He subsequently earned both a master's and a PhD in Mechanical Engineering at MIT. Since 1978, he has been a professor of Mechanical Engineering at MIT. Professor Kamm was one of the founding members of the Biological Engineering department when it was created in 1998.

The Kamm lab has been developing microfluidic platforms over the past 15 years with the aim of studying various aspects of vascular disease, cancer, and neurological disease. The basic platform technology that facilitates simultaneous 3D, multi-cell type cultures has been applied to investigations of vascular barrier function, cancer, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease, among others. In particular, Kamm's Mechanobiology Lab has contributed significantly to our general understanding of the fundamental processes leading to metastatic cancer and the mechanisms by which circulating tumor cells arrest in the microcirculation, then extravasate into the organ tissue. He also focusses on the role of different circulating or resident immune cells in cancer progression and treatment. Kamm's work in disease models has led to collaborations with several biotech and pharmaceutical companies, as well as formation of a start-up company, AIM Biotech. Administratively, Kamm has served as PI on several multi-investigator programs, including a Program Project Grant on mechanotransduction (NHLBI), a Biomechanics Training Grant (NHLBI), an Interdisciplinary Research Group in Singapore, and currently directs an NSF Science and Technology Center on Emergent Behaviors of Integrated Cellular Systems and a U54 Center on Metastatic Cancer funded by the NCI. Kamm has also been instrumental in DEI efforts at MIT through the formation of BEEAM, a program for URM high school students in the Boston area that brings them into MIT labs to gain research experience in preparation for a college education in STEM. The program is now in its 4th year and continues to grow.

JAMES KOZLOSKI, IBM James Kozloski joined IBM Research in May, 2001, where he has worked in the Computational Biology Center at the T.J. Watson Labs in Yorktown Heights, NY. He leads and manages IBM's department of Hybrid Biological-AI Modeling, which collaborates with researchers worldwide in the fields of Computational Neuroscience and Cardiology, modeling brain and heart from synaptic plasticity in neural circuits to functionally active heart and brain tissues. At IBM, he created the

Model Graph Simulator, a high-performance computing tool that's been applied to Neural Tissue Simulation on supercomputers and on IBM's Hybrid Cloud. Parts of this technology were adopted by the European Blue Brain and Human Brain Project. As manager, James oversees efforts to merge AI and biophysical models. His team focuses on quantitative and systems solutions for model-based brain and heart disease therapeutic design by applying generative models to solving parameter inference problems. In addition to several dozen coauthored papers, James has over 250 issued patents in the areas of neuroscience, neurotechnology, and computer science. In 2010, he was named an IBM Master Inventor, and in 2017 he was inducted into IBM's Academy of Technology.

MAÏLYS DE SOUSA MENDES, PHD, Certara Dr Maïlys De Sousa Mendes is a senior research scientist in the Simcyp division of Certara. During her PhD she modelled the transfer of drug across placentas in order to predict foetal exposure using physiologically based pharmacokinetic (PBPK) models. Maïlys joined the Translational Science in DMPK team at Simcyp in 2016 and has worked in several areas including developing and updating compound files and incorporating the pathway mass balance and back conversion. She is part of the transporter team and is therefore highly involved in updating the transporter models in Simcyp as well as developing the analyse of in-vitro data using the Simcyp In Vitro Analysis (SIVA) toolkit in order to obtain more robust parameters.

RYAN PELIS, Novartis Ryan Pelis is a Sr. Principal Scientist in the In vitro ADME group (Drug Disposition, Pharmacokinetic Sciences) at the Novartis Institutes for Biomedical Research (NIBR) in Cambridge, MA. Prior to NIBR Cambridge he was a Professor at SUNY Binghamton and Dalhousie University in Halifax, NS, Canada. Ryan's expertise is in drug transport and metabolism, and understanding their roles in disposition. His role in this space is supporting compounds from discovery to clinical registration.

BROOKE ROCK, PHD, Amgen Dr. Rock is an Executive Director in the Pharmacokinetics and Drug Metabolism department at Amgen. Prior to joining Amgen, Brooke worked at multiple start-up biotechnology companies, after receiving her PhD degree from University of Washington, in medicinal chemistry. Brooke's research interest focuses on translational pharmacology, specifically in the field of enzymology. At Amgen, she has applied that interest across the portfolio in developing novel analytical tools to aid in understanding drug disposition. Brooke has contributed to more than 40 peer-reviewed research papers, and multiple book chapters, as well as numerous presentations at international conferences.

HAOJING RONG, PHD, Kymera Therapeutics Dr. Rong is the Vice President of Preclinical Development at Kymera Therapeutics. She earned her Ph.D. of Pharmacognosy and Phytochemistry from Faculty of Pharmaceutical Science at Ghent University in Belgium. Prior to joining Kymera in 2018, Haojing worked at Amgen, Merck, Amira Pharmaceuticals, Pfizer, and Shire. Haojing's interest and expertise include ADME and Tox knowledge integration for both small molecule and biotherapeutics such as protein therapeutics, oligonucleotides, and gene therapy, specifically human PK and dose projection, drug interactions using modelling and simulation, and applying integrated PK/PD modelling approach in drug development to optimize efficacy and safety.

DIPEN VYAS, PHD, BioIVT Dr. Vyas' career has focused on development of bioengineered liver tissue using a tissue engineering approach. He received his doctoral training in liver tissue physiology and pharmacology at Wake Forest University Institute for Regenerative Medicine and has extensive experience in liver stem cell biology and various hepatocyte models. From 2015-2020, Dr. Vyas led the development of a liver organoid model for applications in drug metabolism and toxicity at Biorg, a biotech startup focused on developing advanced 3D multicellular models. He joined BioIVT in 2020 as a Study Director where he designs and implements sponsored research studies investigating metabolic stability, metabolite identification and hepatoxicity, in various in vitro systems including using the HEPATOPAC model.

YUANXIN XU, MD, PHD, Intellia Dr. Xu is Sr. VP at Intellia to advance breakthrough curative in vivo and ex vivo gene editing therapeutic development. She serves as Head of Early Development and Translational Medicine since January 2020, responsible for pharmacology, toxicology, DMPK, and bioanalysis.

She joins Intellia from Alnylam (5 years) as Senior Director of Bioanalytical Sciences for RNAi development to treat genetic diseases, including patisiran, the 1st approved RNAi therapy. During her 13 years with Clinical Laboratory Sciences at Genzyme/Sanofi, she was most recently Senior Scientific Director and supported clinical studies and approvals of biologics, antibodies, small molecules, as well as cell and gene therapies in many disease areas. Her early experience was at BioTransplant-Immerge (9 years) investigating xenogeneic and allogeneic stem cell and organ transplantation and induction of immune tolerance.

Yuanxin received her B.M. (M.D. equivalent) from Beijing Medical University (Peking University Health Science) and her Ph.D. in Biochemistry from Iowa State University.

POSTER ABSTRACTS

Development of a High-Throughput and Efficient Automated Workflow to Determine Blood to Plasma Ratio

Sravani Adusumalli, Megan Metallides, and Yau Yi Lau
Cyprotex, 313 Pleasant Street, Watertown, MA 02472

PURPOSE

The blood to plasma ratio of a compound is an important parameter in drug discovery and development. Pharmacokinetic parameters are typically determined by analysis of plasma drug concentrations but they should ideally be based on blood; blood drug concentrations can be estimated by correcting plasma levels using this ratio. The blood to plasma ratio determines the concentration of the drug in whole blood compared to plasma and provides an indication of drug binding to red blood cells. Different methods are available to determine the blood to plasma ratio of compounds which are low throughput, laborious and time consuming. A literature survey revealed that currently there is no high-throughput approach developed. We developed a high throughput technique utilizing plasma fractions obtained from whole blood to back calculate the binding of drug to erythrocytes. The technique facilitates running 96 compounds (n=4) in 384 well plate requiring one scientist and half a day to run the assay.

METHODS

Fresh blood with Sodium Heparin as anti-coagulant was used for the assay on the day it was delivered. Hematocrit values were obtained from the vendor and were used for data analysis. Whole blood was centriguged to obtain reference plasma. Blood and reference plasma were aliquoted (147 µL) using Tecan Freedom Evo (liquid handler) into 2 labeled 384-well deep well plates. Compounds (Verapamil, Diltiazem, Midazolam, Chlorthalidone and Methazolamide) were spiked (n=4) into blood and reference plasma to acheive the desired test concentration using Bravo (liquid handler). The spiked blood and reference samples were incubated at 37°C for 60 minutes. At the end of the incubation period, 30 µL of reference plasma samples were precipitated by the addition 130 µL internal standard solution. The incubated blood samples were centrifuged at 6,102 x g for 30 minutes at 4°C to separate the plasma. A 30 µL of the resulting plasma (separated from blood sample) was removed using Bravo and precipitated by the addition 130 µL internal standard solution. Samples were filtered and transfered to clean 384-well plates for analysis by LC-MS/MS.

RESULTS

Blood to plasma ratio was calcuated using the ratio of peak area ratio in reference plasma and plasma separated from blood. No complicated extraction of compounds from whole blood simplified the assay. Blood to plasma ratios of verapamil, diltiazem, midazolam, chlorthalidone and methazolamide were found to be comparable with literature values as well as the manual 96-well plate assay. Interday assay variablity was found to be less than 20% for all compounds except for chlorthalidone which had moderate variability of 34%. This variablity was observed in the manual 96 well plate assay as well.

CONCLUSIONS

The high throughput screening method developed to determine blood to plasma ratio makes it easy for adoption of the assay as routine screening in early drug development to screen large number of compounds efficiently. A highly automated and high throughput unique method developed in 384 well plate to determine the blood to plasma ratio which significantly lowers labor, time and cost using minimal reagents in comparison to current available methods.

LIPID LOADING IN MICROPATTERNED PRIMARY HEPATOCYTE AND KUPFFER CELL CO-CULTURE: NAFLD DISEASE MODELING FOR DRUG TOXICITY SCREENING

Karissa E. Cottier[1], Jeannemarie Gaffney[2], Candice Lewis[1], and Scott Heyward[1].
[1]BioIVT, Baltimore, MD
[2]BioIVT, Medford, MA

PURPOSE

Better in vitro systems are required to model non-alcoholic fatty liver disease (NAFLD), a disorder characterized by hepatic steatosis which can advance to non-alcoholic steatohepatitis (NASH). Here, we show lipid loading and lipid induced toxicity capabilities in HEPATOPACÆ and HEPATOMUNE® cultures, systems that can retain physiologic hepatic function for at least 28 days.

METHODS

Micropatterned Hepatocyte Cultures: HEPATOPAC cultures were created using microfabrication tools to generate an organized co-culture containing cryopreserved human hepatocytes and 3T3-J2 murine embryonic fibroblasts. HEPATOMUNEÆ cultures were generated by adding Kupffer cells to HEPATOPAC cultures that were stabilized in serum-supplemented medium for 7 days prior to addition of Kupffer cells. The hepatocyte: Kupffer cell ratio was of 1:0.4, mimicking an inflamed liver state.

Lipid Loading: To mimic diets linked to development of NAFLD, the media was supplemented with 0.5mM free fatty-acids (FFA) or high glucose (10g/ L) and fructose (1g/L) (HGF). Both FFA and HGF resulted in lipid loading. In parallel, cultures were returned to control media for an additional 3-days to assess reversal of lipid loading. While FFA treated cells unloaded lipid, HGF treated hepatocytes retained their lipid stores. In all conditions, lipid loading could be prevented or reversed using ACC1/2 inhibitors.

Drug Induced Steatosis Assessment: The cultures were incubated with escalating concentrations of either tamoxifen or valproic acid +/- 0.5 mM FFA (Oleate/ Palmitate 1:2) for 10 days. Media was exchanged once per day including addition of fresh drug. On day 10 supernatant was collected and plates were fixed for imaging.

RESULTS

Drug induced steatosis was observed using the HEPATOPAC system. Treatment with valproic acid, but not tamoxifen, induced lipid loading which was more pronounced under steatotic conditions.

Under HGF and FFA loading HEPATOMUNE cultures had reduced viability when given a strong inflammatory signal (LPS) as compared to HEPATOPAC cultures or HEPATOMUNE cultures with no LPS or steatosis stimuli.

CONCLUSIONS

HEPATOPAC and HEPATOMUNE cultures can serve as an easy-to-use long-term system capable of examining the interplay between FFA, HGF, genotype, and immune cell activation on hepatic steatosis and hepatotoxicity- making it a useful platform to study NAFLD related drug toxicity and screen therapeutic modalities.

ABOUT OUR SPONSORS

BIOIVT is the leading provider of high-quality hepatic products. For over 25 years, we have set the standard for hepatocytes and offer leading brands including TRANSPORTER CERTIFIED® and LIVERPOOL® human hepatocytes. Our product offerings also include INVITROCYP™ human liver microsomes, animal hepatocytes and microsomes and INVITROGRO™ hepatocyte media. These research tools are used in in vitro hepatic modeling to analyze the ADME-Tox properties of New Chemical Entities (NCEs). Our research services team works collaboratively with clients to implement studies and provide technical expertise and guidance to advance drug development programs.

CYPROTEX (www.cyprotex.com) was founded in 1999 and specialises in in vitro and in silico ADME-Tox. The company has sites in the UK and the US. In 2016, Cyprotex was acquired by Evotec AG (www.evotec.com). As a whole, the Group offer integrated and stand-alone drug discovery capabilities as well as full CMC and IND-enabling services, allowing the company to provide expert support across the value chain from early discovery through to preclinical development and beyond.

EMIT IMAGING was founded in 2018, by Jack Hoppin, PhD, and a group of imaging researchers, that discovered the need for a high-resolution quantitative fluorescent imaging platform to significantly complement in vivo studies.

Understanding the need for quantitative 3D fluorescent capabilities, these researchers developed Cryo-Fluorescence Tomography (CFT) technology for molecular imaging, biodistribution, drug discovery, understanding of genetic models and development of molecular probes. Starting with a camera, Emit Imaging integrated a cryo-microtome and proprietary software, to build a comprehensive easy to use platform, Xerra. Emit Imaging's platform suite provides the most all-inclusive solution for high-resolution quantitative 3D fluorescence. The company is driven to support and develop technology to best meet their customers' needs. The company is actively working to develop new software tools to increase Xerra's utility and applications.

Emit Imaging has installed systems in leading institutions across North America. The company is based in Baltimore, Maryland.

GUBBS, INC. is a company dedicated to providing software, service and consulting solutions to the Biotechnology and Pharmaceutical industries. Gubbs Inc staff are completely knowledgeable in discovery, development, and bioanalytical workflows typical of laboratories conducting these types of studies, including 21 CFR Part 11 compliance.

HYPHA DISCOVERY is a specialist CRO supporting pharmaceutical and agrochemical companies worldwide through the production of metabolites and late-stage derivatives of drugs and agrochemicals in discovery and development.
We are experts in the scalable synthesis, purification and identification of drug metabolites and oxidised derivatives of lead compounds, and also possess a wealth of experience in the production, purification and structure elucidation of natural products and drug derivatives.

Our "One-Stop Metabolite Shop" concept comprises a comprehensive suite of technologies to enable synthesis of even the most difficult-to-synthesise metabolite. Human and other mammalian phase I and phase II metabolism of drugs and agrochemicals can be synthesised using a variety of methods to maximise the chances of success, including chemical synthesis, microbial biotransformation, mammalian liver fractions (S9/microsomes), recombinant enzymes such as PolyCYPs, as well as the purification of metabolites from biological matrices. Hypha scientists are also experts in structural elucidation via NMR spectroscopy.

Clients regularly ask Hypha to produce metabolites for unambiguous identification, for use as quantitation standards or larger amounts for pharmacology and toxicology studies to satisfy regulatory guidelines, including MIST guidelines. Metabolites arising from a variety of pathways are accessible, including both CYP and non-CYP derived phase I mechanisms, phase II conjugates and metabolites arising from mixed and multi-step pathways.
www.hyphadiscovery.com

MEDICILON offers fully integrated pharmaceutical services for the global scientific community. We focus on providing an exceptional client-centered experience and advancing the drug discovery process.

Since the founding of our company in 2004, our integrated services across biology, chemistry and preclinical services are uniquely designed to help clients developing their research and discovery programs from the initial idea stage to the IND filing phase.

Our headquarters is located in Zhangjiang High-Tech Park in Shanghai, China, with an additional facility in Chuansha Economic Park, Shanghai, China. We occupy over 300,000 sq. ft. in lab space and have over 1,200 employees cross biology, chemistry and preclinical research. Over 50% of our employees have M.S. and Ph. D., over 10% of them have foreign education and/or working experiences.

Medicilon has been recognized as one of the top drug discovery contract research organizations (CRO) in China and is managed by a team of scientists with a wealth of experience in US-based pharmaceutical and biotechnology companies. As our areas of expertise and service capabilities continue to expand, more and more pharmaceutical and biotechnology companies have taken advantage of our integrated drug discovery and development services.

SCIEX Whether you are optimizing your labs current capabilities or scaling up for future demands, SCIEX enables you to be more productive with end-to-end CE and LC-MS solutions. SCIEX solutions provide high levels of sensitivity and dynamic range. This makes even the most complex workflows easier and more efficient, while delivering you the utmost in data quality.

SCIEX is here to help. Whether it is to repair your instrument, assist with your workflows, or help you maximize productivity in your lab – we've got you covered.

SHIMADZU is a world-leading manufacturer of analytical instrumentation. With its technologies, Shimadzu enables its customers from the most diverse industries as well as in the medical field to develop new products and solutions to promote and protect people's health and lives. With our Corporate Philosophy "Contributing to Society through Science and Technology", Shimadzu promises customers worldwide to realize our wishes for the well-being of mankind and the Earth. Shimadzu has been providing solutions in ADME for its wide instrumentation portfolio. Within that portfolio two techniques in particular cover a wide range of experiments for DMPK departments around the world. Chromatography and Mass Spectrometry. Shimadzu has made themselves a global leader in liquid chromatography for 30+ years by producing extremely sensitive and durable systems complimented by powerful software. Within a decade, Shimadzu went from providing only a single quadrupole MS, to having a QQQ, MALDI-ToF, and QToF that can exceed the specifications of top competitor instruments. Our presence in mass spectrometry is growing rapidly and today we are excited to present a novel solution for drug distribution studies: the fastest and first ever integrated Optical Mass Imaging Microscope.

SEKISUI XENOTECH is a global CRO for ADME/DMPK/DDI testing and test systems. A comprehensive array of services including in silico, screening, in vitro and in vivo are employed, with specialties in Enzyme Inhibition and Induction, Drug Transporters, Metabolite ID and Metabolic Stability testing, as well as Radioisotope labeling and QWBA distribution studies. Along with an extensive selection of Hepatocytes and DMPK-focused test systems, we are your trusted partner to efficiently move your drug through development.

NE-ADME
NEW ERA OF ADME & BEYOND

Thank you to all of our Organizers, Speakers, Sponsors and Delegates! Without your dedication, support and participation NE-ADME 2022 would not be possible. We greatly value your comments regarding NE-ADME 2022 as well as thoughts or suggestions for improving future conferences. Please take the time to fill out our online survey when we send it to you next week.

Sincerely,

The Boston Society