APPLIED PHARMACEUTICAL ANALYSIS

OCTOBER 1-3, 2018

SHORT COURSES - SEPTEMBER 30

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ORGANIZERS' WELCOME

Welcome to the 2018 Applied Pharmaceutical Analysis Conference.

Our organizers have gathered another excellent group of speakers for the annual APA 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 APA experience. Thank you for your participation.

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Chair-Elect: Mehran Moghadam, OROX Bioscience

REGULATED BIOANALYSIS

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Chair: Hongying Gao, Innovo Bioanalysis LLC
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MECHANISTIC ADME

Chair: Mehran Moghaddam, OROX Biosciences
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APA 2018 CONFERENCE AGENDA

MONDAY, OCTOBER 1

REGULATED BIOANALYSIS WORKSHOP - Back Bay Ballroom C

7:00 am - 8:00 am
Registration and Breakfast

Regulated Bioanalysis Attendees are Invited to Attend the Plenary Lecture in Back Bay Ballroom D

Session I: Regulatory Guidance

9:10 am - 9:20 am
Workshop Introduction - **C.T. Viswanathan**, C.T. Viswanathan and Associates

9:20 am - 9:25 am
Session Introduction - **C.T. Viswanathan**, C.T. Viswanathan and Associates & **Eric Woolf**, Merck

9:25 am - 9:55 am
Common BA/BE Inspection Observations and their Mitigation Strategies, **Arindam Dasgupta**, FDA

9:55 am - 10:25 am
Recent Inspectional Observations on Immunogenicity, **Michael Skelly,** FDA

10:25 am - 10:55 am
Unique Aspects and Case Studies of Bioanalysis in Support of Veterinary Products, **Valerie Kvaternick**, Merial

MECHANISTIC ADME WORKSHOP - Back Bay Ballroom D

7:00 am - 8:00 am
Registration and Breakfast

8:00 am - 8:10 am
APA 2018 Conference Introduction: **Johanna Mora**, BMS; Workshop Introduction - **Mehran Moghadam**, OROX Biosciences

Session I: Toxicology, Clinical Safety Prediction & SM Biomarkers

8:10 am - 8:15 am
Session & Plenary Introduction - **Cindy Xia**, Takeda & **Chandra Prakash**, Agios

8:15 am - 8:55 am
PLENARY TALK - Donna Dambach, Genentech - Translation Insights in Nonclinical Safety Assessment

8:55 am - 9:25 am
Systems Approaches to Translation of Preclinical Safety Signals to Inform Compound Discovery and Early Development, **Jay Mettetal** , AstraZeneca

9:25 am - 9:55 am
An ADME Perspective on DILI Hazard Identification, **Debra Luffer-Atlas**, Eli Lilly

9:55 am - 10:25 am
Discovery Toxicology: Input and Impact in Early Drug Development, **Yvonne Dragan**, Takeda

10:25 am - 10:50 am
Break

10:55 am - 11:20 am
Break

11:20 am - 12:00 pm
Panel Discussion

12:00 pm - 1:20 pm
Lunch

Session II: Bioanalytical Support for Novel Therapies

1:20 pm - 1:25 pm
Session Introduction - **Andre Iffland**, Vertex & **Jakal Amin**, CRL

1:25 pm - 1:55 pm
CART-ography: Lessons Learned from the Analytical Characterization of the First Two FDA Approved Chimeric Antigen Receptor Therapies, **Sadik Kassim**, Mustang Bio

1:55 pm - 2:25 pm
When the Cell is the Drug, Challenges for Bioanalysis, **Keith Sutton**, CRL

2:25 pm - 2:55 pm
Bioanalytical Support for Gene Therapy Program Using AAV Delivery Vectors, **Lilia Macovei**, Pfizer

2:55 pm - 3:25 pm
Development of a Robust Neutralizing Antibody (NAb) Assay with High Drug and Target Tolerance to Support Clinical Development of an anti-TFPI, **Yuhong Xiang,** Pfizer

Session II: Unusual Metabolic Pathways: Time to Expect the Unexpected?

10:50 am - 10:55 am
Session Introduction - **Eric Ballard**, Amgen & **David Stresser,** AbbVie

10:55 am - 11:25 am
Characterization of Uncommon Metabolites: Case Studies with Ombitasvir and Paritaprevir, **Jianwei Shen**, AbbVie

11:25 am - 11:55 am
Late-occurring and Long-circulating Major Metabolites Formed by Metabolic Cyclization and Aromatization from a CNS Candidate Drug of AstraZeneca, **Chuck Gu**, Biogen

11:55 am - 12:25 am
N-methylation of BI 187004 by thiol S-methyltransferase, **Holly Maw**, Boehringer Ingelheim

12:25 pm - 1:45 pm
Lunch

Session III: Specialty Molecules; Going Beyond Rule of 5

1:45 pm - 1:50 pm
Session Introduction - **Greg Walker**, Pfizer & **Mehran Moghadam**, OROX Biosciences

1:50 pm - 2:20 pm
Drug Discovery in "Rulebreaker" Chemical Space: Ledipasvir and Velpatasvir, **John Link**, Gilead

2:20 pm - 2:50 pm
Working Beyond Rule of 5: Designing Cyclic Peptide C5a Antagonist with Oral Bioavailability, **Kevin Beaumont**, Pfizer

2:50 pm - 3:20 pm
ADME Screening Paradigm for Covalent Inhibitor Drugs – Role of Traditional vs Non-Traditional Assays, **Ashutosh Kulkarni**, Allergan

3:25 pm - 3:50 pm
Break

Session III: Challenges of Immunogenicity in Regulated Environment

3:50 pm - 3:55 pm
Session Introduction - **Darshana Jani**, Pfizer & **Ang Liu**, BMS

3:55 pm - 4:25 pm
Monitoring the Function of Single Immune Cells – How to Tackle this Daunting Task and Apply Cutting-Edge Developments for Obtaining Meaningful Data, **Sylvia Janetzki**, Zellnet Consulting

4:25 pm - 4:55 pm
Building Character in Cell Lines, **Cynthia Inzano**, BMS

4:55 pm - 5:25 pm
Navigating the Development and Validation of Neutralizing Antibody Assays in Support of Biosimilar Assessment, **Corinna Krinos-Fiorotti**, BioAgilytix Labs

5:25 pm - 6:40 pm
Evening Reception & Poster Session

3:20 pm - 3:45 pm
Break

BEYOND ADME & BIOANALYSIS WORKSHOP - GLOBAL HEALTH SESSION

3:45 pm - 3:50 pm
Session Introduction - **Mehran Moghaddam**, OROX Biosciences

3:50 pm - 4:30 pm
Antiparasitic Drug Discovery for Marine Cyanobacteria, **William Gerwick**, Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego

4:30 pm - 5:10 pm
Reversing Neglect: Pharmaceutical Research and Development for Global Health, **Joseph Camardo**, Celgene

5:10 pm - 6:40 pm
Evening Reception & Poster Session

TUESDAY, OCTOBER 2

REGULATED BIOANALYSIS WORKSHOP - Back Bay Ballroom C

7:00 am - 8:00 am
Mentoring Breakfast!

8:00 am - 8:05 am
Plenary Introduction - **Johanna Mora**, BMS

8:05 am - 8:45 am
PLENARY TALK - **Binodh DeSilva** , BMS, The Impact of a Reagent Center of Excellence on Bioanalysis, and a Company's Strategy Around Variabilization

Session IV: Utility of Clinical Biomarkers to Support Drug Development

8:45 am - 8:50 am
Session Introduction - **Fumin Li**, PPD & **Yongjun Xue**, Celgene

8:50 am - 9:20 am
"Total" or "Free" PD Biomarker Assay in Biotherapeutic Drug Development: From Platform Selection to Methodology Evaluation, **Jenny Zhang**, Pfizer

9:20 am - 9:50 am
Target Engagement and Receptor Occupancy Based Translational Strategy for Biologics, **Adrienne Clements-Egan**, J&J

9:50 am - 10:20 am
Sensitive and Robust Trapping-Micro-LC-MS for the Quantification of Biomarkers and Antibody Biotherapeutics, **Jun Qu**, SUNY-Buffalo

10:20 am - 10:45 am
Break

DISCOVERY BIOANALYSIS & NEW TECHNOLOGIES WORKSHOP - Back Bay Ballroom D

7:00 am - 8:00 am
Mentoring Breakfast!

Discovery Bioanalysis and New Technologies Attendees are Invited to Attend the Plenary Lecture in Back Bay Ballroom C

8:50 am - 8:55 am
Workshop Introduction - **Hongying Gao**, Innovo Bioanalysis

Session I: New Technologies

8:55 am - 9:00 am
Session Introduction - **Mark Qian**, Takeda & **Mark Cancilla**, Merck

9:00 am - 9:30 am
Biomarkers of neurodegeneration – a single molecule approach via Quanterix platform, **Krishna Midde**, PPD

9:30 am - 10:00 am
"Immunocapture-LC/MS Based Target Engagement Measurement in Tumor Plasma Membran, **Hiroshi Sugimoto**, Takeda

10:00 am - 10:30 am
Insights into the Insulin Receptor activation: the Cryo-EM Structure of the IR in complex with Insulin, **Giovanna Scapin**, Merck

10:30 am - 10:55 am
Break

Session V: New Technologies and Applications

10:45 am - 10:50 am
Session Introduction - **Lori Payne**, Intertek & **Joseph Tweed**, Pfizer

10:50 am - 11:20 am
Enhanced Immuno-affinity for Hybrid LBA–LC-MS: Considerations and Challenges, **Ang Liu**, BMS

11:20 am - 11:50 am
Bioanalysis of Intact Biologics Therapies, **Elizabeth Groeber,** CRL

11:50 am - 12:20 pm
New Developments in Patient Centric Sampling and Their Application in Clinical Trials, **Melanie Anderson**, Merck

12:20 pm - 1:40 pm
Lunch

Regulated Bioanalysis Attendees are Invited to Attend the Plenary Lecture in Back Bay Ballroom D

2:35 pm - 2:50 pm
Break

MECHANISTIC ADME WORKSHOP

Session IV: Software for Met ID

2:50 pm - 2:55 pm
Session Introduction - **Silvi Chacko**, BMS & **Hongbin Yu**, Boehringer-Ingelheim

Session II: Predictive Tools and Novel Approaches to Evaluate Immunogenicity Response of Biotherapeutics

10:55 am - 11:00 am
Session Introduction - **Monserrat Carrasco Triguero**, Genentech & **Dieter Drexler,** BMS

11:00 am - 11:30 am
Preclinical minimization of Immunogenicity Liabilities for High Quality Bio Therapeutics, **Jochem Gokemeijer**, BMS

11:30 am - 12:00 pm
Current Perspectives on Preclinical Predictive Tools for Immunogenicity Risk Assessment and their Clinical Translation, **Vibha Jawa**, Merck

12:00 pm - 12:30 pm
Human Leukocyte Antigen Class II-associated Immunopeptidomics for Immunogenicity Studies of Biologics, **Andrea Casasola-LaMacchia**, Pfizer

12:30 pm - 1:50 pm
Lunch

1:50 pm - 1:55 pm
Plenary Introduction - **Hongying Gao**, Innovo Bioanalysis LLC

1:55 pm - 2:35 pm
PLENARY TALK - **Dick Smith**, PNNL, "New Developments in Ion Mobility-Mass Spectrometry Enabling Improved Higher Throughput and More Sensitive Proteomics and Pan-Omics Measurements for Clinical Diagnostics and Personalized Medicine

2:35 pm - 2:50 pm
Break

Session III: Omics

2:50 pm - 2:55 pm
Session Introduction - **Hongying Gao**, Innovo Bioanalysis LLC & **Mark Cancilla**, Merck

2:55 pm - 3:25 pm
Automated Metabolite Identification for Design Ideation, **Christopher Kochansky**, Merck

3:25 pm - 3:55 pm
Accelerating the Delivery of Small Molecule High Resolution Accurate Mass Structure Elucidation Results Through the Use of Thermo Scientific™, **Jonathan Bauman**, Pfizer

3:55 pm - 4:25 pm
Fast and Comprehensive Detection and Characterization of Cyclic Peptide Metabolites using Software-aided Data Processing Tools, **Ming Yao** , BMS

4:25 pm - 5:40 pm
Evening Reception & Poster Session

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2:55 pm - 3:25 pm
Toward Understanding Compound Protein Interaction at a System Biology Scale, **An Chi**, Merck

3:25 pm - 3:55 pm
Application of Targeted Mass Spectrometry to the Quantitative Assessment of Internalization Kinetics for Cell-Surface Membrane Protein Targets, **Dhiman Ghosh**, Takeda

3:55 pm - 4:25 pm
Seeing Diseases Clearly through Metabolism, **David Pirman**, Agios

4:25 pm - 5:40 pm
Evening Reception & Poster Session

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WEDNESDAY, OCTOBER 3

Back Bay Ballroom D

7:30 am - 8:30 am
Breakfast

8:30 am - 8:40 am
Plenary Introduction - **Scott Fountain**, CRL & **Hongying Gao**, Innovo Bioanalysis LLC

8:40 am - 9:20 pm
PLENARY TALK - **Joe Cornicelli**, CRL, Research Models for Non-Alcoholic Steatohepatitis: The Need and Challenges for Evaluating Candidate Therapies

DISCOVERY BIOANALYSIS AND NEW TECHNOLOGIES WORKSHOP

Session IV: IT/Information/Knowledge

9:20 am - 9:25 am
Session Introduction - **Elizabeth Groeber**, CRL & **Scott Fountain**, CRL

9:25 am - 9:55 am
Acquiring and Managing Large Data Sets in Toxicology and Pharmacology Models: Considerations, Challenges, and Opportunities, **Scott Fountain**, CRL

9:55 am - 10:25 am
Data informed Quantitative Systems Pharmacology Model Development, **Fei Hua**, Applied BioMath

10:25 am - 10:45 am
Break

10:45 am - 11:15 am
Progress in Proteogeomics, **Daniel Liebler**, Protypia

11:15 am - 11:45 am
VENDOR TALK
Perspectives on Competitive Advantage in the Contract Bioanalysis Market
Graeme Dennis, IDBS

11:45 am - 12:45 pm
Lunch sponsored by IDBS

DISCOVERY & MECHANISTIC ADME COMBINED WORKSHOP

Session V: Role of DMPK in New Modalities

12:45 pm - 12:50 pm
Session Introduction - **Liyu Yang**, Vertex & **Mark Milton**, Novartis

12:50 pm - 1:20 pm
Gene Delivery: What Role does DMPK Have?, **Nag Chemuturi**, Novartis

1:20 pm - 1:50 pm
Mechanistic ADME of siRNA-GalNAc Conjugates, **Jing Tao Wu**, Alnylam

1:50 pm - 2:20 pm
Drug Distribution Analysis in the Preclinical Development of Gene and Cell Therapy Products, **Sarah Voytek**, BluebirdBio

2:20 pm - 2:40 pm
Break

Session VI: Biomarkers: Discovery and Implementation

2:40 pm - 2:45 pm
Session Introduction - **Lisa Christopher**, BMS & **Jing Tu**, PPD

2:45 pm - 3:15 pm
Use of In Vitro Tools and Genetic Data to Identify
Transporter Endogenous Biomarker, **Xiaomin Liang**, Gilead

3:15 pm - 3:45 pm
Evaluation of Endogenous Biomarkers for Transporter Inhibition:
Current State and Future Considerations, **Xiaoyan Chu**, Merck

3:45 pm - 4:15 pm
Absolute Quantitation of 2-HG, an oncometabolite, in Human Brain
Tumors to Support Clinical Development, **Guowen Liu**, Agios

4:15 pm - 4:20 pm
Raffle Drawing

4:20 pm - 4:25 pm
Closing Remarks for the 14th Annual Applied Pharmaceutical Analysis Conference

APA ABSTRACTS

Regulated Bioanalysis Workshop

SESSION I: Regulatory Guidance

Common BA/BE inspection observations and their mitigation strategies

Arindam Dasgupta, FDA

Simple mistakes in drug development can compromise study data reliability in a complex way:

- A drug packager mislabeling drug bottles for a blinded pivotal bioequivalence study.
- A clinical site retains reserves from only one drug shipment, though it received multiple shipments
- A laboratory conducts stability experiments without comparator samples made from fresh stock solutions.

Reviewers from the Office of Study Integrity and Surveillance (OSIS), Food and Drug Administration (US FDA), often identify problems at BA/BE testing sites and try to mitigate them by evaluating data available during site inspections. While mitigation strategies may not prevent a Form FDA 483 observation, the strategies may help confirm that study data are still reliable to support a regulatory decision.

This presentation will review common inspection findings in clinical and analytical portions of bioavailability/bioequivalence studies and potential mitigation strategies based on scientific approaches.

Recent Inspectional Observations on Immunogenicity

Michael Skelly, FDA

FDA inspections of immunogenicity studies can reveal novel and unexpected features in the study records, in addition to confirming or denying observations at other sites and theoretical concepts. The presentation will describe recent findings that may suggest strategies for future studies and for future inspections of immunogenicity studies. The examples intend to invite challenges or alternative explanations. FDA inspectors may explore similar questions in other situations. The observations do not necessarily result in regulatory decisions or formal actions. It is hoped that all can benefit from the experiences.

Unique Aspects and Case Studies of Bioanalysis in Support of Veterinary Products

Valerie Kvaternick, Boehringer Ingelheim (Merial Inc)

Animal health/veterinary pharmaceuticals present some unique opportunities and challenges compared to human health/pharmaceuticals. One unique attribute of drugs developed for animals is the opportunity to evaluate the drug in the target species during drug development including pre-clinical work and target animal safety. Target species include companion animals such as dogs and cats, and production animals such as pigs, cattle, and poultry. Regional differences may impact the classification of an animal and the required testing programs required for approval, for example, the horse is considered a companion animal in the US and a food production animal in the EU. This creates a unique challenge for drugs developed for production animals as extensive testing is required to ensure the safety of food for human consumption. The human pharmaceutical industry is concerned with safety and efficacy in humans. The animal health pharmaceutical industry is concerned not only with the safety and efficacy in the target species (e.g., dog or cattle), but also user and human food safety as well. Human food safety assessments ensure that edible tissues (meat) from treated production animals, such as cattle, do not pose a risk to human health. This is ensured by the conduct of residue studies which require GLP validated bioanalytical methods, that is, tissue residue methods, and the establishment of appropriate withdrawal periods, or time from drug administration to time the animal enters the food chain. Validated bioanalytical methods are also used in bioequivalence (BE) or relative bioavailability (RBA) studies that may be conducted to allow approval of a new product, formulation, or route of administration, or expand the label to other species.

SESSION II: Bioanalytical Support for Novel Therapies

CART-ography: Lessons Learned from the Analytical Characterization of the First Two FDA Approved Chimeric Antigen Receptor Therapies

Sadik Kassim, Mustang Bio

Chimeric antigen receptor T (CAR-T) cell therapies have produced remarkable clinical outcomes in relapse/refractory cancer patients. These therapies have also led, in certain cases, to life-threatening side effects. The field of cellular immunotherapy is now at a critical juncture where a number of CAR-T cell therapies have received FDA approval; this includes tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta), both for patients with advanced B cell malignancies. However, little is known about how to characterize these 'living' drug products before administration and more needs to be understood about how different manufacturing strategies can affect safety and efficacy. Moreover, the interplay between the CAR-T cell therapy drug product and the host-specific microenvironment remains incompletely understood. This talk will review some of the most common analytical characterization challenges encountered during CAR-T cell therapy drug product development and will propose an integrated strategy that may enable for the rapid and comprehensive development of safer and more effective CAR-T therapies for both liquid and solid tumors.

When the Cell is the Drug, Challenges for Bioanalysis

Keith Sutton, CRL

The rapid progression of cell based therapies into the clinic presents challenges to the current paradigms for bioanalysis as applied to small molecule, gene and protein therapies. Measuring the systemic exposure or biodistribution of the cell therapy is normally achieved through the use of the quantitative polymerase chain reaction (Q-PCR) to specifically detect genetically modified cells. However this methodology does not capture key metrics that can contribute to clinical outcomes. Namely the number of cells expressing the encoded protein and the level of expression of that protein. The measurement of protein expression and distribution amongst cell populations can be readily assessed using flow cytometry. However this technical platform is generally not used as a quantitative tool. Cell therapies are challenging our uses of this technique by demanding answers to more complex questions; can it be used for exposure measurement, and can it generate data of sufficient robustness to be used as a basis for pharmacokinetic measurement and modelling?

Bioanalytical Support for Gene Therapy Program using AAV Delivery Vectors

Lilia Macovei, Pfizer

Recombinant adeno-associated viral vectors (rAAV) have been reliable tools for basic research for many years and now making their way into translational use, with the use of rAAVs in early clinical trials as delivery platforms. Their tissue tropism and relative safety due to their low rate of genomic integration represent key features making rAAVs promising instruments as vectors for future gene therapy drugs.

However, one of the main hurdles in using rAAVs is the prevalence of neutralizing antibodies in the general population. In this presentation we will discuss the bioanalytical assay development for the adeno-associated viral vectors used for an AAV-based investigational product. The anti-capsid ADA and Nab assay development, unique challenges and immunogenicity strategy to detect anti-capsid neutralizing antibodies will be presented.

Development of a Robust Neutralizing Antibody (NAb) Assay with High Drug and Target Tolerance to Support Clinical Development of an anti-TFPI Therapeutic Monoclonal Antibody (mAb)

Yuhong Xiang, Pfizer

Objective: Our drug is a fully human monoclonal antibody against Tissue Factor Pathway Inhibitor (TFPI), a natural inhibitor of extrinsic coagulation pathway. Blocking of TFPI function can increase coagulation activity in hemophilia patients. Endogenous TFPI exhibits different isoforms and can bind to lipoproteins and proteoglycans. Circulating target and residual drug generate significant concerns when developing robust NAb assay. MSD competitive ligand binding NAb assay with high target and drug tolerance was requested to support ongoing clinical studies.

Methods: Double acid affinity capture elution approach was used to mitigate drug interference and a robust target removal strategy was employed to enhance target tolerance. Assay sensitivity, drug tolerance, target interference, selectivity and precision were evaluated.

Results: The validated NAb assay sensitivity was 313 ng/mL based on PC performance (mouse anti-drug monoclonal antibody). The drug tolerance and target tolerance were established at 50 µg/mL and at least 1200 ng/mL of TFPI,

respectively, with a positive control at the 1 µg/mL level. The screening cutpoint factor is 0.78. Other assay performance characteristics, including precision and selectivity, were evaluated with acceptable results.

Implications: This validated method demonstrated a superior drug and target tolerance to warrant specific and precise characterization of the ADA responses in support of ongoing clinical studies.

SESSION III: Challenges of Immunogenicity in Regulated Environment

Monitoring the Function of Single Immune Cells – How to Tackle this Daunting Task and apply Cutting-edge Developments for Obtaining Meaningful Data

Sylvia Janetzki, Zellnet Consulting

The assessment of the functional state of single cells is often referred to as the holy grail of immune monitoring in a wide area of applications. Flow cytometry and other assays are being used to obtain data that provide insight into the responsiveness of cells to specific stimuli before and after interventions. Those assays are hampered by an inherent variability connected to the samples tested, the assay performed and the acquisition and analysis of data. The presentation will highlight those challenges and revisit an assay established more than 30 years ago, the Elispot and related fluorescent-based techniques. Latest developments have rendered the highly sensitive Elispot to a poly-functional assay that can provide a vast amount of data related to the functional response of a single cell that have previously not been obtainable. In connection with the ease of assay and validation performance, the availability of harmonization guidelines for the assay and analysis as well as statistical tools the technique is a prime example for proficient and reliable single cell immune monitoring that is also affordable and easy to implement.

Building Character in Cell Lines

Cynthia Inzano, BMS

Over the last decade an increase in pharmaceutical development of I/O drugs has required a better understanding of the complexities of co-stimulatory signaling in cell lines used for bioassays and neutralizing antibody (NAb) bioassays. These complex in vivo signaling systems used by this class of drugs pose a unique challenge in developing in vitro cell based assays. Identifying and correlating key markers on the cell line of interest and understanding the impact certain aspects such as passage, freezing and growth reagents have on these markers, can be important factors for assay consistency, reproducibility and evaluating the appropriateness of the cell lines used.

What is presented herein is a discussion of issues related to bioassay methods, case studies involving the impact of freezing/cell passage, fetal bovine serum and age of complete media on two specific cell lines and their markers and strategies in characterizing cell lines early in development to avoid potential bioassay issues post validation.

Navigating the Development and Validation of Neutralizing Antibody Assays in Support of Biosimilar Assessment

Corinna Krinos-Fiorotti, BioAgilytix Lab

This will be a presentation on biosimilar development the best bioanalytical approaches for evaluating differences between an original compound and biosimilar. An increasing number of patents for originator biologic products are due to expire, creating more industry opportunity in the area of biosimilars, and it is therefore important that we all align on best practices for their development.

SESSION IV: Utility of Clinical Biomarkers to Support Drug Development

Target Engagement and Receptor Occupancy Based Translational Strategy for Biologics

Adrienne Clements-Egan, J&J

The pharmacodynamic effect of a biotherapeutic is driven by its interaction with the therapeutic target, i.e. soluble target engagement (sTE) when the target is a soluble protein or receptor occupancy (RO) when it is a cell surface receptor. These sTE/RO assessments can play a central role in translational pharmacology, not only because they reflect the dynamics of drug engagement and corresponding target modulation after drug treatment, but also because they can provide a mechanism to extrapolate the drug effect between preclinical species and humans, and between healthy and disease bearing populations. For biotherapeutics, nonclinical studies can be designed to examine the relationships between

PK and sTE/RO in healthy cynomolgus monkeys and disease models; and the relationships between PK, sTE/RO and PD can be extrapolated into different systems with potential differences in drug exposure or target expression levels. Additionally, the relationship of PK and sTE/RO between peripheral blood and at the tissue site of action, when appropriate, may be extrapolated. This information can be integrated with relevant human physiological parameters for the development of a mechanistic PK/PD model to predict the desired efficacious drug concentration and levels of sTE/RO in humans. In this presentation, a brief overview of sTE/RO assay methodologies and the application of sTE/RO-based translational strategies will be presented.

Sensitive and Robust Trapping-Micro-LC-MS for the Quantification of Biomarkers and Antibody Biotherapeutics

Jun Qu, SUNY-Buffalo

For LC-MS-based targeted quantification of biotherapeutics and biomarkers in clinical and pharmaceutical environments, high sensitivity, high throughput, and excellent robustness are all essential but remain challenging. Furthermore, high chemical noise in protein bioanalysis typically limits the sensitivity. We describe a novel trapping-micro-LC-MS (T-μLC-MS) strategy for targeted protein bioanalysis, which achieves high sensitivity with exceptional robustness and high throughput. A rapid, high-capacity trapping of biological samples is followed by μLC-MS analysis; dynamic sample trapping and cleanup are performed using pH, column chemistry, and fluid mechanics separate from the μLC-MS analysis, enabling orthogonality, which contributes to the reduction of chemical noise and thus results in improved sensitivity. As observed, a significant sensitivity gain (up to 25-fold) compared with that of conventional LC-MS was achieved, although the average run time was only 8 min/sample with exceptional robustness. This strategy is valuable when highly sensitive protein quantification in large sample sets is required, as is often the case in typical biomarker validation and pharmaceutical investigations of antibody therapeutics. Applications in analysis of biotherapeutics, such as tissue dispositions of mAb, ADC and bs-Ab and time courses of biomarkers, are demonstrated.

SESSION V: New Technologies and Applications

Enhanced Immuno-affinity for Hybrid LBA-LC-MS: Considerations and Challenges

Ang Liu, BMS

Immuno-affinity (or immuno-capture, immuno-extraction) techniques linked to LC-MS have been subject to tremendous interest and rapid growth in quantitative bioanalysis of therapeutic proteins and peptides, monoclonal antibodies, new therapeutic modalities such as antibody-drug conjugates (ADCs), protein biomarkers, as well as unwanted immunogenicity. Immuno-capture employs a specific capture reagent (e.g. antibody) to extract the analyte of interest from biological samples. In this procedure, the analyte of interest binds specifically and tightly to the capture reagent which is usually immobilized on a solid support, and thus can be separated from the potentially interfering endogenous components in matrix. It is an effective approach to simplify biological samples as well as to enrich the target analyte, and thus improves assay sensitivity. In addition, its unique selectivity also provides important information of the availability of specific epitopes on the analyte of interest. This technique takes advantage of the specific immuno-affinity between the capture reagent and the analyte of interest (e.g. therapeutic protein or antibody), which is similar to the capture step used in ligand-binding assays (LBAs). Therefore, immunocapture-LC-MS assays are also known as hybrid LBA-LC-MS assays. The hybrid approach, which couples the complementary analytical benefits from ligand-binding techniques and quantitative instrumentation, plays an increasingly vital role in the development of quantitative PK and PD assays. In this presentation, the practical considerations on the selection of capture reagents, platforms, and internal standards, the limitations and challenges, are described. In addition, case studies and the recommended validation criteria for immunocapture-LC-MS assays are also discussed herein.

Bioanalysis of Intact Biologics Therapies

Beth Groeber, CRL

Advances in the sensitivity of mass spectrometers and new hybrid, high resolution systems permit analysis of biologic-based therapies as intact structures. Bioanalytical methods that measure intact structures have more information content, can be developed faster, and can reveal more relevant pharmacokinetic/toxicokinetics data. The selection of instrumental platform and key optimization parameters

for intact structures will be discussed in the context of case studies including oligo nucleotides, peptides, proteins, antibody drug conjugates, nano-particles, and hybrid biopolymer therapies. The method development work flow for intact structures requires consideration of the interplay between extraction technique and the optimal scan function and resolution of the parent mass isolation and the resolution of the product ion readout. Examples of different optimization pathways will be reviewed. Methods that incorporate high resolution mass analysis have the benefits of differentiating between endogenous/recombinant or wild type/mutant proteins. Transthyretin (TTR) is a protein that, when over expressed, can cause the disease state transthyretin amyloidosis. An immuno-capture high resolution mass spec method was developed to measure the knock down of TTR subsequent to administration of an oligo nucleotide. This method was able to measure wild type and twelve unique mutation variants of the TTR with mass differences as little as 2 AMU. The use of intact mass analysis mitigated the need to create signature peptides for every known mutation variant of TTR, resulting in a method that was created in approximately one tenth the time of a bottom up method.

New Developments in Patient Centric Sampling and There Application in Clinical Trials

Melanie Anderson, Merck

Smart Trials is a cross-functional innovation project at Merck aimed at enabling a more patient-centric clinical trial approach. As part of this initiative, clinical pilot studies are conducted to test feasibility of new technologies/methods for future clinical use in 3 areas: smart sampling (technologies for collection of date/time stamped outpatient PK and PD samples), smart dosing (technologies to accurately record and transmit dosing information), and smart analytics (methods to collect, integrate, and visualize data in real-time). For smart sampling, the use of fingerstick samples collected on DBS cards has several limitations for routine use for at home sampling in clinical development. To address these limitations we have been developing automated approaches to collect date and time of sample collection, evaluating less invasive methods for sample collection as well as integration of data capture with micro-needle based sampling technologies. This talk with provide a status update on these new sampling approaches as well as data collected during use in clinical trials.

Mechanistic ADME Workshop

SESSION I: Toxicology, Clinical Safety Prediction & SM Biomarkers

Systems Approaches to Translation of Preclinical Safety Signals to Inform Compound Discovery and Early Development

Jay Mettetal, AstraZeneca

Early and accurate assessment of drug safety and efficacy would greatly increase the rate of success in clinical development. Not only is it important to select a compound with a good therapeutic index defined by the safety and efficacy profiles, but also one must determine appropriate dose schedules and biomarker interpretations. The clinical implications of preclinical data are often difficult to directly assess in the discovery and early development stages due to a number of factors including differences between species sensitivity and pharmacokinetics, as well as uncertainty and variability. Assessment and translation of these preclinical signals can be enhanced through utilization of modeling and simulation methodologies in order to make assessments and predictions. In this talk, I will describe the application of system pharmacology techniques to understand compound activity, mechanism of action, and translation of preclinical findings to the clinic. I will discuss the use of systems models to assess common on-target safety endpoints for oncology compounds such as gastrointestinal toxicities. In addition, I will describe how systems models can be utilized to understand and mitigate undesirable off-target pharmacology.

Discovery Toxicology: Input and Impact in Early Drug Development

Yvonne Dragan, Takeda

Nonclinical safety assessment is an integral member of the core drug discovery teams. Working in partnership with DMPK, Pharmacology, Medicinal Chemistry or Protein Design, and CMC, nonclinical safety assessment can help to drive the quality of the assets that we take forward into development and toward the clinic. This focus on quality helps to focus discovery assets on selecting agents with the best set of attributes including safety for progression. This is accomplished by actively working across departmental and disciplinary boundaries to define criteria within each project context for success.

SESSION II: Unusual Metabolic Pathways: Time to Expect the Unexpected?

Characterization of Uncommon Metabolites: Case Studies with Ombitasvir and Paritaprevir

Jianwei Shen, AbbVie

Viekira Pak is an approved oral combination therapy of three direct-acting antiviral agents (DAAs) for genotype 1 chronic hepatitis C infections. It includes ombitasvir, paritaprevir/ritonavir and dasabuvir. Ombitasvir is an inhibitor of HCV nonstructural protein 5A (NS5A) with picomolar activities against HCV genotype 1a and 1b subgenomic replicons. Paritaprevir is a potent macrocyclic noncovalent peptidomimetic inhibitor (identified by AbbVie and Enanta) of HCV NS3/4A protease. Dasabuvir is a non-nucleoside inhibitor of HCV NS5B polymerase. This presentation describes the metabolism and disposition of ombitasvir and paritaprevir in humans. Disproportionate human unique metabolites of ombitasvir were characterized and the biotransformation pathway of a rare CYP2C8 mediated C-demethylation is proposed. In the case of paritaprevir, structural characterization of the metabolite M2 using multiple techniques including high resolution LC-MS, isotope labeling, and advanced NMR techniques is presented. A CYP3A4-mediated oxidation followed by an intra-molecular cyclization is proposed for the formation of the M2 metabolite.

Late-occurring and Long-circulating Major Metabolites formed by Metabolic Cyclization and Aromatization from a CNS Candidate Drug of AstraZeneca

Chungang (Chuck) Gu, Biogen

This oral presentation based on recently published drug metabolism of the selective $GABAA_{\alpha 2,3}$ receptor modulator AZD7325 will focus on the thought process and approaches in the identification of three unusual major metabolites involving metabolic cyclization and aromatization. Consideration of the likely biotransformation mechanism was an integral part of the structure elucidation process. While other unusual metabolism in the literature may be mediated by an unusual metabolic enzyme; in this case the unusual metabolites appeared to be generated by plausible metabolic pathways that are known although not intuitive. These three metabolites were either minor or absent in plasma samples after a single dose; however, all became major metabolites in human and preclinical animal plasma after repeated doses and remained detectable in humans for more than 48 hours after the end of seven repeated doses. The observation of the late-occurring and long-circulating major metabolites demonstrates the need to assess the circulating metabolite profile at steady state as opposed to after a single dose. Together with an identified nonintuitive metabolic pathway, this case may serve as a useful cautionary example to others who engage in drug development.

N-methylation of BI 187004 by thiol S-methyltransferase

Holly Maw, Boehringer Ingelheim Pharmaceuticals, Inc.

Methyltransferases are a group of polymorphic drug metabolizing enzymes located in hepatic and extrahepatic tissues. These enzymes catalyze the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to xenobiotic substrates containing an -O, -S, or -N heteroatom. During a clinical metabolite identification investigation with BI 187004, benzimidazole-N-methylation was identified as the major biotransformation pathway. In four patients, the plasma exposure of an N-methylbenzimidazole metabolite (M1) was 7-fold higher than the remaining four patients. The objective of this study was to identify which methyltransferase enzyme was responsible for the benzimidazole-N-methylation of BI 187004. BI 187004 was incubated with human liver microsomes (HLM), human kidney microsomes (HKM), and their respective cytosolic preparations in the presence and absence of isoform-selective chemical inhibitors. Additionally, BI 187004 was incubated with several human recombinant methyltransferases: catechol O-methyltransferase (rhCOMT), histamine N-methyltransferase (rhHNMT), nicotinamide N-methyltransferase (rhNNMT), glycine N-methyltransferase (rhGNMT), and thiopurine S-methyltransferase (rhTPMT). M1 was principally observed in HLM and HKM incubations, and not formed during incubations with recombinant methyltransferases. In all microsomal and cytosolic incubations, formation of M1 was only inhibited by 2,3-dichloro-α-methylbenzylamine (DCMB), an inhibitor of thiol S-methyltransferase (TMT), providing evidence that TMT catalyzed the formation of M1. Interestingly, the N-methylbenzimidazole regioisomer (M14) was only observed in vitro, predominantly during incubations with human kidney cytosol and rhHNMT. The formation of M14 was inhibited by amodiaquine (an HNMT inhibitor) and DCMB, providing additional evidence that both HNMT and TMT catalyzed M14 formation. Overall, this

study demonstrates a novel TMT-mediated N-methylation biotransformation and an HNMT-mediated regioselective N-methylation.

SESSION III:
Specialty Molecules; Going Beyond Rule of 5

Working Beyond Rule of 5: Designing Cyclic Peptide C5a Antagonists with Oral Bioavailability

Kevin Beaumont, Pfizer

The Lipinski Rule of 5 revolutionized the medicinal chemistry approach to drug discovery. Oral absorption potential was rationalized to physicochemical properties which could be modulated to provide improved ADME. This approach has enabled large numbers of small molecule drugs to be registered. Fundamental to this approach is the ability to accommodate physicochemical properties for potency versus the pharmacological target with those required by the Rule of 5. However, an increasing number of pharmacological targets are not amenable to modulation with Rule of 5 space. A good example is the C5a receptor, which requires significant MW, lipophilicity and hydrogen bonding to potently modulate. A series of highly potent cyclic peptide C5a antagonists were identified. This presentation will highlight the changes in optimization strategy that were employed to discover orally bioavailable molecules with optimal properties for progression.

ADME Screening Paradigm for Covalent Inhibitor Drugs – Role of Traditional vs Non-Traditional Assays

Ashutosh (Ash) Kulkarni, Allergan

ADME screening in drug discovery is essential to ensure selection of lead compounds with the most desirable drug-like properties. Tailoring of this early stage ADME screening paradigms to the nature of the therapeutic modality is critical to ensure that relevant and decision-informing ADME data is obtained for advancement of these new chemical or biological entities. A 'one paradigm fits all' approach cannot be used. The differences in ADME and the corresponding screening assays between small molecule and large molecule therapeutics are well defined. However, within the small molecule therapeutics/inhibitors, variations may exist based on the nature of their interaction with the target. Some act as reversible inhibitors while others as covalent/irreversible inhibitors. We were interested in evaluating whether the same ADME screening criteria could be used to evaluate these 2 different types of small molecule inhibitors. Since the screening criteria for reversible inhibitors has been well defined and applied relatively successfully in drug discovery setting, we evaluated the in vitro and in vivo preclinical ADME properties of 10 clinically late stage or marketed covalent inhibitors to define advancement criteria for discovery of future drugs in this arena. We then compared this to the reversible inhibitors to determine whether the same criteria applies for both. In conclusion, the wide range in in vitro and in vivo ADME data makes these traditional ADME assays non-discriminatory in the selection of promising covalent/irreversible compounds. In our opinion, non-traditional assays such as target mass modification, target confirmation by amino acid sequencing, cellular target occupancy, and target turnover rate data in combination with the pharmacokinetic profiles are the critical considerations for progression of irreversible compounds in early discovery.

Beyond ADME & Bioanalysis Workshop - Global Health Session

Anti-parasitic Drug Discovery from Marine Cyanobacteria

William Gerwick, Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego

Parasitic diseases wreak enormous damage to human health and disrupt society function. Moreover, they mainly afflict those populations least able to treat or otherwise overcome these devastating illnesses. Because these populations lack the resources to purchase expensive medications, there is little private sector motivation to discover, develop or bring to market new anti-parasitic drugs. Thus, educational institutions and foundations are the primary sites for research and development of new medications for these neglected diseases. The Skaggs School of Pharmacy and Pharmaceutical Sciences has a strong focus on anti-parasitic drug discovery, and possesses an extensive state-of-the-art screening facility with capacity to screen to 11 different human parasites, including Chagas disease (Trypanosoma cruzi), Human African Trypanosomiasis (Trypanosoma brucei), Leishmaniasis-cutaneous and visceral, Schistosomiasis, Lymphatic filariasis, Amebiasis, Giaridiasis, Cryptosporidiosis, Malaria, Toxoplasmosis and Naegleria/Acanthameba. We have screened approximately 4500

samples of tropical marine cyanobacterial extracts, fractions and pure compounds to most of these parasites, and to date have discovered two significant lead compound classes: the proteasome inhibitory carmaphycins to Plasmodium falciparum (Malaria) and the cysteine protease gallinamides to Trypanosoma cruzi (Chagas disease). In both drug classes we have developed efficient total syntheses and have produced numerous analogs with improved selectivity, potency and pharmaceutical properties. The process of their discovery and lead generation will be discussed.

Reversing Neglect: Pharmaceutical Research and Development for Global Health

Joseph Camardo, Celgene

A major effort is underway to develop new drugs for parasitic and other infectious diseases that affect millions of people. Pharmaceutical companies are now working with NGO and Government partners to apply industry-based drug development approaches for such diseases as malaria, leishmania, onchocerciasis, filariasis, and other diseases that are endemic in the poorest regions of the world. The challenges in drug development for these diseases include their biologic complexity, gaps in the knowledge of pathophysiology, the limitations of animal models, the modest number of identified targets and biomarkers, and the challenges of clinical studies. Specific challenges to medicinal chemistry and pharmaceutics include the need for low dose high potency drugs, high efficacy and safety for mass drug administration, low cost, ease of formulation, tolerance of high heat and humidity, and highly predictable pharmacokinetics. This is an area of strength for the industry and is an important part of our contribution to an important effort to address the problem of neglected diseases.

SESSION IV: Software for Met ID

Accelerating the Delivery of Small Molecule High Resolution Accurate Mass Structure Elucidation Results Through the Use of Thermo Scientific™ Compound Discoverer Software™

Jonathan Bauman, Pfizer

Characterizing the metabolic clearance pathways through the structure elucidation of small molecules is an essential part of the drug discovery and development process. This data helps teams make strategic decisions regarding the safety of molecules. The structure of a metabolite may provide insight to metabolic liabilities such as drug-drug interactions, variable exposure or reactive metabolite formation. Collating in vitro data across several species and integrating results from companion in vivo experiments can create significant accounting challenges to creating a comprehensive picture of the biotransformation pathways. While generating the data needed for these studies does not require significant investments in time, data analysis, documentation and presentation can often be the bottleneck in being able to deliver meaningful results in a timely fashion. High resolutions mass spectroscopy (HRMS) has revolutionized the way structure elucidation is conducted and has become the gold standard for conducting biotransformation work. Software solutions are available for different mass spectrometry platforms, including mostly proprietary and a few platform agnostic options. Using the combination of HRMS data and data analysis software can provide a streamlined process for reporting complex metabolite profiling data sets. In this seminar, I will discuss Compound Discoverer™ by Thermo Scientific™ and how we at Pfizer use this software to help speed up our data analysis and reporting to enable teams us to deliver biotransformation knowledge to our teams.

Fast and Comprehensive Detection and Characterization of Cyclic Peptide Metabolites using Software-aided Data Processing Tools

Ming Yao, BMS

Cyclic peptides (CPs) have various biological activities, they can cross the cell membrane in some cases, and they are often resistant to quick hydrolysis. Therefore, CP has emerged as therapeutic agents. Identification of CP metabolites using HRMS is often required, which, however, represents great analytical challenges. First, commonly employed data processing tools for finding metabolites of small molecule drugs are not well suited for analysis CP metabolites. Secondly, CP metabolites are difficult to generate high quality and interpretable fragments due to their unique structures. Objective of this study was to apply and test recently expanded tool, MetabolitePilot™ software, for automated detection and structural characterization of cyclic peptide metabolites using insulin and atrial natriuretic peptide (ANP) as model compounds.

Insulin and ANP were spiked in rat plasma (20 µM) and incubated at 37°C for 0, 1h and 3h. After proteins were precipitated and removed, incubation superannuants were

analyzed by HPLC coupled with TripleTOF® 5600 system with Acquity BEH C18 column (2.1 x 100 mm). Accurate MS and MS/MS datasets of components with positive charges from 2 to 5 were acquired using dynamic background subtraction, an information dependent acquisition method. The mobile phases of HPLC were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 400 µl/mL and the total run time was 60 min.

Detection and sequence elucidation of ANP and insulin metabolite were accomplished by processing the accurate MS and MS/MS datasets using advanced MetabolitePilot™ software. The LC-MS peak finding strategies traditionally used in small molecule metabolism were expanded to handle larger molecules, especially, molecules where base peak of the isotope cluster is not mono-isotopic peak and finding and confirmation logic relies on the base peak. A combination of targeted and untargeted detection strategies, which were developed based on the prediction of hydrolytic metabolites and the product ion similarity between the peptide parent and its metabolite, and the absence or lower levels of peptide metabolites in control samples, were utilized to find and confirm unknown metabolites of cyclic peptides. For example, 27 potential hydrolytic insulin products covering molecular masses from 850 to 5800 were detected based on selected known peptide b or y fragments. Sequences of 25 incubation products were further assigned based on their accurate mass and further confirmed with MS/MS annotation. For example, 66% of MS/MS signal from insulin (5+) could be assigned with sub 5 ppm error as a,b,y and internal fragments. Although most of the signal came from low m/z fragments, multi-chain fragments preserving disulphide bond were observed. Scoring and ranking of elemental putative sequences pointed to predicted insulin digest products. In the same manner, we found and confirmed two major and one minor metabolites of ANP that is a non-linear peptide with one di-sulphide bond. The results were in agreement with previously published data.

Discovery Bioanalysis & New Technologies Workshop

SESSION I: New Technologies

Insights into the Insulin Receptor Activation: The Cryo-EM Structure of the IR in Complex with Insulin

Giovanna Scapin, Merck

The insulin receptor (IR) is a homodimeric protein that plays a crucial role in controlling glucose homeostasis, regulating lipid, protein and carbohydrate metabolism, and modulating brain neurotransmitter levels. IR dysfunctions have been associated with a variety of diseases, including diabetes, cancer and Alzheimer's. The amino acid sequence of the IR monomer has been known since the 1980s; it is composed of an extracellular portion (ectodomain), a single transmembrane helix and an intracellular tyrosine kinase domain. Insulin binding to the dimer ectodomain triggers a signal leading to the kinase auto-phosphorylation and subsequent recruitment of downstream signaling molecules. Biochemical and mutagenesis data have identified two putative insulin binding sites (S1 and S2); S1 has also been characterized by structural studies. The monomeric ectodomain structure has been solved by x-ray crystallography. However, the details of insulin binding to the full receptor and the mechanism of signal propagation are still not understood. We recently solved the single particle cryoEM reconstructions for the 1:2 (4.3 Å) and 1:1 (7.4 Å) IR ectodomain dimer:Insulin complexes, the first structures elucidating the binding mode of insulin to its receptor. The symmetric 4.3 Å structure shows two insulin molecules bound per dimer; each insulin is bound between the Leucine-rich domain L1 of one monomer and the first fibronectin-like domain (FnIII-1) of the other monomer, and makes extensive interactions with the alpha-subunit C-terminal helix (α-CT helix). Additional interactions are present between the insulin, the α-CT helix and the second leucine-rich domain (L2). The asymmetric 7.4 Å structure shows only one insulin molecule bound per dimer and only one visible α-CT helix. In the presentation we will discuss the issues associated with sample preparation, data collection and processing, as well as the two structures and the suggested mechanism for downstream signal propagation.

SESSION II: Predictive Tools and Novel Approaches to Evaluate Immunogenicity Response of Biotherapeutics

Preclinical Minimization of Immunogenicity Liabilities for High Quality Bio Therapeutics

Jochem Gokemeijer, BMS

All bio therapeutics have the potential to elicit an immune response which can result in decreased efficacy for patients. Preclinical strategies to predict. A preclinical assay toolbox with orthogonal technologies to predict and de-immunize bio therapeutics can aid development teams designing and optimizing drug candidates with reduced immunogenicity liabilities. Here we will discuss the pre-clinical immunogenicity strategy at BMS, assay challenges and gaps as well as clinical correlation.

Current Perspectives on Preclinical Predictive Tools for Immunogenicity Risk Assessment and their Clinical Translation

Vibha Jawa, Merck

A significant effort has been made in the development of preclinical immunogenicity risk assessment tools and their utility in both preclinical and clinical development. The approaches include algorithm driven outputs as well as innovative methods to understand antigen processing and presentation using human immune cells. The pros and cons of these tools and their outputs will be brought forth with this talk. Additionally, some examples where use of such risk assessments have been used to drive the clinical strategy will be shown.

Human Leukocyte Antigen class II-associated Immunopeptidomics for Immunogenicity Studies of Biologics

Andrea Casasola-LaMacchia, Pfizer

Advances in our knowledge of CD4+ T cell-mediated immunity in response to immunogenic peptide sequences has led to a better understanding of immunogenicity, which in vivo also relies on antigen processing and antigen presentation by the Human Leukocyte Antigen (HLAII). Our work focuses on the characterization of the HLAII-associated immunopeptidome by mass spectrometry in specialized antigen-presenting cells (Dendritic cells, DCs) in response to administration of biotherapeutic compounds. The aim is to identify biotherapeutic derived peptides presented to the HLAII complex as a potential T-cell epitopes in order to understand, and potentially predict, the immunogenicity response in humans. While several immunopeptidome workflows have been established for this purpose, some presented methodologies vary significantly from the HLA-purification strategy to the data analysis pipeline, raising the possibility that they also vary in their ability to enrich for the associated immunopeptidome. Therefore, we are evaluating specific critical factors at the technical level, which have increased the HLAII-immunopeptidome recovery and the identification of biotherapeutic-derived peptides in primary human DCs.

PLENARY TALK

New Developments in Ion Mobility-Mass Spectrometry Enabling Higher Throughput and More Sensitive Proteomics and Pan-Omics Measurements for Clinical Diagnostics and Personalized Medicine

Richard D Smith, PNNL

Advances in MS-based proteomics and related 'omics' measurements (e.g., metabolomics and lipidomics) are having increasing impacts on biomedical research, and with the potential for major impacts upon the future of clinical diagnostics and personalized medicine. These MS-based proteomics and related omics capabilities can greatly facilitate disease diagnosis and treatment. Several examples from our laboratory will be used to illustrate present capabilities in the context of large scale cancer research studies, and efforts to identify biomarkers that will facilitate more effective and personalized patient treatments. This presentation will describe the large increases in the throughput and effectiveness enabled by the introduction of ion mobility (IM) separations with MS-based omics approaches. IM provides high speed separations, generally within mseconds to seconds, providing additional separation power that can be used to either augment that of liquid chromatography or to displace it for many applications. Combined IM-MS can address previous proteomic 'blind spots', and also generates biomolecule structure-related information via the IM measurement of collision cross sections, enabling more complete and effective proteome characterization. This presentation will discuss these advances both broadly and in the context of new developments based upon Structures

for Lossless Ion Manipulations (SLIM) that provide large advances in IM resolution, IM-MS throughput, and sensitivity, addressing current challenges for IM-MS-based proteomics and other 'omics' measurements, and making practical a wide range of new applications for biomedical research.

SESSION III: Omics

Toward Understanding Compound Protein Interaction at a System Biology Scale

An Chi, Merck

Understanding drug-target interactions at both protein and pathway signaling network level in healthy vs. disease states is critical for the success of drug discovery and development. In the post-genomic era, quantitative chemical proteomics is emerging as a powerful tool to identify and validate novel druggable targets by means of (i) deconvolution of the molecular mechanism of action (MMoA); (ii) proteome selectivity assessment of bioactive molecules and (iii) druggability assessment for proteins of therapeutic interest with unclear MMoA identified through diverse approaches. To maximize the value of various chemical biology data, to fuel in the hypothesis generation-testing cycle in Target Identification and Validation (TIDVal), we invested in a Chemical Biology Data Management System (CBDMS) as the infrastructure foundation to capture systems-biology perspectives of drug-proteome dynamics, on-target engagement, off-target effects and polypharmacology. Herein we report current progress of this endeavor, particularly in chemical proteomics data handling, analysis and visualization in the context of several exemplary chemical proteomics experiments to identifying novel targets.

Application of Targeted Mass Spectrometry to the Quantitative Assessment of Internalization Kinetics for Cell-surface Membrane Protein Targets

Dhiman Ghosh, Takeda

More than 70% of current-day biopharmaceuticals target cell-surface membrane proteins (CMPs), and as new or existing CMPs enter the drug discovery and development program, it becomes essential to bring them under quantititative pharmacological framework to accelerate the drug development process. Evaluation of CMP properties such as target expression, density, modification/s, internalization rate and turnover mechanisms is central to the design of better therapeutics e.g. antibody-drug-conjugates (ADCs), extracellular drug conjugate (EDCs) or modalities that target CMPs. Currently, target properties have been evaluated through semi-quantitative approaches such as flow cytometry, Western blotting or fluorescent microscopy. While mass spectrometry analysis holds greater promise to the evaluation CSP properties, conventional data-dependent-analysis (DDA) mode in mass spectrometry has been fraught with difficulties in the identification of CMPs due to their low abundance. We believe application of front-end enrichment method in conjunction with targeted mass spectrometry will alleviate the requirement of high sensitivity for low abundance CMP protein detection and quantitation. Keeping in mind the aforementioned problems, here we present a mass spectrometry based-targeted analytical strategy that is capable of evaluating several CMP properties together including, 1) density assessment, 2) rate of internalization, and 3) separation of in-bound traffic from nascent and outbound ones using differential mass tagging. With the multiplexing capabilities, this platform technology will be useful in investigating single or multiple targets simultaneously and can be integrated for most new modalities that target CMPs.

Seeing Diseases Clearly through Metabolism

David Pirman, Agios

Understanding metabolism early on and throughout the drug discovery process has proven to be critical in establishing translatable research, especially for metabolism-based drug discovery programs. Throughout our discovery programs at Agios, we often develop a comprehensive metabolic understanding of not only our targets for a specific disease, but of every biological model we use to study such diseases. This approach allows us to develop a translational set of biomarkers which are continuously characterized and fit-for-purpose biomarkers to solve specific challenges during the drug discovery process. This approach keeps programs moving forward, and has led to the development of biomarkers which have translated from early in vitro disease models to the clinical characterization of our drugs. This discussion will broadly cover our approach to employing metabolomics analysis in drug discovery, disease characterization, and research models.

PLENARY TALK

Research Models for Non-Alcoholic Steatohepatitis: In Vivo Convergence of Metabolic Context, Pathology and Targeted Pathways

J. A. Cornicelli, CRL

The worldwide obesity and overweight epidemic have given rise to a host of comorbidities. Chief among these is the potential for the development of non-alcoholic fatty liver disease (NAFLD). A relatively benign condition, NAFLD can lead to non-alcoholic steatohepatitis (NASH); a more serious disorder that can progress to cirrhosis and liver cancer. With an estimated prevalence of 20% of the Western world adult population affected, the pharmaceutical and biotech industry has mounted vigorous programs to find safe and effective treatments for this disorder.

This presentation focuses on the development and qualification of in vivo animal models for the assessment of NAFLD/NASH. The discussion includes an assessment of the relevance of various acute and chronic disease models for candidate evaluation, translatability to the clinic, differences between pathology observed in humans and animals, and the need for translatable biomarkers for following potential therapies. The ability to recapitulate the metabolic context of the disease coincident with robust pathology while engaging appropriate targets forms the backdrop for the conversation.

SESSION IV: IT/Information/Knowledge

Data informed Quantitative Systems Pharmacology Model Development

Fei Hua, Applied BioMath

Quantitative systems pharmacology models (QSP) mechanistically describe the dynamics of biological system, disease processes, and drug pharmacology. It is increasingly being used in the drug R&D process and can help address questions ranging from early research to late stage development. For example, QSP models can help with target feasibility assessment, translation from animal to human, human efficacious dose projection, and biomarker identification. QSP models normally are composed of series of ordinary differential equations which require initial concentrations and kinetic rates as model inputs. The model simulations are then calibrated or validated against time course data. One major challenge in QSP model development is acquiring the data needed. In this talk, I will discuss the types of data that are useful for QSP model development, new technologies used to provide these data information, and gaps in the technology to provide the data to facilitate QSP model development.

Discovery & Biotransformation Combined Workshop

SESSION V: Role of DMPK in New Modalities

Drug Distribution Analysis in the Preclinical Development of Gene and Cell Therapy Products

Sarah Voytek, BluebirdBio

Cell and gene therapy products offer the potential to be a one-time time treatment for patients with severe genetic diseases and cancer. Development of these novel drugs follows a paradigm distinct from biologics and small molecules due to the lack of applicability of most traditional preclinical models. This presentation will address the role of drug distribution analysis in the preclinical development of cell and gene therapy products, including current opportunities and challenges in this emerging field.

SESSION VI: Biomarkers: Discovery and Implementation

Use In Vitro Tools and Genetic Data to Identify Transporter Endogenous Biomarkers

Xiaomin Liang, Gilead

Transporters play significant roles in the absorption, disposition, elimination, and effects of drugs. Transporter-mediated drug-drug interactions (DDIs) are a major cause of drug toxicities and therefore, regulatory agencies required sponsors to conduct clinical DDI studies to evaluate the potential DDI effect of drug candidates. Currently, in vitro inhibition studies via cell-based systems are used to predict the risk of DDI and triggered the conduct of resource-intensive clinical DDI studies. However, these in vitro assays often result in many false-positive/negative results due to non-specific binding, inter-system/ laboratory variability, and the limitation of in vitro- in vivo extrapolation. Therefore, both academia and pharmaceutical industry have high

interests in identifying endogenous biomarkers that can be supplemented in vitro assays and used to better predict clinical DDI. Using metabolomics and genetic data coupled in vitro uptake studies are useful approaches for discovering endogenous substrates for transporters, such as thiamine for organic cation transporter 1 (OCT1) and tetradecanedioate (TDA) and hexadecanedioate (HDA) for organic-anion-transporting polypeptide 1B1 (OATP1B1).

APA BIOGRAPHIES

ORGANIZERS

Eric Ballard, PhD, Pfizer: Dr. T. Eric Ballard is a Senior Scientist and Lab Head of the Biotransformation Group in the Pharmacokinetics and Drug Metabolism department at Amgen (Cambridge, MA). In his current role, Eric investigates the clearance mechanisms, metabolism and in vivo dispositions of small molecules in support of early and late stage research and development. Eric has a particular interest in investigating tools to better detect reactive metabolites and their potential toxicity mechanisms. Prior to his current position, Eric was a Principal Scientist in the Biotransformation Group at Pfizer and investigated both small and large molecule drug metabolism where he was a contributor to the now approved ADC drug Inotuzumab Ozogamicin. Eric was previously a Postdoctoral Fellow in the Worldwide Medicinal Chemistry Group at Pfizer with Dr. Doug Johnson and Dr. David Gray and a Postdoctoral Associate with Prof. Timothy MacDonald at the University of Virginia. Eric received his Ph.D. in Organic Chemistry in 2008 from North Carolina State University under the direction of Prof. Christian Melander (now at the University of Notre Dame). He has coauthored over 30 publications in the fields of organic synthesis, bioorganic chemistry, chemical biology, and biotransformation/metabolism.

Mark T. Cancilla, PhD, Merck: Dr. Cancilla is currently the Director of Merck's Metabolite Identification & Tissue Distribution Group within the Pharmacokinetics, Pharmacodynamics and Drug Metabolism Department at Merck. He received his B.S. in chemistry from the California Polytechnic State University in San Luis Obispo, CA, and then completed his Ph.D. in analytical chemistry from the University of California at Davis under Professor Carlito B. Lebrilla. Next, he performed two years postdoctoral research at the University of California, Berkeley Chemistry Department with Dr. Julie A. Leary. Mark has been in the pharmaceutical industry for over 18 years, has over 25 authored and co-authored publications and book chapters, including three patents.

Montserrat Carrasco-Triguero, PhD, Genentech: Dr. Carrasco-Triguero, Ph.D. is a Senior Scientist in the BioAnalytical Sciences Department and project lead for multi-functional teams at Genentech. She is responsible for developing bioanalytical strategies and assays to assess the pharmacokinetics, pharmacodynamics and biotransformation of biotherapeutics. Prior to Genentech, she held positions at Chiron and Genitope. Dr. Carrasco has been a member of the organizing committee for the Applied Pharmaceutical Analysis (APA) Discovery Workshop since 2013 and was the chair elect in 2016. She has been providing her expertise to the Predictive Safety Testing Consortium since 2016. Dr. Carrasco's interests include applying novel technologies for bioanalysis and using emerging biomarkers of drug-induced organ injury.

Lisa Christopher, PhD, Bristol-Myers Squibb: Dr. Christopher is a Research Fellow in the Department of Metabolism & Pharmacokinetics at Bristol-Myers Squibb in Princeton, NJ. Her group provides biotransformation support for drug candidates. In addition, Dr. Christopher leads several multi-disciplinary teams that establish and execute the ADME strategy for drug candidates in early clinical development and provide translational support for issue resolution. Her research interests include use of microtracers in ADME and Absolute Bioavailability studies and application of accelerator mass spectrometry for the bioanalysis of samples from these studies, as well as assessment of biomarkers to gain a better understanding of a drug's ADME properties. Dr. Christopher received her PhD degree in Analytical Biochemistry from Georgia Institute of Technology and postdoctoral training at the University of South Alabama and University of North Carolina, Chapel Hill.

Dieter Drexler, PhD, Bristol-Myers Squibb Company: Dr. Drexler is a Research Fellow in the Pharmaceutical Candidate Optimization - Analytical Sciences Department at the R&D site in Wallingford, CT where he leads a group providing analytical and mass spectrometric support for Discovery Chemistry and Biology. The projects include the qualitative and quantitative

analysis of endogenous and exogenous small molecules, peptides/proteins, and biologics in various matrices utilizing a variety of analytical techniques. His research interests involve the development of innovative techniques and methodologies applied to the analysis of novel biopharmaceutical modalities and biomarkers.

Dr. Drexler received his Doctorate in Analytical Chemistry at the University of Ulm, Germany. He joined BASF Corporation in Research Triangle Park, NC as a Postdoctoral Research Fellow to investigate pesticide analysis in soil and water. He then moved to Finnigan Corporation in San Jose, CA as a Product Specialist for ion trap and triple quadrupole mass spectrometers supporting research and marketing efforts. During his career at BMS he has taken on projects and positions with increasing responsibility and is currently the laboratory manager for bioanalytical support.

He has authored or co-authored over 100 journal articles, book chapters, oral and poster presentations.

James P. Driscoll, MyoKardia: Mr. Driscoll is an accomplished Scientist and Project Leader in the Drug Metabolism and Pharmacokinetics department at MyoKardia, Inc, a clinical stage biopharmaceutical company focused on genetic heart disease. He has over 16 years of industry experience with Pfizer, Genentech, Theravance and MyoKardia. His expertise includes reactive metabolite identification, drug metabolism, and bioanalysis of small molecules. His responsibilities include driving early stage projects forward by identifying structure activity relationships, avoiding off-target toxicity, interrogating potential new targets in drug discovery, and leading a team focused on in vitro compound optimization. James received his B.S. in Human Biology from the State University of New York at Albany.a

Scott Fountain, PhD, Charles River Laboratories: Dr. Scott Fountain is currently Executive Director of Toxicology and Pharmacology & Site Lead at Charles River Laboratories, Shrewsbury MA, and most recently served as North American Laboratory Sciences & Clinical Support Services at Charles River Laboratories, overseeing the development and tactical execution of the laboratory sciences strategy within North America. Prior to joining Charles River, Scott was Executive Director of Biotherapeutics and Translational Research in the Department of Pharmacokinetics, Dynamics, and Metabolism (PDM) at Pfizer San Diego, where he established and led a core group integrating quantitative bioanalysis and mathematical modeling & simulation to advance Pfizer's large molecule portfolio. Dr. Fountain earned his BS in Chemistry from Central Michigan University and his PhD in Analytical Chemistry from The University of Michigan in Ann Arbor. After graduation, Scott worked for Waters Corporation as a Senior Applications Chemist in Beverly, MA. In 1998, he joined Pfizer Global Research & Development in Michigan to establish a quantitative LC/MS/MS laboratory in the PDM research line supporting small molecule PK and biomarker research.

Hongying Gao, PhD, Innovo Bioanalysis: Dr. Gao is the CEO and founder of Innovo Bioanalysis, LLC at Mansfield, MA. She founded the startup in 2017 to develop innovative bioanalytical methodology and technology using advanced mass spectrometry for identification of biomarkers in diseases with unknown mechanism in unmet medical needs as well as collaborations with scientists to address DMPK issues in drug discovery and development. Her company also provides contract bioanalytical research service for bioanalysis of biomarkers, drugs and metabolites in biological samples for DMPK, PK/PD, and TK. Dr. Gao has more than 18 years experiences with a track record of innovation and publications in bioanalytical science in pharmaceutical and biotech industry. Prior to her startup, she worked as a scientific and group leader in non-regulated bioanalysis in Pharmacokinetics, Dynamics and Metabolism at Pfizer, Inc for 8-9 years where she was the point of contacts in discovery bioanalysis for antibacterial, neuroscience, and indication discovery research units. She initiated collaborations with key scientists to introduce a novel bioanalytical methodology for early assessment of exposure coverage of human metabolites in animal species and successfully influenced FDA accepting the methodology. She also collaborated with scientists in transporter group at Pfizer for identifying novel OATP transporter biomarkers. She was the lead of the mass spectrometry networking

group across multiple function lines at Groton, CT between 2015-2016. Before she joined Pfizer in 2008, she worked as staff investigator/research scientist II leading a small group of scientists supporting drug metabolism at Vertex Pharmaceuticals, Inc in MA for 5 years. Dr. Gao obtained her Ph.D. from University of Cincinnati in 1998 and completed her postdoctoral researches in Dick Smith lab at Pacific Northwest National Laboratory in 2000. She is the author or co-author of over 20 publications and invited speaker on MIST subject. She initiated and organized 2012 AAPS MIST symposium, 2016 ASMS next generation LC/MS workshop, and co-chaired several APA workshops. She currently serves as the chair for Discovery Bioanalysis and New Technologies of the Applied Pharmaceutical Analysis meeting.

Darshana Jani, MSc, Pfizer: Darshana Jani is Scientific Associate Director at Pfizer, Cambridge, MA, USA, where her role is to serve as scientific and technical lead for both CROs and internal teams for development, validation, and application of bioanalytical assays to comply with scientific and regulatory requirements. Darshana has over 25years of experience in supporting preclinical to clinical studies holding positions with Sanoffi, MedImmune, Biogen and Pfizer. Darshana has risen from the ranks, at the outset developing and applying boianalytical methods, while assuming numerous responsibilities germane to regulatory compliance of the validation and application of group procedure. She has guided bioanalytical lab groups, held responsible for overseeing any and all phases of drug recovery and development; a task which incorporated product characterization, potency determination, immunogenicity testing, as well as surrogate biomarker assay development, validation and sample testing. Darshana has published several recommendation white papers with industry, academic and regulatory peers in the area of biomarkers as well as immunogenicity.

Fumin Li, PhD, PPD: Dr. Fumin Li is the Associate Director, R&D and Discovery, for PPD Bioanalytical Laboratories in Middleton WI, USA. His responsibilities include daily operations in the laboratory to ensure compliance with company SOPs, policies and client timelines and deliverables; providing mentorship, guidance and development to laboratory management and research & development team; allocating personnel, analytical instrumentation and other resources to meet business needs; and collaborating among different functional groups within the division to drive efficiency and implement new technologies. Dr. Li has more than 20 years' experience in analytical chemistry and 13 years' experience in the pharmaceutical contract research organization (CRO) industry with a focus on regulated bioanalysis and biomarker bioanalysis of small molecules using LC-MS. Dr. Li worked at Covance Laboratory in Madison WI, as Staff Scientist, Senior Staff Scientist, and Technical Operation Lead in the LC-MS method development group for regulated bioanalysis from 2006 to 2013. From 2004 to 2006, Dr. Li was a postdoctoral fellow at Pacific Northwest National Laboratory (PNNL), applying ion mobility mass spectrometry (i.e., IMS-TOFMS or FAIMS-TOFMS) for high throughput proteomics. Fumin obtained his Ph.D. in analytical chemistry from Iowa State University in 2004 under the direction of Dr. Robert S. Houk. Dr. Li's research focuses on developing high throughput and reliable LC-MS or LC-MS/MS methods for quantitative measurement of both small molecules and large therapeutic biomolecules (e.g., peptides, proteins, and oligonucleotides) in biological matrices. Dr. Li has over 50 publications (including three book chapters) and external presentations. He currently serves Chair-Elect for Regulated Bioanalysis for Applied Pharmaceutical Analysis (APA).

Ang Liu, PhD, BMS: Dr. Ang Liu is currently a Senior Research Investigator at Bristol-Myers Squibb. Ang joined the Department of Bioanalytical Sciences at BMS in 2012 with experience in regulated bioanalysis at Tandem Labs and experience in protein bioanalysis at Yale University. Ang received her Ph.D. in Pharmacognosy from University of Illinois at Chicago in 2009. Ang has extensive experience in regulated LC-MS based bioanalysis of small-molecule and large-molecule drugs, new treatment modalities, and biomarkers. Currently Ang is broadening her expertise in LBA based bioanalysis in support of PK and immunogenicity. Ang has authored or co-authored over 15 scientific publications in peer-reviewed journals. Ang serves as a reviewer of a number of journals and also serves on the organizing committee of Applied Pharmaceutical Analysis Conference.

Johanna R. Mora, PhD, Bristol-Myers Squibb: Dr. Mora is currently a Group Leader in the Department of BioAnalytical Sciences at Bristol-Myers Squibb (BMS). She received a Bachelor of Science degree in Chemistry from the University of Costa Rica in 1999 and a Ph.D. in Analytical Chemistry from the University of Kansas in 2004. At BMS she leads a team of scientists in the development of methods to support bioanalysis and bioanalytical strategy of small, macromolecular, and new modality therapeutics for PK and immunogenicity assessments. She is a mentor to her team, is an author of over 15 scientific publications and has served in several cross functional teams within BMS. She is an active member of AAPS, serves in the Emerging Technology Discussion Group within AAPS' LBABFC and is Presiding Chair-Elect of the Applied Pharmaceutical Analysis meeting.

Mehran Moghaddam, PhD, OROX BioSciences: Dr. Moghaddam obtained his PhD in medicinal chemistry from Oregon State University-School of Pharmacy and completed his post-doctorate research in lipid metabolism from UC-Davis. Later he completed an executive MBA program at University of Southern California. Mehran has over 22 years of corporate experience in discovery and development of small and large molecules in DuPont, Pfizer, and Celgene organizations. In Celgene, he served as the head of Discovery DMPK. In that capacity, he played a major role in discovery and nomination of 19 small-molecules and 1 antibody into development. Additionally, he led the DMPK team responsible for generation of discovery DMPK data for Revlimid®, Pomalyst® and Otezla®; approved therapies for multiple myeloma and psoriatic arthritis. He is the author/coauthor on nearly 50 publications and several patents. Mehran recently retired from Celgene to found a start-up company, OROX BioSciences Inc., dedicated to efficient and accelerated discovery of small-molecule drugs for treatment of fibrotic ailments.

Chandra Prakash, PhD, Agios: Dr. Prakash is a Senior Research Fellow in Drug Metabolism, Pharmacokinetics and Clinical Pharmacology department at Agios, Cambridge, MA. He obtained his Ph.D. in synthetic organic chemistry. He held several academic appointments prior to joining the pharmaceutical industry. He worked for 16 years at Pfizer Global Research and Development, Groton, CT and 6 years at Biogen, Cambridge where he managed a metabolism group. He joined Agios in June 2015. For the last 30 years, Dr. Prakash has been involved in the drug metabolism and clinical pharmacology studies to support drug discovery, development and registration. His research is primarily focused on the development and utilization of novel approaches and techniques which include in vitro methods using human and animal hepatic cellular and subcellular systems, recombinant human drug metabolizing enzymes, sensitive analytical technologies and in silico computational models to assess the metabolism and toxicological aspects of the new chemical entities. He is the author of more than 265 manuscripts, book chapters, presentations and patents. He also coedited five volumes of Handbook of Metabolic Pathways of Xenobiotics.

Mark Qian, PhD, Takeda: Dr. Mark Qian is Director of Bioanalytical Chemistry at Takeda Pharmaceuticals in Cambridge, MA and has been working in the pharmaceutical industry for more than twenty years in small and large molecule bioanalysis for supporting drug discovery, preclinical, and clinical development. One of focuses of his group is to provide bioanalytical strategies and assay support based on multiple technology platforms for developing targeted therapeutics for cancer treatment. Mark got his Ph.D. in analytical chemistry from University of Michigan, Ann Arbor. Previously, he served Chiron Corp., DuPont Pharmaceuticals, and Millennium Pharmaceuticals (acquired by Takeda). He is the author or co-author of more than 30 publications.

David Stresser, PhD, AbbVie: Dr. Stresser is a Principal Research Scientist in the DMPK-Translational Modeling department at AbbVie. Prior to joining AbbVie in 2016, he held research, management and business development positions at Gentest Corporation, BD Biosciences and Corning Life Sciences in Woburn, MA. He received post-doctoral training in the laboratory of David Kupfer at the University of Massachusetts Medical School in Worcester, Massachusetts and graduate work in the laboratories of David E. Williams and George S. Bailey at Oregon State University in Corvallis, OR, receiving a Ph.D. in toxicology in 1994. Dr. Stresser has authored or co-authored > 40 articles or book chapters in the field of drug metabolism and has been an invited speaker at various national and international meetings, pharmaceutical companies and universities.

C.T. Viswanathan, PhD, CT Viswanathan & Associates, Inc.: Dr. Viswanathan is currently affiliated with CT Viswanathan & Associates, Inc. specializing in Biopharmaceutics, Clinical Pharmacology and GLP, providing Quality Regulatory consulting services to Pharmaceutical Industry. He is the former Associate Director in the Division of Scientific Investigations, Office of Compliance, CDER, FDA. He had policy and scientific oversight in the regulatory administration of GLP and Bioequivalence Inspectional programs as well as Regulated Bioanalysis. He has issued Warning Letters and pursued administration actions with non-compliant laboratories and has made regulatory decisions in the acceptability of data. He received his B.Sc and M.Sc degrees in Chemistry from the Presidency College, Madras, India. He received a M.S. in Organometallic Chemistry from Marquette University, Milwaukee, and M.S in CNS Pharmacology and a Ph.D in Pharmacokinectics from the University of Wisconsin, Madison. Following post-doctoral research at the University of Georgia and the University of Washington, Seattle, he joined the FDA. During his tenure at FDA, Dr. Viswanathan served as a primary reviewer of clinical pharmacology, then became the Chief of Pharmacokinetics Branch and subsequently the acting Director of the Division of Biopharmaceutics for over two years (currently known as Clinical Pharmacology) prior to joining the Office of Compliance.

Yongjun Xue, PhD, Celgene: Dr. Y-J Xue is a director at Celgene's DMPK group in Summit, NJ and is involved in exploratory and development bioanalytical support. He previously held positions within development bioanalytical organizations at Bristol-Myers Squibb in Lawrenceville/New Brunswick, NJ, and at Procter & Gamble Pharmaceuticals, Norwich, NY. Y-J completed his Ph.D. degree in 1994 at Iowa State University under the direction of Dr. Edward Yeung. Y-J's research interests include bioanalytical method development/validations for challenging drug candidates/metabolites (e.g. chiral compounds), tissue sample analysis, therapeutic protein/antibody drug conjugate analysis by LC-MS/MS, and small molecule/protein biomarker analysis by LC-MS/MS.

Liyu Yang, PhD, Vertex: Dr. Liyu Yang is currently Director of Bioanalysis at Vertex Pharmaceuticals Inc. Dr. Yang is a scientific leader with more than 20 years of experience in drug discovery and development especially in the areas of bioanalytical, biomarker, and DMPK. She has demonstrated ability at leading multiple projects for successful R2Ds (research to development transition), INDs (Investigational New Drug Application), and NDAs (New Drug Application). Prior to Vertex, Dr. Yang had tenures at Biogen as Director of Global Biomarker Discovery and Development, Director of Bioanalysis DMPK, at Schering-Plough Research Institute as Principal Scientist of DMPK, and at Pacific Northwest National Laboratory as visiting scientist. Dr. Yang obtained her BS in Chemistry from Nanjing University, MS, and Ph.D. in Chemistry from Iowa State University, and MBA from MIT. Dr. Yang is author and co-author of more than 25 publications. She also volunteers in education related non-profit organizations.

SPEAKERS

Melanie Anderson, Merck: Melanie Anderson is a principal scientist at Merck Research Laboratories (NJ, USA) and manages a Clinical Regulated Bioanalysis group. Melanie has over 15 years' experience in both pharma and CRO settings, conducting LC–MS/MS analysis for quantitation of small and large molecules. Melanie has participated in FDA inspections and contributed to regulatory filings for multiple programs. Additionally, Melanie has experience in non-compartmental pharmacokinetic analysis of atypical compounds.

Melanie's scientific interests include analysis of unique matrices, unstable compounds, challenges in chromatographic separations, and patient centric microsampling approaches for regulated clinical trials. Recently, Melanie has evaluated and helped implement microsampling devices in animal and human regulated studies. She received her BA in Chemistry from Hasting College (NE, USA) in 2002, and an MS in Chemistry from Lehigh University (PA, USA) in 2007.

Jonathan Bauman, Pfizer: Jonathan N. Bauman is a Principal Scientist at Pfizer Inc. with 27 years of experience in drug metabolism. Currently, he is a Biotransformation team leader at Pfizer's research headquarters in Groton, CT. Throughout his career he has worked in Drug Metabolism and Pharmacokinetics groups at G. D. Searle & Co., Parke Davis and Pfizer (Ann Arbor, MI and Groton, CT). During his career, Jon has specialized in enzyme kinetics, enzymology, chromatography, mass spectrometry, and drug metabolism of small molecules. More recently he has focused on reactive metabolites, bioactivation and evaluating metabolite identification software including developing and running a high throughput metabolite identification assay. Jon has contributed to 35+ external publication and presentations and has been a member of the International Society for the Study of Xenobiotics (ISSX) for 25 years.

Kevin Beaumont, Pfizer: Throughout 35 years in the Pharmaceutical industry, Kevin has worked extensively in the drug metabolism field. His major area of expertise is in the modulation of physicochemistry to affect drug disposition and prediction of human pharmacokinetics. He is author on approximately 50 peer reviewed publications.

Kevin has worked on many Discovery and Development projects throughout his career. He has been responsible for the DMPK input to at least 30 FIH studies as well as 10 Phase II compounds, including 1 marketed agent. Kevin now provides DMPK input to the Inflammation and Immunology Rare Disease Research Unit, based in Cambridge Massachusetts.

Joseph S. Camardo, MD, Celgene: Dr. Camardo is Senior Vice President at Celgene Corporation. He earned an MD from University of Pennsylvania School of Medicine in 1979 and completed residency training for Internal Medicine. Dr. Camardo was a fellow in Neuroscience at Columbia University and at the University of Pennsylvania, where he worked on ion channels and behavior. In 1989 he joined the Clinical Research division of Wyeth-Ayerst Laboratories, ultimately became head of Clinical Research and Medical Affairs, at Wyeth. Dr. Camardo is chair of the Board of MaliHealth, an NGO that works with mothers and children in Bamako, Mali, a fellow at the College of Physicians of Philadelphia, a member of the Institute for Ethical Leadership and Social Responsibility at Rosemont College, and a board member of Anyone Can Fly, an arts foundation in NY.

Andrea Casasola-LaMacchia, PhD, Pfizer: Dr. Casasola-LaMacchia is a Postdoctoral scientist at Quantitative Biomarkers & Biomeasures in BioMedicine Design, Pfizer Inc., USA. She did her initial postdoctoral work in the Stirewalt Laboratory within the Clinical Research Division at the Fred Hutchinson Cancer Research Center (Fred Hutch) in Seattle WA. Her studies included characterization of the Acute Myeloid Leukemia proteome and the Human Leukocyte Antigen I-immunopurification in order to study the associated immunopeptidome as a collaborative effort at the Fred Hutch. She was awarded her Biochemistry PhD from Universidad Nacional Autónoma de México (UNAM) in Mexico City while studying epigenetic mechanisms of hemoglobin genes regulation in the Recillas-Targa Laboratory at the Instituto de Fisiología Celular, UNAM. She joined the Groudine Laboratory within the Basic Sciences Division at the Fred Hutch as a visiting graduate fellow, where she concluded her PhD research focusing on the characterization of the nuclear envelope proteome during hematopoietic differentiation. Dr. Casasola-LaMacchia received her BA in biology from the Facultad de Ciencias UNAM, while studying antigenic kindship within snake venoms and antivenoms in the Stock Lab at the Biotechnology Institute (IBT, UNAM), Cuernavaca Mor. Dr. Casasola-LaMacchia is a former board member of Hutch United, a grassroots organization that was founded in 2013 by researchers at Fred Hutch to foster an inclusive community and to promote the success of underrepresented and self-identified minority scientists at Fred Hutch. She is currently a Pfizer representative for the Massachusetts Industry Postdoc Association.

An Chi, PhD, Merck: Dr. An Chi has been involved in understanding the MOA of therapeutics functional effect in healthy and disease biological system at Merck Research Laboratories, Boston since 2006. Her expertise is in proteomics, system biology and translational biomarker discovery and development. An obtained her Ph.D. from Dr. Donald Hunt's lab at the University of Virginia, VA, contributing to the development and application of Electron Transfer Dissociation mass spectrometry to enhance

the identification of low abundance peptide/protein post-translational modifications and Top-Down characterization of whole proteins at a global scale. Presently An is focusing on the build-out of chemical proteomics capabilities and data analysis infrastructure to enable target ID/Validation and mechanism deconvolution for phenotypic screens.

Xiaoyan Chu, PhD, Merck: Dr. Xiaoyan Chu is a Senior Principle Scientist in the Department of Pharmacokinetics, Pharmacodynamics & Drug Metabolism (PPDM), Merck & Co. Inc. Rahway, NJ. She received her Ph.D. from the Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan. After completing her post-doctoral research at the Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, she joined the Department of PPDM at Merck & Co. As the scientific leader for Transporter & In Vitro Technology Group, she is responsible for developing the strategies to support drug discovery and development programs to better understand the role of transporters/enzymes in drug disposition and drug-drug interactions, and for evaluating new technologies to establish in vitro to in vivo extrapolation and translational modeling approach to predict drug transporter/metabolic enzyme related drug disposition and interactions. She has over 50 peer-reviewed research and review articles/ book chapters in the area of drug transporters/metabolic enzymes and pharmacokinetics. She is a member of the International Transporter Consortium (ITC) and has been leading several ITC working groups for preparation of ITC whitepapers related to the roles of transporters in intracellular drug concentrations, endogenous biomarkers and drug probes for transporter inhibition. She is also an invited speaker, organizer and steering committee member of various scientific meetings.

Adrienne Clements-Egan, PhD, J&J: Dr. Adrienne Clements-Egan is a Scientific Director of Bioanalytical Sciences within the Biologics Development Sciences (BDS) Department of Janssen Research & Development, LLC (Johnson & Johnson). Dr. Clements-Egan has over ten years of experience in various aspects of biotherapeutic development. Currently her team supports PK/PD assessments for the global biotherapeutic portfolio within Janssen R&D by developing soluble target engagement and receptor occupancy assays. Dr. Clements-Egan is also responsible for bioanalytical CRO management on behalf of the BDS. Dr. Clements-Egan serves as a steering committee member of the Therapeutic Protein Immunogenicity Community within the American Association of Pharmaceutical Sciences (AAPS). Within AAPS, she has previously served as the chair of the eLearning committee for the development of educational webinar and e-course content. She is also a co-author on several publications about critical reagent development and immunogenicity assays. Dr. Adrienne Clements-Egan received her Bachelor of Science degree from the University of Nevada at Reno, and her Ph.D. from the University of Pennsylvania in Biochemistry and Molecular Biophysics.

Joseph A. Cornicelli, PhD, Charles River: Dr. Cornicelli is the Senior Director of In Vivo Pharmacology for Charles River Discovery Services for the cardiometabolic diseases therapeutic area. He has over 30 years of experience in drug discovery and development. Trained at the University of Cincinnati, Dr. Cornicelli completed post-doctoral fellowships at the Mayo Clinic Foundation, and Columbia University, where he also held an appointment on the research faculty in the Department of Medicine. He joined Warner-Lambert in 1985 where he later became a Research Fellow working in the atherosclerosis, inflammation and cardiovascular diseases therapeutic areas. During his tenure with Warner-Lambert, and later with Pfizer, Dr. Cornicelli served on the atorvastatin discovery team, and the troglitazone and pregabalin development teams. All of these entities became marketed products. He chaired several discovery teams in the areas of atherosclerosis, inflammation and inflammatory pain. In 2007, Dr. Cornicelli joined Charles River (Molecular Imaging Research) as the Director of Inflammation and Cardiovascular Pharmacology Services. He is responsible for the scientific oversight for the products and services offered by Charles River for cardiometabolic diseases, and for assisting clients with their drug discovery and development efforts. He is the author of over 30 publications and 4 patents, and serves as a reviewer for several scientific journals. Dr. Cornicelli is a Fellow of the American Heart Association's Council on Atherosclerosis, Thrombosis and Vascular Biology, and a member of the American Physiological Society.

Donna Dambach, PhD, Genentech: Dr. Dambach is currently Senior Director, Head of Toxicology at Genentech, Inc. The Toxicology group provides portfolio and program strategic and technical expertise from discovery through post-marketing phases including safety lead optimization and development candidate identification, the design and implementation of non-clinical development strategies for INDs/CTAs through NDAs/MAAs, and investigative toxicology support for programs through an Investigative Toxicology Laboratory.

Donna has been with Genentech for 12.5 years and has a combined 26 years of experience in both academia and industry as a comparative anatomic pathologist and as a discovery, investigative and regulatory toxicologist.

Donna received her Ph.D. from the Department of Toxicology from Rutgers University/University of Medicine and Dentistry of New Jersey. Her doctoral research examined the role of inflammatory mediators and NFκB in the acetaminophen-induced model of acute oxidative-stress-mediated hepatotoxicity. Donna received her veterinary degree (V.M.D.) from the University of Pennsylvania, completed a residency in comparative anatomic pathology at the University of Pennsylvania and attained board certification (American College of Veterinary Pathology) as a diplomate in the subspecialty of Comparative Anatomic Pathology. She is a Fellow of the Academy of Toxicological Sciences and has authored or co-authored over 50 peer-reviewed publications/book chapters and holds two patents for safety biomarkers.

Arindam Dasgupta, PhD, FDA: Dr. Dasgupta is currently the Deputy Director of the Division of New Drug Bioequivalence Evaluation (DNDBE) in the Office of Study Integrity and Surveillance (OSIS) located in the Office of Translational Sciences (OTS), Center for Drug Evaluation and Research (CDER) at FDA. OSIS directs and conducts inspections of in-vivo and in-vitro bioavailability/bioequivalence studies and non-clinical studies submitted in support of pharmaceutical development, as part of the Agency's Bioresearch Monitoring (BIMO) program. Before joining OSIS, Dr. Dasgupta completed postdoctoral fellowships at Department of Biochemistry and Molecular Genetics, University of Virginia and National Cancer institute (NCI) at National Institutes of Health (NIH). After completing his fellowship, Arindam joined the Center for Drug Evaluation and Research in 2008 as a Pharmacologist in the Division of Scientific Investigations, GLP and BE Investigations Branch. He served as acting Team Lead and lead Pharmacologist positions before talking up the current position of Deputy Director position. Arindam received his BS in Zoology from the University of Calcutta, Calcutta, MS in Biophysics, Molecular Biology, and Genetics from the University of Calcutta, Calcutta, and Ph.D. in Molecular Biology from Jadavpur University, Calcutta. He has authored numerous scientific publications in prestigious peer-reviewed journals.

William H. Gerwick, PhD, UC San Diego: Dr. Gerwick received his B.S. in Biochemistry (UC Davis) and Ph.D. in Oceanography (Scripps Institution of Oceanography), did postdoctoral studies at the U. Connecticut, was Professor of Chemistry at the U. Puerto Rico and then moved to the College of Pharmacy, Oregon State U. as Professor of Pharmaceutical Sciences. In 2005 he became Professor of Oceanography and Pharmaceutical Sciences at Scripps Oceanography and the Skaggs School of Pharmacy, UC San Diego, and in 2011 was promoted to Distinguished Professor. He has received several awards and prizes, and is a Fellow of the American Association for the Advancement of Science (AAAS) and the American Society of Pharmacognosy. His research focuses on the discovery of novel natural products from marine cyanobacteria and algae, their biomedical applications, and investigations of their biosynthesis.

Dhiman Ghosh, PhD, Takeda: Dr. Ghosh is working as a Scientist at Takeda Pharmaceuticals, Boston with core expertise in Bioanalysis, Proteomics and Systems Biology. Dhiman received his PhD in Biochemistry from the University of Manitoba, Canada for his research work on Membrane Proteomics and completed his postdoctoral training at the Institute for Systems Biology, Seattle under the supervision of Dr. Leroy Hood. His current research focuses on the development of mass spectrometry based platform technology for the identification and characterization of cell-surface membrane proteins to support the drug discovery process.

Jochem Gokemeijer, BMS: Jochem Gokemeijer has been working at Bristol Myers Squibb for 15 years in various roles on Immunogenicity, PK PD and biologics drug discovery. Before that he was involved in two alternative scaffold biotech companies Phylos and Adnexus Therapeutics. Jochem did his undergraduate work in the Netherlands at the University of Groningen and graduate work at the Dana Farber Cancer Institute.

Fei Hua, PhD, Applied BioMath: Dr. Fei Hua is Senior Director of Modeling and Simulation and Clinical Pharmacology at Applied BioMath, a consulting company based in Concord MA. Before joining ABM, she was Director of clinical pharmacology at Pfizer. Dr. Hua received her BS in biology from Tsinghua University, Beijing, China and her Ph.D. from Department of Physiology at Cornell University followed by postdoctoral training at MIT in Department of Biomedical Engineering. Throughout her training, Dr. Hua has always worked in the lab in parallel with model building to generate data that feeds into the model. Working in pharmaceutical industry, Dr. Hua has developed mathematical models to support both preclinical programs and clinical programs and in many different disease areas including oncology, CVMED, immunology and rare disease. Over the years, Dr Hua developed skills to integrate all the available information and build fit-for-purpose models to drive business decisions in a timely manner.

Cynthia Inzano, PhD, BMS: Dr. Inzano is a Senior Scientist at Bristol-Myers Squibb working in the Bioanalytical Sciences group. Mrs. Inzano has worked in the pharmaceutical industry for the last 18 years with experience in immunogenicity and bioassay development, quality control and manufacturing areas. She has her Bachelors degree and Masters degree in Biology from Georgian Court University and is currently working towards her Ph.D in Molecular Biosciences at Seton Hall University.

Sylvia Janetzki, MD, ZellNet Consulting: Dr. Janetzki is founder and president of ZellNet Consulting. The focus of her work during the past >20 years has been immune monitoring approaches for clinical studies with emphasis on Elispot and sample integrity. Her work led her to a tight collaboration with the Cancer Immunotherapy Consortium (CIC/CRI), for which she initiated and lead a world-wide proficiency panel program for assays like Elispot, Multimer staining, Intracellular Cytokine Staining (ICS), Luminex and others. Multiple guidelines for harmonized assay performance have been published as a result of this work. Sylvia is the leader of the MIATA core team for the enhancement of structured and transparent reporting of immune monitoring results (miataproject.org). She is the author of numerous publications, books and book chapters, including a recent Nature Protocols publication on guidelines for Elispot evaluation. She received her MD from Humboldt University, Berlin, and her post-doctoral training in Immunology from Fordham University and MSKCC.

Vibha Jawa, PhD, Merck: Dr. Jawa is currently a Director, Biologics and Vaccine Bioanalytics at Merck where she is responsible for developing a strategy and provides oversight and management of scientific programs for discovery, development and optimization of biologics and vaccines. In this role, she is partnering with discovery and development groups to design better molecules. Dr Jawa received her bachelors in Biochemistry (1991) from Delhi University and her doctorate in Biochemistry/ Immunology (1998) from All India Institute of Medical Sciences, New Delhi, India with a thesis work studying the immune mechanisms behind autoimmune diseases followed by a postdoctoral fellowship at University of Pennsylvania on monitoring the immune response to viral vectors. She continued to work in the field of gene therapy company evaluating viral vectors for hemophilia therapy in the Bay area followed by City of Hope Cancer Centre Stem Cell and Gene Therapy group and at Amgen from 2003-2016.

Her current research interests include evaluating immune response biomarkers for early drug development and efficacy, immunogenicity prediction using in silico, in vitro and in vivo technologies, modeling impact of immunogenicity on PK and PD and their application to a systems based approach, antigen processing and presentation and the role of T cells in immune response to drug products. Vibha is a member of professional organizations like American Association of Pharmaceutical

Scientists (AAPS) and American Association of Immunology (AAI) and Federation of Clinical Immunology Society (FOCIS). Within AAPS, Vibha has been actively involved as a Steering Committee member of the Therapeutic Protein Immunogenicity Focus Group (TPIFG) and is currently leading the Immunogenicity Prediction Action Program Area (IPAPA). She has been an invited speaker in multiple sessions at AAPS for past few years and has successfully moderated and organized programming at AAPS NBC (National Biotechnology Conference) and AAPS Annual Meetings. She has also organized and participated in AAPS webinar and e forum to propagate learning and sharing information among AAPS members. Dr. Jawa also serves as a manuscript reviewer for *The AAPS Journal* and *J.Pharm Sci Journal*. Dr. Jawa has published over 50 papers and her articles in the Nature Genetics, Annals of Hematology and Clinical Immunology journals have been cited over 2500 times. She is the recipient of the 2015 Ebert Prize from the American Pharmacists Association for her work on assessing risk of critical quality attributes in a humanized mouse model system.

Chris Kochansky, PhD, Merck: Dr. Kochansky received a B.S. in Chemistry from Elizabethtown College in Pennsylvania followed by a M.S. from Bucknell University with a concentration in Analytical Chemistry. He subsequently joined the Department of Drug Metabolism of Merck & Co., Inc. in Rahway, NJ. Over the last 18-years at Merck, Chris has spent time in several DMPK groups focused on preclinical development, drug discovery, in vitro technologies, and therapeutic siRNA. He is currently an Associate Principal Scientist in the Metabolite Identification and Tissue Distribution group located in West Point, Pennsylvania. He has authored or co-authored 18-publications along with obtaining a Ph.D. from the University of the Sciences in Philadelphia.

Ashutosh Kulkarni, PhD, Allergan: Dr. Kulkarni is currently a Director of Clinical Pharmacology in the Nonclinical and Translational Sciences Group at Allergan plc where he leads a team of PhD Scientists supporting multiple therapeutic areas such as medical dermatology, CNS, GI, ophthalmology and urology. Ashutosh graduated from the University of Southern California with a PhD in Pharmaceutical Sciences under the mentorship of Dr Vincent H. L. Lee. After graduating, he joined Neurion Pharmaceuticals as a Scientist in their Molecular Neurosciences group. After 2 years at Neurion, he joined the Pharmacokinetics and Drug Disposition group at Allergan Inc and was instrumental in devising nonclinical strategies as a member of project core teams, conducting nonclinical studies, writing regulatory submissions (INDs, NDAs, IBs etc) and supporting various ophthalmology and dermatology programs. He also led nonclinical sub teams which comprised of various other functional team members. In 2011, Ashutosh joined the Celgene discovery DMPK group in San Diego. As part of this group, he led discovery sub teams primarily in the oncology and inflammation & immunology therapeutic areas. He also led a group of junior scientists and helped implement efficient discovery DMPK screening paradigms to support lead identification and lead optimization efforts leading to nomination of new chemical entities (small molecules and biologics) as development candidates. In 2016, Ashutosh rejoined Allergan as a Director in their Global Clinical Pharmacology group. His team now supports nonclinical as well as clinical studies (PK and Clinical Pharmacology), discovery through Phase 3 and beyond, for all therapeutic modalities. In addition, Ashutosh has published several peer reviewed articles throughout his career and has also served in a teaching role (more recently as Adjunct faculty) at the USC School of Pharmacy.

Valerie Kvaternick, Merial, Inc.: Ms. Kvaternick's research experience is in the areas of absorption, distribution, metabolism, and excretion of veterinary drugs and agrochemical compounds, as well as the environmental fate and worker exposure of agrochemical compounds. She has expertise in support of pharmacokinetic and tissue residue studies, and in drug development programs for companion and food-producing animals as a project team representative. She has been study director or principal investigator for over 50 studies in these areas, including a tissue residue depletion study to determine the withdrawal period of a veterinary drug, and contributed to numerous study designs in support of veterinary drug development to meet pharmacokinetic/bio-analytical and regulatory matters. She has served as an internal residue expert and written residue expert reports and other technical documents for submission to the agencies. She has experience with a wide array of specialized laboratory equipment and techniques, data analysis and interpretation, and various industry regulations. She holds various

leadership roles in the company, has supervised a diverse work force, is a member of the American Chemical Society, and is a peer-reviewer for journals such as *The Veterinary Journal*, *BMC Veterinary Research*, *Journal of Chromatography B*, *Medicinal Chemistry: Current Research*, *Talanta*, and *Iranian Journal of Applied Animal Science*.

Xiaomin Liang, PhD, Gilead Sciences: Dr. Liang is currently a research scientist in the Department of Drug Metabolism at Gilead Sciences Inc. She holds a Bachelor of Science degree in molecular toxicology from University of California, Berkeley (UC Berkeley). She earned her Ph.D. from the program of pharmaceutical sciences and pharmacogenomics at University of California, San Francisco (UCSF), 2016. After doing fellowship training in the Office of Clinical Pharmacology at FDA, she joined Gilead Sciences in 2017. The main focus of her graduate and post-graduate research is to study membrane transporter biology and transporter-mediated drug disposition and pharmacokinetics.

John O. Link, PhD, Gilead Sciences: Dr. Link is a Vice President, Medicinal Chemistry at Gilead Sciences. John completed his bachelor's degree in chemistry from the University of Minnesota in 1985 working in the labs of Thomas R. Hoye. He received his PhD in Chemistry under the direction of EJ Corey at Harvard University in 1992 working on CBS reduction chemistry and a novel synthesis of amino-acids, now a name reaction (Corey-Link Reaction). From 1992-1996 John worked at Syntex/Roche Palo Alto in the therapeutic area of inflammation and immunology. There John elucidated the mechanism of inosine monophosphate dehydrogenase, the rate-limiting enzyme in GTP biosynthesis and target of CellCept® and the mechanism of the IMPDH inhibitor mycophenolic acid (active form of the immunosuppressant CellCept®). From 1996-2006 John worked on protease inhibition at Celera (South San Francisco) in inflammation, cardiovascular and antiviral areas with three compounds entering clinical trials. Since 2006 John has been at Gilead Sciences focusing on antiviral programs in hepatitis C and HIV. He led the project teams that discovered the NS5A inhibitors ledipasvir and velpatasvir (Harvoni® Epclusa®). The NS3 protease inhibitor voxilaprevir was discovered in his organization (Vosevi®). He is co-inventor on those three drugs. He is also a co-inventor and was an early project leader on the first-in-class HIV Capsid inhibitor GS-6207 now in Phase 1 clinical trials. John was awarded the American Chemical Society's 2015 "Heroes of Chemistry Award" for his contributions to the discovery of Harvoni®.

Guowen Liu, Agios Pharmaceuticals: Guowen is currently the Director of bioanalysis in Agios Pharmaceuticals, where he is responsible for both Discovery and Development bioanalysis for drugs and biomarkers. Prior to joining Agios, he had worked for Bristol-Myers Squibb Co. in New Jersey for more than 10 years in the regulated bioanalysis field. Guowen has published more than 20 manuscripts and book chapters. He was also invited to speak at different conferences as well as by FDA on different topics related to Bioanalysis.

Debra Luffer-Atlas, PhD, Eli Lilly and Co.: Debra is a Senior Research Fellow with 26+ continuous years of drug discovery and development experience at Merck (1992-97) and Lilly (1997-present). She has deep expertise in nonclinical and clinical drug disposition (ADME) and she is widely regarded as an expert in the area of metabolites in safety testing (MIST). She and an FDA reviewer recently co-authored an editorial in *Expert Opinion on Drug Metabolism & Toxicology* entitled "A decade of drug metabolite safety testing: industry and regulatory shared learning."

Debra previously served as team leader for the ADME/Toxicology Screening Group, which serviced the entire Lilly discovery portfolio to optimize ADME properties of lead series. She was accountable for more than 20 screening assays, including solubility, metabolic stability, passive cell permeability, and CYP inhibition. She was also responsible for implementing ADME screening assays and developing DMPK capabilities with several CRO partners in China and India. For her leadership in this space, she received the prestigious Lilly Quality Pinnacle Award in 2007.

Debra currently leads a Lilly discovery team focused on neuropathic pain. She also serves as co-chair of the Lilly ADME/

Toxicology/PKPD Drug Induced Liver Injury (DILI) Working Group and she is a member of the Lilly Liver Safety Committee. She is a member of the cross-industry IQ-DILI Consortium Steering Committee, she serves as co-chair of the IQ-DILI Nonclinical Translation Working Group, and she recently joined the IQ Drug Metabolism Leadership Group.

Debra frequently gives external presentations on the drug discovery process. In 2007 she was invited by PhRMA to serve as a panelist at a Congressional briefing on "The Making of Medicines: A Look into the Pharmaceutical R&D Process." In 2018 she was invited by IQ-DruSafe to present on DILI screening strategies at FDA Pharmacology and Toxicology Reviewer Training: Small Molecule Safety Lead Optimization and Candidate Identification Practices.

Hlaing (Holly) Maw, Boehringer Ingelheim: Hlaing (Holly) Maw is Senior Scientist of Drug Metabolism and Pharmacokinetics at Boehringer Ingelheim Pharmaceuticals, Inc., in the USA. Holly is a member of the International Society for the Study of Xenobiotics (ISSX). Holly received her B.Sc. in chemistry from Eastern Nazarene College and M.Sc. in chemistry/biotechnology from Tufts University. She is a highly motivated drug metabolism scientist with over 10 years of pharmaceutical industry experience in metabolite identification and drug metabolites safety (MIST) assessment, in addition to 7 years of the peptide synthesis and analytical experiences. Holly has presented and published extensively in the area of SAR E-state modeling, boronic acid peptide inhibitors and unusual drug metabolism.

David Pirman, Agios: David Pirman is currently a Senior Scientist at Agios working in the metabolism group. He and his team support numerous pre-clinical programs at varying stages across the portfolio. He completed his training under Professor Richard Yost at the University of Florida developing quantitative MALDI MS imaging methods. He then spent a year at MD Anderson Cancer Center working on cancer lipid metabolism followed by a postdoctoral position at Pfizer further studying metabolic disease. David has been developing and using mass spectrometry methods to study disease metabolism applied to the drug discovery setting for 7+ years.

Jun Qu, SUNY-Buffalo: Jun Qu is the group leader of the Proteomics and Pharmaceutical Analysis lab of SUNY-Buffalo and a professor in the Department of Pharmaceutical Sciences. His research is focused on the study of Clinical and Pharmaceutical Proteomics and Pharmaceutical Analysis using LC/MS-based strategies. His research programs include i) highly sensitive and accurate characterization of biotherapeutics in pharmaceutical and clinical systems using LC/MS; ii) high-resolution and large-scale expression profiling of pathological proteomes, for the discovery of novel disease/therapeutics biomarkers using MS1-based proteomic methods. iii) Sensitive identification, localization and quantification of post-translational modifications in tissue proteomes; iv) targeted investigation of marker proteins that are of high interests for clinical and pharmaceutical study. He has ~100 peer reviewed publications and >60 grants funded as PI or co-I.

Giovanna Scapin, PhD, Merck: Dr. Scapin is a Principal Scientist at Merck & Co., Inc. She graduated Magna cum Laude in 1985 from Padova University (Italy) with a degree in Organic Chemistry, and 1989 she received her PhD from the same university with a thesis in Structural Biology. In February 1990 Giovanna joined the laboratory of Dr. James C. Sacchettini at the Albert Einstein College of Medicine, Bronx (NY), as postdoctoral fellow, and subsequently as Instructor. In 1997 Giovanna joined Merck and Co., Inc, where she provided crystallography support for diabetes, inflammation, anti-infective and oncology targets. In 2016 she joined, as visiting scientist, the Simons Electron Microscopy Center at the New York Structural Biology Center. She is now back full time at Merck, to introduce and apply CryoEM techniques to drug discovery projects.

Since 2010, Giovanna is an active member of the organizing committee for the International School of Crystallography.

Jianwei Shen, PhD, AbbVie: Dr. Shen currently worked as principal research scientist in the department of biotransformation and bioanalysis, Abbvie Inc., mainly to provide ADME characterization of small molecule drugs and antibody-drug conjugates from preclinical to clinical development. Prior to joining the biotransformation group, worked as a senior scientist in department of structural chemistry, Abbott Lab, to provide LC-MS support for proteomics, characterization of recombinant proteins and therapeutic biologics.

Michael F. Skelly, PhD, FDA: Dr. Skelly is a Lead Pharmacologist in the Division of Generic Drug Bioequivalence Evaluation, within the Office of Study Integrity and Surveillance, Office of Translational Sciences, Center for Drug Evaluation and Research, at FDA. His work involves audits of bioavailability/bioequivalence studies of human drugs, and also PK-PD-immunogenicity studies of biologics. After earning a B.A. from the University of Virginia and Ph.D. in Pharmacology from the George Washington University, he took postdoctoral training in Toxicology and Geriatrics, both at the University of Cincinnati. Prior to joining FDA in 1995, Dr. Skelly held positions in contract laboratories, with responsibilities in toxicology, bioanalytical chemistry, and pharmacokinetics.

Richard D. Smith, PhD, PNNL: Dr. Smith is Chief Scientist for the Biological Sciences Division at Pacific Northwest National Laboratory. He is the author or co-author of more than 1000 peer reviewed publications, holds 60 US patents, the recipient of the 2003 American Chemical Society Award for Analytical Chemistry, the 2009 HUPO Discovery Award in Proteomics Sciences, and the 2013 ASMS Distinguished Contribution in Mass Spectrometry Award. His early research included the development of the combinations of both supercritical fluid chromatography and capillary electrophoresis separations with mass spectrometry. His group led in the early development and use of high resolution capillary LC and high resolution mass spectrometry for applications in proteomics. A key part of his research over the last decade has involved the development and application of approaches for improving ion mobility separations and sensitivity in conjunction with mass spectrometry for proteomics, metabolomics, and other 'pan-omic' measurements. More recently this has included the development of Structures for Lossless Ion Manipulations for achieving much higher resolution ion mobility separations with mass spectrometry, as well as for increasing the speed and sensitivity of biological applications.

Hiroshi Sugimoto, PhD, Takeda: Dr. Sugimoto is Senior Scientist I of DMPK at Takeda Pharmaceuticals International Co. in Cambridge, MA, USA mainly focusing on the quantitative bioanalysis of biotherapeutics and protein biomarker for developing state-of-art cancer drugs. He and his colleagues are focusing on the immunocapture-LC/MS and LBA based biotherapeutic target assays and therapeutic fusion protein assay for the project supports. Previously, he was serving as the PK representative at the drug discovery stage in Takeda Pharmaceutical Company in Japan mainly for oncology and neuroscience arena. Dr. Sugimoto received his BA and MS in pharmaceutical science from the Kanazawa University and Ph.D. in pharmaceutical science from Shizuoka University in Japan with dissertation titled "Development of novel analytical methods for the determination of disease-related small molecule biomarkers by LC/MS/MS." He has contributed to multiple pharmaceutical research frontiers including transporter-mediated drug interaction study and LC/MS-based quantitative biomarker assay.

Sarah Voytek, PhD, bluebird bio: Dr. Voytek is an Associate Director in Preclinical Development at bluebird bio in Cambridge, MA. Sarah leads the preclinical development strategy for several gene therapy, cancer immunotherapy, and gene editing programs. Previously, Sarah worked at Novartis where she headed a laboratory employing molecular and cell biology techniques to study mechanisms of toxicologic drug effects. At Novartis, Sarah also led the preclinical development strategy for several non-conventional therapeutic approaches, including oligonucleotides and gene-edited cell therapies. Prior to joining Novartis, Sarah was a Scientist at Intelligent Bio-Systems, a next-generation sequencing company. She obtained her Ph.D. from The Scripps Research Institute, where she studied the in vitro evolution of catalytic RNAs, and her bachelor's degree in Biochemistry from Brown University, where she investigated polymer drug delivery systems.

Yuhong Xiang, MS, Pfizer: Bioanalytical Scientist at BioMedicine Design, Pfizer, has been working with Pfizer since early 2016. Her role is to develop and validate regulated bioanalytical assays in PK, ADA and NAb for preclinical and clinical studies in various therapeutic areas. Prior to joining Pfizer, she worked at Biogen for 8 years and supported numerous bioanltyical method development and optimization of Biomarker, Target Engagement, PK and ADA assays using various technologies such as FACS, ECL, and iPCR. Positions held prior to joining Biogen Idec included Research Associate with BD Biosciences, Immunoassay Specialist with Harvard School of Public Health, Technical Assistant and Database Administrator in the Division of Bioengineering and Environmental Health, M.I.T. She published lead authored and coauthored manuscripts in major journals and presented scientific posters at AAPS, WRIB, Land O'lake Bioanalytical conferences. She received the M.S. degree in Computer Science from Boston University and the M.S. degree in Molecular Biology from Xi'an Jiaotong University.

Ming Yao, BMS: Ming Yao received his B.S. from Henan University, China and his M.S. from Chinese Academy of Preventive Medicine. After graduation, he work at institute of occupational medicine as associate professor. As a visiting scientist, he studied bioactivation of chemicals at Merck as well as University of Western Ontario. In 1997, he joined BMS and responsible for designing and conducting in vitro and in vivo biotransformation studies for multiple programs to support both development and discovery projects. In most recent year, he was also involved for development of protein drug, such as the antibody drug conjugate (ADC), biotransformation of biologics. Ming has over 22 years of experiences in pharmaceutical industry and has over 50 publications and 5 book chapters covering new technologies of mass spectrometry and its applications in drug metabolism.

POSTER ABSTRACTS

Rapid Development of anti-idiotypic binders using a novel affinity scaffold

Alex Wignall, Alex Davidson, Helen Curd, Rob Ford, James Nuttall, Amanda Nicholl, Matt Johnson
Avacta Life Sciences, Wetherby, UK

Purpose:

Affimer® proteins are next-generation affinity scaffolds with great potential for the generation of both novel biotherapeutics and renewable research and diagnostics tools. The availability of capture reagents for therapeutic antibody bioanalytical development can be rate limiting. Antibodies have long represented the gold standard reagent in this field, yet themselves present with many issues. We have applied Affimer technology to the generation of anti-idiotypic Affimer binders to offer rapid and reliable anti-idiotypic reagents for drug development.

Methods:

Based on the Type 1 cystatin protein fold, the Affimer scaffold has been engineered to accept peptide loops such that target-specific Affimer proteins can be selected from highly diverse libraries of random loop sequences. Using phage display, it is possible to generate highly-specific anti-idiotypic Affimer binders using a 12 week development process. The resulting Affimer® binders can be used as reagents in typical pharmacokinetic and immune response assays, such as bridging assays or Fc detection assays, for characterisation of monoclonal biotherapeutics.

Results:

Four specific anti-idiotypic Affimer binders for trastuzumab, rituximab, adalimumab and ipilimumab antibodies were developed and characterised. Performance analysis via Fc detection sandwich assay showed all binders exhibit low matrix effect, minimal batch variation, offer a broader dynamic assay range compared to current antibody-based assays and meet the regulatory requirements for critical assay reagents. In addition, the ease of use, rapid development and security of supply offered by Affimer binders make these anti-idiotypic reagents an antibody alternative solution for bioanalytical assays in drug development.

Conclusions:

The Affimer scaffold has properties (including generation of fusion proteins, ease of production, speed to discovery, specificity and stability and low batch-to-batch variation) supportive for the development of critical reagents that underpin clinical monoclonal drug development programs. Consequently, the use of Affimer binders as anti-idiotypic reagents provides a number of performance improvements for therapeutic antibody capture in bioanalytical assays.

Tip-based solid phase extraction for released nanoparticle quantitation using LC-MS/MS

Amy Rose Boisvert, Zhenhua Gu, Brian Rago, JA Tweed

Purpose:

An area of increasing interest in the bioanalysis discipline is the quantitation of nanoparticles in biological matrix. Nanoparticle medicines are complex drug formulations that encapsulate cytotoxic agents for molecular cell targeting. These formulations when dosed, however, require considerable time and effort to develop robust and reliable bioanalytical assays for the different quantitative endpoints needed to assess the pharmacokinetics, blood distribution and cellular and/or tumor uptake. In our laboratory, we have developed a comprehensive automated SPE tip extraction technique that allows us to generate released nanoparticle concentration data from biological matrix samples. When compared with our GLP validated LC-MS/MS bioanalytical assay that employs plate-based SPE, this new tip-based SPE procedure reduces our sample extraction time from 2.5 hours to 20 minutes.

Methods:

The extraction procedure compares two different types of SPE Tips extracted on the Hamilton Microlab STAR robotic liquid handling platform. The SPE Tip sample extraction procedures compare a fixed monolithic sorbet to a dispersive SPE stationary phase. The LC system employed was a Shimadzu UFLC comprised of an autosampler, column oven, three HPLC pumps, and a solvent degasser. A Phenomenex Kinetex HILIC column in combination with buffered acetonitrile and water mobile phases were employed for gradient separation of analytes. The mass spectrometer systems employed were Applied Biosystems API5500 and API6500+. In LC-MS/MS, MRM detection of the singly charged analyte was used in positive-ion electrospray mode. Calibration standards for quantitating the released nanoparticle analytes were prepared in a biological matrix (blood plasma) by using serial dilution.

Results:

Quantitative preclinical and clinical concentration data generated for a small molecule drug derived from a targeted nanoparticle has been typically carried out via an involved sample preparation and plate based SPE extraction procedure coupled with LC-MS/MS. Application of a novel SPE Tip method for preclinical portfolio support using 25 uL of biological matrix over a range of 10 – 500 ng/mL resulted in intra-assay analytical precision (%CV) and accuracy (%RE) ranged from 1.0 to 8.6% and -7.9 to 16%, respectively. Inter-assay analytical precision (%CV) and accuracy (%RE) ranged from 1.9 to 10.4% and -0.4 to 5.2%, respectively with demonstrated linearity of R2 = 0.9985 (linear 1/x2) averaged over the 3 validation batch runs. Through the use of our robotic liquid handler, we have reduced our extraction time of our released nanoparticle extraction assays from 2.5 hours to 20 minutes while maintaining adequate quality and performance that meets GLP validation guidance acceptance criteria.

Conclusions:

This novel approach allows for the overcoming of challenges associated with time consuming and laborious sample preparation and extraction procedures used for released nanoparticle LC-MS/MS bioanalytical assays.

PRESENCE OF DRUG METABOLIZING ENZYME INDUCER ARTIFACTS IN COMMERCIALLY AVAILABLE HUMAN LIVER SAMPLES INCREASES PROTEIN LEVELS OF CLINICALLY RELEVANT ENZYMES

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Purpose:

One of the most commonly administered anticonvulsants on the market is phenytoin. It is known to be a potent cytochrome P450 (CYP) 3A4 inducer and a substrate for CYP2C9 and 2C19. In this study, we measure mRNA, protein abundance and activity levels of CYP3A4 and other drug metabolizing enzymes (DMEs) in human liver donor tissue. Sequential-window analysis of all theoretical mass spectra (SWATH-MS) was used to achieve a global proteomic analysis of the effect of phenytoin on all DMEs.

Methods:

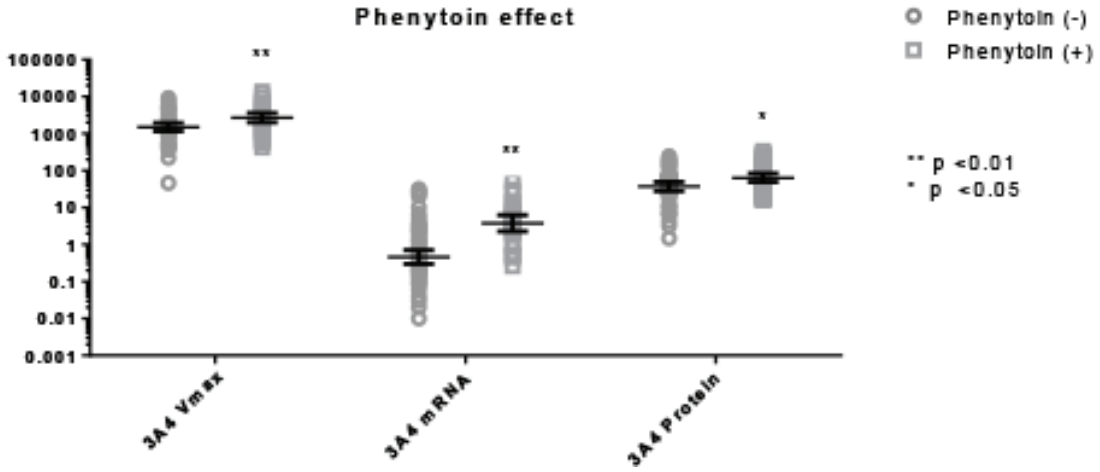
One-hundred and six human liver samples were screened for the presence of phenytoin and other drugs with known inductive or inhibitive effects on DMEs. Samples containing phenytoin were then quantified using LC-MS. Protein levels for 38 DMEs including 18 CYPs were measured using SWATH-MS. Activity levels for CYP3A4 were conducted in house as well as provided by the vendor. mRNA levels for all CYPs and certain nuclear transcription factors were also measured. Non-parametric independent sample tests were run in SPSS and comparisons with a p-value <0.05 were considered significant.

Results:

Presence of phenytoin was found in 34 of the 106 human liver samples. Additionally, phenytoin was found in prepared human liver microsomes purchased from the vendor. **Figure 1** shows that the mRNA, Vmax and protein level of CYP3A4 were all found to be significantly increased in samples containing phenytoin. mRNA of CYP2A6, 2B6, 2C8, 2C9 and 2C19 were all significantly increased as well as the protein levels of CYP2B6, 3A5 and CYP2J2.

Conclusions:

Caution should be taken when performing incubation studies with CYP3A4 substrates as results could potentially be swayed due to an increased abundance of CYP3A4. The use of ambiguous protein expression values due to induction from phenytoin may decrease the accuracy of PBPK models using the data. The presence of phenytoin and other CYP inducers may also be responsible for the variability and reproducibility issues that arise between labs. Livers and microsomes should be screened prior to studies to prevent swayed results due to the induction of DMEs.



LC-MS/MS assay for N1-methylnicotinamide to assess the activities of multiple renal cationic transporters in a first-in-human clinical trial

Lina Luo[1]; Jared Kay[1]; Ragu Ramanathan[1]; Christopher L Holliman[1]; David Rodrigues[1]
[1]Pfizer; Groton, CT

Purpose:
Recently, there has been an increased focus on biomarkers associated with drug-drug interactions (DDIs) linked to organic anion transporters (OATs), multidrug and toxin extrusion proteins (MATEs), and organic cation transporters (OCTs). One such potential biomarker, N1-Methylnicotinamide (1-NMN), has been identified as a possible candidate for renal transporters involving OCT2 and MATEs. Utilizing such endogenous probes, in conjuction with DDI decision tress from the regulatory agencies, can help to discharge DDI risk quickly in Phase I studies.

Methods:
1-NMN is present in native plasma and urine, thus 3-times charcoal stripped surrogate matrix was employed for this assay. Hydrophilic Interaction Chromatography (HILIC) was employed to obtain separation of desired analytes on an Acquity UPLC HILIC Column (1.6 uM, 2.1 x 50 mm) utilizing gradient conditions. Mobile phase A consisted of 20 mM ammonium formate, 0.1% formic acid in 90:10 (water: acetonitrile), and mobile phase B consisted of 20 mM ammonium formate, 0.1% formic acid in 90:10 (acetonitrile : water). Samples were analyzed using tandem mass spectrometry (MS/MS) on a Sciex API6500 and a Waters Acquity i-Class UPLC.

Results:
1-NMN was quantified in positive ion mode using MRM transitions of m/z 137 → 93.9, and a D3-NMN internal standard. A wide dynamic range was established (0.1 – 10,000 ng/mL), and the assay was validated across analysts and various mass spectrometers to evaluate assay reproducibility. Using the described assay, analysis of clinical samples from subjects dosed with a drug candidate identified as an in vitro inhibitor of OCT2, MATE1, and MATE2-k. Changes in AUC values of 1-NMN were observed ranging from 2 to 4 fold in treatment groups relative to placebo dosed groups. Samples were also evaluated utilizing an AUC pooling approach to evaluate proportional NMN changes utilizing a single sample for each subject, decreasing the number of samples from 1000s to 100s.

Conclusions:
The fit-for-purpose validated assay was successfully used to measure 1-NMN levels in human plasma and urine samples. The assay was very simple and reliable with good selectivity and sensitivity, which offers options for earlier characterization and clinical safety projections for OCT2 and MATEs mediated DDIs.

A Bottom-up Approach to Characterize CYP Enzyme-Drug Conjugates

Jingzhou Liu, Sean Han and Thomas Eric Ballard

Purpose:

In drug research and development, minimizing toxicity and DDI becomes a top priority. Mechanism-based inactivation (MBI) of CYP enzymes may lead to DDI and toxicity, therefore identifying the mechanism could aid in the development of safer drug candidates. This study aims to characterize a small molecule-CYP enzyme covalent adduct and describes efforts to identify the binding site.

Methods:

A test compound, raloxifene, was incubated with CYP3A4 enzyme and the modified enzyme was digested with trypsin (and/or a similar enzyme). The digested mixture was further processed and run on a Thermo Orbitrap Fusion LCMSMS system to acquire MS and MS/MS data for peptide mapping. Data were processed with Thermo BioPharma Finder software to identify each unmodified and modified peptide. The potential binding sites can be identified by peptide sequencing based on MS/MS data.

Results:

Raloxifene was used as standard to validate this method and workflow. Over 95% of CYP 3A4 sequence coverage was achieved. A raloxifene adduct on cysteine-239 of CYP 3A4 was identified and confirmed by peptide sequencing.

Conclusions:

A bottom-up method and work-flow was developed to characterize drug-CYP enzyme adducts contributing to our understanding of mechanism-based inhibition. Investigation of additional CYP3A4 inhibitors and examination of other CYP enzymes is ongoing to better understand the versatility and applicability of this approach in drug discovery.

Quantitation of the Monoclonal Antibody Rituximab Using Volumetric Absorptive Microsampling, Impact-Assisted Extraction, Trypsin Digestion and LC-MRM

Jean-Nicholas Mess, Nikolay Youhnovsky, Kevork Mekhssian and Anahita Keyhani
Altasciences, Laval, QC, CANADA

Novel Aspect:

To demonstrate the applicability of volumetric absorptive microsampling for the quantitation of biotherapeutic monoclonal antibodies using a bottom-up LC-MRM approach.

Introduction:

Volumetric absorptive microsampling (VAMs) has emerged as an alternative approach for blood sampling during clinical and preclinical studies. It enables precise and accurate collection of a determined blood volume, therefore reducing the hematocrit effect associated with the dried blood spot (DBS) technique. Nevertheless, the sample hematocrit level can still bias drug measurements by affecting the desorption of the analyte(s) from the VAMs device. The recently established impact-assisted extraction (IAE) approach has proven to overcome such bias for small molecules and peptides, but its applicability to biotherapeutic proteins quantitation has yet to be verified. The usefulness of this approach for the bottom-up LC-MRM quantitation of the monoclonal antibody Rituximab in human blood is herein demonstrated.

Methods:

Human whole blood samples fortified with Rituximab were absorbed onto 20 µL MitraTM microsamplers (Neoteryx) and dried at room temperature in the presence of desiccant for at least 24 hours. The tips were then transferred into a 96-well plate followed by a grinding stainless steel ball and extraction solvent. After aggressive mixing, proteins were reduced, alkylated and digested with trypsin for 2 hours at 37 °C. The resultant peptides were separated on a Waters XBridge Peptide BEH C18 column (50 x 2.1mm, 3.5 µm) using gradient elution with 0.2% acetic acid in water and acetonitrile as mobile phases. The Rituximab-specific peptide GLEWIGAIYPGNGDTSYNQK and corresponding heavy-labeled peptide were monitored by MRM on a SCIEX Triple Quad 6500+.

Results:

In this research, the applicability of VAMs combined with impact-assisted extraction was demonstrated for the quantitation of the monoclonal antibody Rituximab in human blood using a bottom-up LC-MRM approach. Optimization of the extraction conditions included screening of multiple solvent mixtures to achieve optimal desorption of Rituximab from the VAMs sorbent. Protein solubility and compatibility with trypsin digestion were the primary drivers to select the extraction solvent. Optimal Rituximab extraction was obtained using a mixture of ammonium bicarbonate buffer containing 20% acetonitrile and 0.25% octyl beta-glucopyranoside. Desorption of Rituximab from the VAMs sorbent was found to be much slower than for typical small molecules. As such, extraction of Rituximab was greatly improved when the VAMs sorbent remained in solution during the entire sample processing procedure, including reduction with TCEP, alkylation with iodoacetamide and trypsin digestion. This suggests that complete desorption of Rituximab requires prolonged incubation in the extraction solvent at elevated temperatures or that following trypsin digestion, the resultant tryptic peptides are much easier to desorb than the full length native protein. The effect of the blood hematocrit level on Rituximab recovery using impact-assisted extraction was evaluated at 25%, 35%, 45%, 54% and 63%. Rituximab recovery was independent of the hematocrit level and fluctuated between 95.6 and 104.3% across all levels. In contrast, by omitting the impact-assisted extraction step, up to 40% difference in Rituximab recovery were observed between low and high hematocrit levels. A precision and accuracy analytical batch was assayed using the optimal conditions described herein. Intra-day precision of the assay was <5.3% with accuracies between 96.8 and 105.7% for all QCs. Long term stability of Rituximab dried samples for up to one year at room temperature was also demonstrated.

Conclusions:

In this research, VAMs was successfully applied to the analysis of Rituximab in blood. This microsampling technique combined with impact-assisted extraction is suitable for the quantitation of monoclonal antibodies using a generic bottom-up LC-MRM approach.

Optimized Extraction and MFLC-MS/MS Analysis of the Antibody Drug Conjugate SigmaMAb Extracted from Rat Plasma

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Purpose:

Antibody drug conjugates (ADCs) are potent and specific biopharmaceuticals. These proteins are typically analyzed using costly immunoassay methods, which do not have the selectivity to accurately quantify the ADC alone. HPLC-MS/MS analysis of the digested antibody is an alternative approach that provides better selectivity with less method development and cost when compared to immunoassay analysis. Additionally, coupling the mass spectrometer with a microflow LC platform (MFLC) results in a significant increase in the analyte signal. In this abstract we describe an accurate, precise, cost effective extraction and MFLC-MS/MS analysis of the ADC SigmaMAb that can be applied to the extraction and analysis of other ADCs.

Methods:

SigmaMab (Sigma-Aldrich), a commercially available recombinant monoclonal IgG1 human antibody linked to dansyl-fluorophores was analyzed. SILuMAb (Sigma-Aldrich), an isotope labeled antibody arginine (13C6,15N2) was used as the internal standard. SigmaMab was spiked into rat plasma and 50µL was aliquoted into a 96 DWP. Internal standard was then added to the plate followed by acetonitrile precipitation. The plate was vortexed/centrifuged and the supernatant was removed. The pellet was re-suspended and denatured/reduced in RapiGest (Waters) and DTT. The plate was incubated at 60°C for 30 minutes and alkylated with iodoacetamide and digested with porcine trypsin by incubating the plate at 37°C for 2.5 hours. The remaining solution was extracted using an SPE method (strong anion exchange) and analyzed using MFLC-MS/MS. The analysis was performed using an API-6500 mass spectrometer operating in positive ESI mode. The MFLC system was a Waters Acquity M-Class operating with binary gradient methods and a flowrate of 0.050 mL/min. Separation was achieved using a Kinetex Biphenyl column (Phenomenex 5cm x 1.0mm, 1.7µm).

Results:

Sample preparation and instrument parameters were optimized in order to obtain the highest signal for the ADC with optimal chromatographic resolution. For SigmaMab, the most selective peptide sequences were LMIDATK (light chain) for quantitation and ALPAPIEK (heavy chain) for confirmation. The most selective internal standard sequence was YASESMSGIPSR (arginine13C6,15N2). The data indicated that a pellet digested using acetonitrile 0.1% formic acid removed a substantial amount of interfering proteins. Urea, Rapigest, and Octyl-β-d-glucopyranoside were evaluated to provide optimal antibody denaturing. It was found that Rapigest provided superior denaturing to Octyl-β-d-glucopyranoside and is also faster to denature than urea and doesn't require further dilution of the sample to reduce the concentration prior to the trypsin digestion. The digestion time required and the trypsin concentration were also evaluated. The data suggests that the digestion reaches completion after 2.5 hours at 37°C with a trypsin concentration of 0.2 mg/mL. Further clean-up of the sample was evaluated using various SPE phases and it was determined that a simple TCA crash provided the highest recovery of the target peptide. A 16 point calibration curve was extracted and analyzed in duplicate using the optimized methods. The concentration range was 100-10,000 ng/mL. The results of the analysis indicate the method is accurate and precise. Using a 1/X*X linear regression the r value was found to be 0. 9979 with the percent difference of the standard points less than 15% from the nominal concentrations. Using the MFLC system instead of the conventional HPLC (flowrate of 0.700 mL/min) resulted in a >40% increase in peptide signal.

Conclusions:

The extraction and MFLC-MS/MS method developed is accurate and precise and can be used as a starting point for other ADC or large molecule analysis. The extraction method is cost effective since it doesn't require expensive immunocapture products. Additionally the sample preparation can be completed in only a few hours compared to other methods that require overnight digestion.

Quantitation of Potential Lipid Biomarkers, Glycoslyceramide and Glycosylsphingosine, for Parkinson Disease's by Chiral Supercritical Fluid Chromatography-tandem MS

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Purpose:

Glycosylceramide (GlcCer) and its deacylated form glycosylsphingosine (GlcSph) have emerged as potential biomarkers for Parkinson's disease (PD). How ever their isobaric structural isomers, galactosylceramide (GalCer) and galatosylsphingosine (GalSph), may interfere the measurement of these biomarkers. A supercritical fluid chromatography
(SFC)-MS/MS method was developed and optimized for the separation and analysis of GlcCer/GalCer and GlcSph/GalSph in plasma of healthy volunteers and PD patients.

Methods:

An SFC–MS/MS assay for quantitative determination of these potential lipid biomarkers in human plasma using a surrogate matrix approach was developed. GlcCer/GalCer and GlcSph/GalSph were extracted from 100 µL of plasma using a protein precipitation extraction method and the resulting extracts were separated and analyzed by using a Chiralcel OZ3 column (3 x 150 mm). The SFC system was operated with pressurized carbon dioxide with addition of 0.1% ammonium hydroxide in methanol as organic modifier. 13C6-GlcSph and D5-GlcCer were used as internal standards.The standard curves, ranging from 0.1 to 100 ng/mL for all analyzes, were fitted to a 1/x2 weighted quadratic regression model.

Results/Conclusion:

The results showed that SFC-MS/MS can provide high separation efficiency for these isobaric lipid pairs of GlcCer/GalCer and GlcSph/GalSph. SFC is fast (3~5 times faster than LC with reduced column reequilibration time). The total run time for the analysis was 2.5 min. The results showed that the concentration levels of GlcCer and GlcSph in plasma were slightly elevated in the PD patients. Further evaluation of these potential biomarkers in other tissues, such as brain or CSF, could be explored.

Catabolism of peptides using ion mobility enabled high resolution mass spectrometry coupled with Mass-MetaSite data processing

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Purpose:
The pharmaceutical industry is increasingly exploring biotherapeutic molecules as an alternative to conventional small molecule drugs, with the number of biotherapeutic drugs approved each year continuing to outpace that of small molecule compounds. DMPK groups are looking to extend principles and methodologies developed over years of small molecule drug development to the ADME of diverse biotherapeutics. As a consequence, there is active interest in developing the next generation of software tools to help identify and characterize the clearance and metabolic fate of biotherapeutics. Here, we mine ion mobility high resolution mass spectrometric (DIA) data for the analysis of biotherapeutic drug metabolism using the Mass-MetaSite and WebMetabase software platform for processing.

Methods:
14-amino acid analogues of the natural hormone somatostatin where studied. They were incubated in human serum at eleven time points (0, 5 min, 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 30 h and 48 h). Data were collected on an ACQUITY UPLC coupled to a benchtop IMS QToF using HDMSE. HDMSE is a data independent mode of acquisition that provides a straightforward way to both collect all precursors and corresponding fragment ions and resolve them into MS/MS quality spectra. Data were processed using Mass-MetaSite software which is now able to read data directly from UNIFI using the built-in Application Programming Interface (API). The processed data were also uploaded onto the server based application, WebMetabase 3.2.9, where all the samples from the same experiment were analyzed and clustered.

Results:
This study outlines and focuses on the benefits and ease of use of ion mobility for the application of catabolite identification, as well as the use of Mass-MetaSite and WebMetabase for processing these complex data sets. A number of catabolites for the parent peptides were identified over the time course and could be attributed to hydrolysis of the peptide backbone, with the main catabolites identified attributed to hydrolysis of the non cyclic portion of the peptides. Mass-MetaSite and WebMetabase were used to characterize metabolic fate of the somatostatin peptide showing that data can be collected routinely, processed and reviewed efficiently across time courses and treatments. A measure of the ratio of structurally matched to mismatched product ions found by Mass-Metasite provides confidence in catabolite assignment through data acquired by DIA. All peptides found also automatically generated CCS (Collision Cross Section) values to estimate the size of the fragments, help confirm identity across samples in matrix, and enabled high quality DIA spectra to be obtained. Approximately 99 % of the parent was rapidly (<10 min) turned over generating three key catabolites with minor metabolites, possibly attributed to hydrolysis of cyclic potion of the peptide, formed over the incubation time course.

Conclusions:
The use of Ion Mobility DIA approaches (HDMSE) showed an improvement in data quality through alignment of precursor and fragment ions in both RT and DT (drift time) thus leading to improved spectral quality and an increase in identified fragment ions over the number of false positives for several peptides. Samples were processed to identify major hydrolytic cleavages and spectra analyzed with confidence

metrics to provide the user with a high level overview of catabolism. Processing and visualization using Mass-Metasite and WebMetabase using IMS enabled data provided a comprehensive overview of complex catabolic data.

Characterizing KRAS Target Half-life and Target Occupancy Using Stable Isotope Labeling Combined with LC-MS Bioanalysis

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Purpose:

KRAS is a GTP-binding protein linking receptor tyrosine kinase activation to intracellular signaling. The $KRAS^{G12C}$ is a common mutation present in non-small cell lung cancer and pancreatic cancer. Amgen compound 1 is an irreversible inhibitor that covalently binds to cysteine 12 and locks KRAS in an inactive conformation preventing cell growth and division and possibly cancer.

Methods:

Target turnover is measured using SILAC, with incorporation of a C13 amino acid in the peptide containing G12C. Cells are grown initially in heavy media before switching to light media. Both heavy and light peptides are measured, and the relative isotope abundance is calculated which in turn will be used to provide the half-life.
Target occupancy measures both the peptide of interest and the peptide-inhibitor adduct, it is further used to assess the target engagement.

Immunocapture LC-MS Assay used for the measurement involves an initial immunocapture step using streptavidin magnetic beads coupled to biotinylated anti-RAS which captures all forms of RAS (HRAS, NRAS & KRAS). After extensive washes, KRAS is eluted from the beads and reduced/ denatured then digested. The corresponding peptides are then quantified by LC-MS/MS.

Results:

The protein target turn over and drug adduct formation at the cell line level (MIA PaCa-2 and H358) and in mouse tumor xenograft models were measured.
Amgen compound 1 engagement assessment was conducted on multiple PD xenograft studies. The data showed good correlation with the cellular PD (p-ERK) measurement.

Conclusions:

SILAC was used to determine KRAS half-life in multiple cell lines, it is the best in vitro approximation of an in vivo KRAS half-life. Furthermore, measurement of drug-KRAS protein adduction is a direct measurement of target engagement achieved in vivo.

High sensitivity quantification of Salmon Calcitonin from human serum using mixed-mode SPE and LC-MS/MS

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Purpose:

Salmon calcitonin is a cyclic, 32 amino acid synthetic polypeptide, commonly used in the treatment of osteoporosis. The ability to accurately quantify this peptide with high sensitivity and selectivity in support of drug research and development is of significant interest. Usually, cyclic peptides are difficult to analyze using LC-MS, since MS sensitivity is low due to poor transfer of ions into the gas phase and poor fragmentation. Additionally, accurate quantification of calcitonin in serum is particularly challenging, since its pharmacokinetics are characterized by rapid absorption, within 15 minutes, and rapid elimination, with a half-life of <20 minutes, resulting in low pg/mL circulating levels.

Tandem quadrupole instruments are the gold standard for routine LC-MS/MS quantification of small molecules, peptides and digested proteins. However, as the need to quantify intact larger peptides and proteins increases, HRMS instruments are increasingly becoming an attractive orthogonal platform for quantitative laboratories. Previously published LC-MS methods for quantification of salmon calcitonin have involved time-consuming and laborious enzymatic digestion.

Here, we describe a simple method for the quantification of salmon calcitonin from human serum using a fast, selective mixed-mode solid phase extraction (SPE) in a 96-well format, UPLC and tandem quadrupole mass spectrometer to achieve limits of detection of 10 pg/mL and limits of quantification of 25 pg/mL, with QC accuracies within 15 % from 100 µL serum. Additionally, we compare these performance characteristics to a targeted HRMS approach for quantification.

Methods:

Calibrators and quality controls samples (QCs) of salmon calcitonin were prepared using commercially available human serum (25–1500 pg/mL). Spiked samples (100 µL) were precipitated using acetonitrile containing 0.1% formic acid (100 µL) vortexed and centrifuged. The resulting supernatant was diluted with a 4% phosphoric acid solution and extracted on an Oasis WCX µElution plate. LC-MS/MS quantification of the post-extract eluate was performed on a low dispersion ACQUITY UPLC I-Class LC, coupled to a Xevo TQ-XS tandem quadrupole mass spectrometer using positive electrospray ionization (ESI+) and multiple-reaction monitoring mode (MRM). Chromatographic separation was achieved using a sub-2 µm particle Waters CORTECS UPLC C18+ column (2.1 x 50m), at a flow rate of 0.4 mL/min using and a linear gradient with 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Final injection volume was 20 µL. The different acquisition modes available on the HRMS system were evaluated and quantitation was performed using Tof MRM (precursor to precursor) mode.

Results:

In this work, we have developed a complete sample preparation and LC-MS/MS workflow for the sensitive and accurate quantification of salmon calcitonin from human serum. Using analytical scale LC, tandem quadrupole MS, and only 100 µL of extracted serum, a limit of detection of 10 pg/mL was achieved. Using 1/x weighted regression, calibration curves from 25-1500 pg/mL were linear (>0.99), with a mean accuracy across the calibration curve of 100.625 %. For all QC levels, accuracies were between 85-115% with % CVs ≤ 15%, indicating an accurate, precise and reproducible method. For the HRMS system, a LLOQ of 50 pg/mL was achieved and % CV for all calibration curve and QC points was below 10%.

Conclusions:

Use of a simple, yet selective SPE sample preparation strategy (without time-consuming digestion), combined with a fast UPLC analysis (4.5 minutes) and a highly sensitive tandem quadrupole mass spectrometer, enabled a LLOQ of 25 pg/mL with 5x lower sample volumes compared to previously published methods. Additionally, quantitative performance of the HRMS system was comparable to the tandem

quadrupole system, achieving LLOQ of 50 pg/mL.

This fast and simple method removes the need for tedious sample preparation associated with digestion and enables higher sample throughput at a lower cost. For quantitative laboratories looking to diversify their technology platforms, the robust quantitative performance of the HRMS system, in addition to its ability to perform discovery and screening experiments, makes it an attractive compliment to existing TQ instruments.

A multiplex electrochemiluminescence method (ECL) for the analysis of angiogenic markers in monkey plasma

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Purpose:

Angiogenesis is the physiological process through which new blood vessels form using pre-existing vessels. The deregulation of this process is believed to be an important step in malignant transformation and pathological conditions such as diabetic retinopathy, rheumatoid arthritis, and ischemia. The ability to accurately detect and monitor circulating levels of angiogenic markers is of great interest to researchers as they are potential prognostic indicators of disease activity and response to chemotherapy. Since non-human primates are widely used in in-vivo preclinical studies, a method for the simultaneous detection of low levels of these angiogenic markers using small amount of plasma volume is an excellent tool to test new clinical candidates before clinical research can be initiated.

Methods:

A study was undertaken to qualify a multiplex electrochemiluminescence (ECL) immunoassay using the MULTI-ARRAY technology for the quantitation of VEGF-A, VEGF-C, VEGF-D, Tie-2, Flt-1, PlGF, FGF (basic) in Cynomolgus monkey plasma. Standards, quality control samples and monkey plasma matrices were assayed using a human-specific ECL immunoassay kit from Meso Scale Discovery®. Analytical qualification and parameters, including precision, accuracy, parallelism, selectivity, antibody interference, proof of concept and short-term stability were assessed.

Results:

Several EDTA Plasma lots from untreated Cynomolgus monkey were analysed and quantifiable endogenous levels of all markers were observed even though most lots were below the lower limit of detection for VEGF-C and Tie-2. To ensure that the method could detect modulation in the endogenous levels, and in the absence of disease-state non-human primate samples, serum and/or plasma from human patients with gastric cancer, sepsis, and diabetes were evaluated and compared to serum samples from healthy patients. Fold changes of different levels of magnitude were indeed observed for all markers and conditions. Precision and relative accuracy, selectivity and short term stability were successful for all markers even though over- and under-recovery were observed for VEGF-C and Tie-2, respectively. Moreover, parallelism assessment for all analytes was determined and the minimal required dilution of the assay was established at 2-fold. Therefore, only 50µL of plasma to perform duplicate analysis is required. Contradicting data between plasma lots was observed for VEGF-C and Tie-2. An interference test was thus performed which demonstrated the presence of crosstalk between the VEGF-C and Tie-2 detection antibodies.

Conclusions:

The qualification data demonstrated that this ECL immunoassay is suitable for the analysis of VEGF-A, VEGF-D, Flt-1, PlGF, FGF (basic) in monkey plasma from pre-clinical studies. In addition, the results obtained demonstrate that the method would likely be sensitive enough to detect even subtle changes in angiogenic marker profiles in Cynomolgus monkey plasma that may occur due to a test article related effect in a pre-clinical trial. However, the data obtained for VEGF-C and Tie-2 indicates that this assay is unable to accurately detect these analytes.

COMPARISON OF DATA DEPENDENT AND DATA INDEPENDENT ACQUISITION BASED PROTEOMIC APPROACHES FOR QUANTIFICATION OF HEPATIC HUMAN CYTOCHROME P450 ENZYMES

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Purpose:

Data-dependent (DDA) and data-independent acquisition (DIA) are two common modes to acquire global proteomics data using mass spectrometry. Here, we studied the expression of clinically relevant drug metabolism enzymes in human liver homogenate. A correlation analysis was performed on the protein expression determined using total protein approach (TPA) versus relative intensity for DDA and SWATH-MS (Sequential-window analysis of all the theoretical mass spectra), respectively.

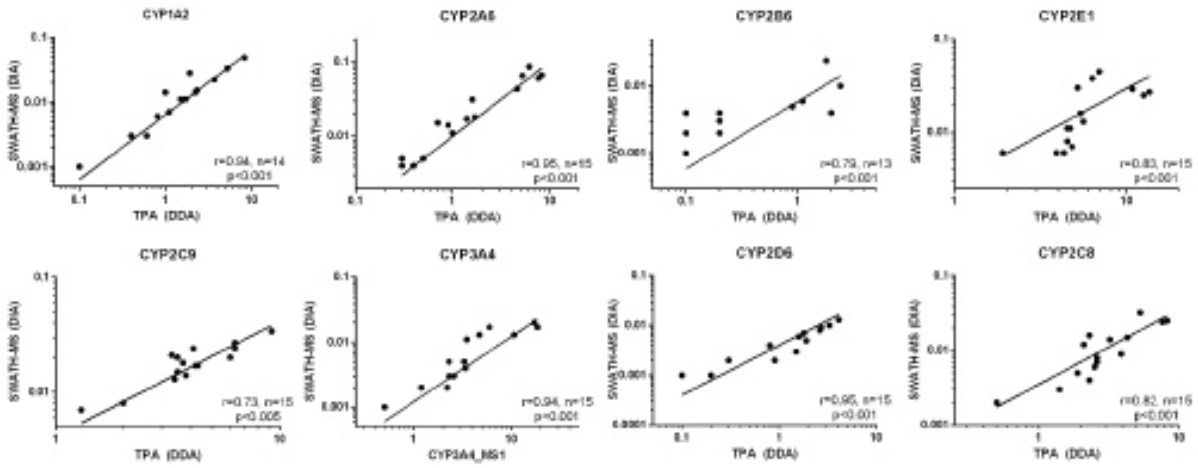
Methods:

Eight clinically relevant cytochrome P450 in whole liver homogenate from 15 human livers were determined using an LC-MS/MS-based label-free relative quantification method. Samples were homogenized in a urea buffer cocktail, and pressure-cycling assisted in-solution digestion was carried out using trypsin. Data acquired in DDA mode was analyzed with MaxQuant and "total protein approach" was used to determine protein concentrations.1 Parallelly, a spectral library was generated from DDA data using Protein Pilot, and intensity of proteins of interest was extracted using Skyline.2 Surrogate peptides were selected as described previously in the literature.3 Bovine serum albumin was spiked to monitor the digestion and instrument variability, and data were normalized before statistical analysis. Non-parametric Spearman correlation analysis was used for the performance of two approaches and correlations with p-value <0.05 were considered significant.

Results:

Collectively, the data suggest a good correlation between the protein intensities from SWATH-MS based experiments and TPA derived protein concentration (Figure 1). The correlation was strong (r>0.90) and significant (p<0.001) for CYP1A2, 2A6, 2D6, and 3A4. While significant, the correlation for CYP2E1, 2B6, 2C8 and 2C9 was moderate (r=0.70-0.90). The discrepancy could be attributed to selection of weak surrogate peptides for these proteins in DIA or an error in a stochastic sampling of precursor ions in DDA. To conclude, SWATH-MS demonstrate an alternative and reasonable global proteomics approach to traditional DDA methods for quantification of proteins.

FIGURE 1:



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The Boston Society

Bioanalytical Regulatory Submission Challenges in INDERAL LA Pivotal Bioequivalence Study

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Purpose:
The objective of this presentation is to describe the challenges and success of the bioanalytical effort for a pivotal bioequivalence (BE) study conducted per the Health Canada (HC) request to support the manufacturing site transfer of INDERAL LA (Propranolol hydrochloride) extended-release capsules and maintain marketing authorization in Canada. Propranolol is extensively metabolized by the liver through 3 primary routes: aromatic hydroxylation (mainly 4-hydroxylation), N-dealkylation followed by further side-chain oxidation, and direct glucuronidation resulting in propranolol, 4-Hydroxy-propranolol and their respective glucuronide conjugated metabolites as the major circulating species. Considering extended release formulation with absorption, distribution and elimination phases overlapped, the total propranolol (measured as sum of free and conjugated forms) was viewed as a representative circulating moiety of the product absorbed. Therefore AUC and Cmax of total propranolol were defined as the primary end points for BE evaluation whereas those for the metabolite total 4-hydroxy propranolol were for informatory purposes only (as outlined in the FDA draft guidance for drug products1). Following submission to HC, Pfizer was requested to re-assess BE based on quantification of free propanol. Thus, in addition to total propranolol, a bioanalytical method for free propranolol was validated and utilized to re-analyze original study samples. Sufficient matrix stability was established from collection to final reanalysis. The method reproducibility was successfully demonstrated using incurred sample reanalysis (ISR).

Methods:
A sensitive and selective HPLC-MS/MS bioanalytical method was validated for the quantification of free propranolol in human plasma containing K3EDTA and sodium metabisulfite for the reanalysis of original clinical study samples. A 50-µL matrix aliquot was fortified with 25 µL of 100 ng/mL propranolol-d7 internal standard working solution. Analytes were isolated through protein precipitation extraction. The final extract was analyzed by electrospray ionization (ESI) liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using positive ionization mode. A linear, 1/concentration^2 weighted, least-squares regression algorithm was used to quantitate the unknown samples. The quality controls (QCs) prepared with glucuronide conjugated propranolol were monitored for free propranolol to assess potential contribution to propranolol as a result of glucuronide hydrolysis. Original QCs prepared with propranolol and with glucuronide to support the initial sample analysis were used to establish long-term stability (LTS) from sample collection to the final reanalysis.

Results:
Calibration curve was linear over the range of 1.00-600 ng/mL. The validation met acceptance criteria with respect to precision, accuracy, sensitivity, selectivity, matrix effects, carryover, dilution, linearity, recovery and tested stability2, 3, 4 and was successfully used to measure free propranolol in clinical samples. Bench top (BT), LTS, and freeze thaw (F/T) stabilities were extended to cover for sample re-analysis. During study sample analysis for free propranolol, the inter-day assay precision, expressed as the inter-run percent coefficients of variation (%CV) of the measured concentrations, was ≤8.34% for the low (2.5 ng/mL), medium low (40 ng/mL), medium high (240 ng/mL), and high (450 ng/mL) QC samples. The inter-day assay accuracy, expressed as percent relative error (%RE) between the nominal and measured concentrations ranged from -2.35% to 1.49% for the low, medium low, medium high, and high QC samples.

Conclusions:
The bioequivalence was demonstrated for both total and free propranolol using fully validated bioanalytical methods. Sample reanalysis for free propranolol followed by the Health Canada approval of new manufacturing and packaging sites (Newbridge and Vega Baja, respectively) eliminated the need to repeat the BE study and ultimately maintain uninterrupted patient access to the only long acting propranolol product in Canada.

IDENTIFICATION OF AN UNEXPECTED CYCLIZED METABOLITE AND EFFORTS TO DELINEATE THE MECHANISM OF FORMATION: EVIDENCE OF A REACTIVE IMINIUM ION

Valerie Kramlinger, Jingzhou Liu, Ben Milgram, Rob Foti

Purpose:

Chronic pain has been estimated to affect over 25 million people in the United States and the search for non-opioid alternatives to treat pain remains an important area of pharmaceutical research. To that end, we synthesized and evaluated a series of sulfamide pyridazine inhibitors of Nav1.7, a genetically validated modulator of chronic pain, using in vitro metabolic assays and in vivo pharmacokinetic studies in preclinical species. Metabolite identification efforts for a lead molecule, Compound A, identified seven metabolites. A major circulating metabolite (Metabolite A1) that was seen in plasma of all preclinical species was estimated to circulate at levels (as high as 1.8-fold of Compound A). In response to these findings, efforts were put toward definitive structural determination of Metabolite A1 and further clarification of the mechanism of Metabolite A1 formation. Because the proposed formation mechanism proceeds through an iminium ion intermediate, additional studies were carried out to assess the reactivity of the proposed intermediate.

Methods:

Preliminary metabolite identification experiments were carried out in liver microsomes and hepatocytes, and eventually in plasma from preclinical species. All samples were analyzed by LC-HRMS/MS. The structure of Metabolite A1 was elucidated through crystallography efforts and confirmed by synthesis and comparison to a synthetic standard. Additional studies aimed at elucidating the mechanism of Metabolite A1 formation were performed using Supersomes containing cytochrome P450 3A4 (CYP 3A4).

Results:

Based on the crystal structure of collected Metabolite A1 and the subsequent synthetic standard, metabolite A1 was proposed to be a product of a cyclization reaction with a mass loss of 2 amu from the parent. Formation of Metabolite A1 was catalyzed by CYP3A4 in an NADPH-dependent manner. Subsequent experiments have shown Metabolite A1 to be reactive towards nucleophilic trapping agents such as potassium cyanide; this is observed both by decreased formation of Metabolite A1 in the presence of potassium cyanide as well as identification of a cyanide adduct by LC-MS/MS. Curiously, when Metabolite A1 was incubated in the presence of CYP3A4 and cyanide, the same cyanide-trapped species was observed. Both Compound A and Metabolite A1 showed time-dependent inhibition of CYP3A4 with a KI of 28 μM and 6.9 μM and a kinact of 0.07 min-1 and 0.03 min-1, respectively.

Conclusions:

The potential for an intermediate in the formation of Metabolite of A1 (and for Metabolite A1 itself) to be reactive towards nucleophilic trapping agents suggests that circulating Metabolite A1 may have the potential to react with endogenous nucleophiles such as cysteine or lysine residues on macromolecules (i.e. proteins). This possibility was of concern due to the body of evidence suggesting that reactive metabolites, including iminium ions, may be involved in idiosyncratic drug reactions. Strategies to reduce the formation of the reactive intermediate by blocking bioactivation were employed and subsequent synthetic efforts identified Compounds B – D with similar potencies and pharmacokinetic properties but without the metabolic liability of Compound A.

An Intact Level Workflow for Sensitive Monoclonal Antibody Monitoring in Preclinical Matrices

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Purpose:

To demonstrate HRMS sensitivity of intact level quantification for three monoclonal antibodies, adalimumab, infliximab, and trastuzumab prepared through affinity purification in mouse plasma.

Methods:

Adalimumab, infliximab, or trastuzumab samples were prepared by spiking formulation solution into mouse plasma. Each mAb was immuno purified from plasma using goat anti-human Fc prepared in a streptavidin coated 96-well plate.1 After elution under acidic conditions, extracted mAb was quantified by UPLC-HRMS. The LC-MS system consists of a binary pump, sample manager, column manager, and a Vion QTof HRMS. The mass range used for data acquisition was 500-4000 m/z, and the system was operated in ESI+ full scan sensitivity mode. A generic gradient was used with a flow rate of 0.2 mL/min. Data was aquired, analyzed and quantified using the UNIFI informatics system.

Results:

Intact level quantification attributes were determined for adalimumab, infliximab, and trastuzumab. The samples were prepared using a streptavidin coated 96 well plate. The streptavidin coated plate offered a cost efficient and automation friendly means of antibody preparation from biological matrix. The quantification limit, amount loaded on the column, linear range, linear dynamic range, and % accuracy were determined. Results show sensitive detection of all three mAb tested. Using 25-50 µL plasma volumes, the detection limit is 0.05 µg/mL, linear dynamic range is from 1.7 to 2.0 and load on column is 250-500 pg. The data show that HRMS can be used for routine and sensitive quantification of mAb in biological matrix.

Conclusions:

HRMS quantification of intact mAbs offers analytical scientists many advantages over traditional approaches. In addition to simpler sample preparation (versus surrogate approaches), structural information at the whole molecule level can provide additional information in understanding the state of mAb in question in biological systems and interpreting quantitative outcomes. In this study, high sensitive detection of adalimumab, infliximab, and trastuzumab in mouse plasma was achieved, with LLOQ of 0.05 µg/mL and ~2 Log linear dynamic range. Results show that the combination of a sensitive mass spectrometer with best practice in sample preparation and data processing parameters are critical in obtaining highest sensitivity.

References:

1. Kellie J., Kehler J., Mencken T.J., Snell R.J., Hottenstein C.S. Bioanalysis 8, 2103 (2016).

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