APPLIED PHARMACEUTICAL ANALYSIS

SEPTEMBER 26-28, 2023

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2023 WORKSHOPS: SEPT 26 - Regulated Bioanalysis | SEPT 27 - Discovery Bioanalysis & New Technologies | SEPT 28 - Mechanistic ADME

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ORGANIZERS' WELCOME

Welcome to the 2023 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.

APA ORGANIZING COMMITTEES

REGULATED BIOANALYSIS

Chair: James Schiller, Merck
Chair-Elect: Joseph Tweed, Bicycle Therapeutics
Committee: Darshana Jani, Moderna; Fumin Li, Biollege Bioanalytical Consulting; Lori Payne, Alturas Analytics; Farhad Sayyarpour, Inotiv; Jenifer Vija, Ajivia, LLC; Yongjun Xue, BMS; Ang Liu, Daiichi Sankyo

DISCOVERY BIOANALYSIS & NEW TECHNOLOGIES

Chair: Christopher Kochansky, Exelixis
Chair-Elect: Hiroshi Sugimoto, Takeda
Committee: Dieter Drexler, BMS; Hongying Gao, Innovo Bioanalysis LLC; Jonathan Josephs, Genentech; Lina Luo, BMS; Jing Tu, Resolian; John Williams, Vertex; Yu Tian, AbbVie; Zachary Parsons, BMS; Jeongsup Shim, Genentech

MECHANISTIC ADME

Chair: Benjamin Johnson, BMS
Chair-Elect: Donglu Zhang, Genentech
Committee: Eric Ballard, Takeda; Nagendra Chemuturi, Eli Lilly; James Driscoll, BMS; Chandra Prakash, Agios Pharmaceuticals; David Stresser, AbbVie; Greg Walker, Pfizer; Cindy Xia, Renegade Therapeutics; Hongbin Yu, Boehringer-Ingelheim

APA 2023 CONFERENCE AGENDA

DAY 1: Tuesday, Sept. 26 | Regulated Bioanalysis Workshop

7:00 - 8:30 AM **Registration, Breakfast, Exhibits**
8:30 - 8:40 AM **Workshop Introduction**

SESSION I: Assessing the Current Landscape of FDA Regulations

8:40 - 8:45 AM **Session Introduction**
Lori Payne, Alturas Analytics & James Schiller, Merck

8:45 - 9:15 AM **Drug-Drug Interaction Assessment for Therapeutic Proteins**
Qin Sun, FDA

9:15 - 9:55 AM **PLENARY: *Merck's Strategy Around Decentralized Trials, Bioanalytical Sampling and Logistics***
Melanie Anderson, Merck

SESSION II: Advances in Sampling Strategies: A Dialogue with Joleen White and Lori Payne

9:55 - 10:25 AM **Patient Centric Sampling in Therapeutic Development Programs**
Joleen White, Bill and Melinda Gates Medical Research Institute

10:25 - 10:40 AM **Space - the Final Microsampling Frontier**
Lori Payne, Alturas

10:40 - 10:55 AM **VENDOR PRESENTATION**
Targeted High-Resolution Mass Spectrometry: Sensitive and Precise Therapeutic Protein Target Quantification in any Tissue
Matt Westfall, Inotiv

10:55 - 11:15 AM **Break & Exhibits**

Session III: Emerging Modalities and New Technologies

11:15 - 11:20 AM **Session Introduction**
Darshana Jani, Moderna & Joe Tweed, Bicycle Therapeutics

11:20 - 11:45 AM **Evaluating a Hybrid LC-MS/MS Approach for the Quantification of siRNA Analytes**
Karan Agrawal, Janssen Research and Development

11:45 - 12:10 PM **Translating Preclinical Findings Into Clinical Biomarker Assays To Support The Phase I/II Study of BT7480, A Bicycle Tumor-Targeted Immune Cell Agonist™**
Heather Cohen, Bicycle Therapeutics

12:10 - 12:35 PM **RT-qPCR Method Development and Validation for mRNA Analysis**
Suresh Paddigari, Moderna

12:35 - 1:55 PM **Lunch, Exhibits & Poster Viewing**

1:55 - 2:15 PM **SPONSOR SHOWCASE**

Session IV: Immunogenicity Applications for Various Drug Modalities

2:15 - 2:20 PM **Session Introduction**
Ang Liu, Daiichi & Fumin Li, Biollege Bioanalytical Consulting

2:20 - 2:45 PM **Qualification for Sars-Cov-2 Neutralization Assay**
Min Zhao, Moderna

2:45 - 3:10 PM **Chasing the Matrix Interference in a Bispecific ADA Assay - Divergent Target Interference Based on a Patient Geography**
Arkadeep Sinha, Upstream Bio

3:10 - 3:35 PM **MAPPs Assay and Key Applications at Novartis**
Anette Karle, Novartis (Switzerland)

3:35 - 3:55 PM **Break & Exhibits**

Session V: Rapid Fire Poster Presentations

moderated by Joseph Tweed, Bicycle Therapeutics

3:55 - 4:00 PM **Session Introduction**

4:00 - 4:45 PM **Poster Presentations**

4:45 - 6:15 PM **Reception, Exhibits & Poster Viewing**

DAY 2: Wednesday, Sept. 27 | Discovery Bioanalysis & New Technologies Workshop

7:30 - 8:30 AM **Registration, Breakfast, Exhibits**
8:30 - 8:40 AM **Workshop Introduction**
Christopher Kochansky, Exelixis

SESSION I: Leveraging Biomarkers & Integrating Multi-Omics for Biomarker Discovery

8:40 - 8:45 AM **Session Introduction**
Jonathan Josephs, Genentech & Zac Parsons, BMS

8:45 - 9:25 AM **PLENARY: *Quantitative Mass Spectrometry-based Multi-omics for Drug Development and Precision Medicine***
Bhagwat Prasad, Washington State University

9:25 - 9:55 AM **Multi-Omics Analysis of Colon Mucosa and Wall from Crohn's Disease Patients**
Liang Jin, AbbVie

9:55 - 10:25 AM **Mass Spectrometry CSF Proteomics and Single-cell Sequencing to Identify Novel Biomarkers in Multiple Sclerosis**
Akshaya Ramesh, Genentech

10:25 - 10:55 AM **Break & Exhibits**

SESSION II: Applications of Precision Medicine in Drug Discovery Research

10:55 - 11:00 AM **Session Introduction**
Hiroshi Sugimoto, Takeda & Yu Tian, AbbVie

11:00 - 11:30 AM **Development of a Targeted, LC-MS Assay to Investigate Collagen Crosslinking Dynamics in IPF Pathogenesis**
Sarah Lloyd, AbbVie

11:30 - 12:00 PM **NanoString-based High Throughput microRNA Biomarker Assay Development**
Saran Ayyadurai, Takeda Development Center Americas Inc.

12:00 - 12:30 PM **ddPCR Technology in Muscular Dystrophy Research to Quantify Human Skipped/Non-Skipped Transcripts**
Brijesh Garg, PepGen

12:30 - 12:40 PM **VENDOR PRESENTATION**
Sensitive Quantitation of Antisense Oligonucleotides in Human Plasma Using Triple Quadrupole & HR Orbitrap Based MS Methods
Hao Yang, Thermo Fisher Scientific

12:40 - 2:10 PM **Lunch, Exhibits & Poster Viewing**

2:10 - 2:20 PM **Rapid Fire Poster Presentations**

SESSION III: Advancing DMPK/Bioanalysis of PROTACs

2:20 - 2:25 PM **Session Introduction**
John Williams, Vertex & Jeongsup Shim, Genentech

2:25 - 2:55 PM **Investigating Permeability for Heterobifunctional Protein Degraders**
Joe Cannon, BMS

2:55 - 3:25 PM **Bioanalytical Strategies for Characterization and PK Assessment of Multi-specific Antibodies**
Yuting Wang, AbbVie

3:25 - 3:55 PM **Break & Exhibits**

3:55 - 4:25 PM **Pharmacokinetic and Pharmacodynamic Modeling of STAT3 Degraders**
Mike Weis, Kymera Therapeutics

SESSION IV: Vendor Session - Latest Advances in LC-MS

4:25 - 4:30 PM **Session Introduction**
Hongying Gao, Innovo Bioanalysis LLC; Christopher Kochansky, Exelixis; Jonathan Josephs, Genentech

4:30 - 5:00 PM **Vendor Presentations: Latest Advancements in LC-MS for Proteomics & BioPharma Discovery**
Matthew Stone, SCIEX; Ismael Zamora, Molecular Discovery, and Jerry Pappas, Thermo

5:00 - 5:05 PM **Closing Remarks**

DAY 3: Thursday, Sept. 28 | Mechanistic ADME Workshop

7:30 - 8:30 AM **Registration, Breakfast, Exhibits**

8:30 - 8:40 AM **Workshop Introduction**
Benjamin Johnson, BMS

SESSION I: ADME of Select Modalities

8:40 - 8:45 AM **Session Introduction**
Nagendra Chemuturi, Eli Lilly & Cindy Xia, Renegade Therapeutics

8:45 - 9:10 AM **Developing Efficacious Preclinical PK/PD Strategies for Bifunctional Targeted Protein Degrader (TPD) Towards Non-Small Cell Lung Cancer (NSCLC)**
Steven Louie, Moderna Therapeutics

9:10 - 9:35 AM **ADME Properties of SOTORASIB, a Covalent Drug for Treatment of KRAS G12C Addicted Tumors**
Upendra Dahal, Amgen

9:35 - 10:00 AM **ADME and DDI Consideration of a Novel LNP Lipid Excipient**
Lei Ci, Moderna

10:00 - 10:25 AM **Break & Exhibits**

10:25 - 10:45 AM **Sponsor Showcase & Rapid Fire Poster Presentation**

SESSION II: Unexpected ADMET Profiles in the Clinic and Investigations into Root Causes

10:45 - 10:50 AM **Session Introduction**
Benjamin Johnson, BMS & Greg Walker, Pfizer

10:50 - 11:15 AM **Characterization of Divergent Metabolic Pathways in Elucidating an Unexpected, Slow-Forming, and Long Half-Life Major Metabolite of Iclepertin**
Tom Chan, Boehringer Ingelheim

11:15 - 11:40 AM **Leveraging ADMET Data to Select a Best in Class LPA1 Antagonist**
Ramola Sane, BMS

11:40 - 12:05 PM **Complex Metabolism of the Novel Neurosteroid, Ganaxolone, in Humans. A Unique Challenge for MIST Assessment**
William Fitch, Stanford University

12:05 - 1:20 PM **Lunch, Exhibits & Poster Viewing**

SESSION III: Applying Cutting-edge ADME Technologies

1:20 - 1:25 PM **Session Introduction**
Donglu Zhang, Genentech & David Stresser, AbbVie

1:25 - 1:30 PM **Plenary Speaker Introduction**

1:30 - 2:10 PM **PLENARY: *The Dynamic Free Fraction: Concept, Methodology, and Use in Clearance Predictions***
Zhengyin Yan, Genentech

2:10 - 2:35 PM **Revealing the Absorption Intricacies of bRo5s and PROTACs Through a Fresh Perspective on Assays and Novel Physiochemical Trends**
Edward Price, AbbVie

2:35 - 3:00 PM **A Novel All Human Hepatic Cell-Based Tri-Culture System for Preclinical Drug Development**
Jessica Weaver & Edward LeCluyse, LifeNet Health

3:00 - 3:25 PM **Mathematical Models to Characterize the Absorption, Distribution, Metabolism, and Excretion of Protein Therapeutics**
Dhaval K. Shah, SUNY Buffalo

3:25 - 3:45 PM **Break & Exhibits**

SESSION IV: Exploring Unconventional Biotransformation Pathways

3:45 - 3:50 PM **Session Introduction**
James Driscoll, BMS & Eric Ballard, Takeda

3:50 - 4:15 PM **Unusual In Vivo Metabolism of Nifurtimox**
Dieter Lang, Bayer AG

4:15 - 4:40 PM **Oxidation-reduction Equilibrium of Thiol-containing Drugs and Metabolites**
Simone Schadt, Roche

4:40 - 4:45 PM **Conference Closing Remarks**

RAPID-FIRE POSTER PRESENTATIONS

REGULATED BIOANALYSIS WORKSHOP

Tuesday, September 26 • 4:00 PM • Auditorium

1. **Bahar Rizi/Eshani Nandita, Sciex:** *Sensitive quantification of the protein targeting chimera (PROTAC) TL 13-112 in rat plasma using an LC-MS/MS workflow*
2. **Nevena Mollova, Gilead:** *Development and validation of a quantitative bioanalytical method for obeldesivir and its major metabolite in plasma*
3. **Papa Makhtar Drame, Keros Therapeutics:** *A Competitive Ligand-binding Assay to Detect Neutralizing Ab to A Multidomain Large Molecule*
4. **Stepanie Pasas Farmer, BioData Solutions:** *New Statistical Approach for CP Determination for Data Sets with Multiple Population Distributions – Use of Deconvolution to Retain All Data Points and Avoid Over-Removal of Data as Outliers*
5. **Matthew Solomon, Merck:** *Overcoming Matrix Interference for an Enzymatic Pharmacokinetic Ligand Binding Assay for Protein X*
6. **Ramakrishna Reddy Voggu, Labcorp:** *Analysis of the Monoclonal Antibody Pembrolizumab in Human Blood Collected via Volumetric Absorptive Microsampling (VAMS) Technology and Utilizing LC-MS/MS Detection*

DISCOVERY BIOANALYSIS WORKSHOP

Wednesday, September 27 • 2:10 PM • Auditorium

1. **Robert Plumb, Waters:** *Prostate Cancer Patient Stratification and Monitoring Using LC/MS/MS-Based Targeted Lipidomics and Discovery Proteomics*
2. **Anahita Keyhani, Altasciences:** *Sensitive Quantitation of Antisense Oligonucleotides Using Capitainer© qDBS Microsampling Device Coupled with Hybridization LC-MS/MS*

MECHANISTIC ADME WORKSHOP

Thursday, September 28 • 10:25 AM • Auditorium

1. **David Stresser, AbbVie:** *Development of Alkaline Phosphatase Prodrug Bioconversion Assays to Support Drug Discovery*
2. **Robert Plumb, Waters:** *Understanding the Pharmacometabodynamics of Gefitinib Using Ion Mobility MS Based Metabolomics and Microsampling*
3. **Shiny Rajan, Javelin Biotech:** *Development of a Novel Milli-fluidic Liver Tissue Platform for Mechanistic Pharmacokinetic (PK) and Drug-Drug Interaction (DDI) studies*

OTHER POSTERS/POSTER VIEWING

(concurrent with the lunch breaks all 3 days & Reception on Sept. 26th)

1. **Jun Sun, Resolian Bioanalytic:** *Drug protein binding assessment using rapid and high-throughput magnetized silica beads method*
2. **Ismael Zamora, Mass Analytica:** *From MS signal analysis to AI based method: Solutions for metabolism data processing automation*
3. **Pin Jiang, Medicilon:** *The Boost of in vitro ADMET, in vivo PK and Bioanalysis for Drug Discovery and Development*
4. **Robert Ross, ThermoFisher Scientific:** *Comprehensive Characterization of tRNA by Ultra High-Performance Liquid Chromatography High-Resolution Accurate Mass Spectrometry*
5. **Chaoyang Dai, Frontage:** *Method Validation of Phospho-Tau217 (pTau-217) Quantitation Using the Quanterix SimoaTM HD-X Platform and Potential Applications*

APA ABSTRACTS

REGULATED BIOANALYSIS WORKSHOP

SESSION I: ASSESSING THE CURRENT LANDSCAPE OF FDA REGULATIONS

Drug-Drug Interaction Assessment for Therapeutic Proteins
Qin Sun, FDA

The presentation will address the following topics related to drug-drug interaction (DDI) assessment for therapeutic proteins (TPs):

➢ Mechanisms related to proinflammatory cytokine
- TP is a proinflammatory cytokine
- TP is a proinflammatory cytokine modulator

➢ Mechanisms unrelated to proinflammatory cytokine

➢ DDI assessments for antibody-drug conjugates

➢ Additional considerations for TP DDI assessments

PLENARY PRESENTATION

Merck's Strategy Around Decentralized Trials, Bioanalytical Sampling & Logistics ?
Melanie Anderson, Merck

The pharmaceutical industry is increasingly using decentralized clinical trial (DCT) approaches to bring the trial to the patient. DCTs can reduce patient burden, increase enrollment diversity, and increase trial speed by disrupting the site-based paradigm which relies heavily on geographic co-location with brick-and-mortar clinical sites. The value of these approaches to enable remote trial conduct was clear during the COVID-19 pandemic when over 2000 clinical trials were stopped due to pandemic related travel restrictions. A key enabling technology for DCTs is patient centric sampling (PCS). PCS can reduce collection volumes, decrease pain associated with blood sampling, and importantly enable remote collection of samples. We cannot move away from site-based clinical trials without considering how the biological samples, used to establish safety and efficacy, will be collected. Patient centric sampling can decrease patient burden and increase trial efficiency with additional sampling time points. This data can be critical for establishing pharmacokinetic-pharmacodynamic relationships in drug development and is increasingly critical for precision medicine. Although there are many examples of successful implementation in clinical research, widespread PCS adoption has been limited. Within the IQ PCS working group, there were several case studies of Sponsors employing patient centric sampling to support clinical trials and enable the development of new COVID-19 therapeutics. This illustrates that these approaches are mature and can be successfully implemented in every phase of clinical development. Clinical researchers should consider when PCS is appropriate to decrease patient burden and to capture critical data points that may not coincide with scheduled clinical site visits.

Merck has a rich history in patient centric sampling using traditional dried blood spot sampling and novel blood collection devices in over 40 clinical trials to obtain PK, PD, and translational biology data. Building a strategy for operationalizing PCS is a key component of success. In this presentation, we will discuss the value of DCTs and PCS, high light implementation considerations, and share best practices from internal case studies.

SESSION II: ADVANCES IN SAMPLING STRATEGIES: A DIALOGUE WITH JOLEEN WHITE AND LORI PAYNE

Patient Centric Sampling in Therapeutic Development Programs
Joleen T. White, Bill & Melinda Gates Medical Research Institute

- What does patient centric even mean in the context of bioanalytical science?
- Why should I consider pivoting from traditional approaches?
- How have the technology and science evolved over the past 20 years?
- A case study for application to a monoclonal antibody clinical development program

Space – The Next Frontier of Microsampling
Lori Payne, Alturas Analytics, Inc.

Microsampling in space? Yes, this is the next frontier. If science fiction writers are right, humans will soon be living in space. Turns

out there is a dearth of actual data on the effect of microgravity on pharmacokinetic (PK) parameters although it is known that microgravity affects the body's fluid dynamics, intestinal motility, and cardiovascular activity in space. It is important to know how drugs used to treat nausea, headaches, pain, insomnia, and so on work on space travelers and how microgravity PK differs from terrestrial PK. Some data are available from rats and tissue chips in space and studies in simulated microgravity conducted on Earth, but very little actual data compare subjects' PK on Earth to that in Space. That is about to change. Pre-flight blood samples were collected on Neoteryx Mitra® microsampling devices by Trajan from astronauts who will be flying on an upcoming mission. Crew members were orally dosed with 500 mg acetaminophen and then sampled at different time points. The samples were analyzed via LC-MS/MS and PK parameters have been calculated. Those data will be presented along with planned next steps. Learn some new out-of-this-world terms and exciting plans that promise to change our knowledge about PK in space.

VENDOR PRESENTATION

Targeted High-Resolution Mass Spectrometry: Sensitive and Precise Therapeutic Protein Target Quantification in any Tissue

Matt Westfall, Inotiv

Our Target Sufficiency® platform is built on proteomic targeted mass spectrometry (MS), which provides precise, sensitive, and specific measurements and is the gold standard for protein quantitative analysis. Analyses are multiplexed, which enable simultaneous measurements of dozens of proteins per sample. Molar scale measurements by targeted MS enable analysis of the abundance ratios of key system proteins. Inotiv has developed over 200 targeted protein assays and can rapidly configure a new assay for any protein.

SESSION III: EMERGING MODALITIES AND NEW TECHNOLOGIES

Evaluating a Hybrid LC-MS/MS Approach for the Quantification of siRNA Analytes

Karan Agrawal, Janssen Research and Development

Oligonucleotide therapeutics such as anti-sense oligonucleotides (ASOs) and short interfering RNAs (siRNAs) have emerged as a significant therapeutic modality owing to their capability to modulate drug targets that are not tractable by traditional small molecules or protein drugs. However, oligonucleotides present unique bioanalytical challenges due to their complex structure, high molecular weight, and heavily charged nature. Recently, two independent publications demonstrated a hybrid LC-MS/MS approach for ASO therapeutics, where the analyte is isolated from the biological matrix by binding to a magnetic bead-immobilized complement strand probe. This hybrid approach combines the sensitivity benefits of hybrid ELISA with the specificity benefits of LC-MS. We have since adapted this approach for siRNA therapeutics. Unlike ASOs, siRNAs are a duplex of a non-bioactive sense strand and a bioactive anti-sense strand, and the binding of target anti-sense strand to the probe could be compromised by presence of sense strand. In this presentation, we discuss the workflow that led to a successful method development and qualification for siRNA therapeutics using a hybrid LC-MS/MS approach. We discuss the various options available for probes, the optimization of matrix-to-probe ratio to maximize anti-sense strand capture while minimizing probe self-hybridization, the impact of temperature on siRNA denaturing and the integrity of the biotin-streptavidin bond used to immobilize the probe to the magnetic beads, and a method qualification approach. We also discuss the impact of using internal standards either during or after extraction on assay accuracy and precision. We further evaluate higher aliquot volumes and more advanced analytical instrumentation to determine if additional analytical sensitivity can be gained in order to achieve comparability to qPCR methodologies, and evaluate biological samples by SPE-LC-MS/MS, hybrid LC-MS/MS and qPCR to compare each method. Overall, we will present a tool that can be used in future bioanalytical studies to develop effective hybrid LC-MS/MS methods for siRNA oligonucleotide therapeutics of interest.

Translating Preclinical Findings Into Clinical Biomarker Assays To Support The Phase I/II Study of BT7480, A Bicycle Tumor-Targeted Immune Cell Agonist™

Heather Cohen, Bicycle Therapeutics

- BT7480 is a Bicycle TICA™ that binds both CD137 on immune cells and Nectin-4 on cancer cells to deliver a potent anti-tumor immune signal in Nectin-4 expressing tumors
- Sharing a case study relating to the development of fit-for-purpose assays to monitor predictive and pharmacodynamic biomarkers in the clinic including a custom multiplexed immunofluorescence assay using a proprietary Nectin-4 mAb and MultiOmyx technology to quantify the presence of CD137 and Nectin-4+ cells in human tumors, and a custom CD137 receptor occupancy assay to monitor target engagement of drug in human blood.
- Preclinical validation studies to assess biomarker utility to advance clinical biomarker strategies to be shared including ability to monitor immune changes in tumor and blood following BT7480 dosing in mice

SESSION IV: IMMUNOGENICITY APPLICATIONS FOR VARIOUS DRUG MODALITIES

Qualification for Sars-Cov-2 Neutralization Assay

Min Zhao, Moderna

This study focused on the qualification of three pseudotype virus neutralization assays (PsVNAs) to quantify neutralizing antibodies against SARS-Cov-2 variants virus (D614G, Omicron BA.1 or Omicron BA4/5) in human serum. These assays will be used to support clinical sample testing for mRNA-1083-P101 and mRNA-1230-P101. The qualification parameters included the determination of quantitation range (from LLOQ to ULOQ), the specificity of each assay in human serum, robustness and sample stability. The study revealed that samples remained through up to 6 freeze-thaw cycles. Additionally, heat inactivated samples could be stored for up to seven days at 5 ± 3 °C, and serially diluted samples in infection medium could be stored at -80 °C for up to 7 days. These findings offer valuable insights for the handling and storage of samples, contributing to the efficiency and reliability of clinical sample analysis in vaccine development.

Chasing the Matrix Interference in a Bispecific ADA Assay – Divergent Target Interference based on Patient Geography

Arkadeep Sinha, Upstream Bio

Multimeric targets are known to be problematic with ADA assessments, frequently causing false positives by forming a bridge between labeled drugs. However, during the development and validation of a bridging ADA assay for a bispecific molecule against a multimeric target, no target interference was observed at levels higher than the physiological level. Additionally, the distribution of assay signals from commercially available treatment-naïve diseased individual (TNDI) matrices tested during method development did not show any significant variability or selectivity issues hinting towards any potential matrix interference. However, during assay validation, high variability in signal responses from commercially available TNDI matrices was observed, resulting in the validation cut point being over two-fold higher than the one determined during method development, increasing the probability of underestimating ADA-positive individuals. This case study chronicles the identification of the interfering molecule and steps taken for its resolution.

MAPPs Assay and Key Applications at Novartis

Anette Karle, Novartis

Evaluating the immunogenicity potential of biotherapeutics is becoming increasingly important and challenging at the same time due to novel emerging formats and modalities. MAPPs (MHC-associated peptide proteomics) is one of the assays best characterized regarding its value for immunogenicity potential assessment. The presentation will provide an overview over how MAPPs assays can be applied to support the selection of biotherapeutic candidates, to understand mechanistic root causes of immunogenicity, and to evaluate the impact of aggregation and immune complex formation on immunogenicity.

DISCOVERY BIOANALYSIS & NEW TECHNOLOGIES WORKSHOP

SESSION I: LEVERAGING BIOMARKERS & INTEGRATING MULTI-OMICS FOR BIOMARKER DISCOVERY

PLENARY PRESENTATION

Quantitative Mass Spectrometry-based Multi-omics for Drug Development and Precision Medicine

Bhagwat Prasad, Washington State University

Drug metabolizing enzymes and transporters are critical determinants of drug absorption, metabolism, distribution, and elimination (ADME) and influence drug-drug interactions (DDIs) and response. While significant progress has been made to utilize in vitro models to predict drug ADME using physiologically-based pharmacokinetic (PBPK) models, these models require comprehensive physiological data on inter-individual variability. In particular, PBPK models require quantitative information on the levels and activity of individual pathways involved in drug disposition across different tissues and populations (healthy vs. diseased or children vs. adults). A significant lack of quantitative knowledge regarding non-Cytochrome P450 (non-CYP) enzymes and transporters in human is the major limitation towards building PBPK models in the drug development which often results inaccurate in vitro to in vivo extrapolation (IVIVE) and poor prediction of interindividual variability of drug metabolism. Although non-CYP enzymes are expressed in multiple human tissues, differential tissue expression and

interindividual variability in the expression of these enzymes are not well studied. Uncharacterized sub-cellular localization of some non-CYP enzymes is another knowledge gap with respect to the development of quantitatively viable in vitro and in silico models. Similarly, animal to human scaling of non-CYP metabolism is not accurate because of the unknown inter-species differences. To address these issues, we utilize state-of-the-art quantitative proteomics in conjunction with metabolomics and genomics approaches to characterize abundances and activity of drug metabolizing enzymes and transporters in human tissues and biofluids. These data are then integrated into PBPK models to predict variability in drug clearance and DDIs, particularly in underrepresented populations such as children in whom clinical studies are not routinely performed. This presentation will include case studies on the applications of quantitative proteomics and metabolomics in drug development and precision medicine.r

Multi-omics Analysis of Colon Mucosa and Wall from Crohn's Disease Patients

Liang Jin, AbbVie

Crohn's Disease (CD) is the most common type of Inflammatory Bowel Disease (IBD), a class of chronic diseases of the gastrointestinal tract with unknown etiology. The diagnosis, evaluation, and prognosis of Crohn's Disease remain challenging, where novel biomarkers and targets are highly desired. Omics technologies, such as transcriptomics and proteomics, have been commonly used to study bulk colon biopsies. However, the analysis using bulk tissue undermines the molecular signals, because the mucosal layer and the muscular layer of the colon are physiologically distinct. In this study, transcriptomic and proteomic analysis were performed for mucosa and wall layers separated from the same colon biopsies based on histological staining to achieve better molecular resolution. Several strategies were used to integrate the multi-omics data. First, correlations between RNA and protein were analyzed based on the intensities across all samples and effect sizes between CD inflamed and normal samples. Second, the dysregulated biological functions related to CD were compared between transcriptomics and proteomics. Third, a proteogenomic approach was conducted to enable large-scale detection and quantitation of splicing isoforms at the protein level. Fourth, a machine learning based approach was applied for multi-omics feature selection and biomarker identification. Our results suggested strong correlation between transcriptomics and proteomics based on biological functions, and moderate correlation based on abundance and effect size. Differential isoform abundance was observed between different compartments of colon, which may uncover new disease mechanisms. In addition, the features selected from the multi-omics data robustly classify the CD samples. These features can potentially be used as CD biomarkers.

Mass Spectrometry CSF Proteomics and Single-cell Sequencing to Identify Novel Biomarkers in Multiple Sclerosis

Akshaya Ramesh, Genentech

Background: Proteomic analysis of cerebrospinal fluid (CSF) may help identify novel markers of progressive multiple sclerosis (MS) biology and further elucidate mechanisms of action of ocrelizumab (OCR).

Methods: OBOE is a prospective, multicenter, open-label trial in 131 patients with MS. Clinical and MRI CSF measures were examined. CSF proteins from all pts were analyzed using Data Independent Acquisition nano-liquid chromatography tandem mass spectrometry. Baseline associations in the cohort were analyzed by Spearman correlation analysis. Comparisons of pre vs post treatment CSF protein levels were performed by paired Mann-Whitney U test. Cell-specific gene expression was inferred using public MS brain single-nuclei RNAseq data.

Results and conclusions: We identified a total of 1583 CSF proteins across 245 samples from 131 pts. In the combined cohort, 42 proteins correlated with MS relapsing biology, 206 with disease burden and 7 with chronic progressive biology. Proteins correlating with greater disease burden were enriched for brain vascular proteins; while those correlating with lower burden were enriched for oligodendrocyte and neuron-related proteins. Proteins correlating with greater progression primarily reflected astrocyte-related proteins. At post-treatment Week 52, 46 proteins were significantly reduced in pts with MS, and primarily included immunonoglobulin proteins. Validation with targeted assays and independent cohorts would support real-world utility of these findings.

SESSION II: APPLICATIONS OF PRECISION MEDICINE IN DRUG DISCOVERY RESEARCH

Development of a Targeted, LC-MS Assay to Investigate Collagen Crosslinking Dynamics in IPF Pathogenesis

Sarah Lloyd, AbbVie

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fibrotic disease with a 3-year median survival from diagnosis. With high mortality and very few treatment options, there is an obvious unmet need in medical care for IPF. Changes to the extracellular matrix (ECM) are key features and important drivers of this disease. While several groups have made great progress in matrisome and ECM proteomics,

these technologies are still limited in revealing the crosslinking nature of ECM proteins, especially collagen. Thus, we established an improved, targeted LC-MS assay to evaluate crosslinked amino acids. We measured the collagen crosslinking modifications, lysinenorleucine (LNL), hydroxylysylnoreleucine (HLNL), dihydroxylysylnoreleucine (DHLNL), pyridinoline (Pyr), and deoxypyridinoline (DPyr). Typical collagen crosslinking analysis procedure involves extensive washing steps prior to acid hydrolysis and a final solid phase extraction (SPE) purification process before sample analysis. To increase efficiency, we developed a simplified sample preparation procedure that reduced the initial processing steps and eliminated the SPE process. Analysis time was reduced to less than two minutes per sample which provides the potential for high sample throughput and greater flexibility with respect to instrument usage. Using this advanced methodology, we assessed collagen crosslinking in human IPF lung tissue compared to healthy lung. We showed an increase in immature collagen crosslinks, especially DHLNL, in IPF tissue. In addition, preliminary evidence suggests that DHLNL is increased in the bleomycin fibrotic mouse model and this increase is nullified by treatment with the anti-fibrotic compound SM16. Thus, in addition to significant methodology advancements, we have shown the biological relevance of evaluating crosslinking. While this tool has proven to be efficient and applicable, it is still limited in that it assesses crosslinking after complete amino acid breakdown and cannot identify protein of origin or position of crosslinks. Future work will be directed toward advancing proteomics technology for analysis of natural crosslinked proteins.

NanoString-based High Throughput MicroRNA Biomarker Assay Development

Saran Ayyadurai, Takeda Pharmaceuticals

Biomarkers help track disease progression, predict outcomes and treatment effects. MicroRNAs have emerged as a potential biomarker for many diseases, but detecting and identifying them from clinical samples is challenging. Herein, we developed a high-throughput NanoString nCounter miRNA assay combined with an automated KingFisher magnetic particle processor-based small RNA extraction step. This method allows us to detect circulating miRNAs from clinical serum samples of healthy volunteers and individuals with diseases. The NanoString nCounter Analysis System uses a unique molecular barcode to measure up to 800 miRNA gene expression from a single sample. This method is robust, semi-automated, and reliable in detecting multiple serum miRNA biomarkers precisely and requires minimal hands-on processing time and a small sample volume. This method can be a proof of concept for developing high-throughput miRNA biomarker candidate detection for preclinical and clinical biomarker discovery.

Application of Droplet Digital PCR (ddPCR) Technology in Muscular Dystrophy Research to Quantify Human Skipped and Non-skipped Transcripts

Brijesh Garg, PepGen

Background: Exon skipping using antisense oligonucleotides (ASOs) has recently emerged as one of the most promising technologies to address mutations in genes associated with muscular dystrophies including Duchenne muscular dystrophy (DMD). Unconjugated oligonucleotides are not readily distributed to muscle and are not efficiently taken up into cells or into the nucleus. PepGen's enhanced delivery oligonucleotide (EDO) cell-penetrating peptide technology is engineered to optimize tissue delivery and cellular uptake of therapeutic oligonucleotides, to induce exon skipping for open reading frame restoration and to produce shortened, yet a functional form of the protein.

Purpose: to characterize pharmacological effects of the desired ASO molecules, appropriate PCR methods are required to quantify skipped and non-skipped transcripts. Both, conventional RT-PCR and droplet digital PCR (ddPCR) assays, can be used to detect desired skipped/non-skipped transcripts. Here, we demonstrate the development and qualification of research-grade fit-for-purpose ddPCR assays to quantitate % exon skipping.

Results/findings: ddPCR assays were designed, developed, and qualified, to detect and quantify exon skipped and non-skipped transcript levels. Qualification parameters included analytical range, precision, reproducibility, robustness, specificity, selectivity, dilutional linearity, matrix interference, and recovery. All the qualification parameters passed the previously established acceptance criteria including% exon skipping levels. Synthetic constructs, wild-type cell-lines, and healthy volunteer skeletal muscle tissue samples were used to successfully develop fit-for-purpose skipped and non-skipped ddPCR assays.

Conclusions: the development and qualification of the ddPCR assays simplifies precise quantitation of skipped and non-skipped transcripts. A good dynamic range (5000-0.5 copies/µL) for both skip and non-skip assays was established along with% skip signal (1 0%-0.1%). No matrix interference was observed and spiking of select concentrations of synthetic constructs showed expected recovery.

VENDOR PRESENTATION

Sensitive Quantitation of Antisense Oligonucleotides in Human Plasma Using Triple Quadrupole & HR Orbitrap Based MS Methods

Hao Yang, Thermo Fisher Scientific

Over the last decade, antisense oligonucleotides (ASOs) based therapeutics have gained tremendous interests due to their abilities to regulate gene translation through complementary hybridization to their mRNA targets. Since the first approval of Fomivirsen in 1998, nine more ASO-based drugs have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and 38 more are currently undergoing phase III clinical trials. Given the number of drug candidates entering clinical trials, there have been an increased demand for highly sensitive and robust quantitative bioanalytical methods to evaluate their pharmacokinetic and metabolic profiles. In this talk, we will showcase the quantitative performances of Thermo Scientific™ TSQ Altis™ Plus and Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometers for the analysis of ASOs in human plasma matrices.

SESSION III: ADVANCING DMPK/BIOANALYSIS OF PROTACS

Investigating Permeability for Heterobifunctional Protein Degraders

Joe R. Cannon, BMS

Heterobifunctional protein degraders have the potential to make huge strides in the pharmaceutical industry via targeting the 'undruggable' proteome, but they have their own specific challenges that must be overcome first. This modality is characterized by high molecular weight, very low unbound fractions in plasma, and general lack of compatibility with many in vitro assay formats that historically work well with canonical small molecules. To this end, renewed efforts are required to modify existing or create new assays that provide high quality and decisional data for triaging compounds from this promising modality for progression through tiered assay funnels. Presented is a brief overview of preliminary efforts to extract more informative results from permeability assays for heterobifunctional protein degraders.

Bioanalytical Strategies for Characterization and PK Assessment of Multi-specific Antibodies

Yuting Wang, AbbVie

Multi-specific antibodies have become rising stars in the field of biotherapeutics in recent years. They are engineered to bind either two or more targets or two or more epitopes on the same target. Compared to traditional monoclonal antibodies, multi-specifics offer superior functional advantages due to their enhanced specificity and targeting ability. However, their diverse structural variations combined with a complex mechanism of action (MoA) increase both physical and chemical liabilities thereby raising significant bioanalytical challenges at various stages of drug development. Here we will share bioanalytical strategies for characterization and in vivo PK assessment of these multi-specifics using a variety of LCMS-based assays. Further, considerations towards assay platform selection and challenges in assay development will also be discussed.

Pharmacokinetic and Pharmacodynamic Modeling of STAT3 Degraders

Michael Weis, Kymera Therapeutics

Signal Transducer and Activator of Transcription 3 (STAT3) plays an important role in the transduction of signals from growth factors and cytokines in both normal and malignant cells. Aberrant activation of STAT3 has been observed in many cancers including lymphomas and leukemias through activating mutations. STAT3 has been historically considered "undruggable". Heterobifunctional degraders that recruit endogenous ligases to ubiquitinate substrate proteins leading to their degradation by the proteosome represent a promising novel therapeutic modality with great potentials to drug undrugged protein targets. Herein we will discuss discovery of potent and selective STAT3 degraders, with an emphasis of Modeling and Simulation contributions along the discovery/pre-clinical development continuum. These efforts have culminated in the entry of Kymera's KT-333 into the clinic for the treatment of hematologic malignancies and solid tumors

MECHANISTIC ADME WORKSHOP

SESSION I: ADME OF SELECT MODALITIES

Developing Efficacious Preclinical PK/PD strategies for Bifunctional Targeted Protein Degrader (TPD) Towards Non-Small Cell Lung Cancer (NSCLC)

Steven W. Louie, Moderna

Targeted protein degraders (TPDs) or proteolysis-targeting chimera (PROTAC) molecules have been in research development for over 20 years. Recent transition of this newer therapeutic modality from academia to the clinic has generated a lot of excitement and momentum in this field. The ability of TPDs to recruit the cell's ubiquitin-proteosome system to degrade proteins of interest that were formerly identified as "undruggable" targets has highlighted the therapeutic potential of this modality. Undruggable targets include transcription factors, protein targets with mutations or shallow binding pockets, or any target having poor affinity or access by traditional small molecules. This presentation will provide a brief overview and introduction on the mechanism of action by heterobifunctional protein degraders; followed by the preclinical ADME challenges and potential strategies to overcoming these challenges. Current orally bioavailable molecules in clinical Phase I and Phase II will be highlighted on their current successes and how these successes may apply to current discovery and development molecules.

ADME Properties of Sotorasib, A Covalent Drug for Treatment of KRAS G12C Addicted Tumors

Upendra P. Dahal, Amgen

Targeted covalent inhibitor drugs are regaining attraction as a SM drug modality that can be applied to protein targets which are even previously considered undruggable (for example KRAS). KRAS is a mediator of intracellular signaling required for cell growth, proliferation and survival. One mutation in KRAS of particular interest is G12C, which is present in many solid cancers but not in normal tissues. Sotorasib is a firstinclass KRAS G12C inhibitor that binds irreversibly to the P2 pocket through a novel interaction with a cryptic groove at histidine 95 and a precise covalent interaction with cysteine 12, imparting enhanced selectivity for KRAS G12C. This presentation will examine the challenges and learnings for the development of covalent inhibitors through characterization of the absorption, distribution, metabolism and excretion (ADME) properties of sotorasib.

SESSION II: UNEXPECTED ADMET PROFILES IN THE CLINIC AND INVESTIGATIONS INTO ROOT CAUSES

Characterization of Divergent Metabolic Pathways in Elucidating an Unexpected, Slow-Forming, and Long Half-Life Major Metabolite of Iclepertin

Tom Chan, Boehringer Ingelheim

Iclepertin is a glycine transporter inhibitor in Phase III development for the treatment of cognitive impairment associated with schizophrenia. Early metabolite identification studies associated with the first in human studies identified a single major circulating metabolite M530a, which was anticipated from preclinical studies. However, a second major circulating metabolite, M232, was uncovered only after multiple dose studies. Surprisingly, at steady state exposures, M232 was the predominant circulating metabolite with exposure values higher than both M530a and iclepertin. In vitro studies that were conducted to delineate the metabolism of iclepertin revealed that M232 and M530 are secondary metabolites formed from two independent pathways diverging from the initial oxidation of iclepertin. In the pathway leading to M232, markedly slow formation of an intermediate metabolite, M526, resulted in M232 levels that were difficult to detect in preclinical and single dose studies. However, the very long elimination half-life of M232 (243 h) resulted in extensive accumulation of this metabolite following repeated dosing. This contrasts with a more typical formation rate of M530a of which was limited by the initial rate of iclepertin oxidation and possessed an elimination half-life similar to parent iclepertin (~40 h). This case study emphasizes the potential of missing slow forming and low clearance metabolites based on data from preclinical or single dose clinical studies and how in special cases such as this, a prudent, staged approach to metabolite identification, can help identify these metabolites prior to large-scale clinical trials.

Complex Metabolism of the Novel Neurosteroid, Ganaxolone, in Humans. A Unique Challenge for MIST Assessment

William Fitch, Stanford University

The human pharmacokinetics, metabolism, and excretion of [14C]-ganaxolone (GNX) were characterized in healthy male subjects (n = 8) following a single 300 mg (150 µCi) oral dose. GNX exhibited a short half-life of 4 h in plasma, while total radioactivity had a half-life of 413 h indicating extensive metabolism to long-lived metabolites. Identification of the major GNX circulating metabolites required extensive isolation and purification for LC-MS/MS analysis, together with in vitro studies, NMR spectroscopy and synthetic chemistry

support. This revealed that the major routes of GNX metabolism involved hydroxylation at the 16α-hydroxy position, stereoselective reduction of the 20-ketone to afford the corresponding 20α-hydroxysterol, and sulfation of the 3α-hydroxy group. This latter reaction yielded an unstable tertiary sulfate, which eliminated the elements of H2SO4 to introduce a double bond in the A ring. A combination of these pathways, together with oxidation of the 3β-methyl substituent to a carboxylic acid and sulfation at the 20α position, led to the major circulating metabolites in plasma, termed M2 and M17, accounting for 20.5% and 14.7%, respectively, of the drug-related material in plasma up to 720 h postdose. These studies, which led to the complete or partial identification of no less than 59 metabolites of GNX, demonstrated the high complexity of the metabolic fate of this drug in humans, and demonstrated that the major circulating products in plasma can result from multiple sequential processes that may not be easily replicated in animals or with animal or human in vitro systems.

PLENARY PRESENTATION

Dynamic Free Fraction: Concept, Methodology and Use in Clearance Prediction

Zhengyin Yan, Genentech

Fraction of unbound drug (f_u), a measure of drug binding extent in plasma, is commonly used for predicting hepatic clearance, which often leads to systematic under predictions especially for highly bound drugs. We introduce the "dynamic free fraction" (f_D) as a new binding parameter describing drug protein binding kinetics that can be determined experimentally by coupling the drug binding assay with a reporter enzyme in combination with high-resolution mass spectrometry. This methodology circumvents a long-standing challenge inherent in existing methods for determining drug binding kinetics constants such as kon and koff, and enables assessment of the impact of protein binding kinetics on pharmacokinetic properties of drugs. With a large group of diverse drugs, we demonstrated that the well-stirred model incorporating with f_D correctly predicted both hepatic clearance and liver extraction ratio without systematic bias, which is markedly better than those predicted with f_u. The results suggest that dynamic free fraction (f_D) as a measure of protein binding kinetics is a key determinant in hepatic clearance, which is contrary to the currently held view.

SESSION III: APPLYING CUTTING-EDGE ADME TECHNOLOGIES

Revealing the Absorption Intricacies of bRo5s and PROTACs through a Fresh Perspective on Assays and Novel Physicochemical Trends

Edward Price, AbbVie

Understanding absorption is pivotal in early drug development, especially with the shift towards "beyond rule of 5" (bRo5) drugs. While traditional methods offer insight, they may not fully capture the intricacies of these complex compounds. We built on previous findings and evaluated descriptors like Experimental Polar Surface Area (EPSA) and the AbbVie multiparametric score (AB-MPS) against an extensive human and rodent dataset. This led to the discovery of new benchmarks, patterns, and a novel descriptor, ETR (the Ratio of EPSA to TPSA). Our findings reveal specialized strategies for effective bRo5 and PROTAC drug design and absorption prediction. This research aims to guide scientists in the intricate bRo5 landscape and advance in vitro approaches for optimal absorption prediction.

A Novel All Human Hepatic Cell-Based Tri-Culture System for Preclinical Drug Development

Jessica Weaver & Edward L. LeCluyse, LifeNet Health

Finding a reliable, long-term, and convenient human-relevant culture platform for primary human hepatocytes (PHHs) for routine pharmacological and toxicological studies remains a challenge. Current in vitro model platforms are often inconvenient and complex, lack phenotypic stability over time, and do not support a wide range of PHH lots, lacking experimental reliability and flexibility. Recent technical advances in multicell system optimization enabled the development of a novel in vitro co-culture platform for drug metabolism and toxicity applications. TruVivo™, an all-human 2D+ hepatic system (TV2D+), provides human-relevant, reliable results in an easy-to-use format. In this presentation, a description of the basic triculture platform will be provided, including the proper handling and setup of the kit components, as well as the maintenance of TruVivo (TV2D+) over time periods out to 2 or more weeks of culture. This platform takes advantage of the simplicity of standard 2-dimensional culture techniques and equipment, while maintaining the longevity and phenotypic stability over time that typically accompany more complex 3-dimensional systems. Typical results will be shown for key cellular features and functional performance, such as attachment and percent plateability in TV2D+ as a function of PHH seeding density, as well as stable functionality for at least two weeks in culture. When

established properly, the PHHs in TV2D+ organize into hepatocyte colonies, express hepatic-specific markers, and maintain viability, architectural integrity, and physiologically relevant levels of albumin and urea lots from both healthy and diseased donor tissues through at least 2 weeks in culture. Moreover, the system enables greater flexibility in cellular composition, plate format, donor lot selection, plus feeding and treatment regimens to accommodate specific experimental and application requirements. This unique combination of attributes makes the novel TV2D+ system an ideal hepatic model for a variety of pharmacological and toxicological applications.

Mathematical Models to Characterize the Absorption, Distribution, Metabolism, and Excretion of Protein Therapeutics

Dhaval K. Shah, State University of New York at Buffalo

This presentation provides an overview of mechanistic PK models developed to characterize ADME properties of therapeutic proteins. These models can be used to support model-informed discovery and development of these molecules. As the next-generation of therapeutic proteins with diverse physicochemical properties and mechanism-of-action is being developed rapidly, there is an urgent need to better understand the determinants for the ADME of therapeutic proteins and evolve existing platform PK models to facilitate successful bench-to-bedside translation of these promising drug molecules. As such, the presentation will also provide an overview on the platform PBPK models for protein therapeutics.

SESSION IV: EXPLORING UNCONVENTIONAL BIOTRANSFORMATION PATHWAYS

Unusual in vivo Metabolism of Nifurtimox

Dieter Lang, Bayer AG

The oral antiparasitic drug nifurtimox has been used to treat Chagas disease for more than 50 years. Historical in vivo studies determined that the metabolism of nifurtimox is rapid and extensive and very little is excreted unchanged. Attempts to study nifurtimox metabolism have had limited success, yet this knowledge is fundamental to characterizing the pharmacokinetics and pharmacodynamics of the drug. To unravel the metabolic fate of nifurtimox, we conducted in vitro studies using hepatic and renal sources as well as in vivo studies in rats using 14C-labelled nifurtimox. In addition, we analyzed samples of human urine and plasma from phase 1 clinical studies in which participants received a single dose of 120 mg nifurtimox. Surprisingly, nifurtimox turned out to be very stable in in vitro incubations with hepatocytes and subcellular fractions, and only traces of a few metabolites were identified. In contrast, more than 30 metabolites were identified in rat urine at early time points, mostly with atypical mass changes. We developed an HRMS scouting method for analysis of human samples based on the sulfur atom in nifurtimox and the natural abundance of 34S, and a characteristic MS/MS fragmentation of nifurtimox and metabolites. Fragmentation patterns on HRMS/MS were used to propose structures for 18 metabolites (22 including stereoisomers). The six most abundant products were synthesized, the structures confirmed by NMR, and finally quantified in human plasma and urine. Overall, we determined that metabolism of nifurtimox is almost certainly not mediated by typical hepatic and renal drug metabolizing enzymes, and instead is rapidly metabolized mainly by reduction or direct nucleophilic attack, with some evidence of oxidation. In contrast to historical data, we generated evidence that a significant portion of nifurtimox is renally excreted unchanged but seems to be unstable during excretion in vivo.

Oxidation-reduction Equilibrium of Thiol-containing Drugs and Metabolites – the Dalcetrapib Example

Simone Schadt, Roche

In this presentation, we will delve into the oxidation-reduction equilibrium of thiol-containing drugs, using dalcetrapib as a prime example.

We will explore the pharmacokinetics of dalcetrapib, a thioester prodrug, and its active metabolite M1, which forms dimers and cysteine adducts with endogenous thiols.

Furthermore, we will shed light on the extensive metabolic pathway of dalcetrapib, which results in over 80 identified metabolites.

Lastly, we will share the technical challenges we faced during the MIST assessment, providing insights into the complexities of drug metabolism studies.

APA BIOGRAPHIES

Karan Agrawal, PhD, Janssen Research and Development: Dr. Agrawal is a Scientist in the Bioanalytical Discovery and Development Sciences team within the Preclinical Sciences and Translational Safety organization at Janssen Research and Development. In this role, Karan focuses on the development and application of bioanalytical assays for the identification and measurement of biomarkers to support the assessment of target engagement, pharmacodynamics effect, and drug-drug interaction. Dr. Agrawal also supports the quantitative and qualitative assessment of drug candidates and their metabolites in the biological matrix, with a focus on novel modalities.

Prior to joining Janssen, Karan was a Staff Scientist at Covance by LabCorp in Indianapolis, Indiana, where he was responsible for bioanalytical method development and validation for small and large molecule projects. Dr. Agrawal earned a B.A. in Chemistry and Molecular Biology from Cornell University, an M.Sc. in Forensic Science from King's College London, and a Ph.D. in Pharmacology and Toxicology from the University of California-Davis.

Melanie Anderson, Merck: Melanie Anderson is a principal scientist at Merck, Sharp, and Dohme (NJ, USA) with over 20 years' experience in clinical development. In her current role, she evaluates and implements patient centric sampling approaches for drug level quantitation and biomarker testing in clinical research. She recently co-led a cross-functional team at Merck working to operationalize decentralized clinical trial technology. Melanie currently co-chairs the PCSIG working group on diagnostics and co-chairs the CPLG/TALG IQ Patient Centric Sampling Group which published an editorial entitled "Will pateint-centric sampling become the norm for clinical trials after COVID-19?" in Nature Medicine in November 2020. She received her BA in Chemistry from Hasting College (NE, USA), and an MS in Chemistry from Lehigh University (PA, USA).

Saran Ayyadurai, PhD, Takeda: Dr. Ayyadurai is a Senior Scientist in the DMPK genomics and biomarker team at Takeda Pharmaceuticals, Cambridge, MA. His current role focuses on developing ddPCR-based genomic and NanoString-based biomarker assay support for gene and cell therapy programs within Takeda and with CRO partners. Previously, he worked as a qPCR lab scientist in a Quality Control (QC) lab at Cytovance Biologics in Oklahoma City, developing and supporting qPCR-based host cell/residual DNA testing for GMP-manufactured bulk drug substances.

He has a Ph.D. in Infectious disease (2010) with seven years of basic molecular biology/immunology postdoctoral research experience focusing on IBS/IBD-associated tumor research before joining the Pharma/Biotech industry in 2017.

Joe R. Cannon, PhD, BMS: Dr. Cannon received his PhD under Catherine Fenselau at the University of Maryland and proceeded to Post doctoral appointments in Analytical Chemistry at the University of Texas at Austin to study advanced Mass Spectrometry techniques for intact protein characterization and instrumentation, and then subsequently at Harvard Medical School to study Cell Biology and Neurodegeneration using quantitative proteomics. Since then, he has worked in drug metabolism and pharmacokinetics at Merck and currently is an Associate Director of Biotransformation at Bristol Myers Squibb. His work and research focus largely on novel assay development using a variety of analytical techniques for peptides, biologics, and additional 'Beyond Rule of 5' modality compounds.

Tom Chan, PhD, Boehringer Ingelheim: Dr. Chan received his Ph.D. in the University of Toronto in the Department of Pharmaceutical Science and conducted postdoctoral work at the Centre Hospitalier de l'Universite de Montreal. In these roles, he focused on the mechanisms of oxidative stress and impact of ischemia on apoptosis in the liver. He joined Boehringer Ingelheim Pharmaceuticals in 2009 where he currently leads a group of scientists who focus on the use of in vitro tools to assess drug-drug interaction risk in drug development and represents the DMPK department on selected projects in development.

Lei Ci, PhD, Moderna: Dr. Ci is an associate scientific director at Moderna Therapeutics. She has 15 years of experience in drug discovery and development. She received her PhD from Dr. Sugiyama Yuichi's lab at the University of Tokyo. After graduation, Lei worked at biotech companies leading the ADME and DMPK effort to support drug discovery. In her current role, Lei is leading DMPK and clinical pharmacology efforts for multiple

high impact projects in Moderna's mRNA pipeline. She has actively participated in academic and regulatory consortia and professional societies. She recently published papers evaluating ADME, PK, and biodistribution of LNPs.

Heather Cohen, PhD, Bicycle Therapeutics: Dr. Cohen is the Sr. Director of Translational Sciences at Bicycle Therapeutics. Bicycle Therapeutics is a clinical stage biopharma company pioneering a unique class of chemically synthesized medicines based on its proprietary bicyclic peptide (Bicycle®) product platform to address therapeutic needs unreachable with existing treatment modalities. Bicycles are fully synthetic constrained peptides with antibody-like affinities that target selectively, readily penetrate tissue, and can be chemically linked together to generate multifunctional molecules. For the treatment of high unmet need cancers, Bicycle Therapeutics is developing Bicycle tumor-targeted immune cell agonists™ and cytotoxics.

Heather has nearly 20 years of discovery and translational research experience including over 9 years of drug development experience in the biopharma industry. She holds a B.S. from the University of Minnesota in Genetics, Cell and Developmental Biology and a Ph.D. in Immunology from the University of Maryland, where she studied the development of immunosuppressive macrophages in infectious and inflammatory diseases. Prior to joining Bicycle Therapeutics in 2020, she was at EMD Serono and Jounce Therapeutics with increasing roles of responsibility developing oncology assets from target discovery through Phase 2 clinical trials.

Upendra P. Dahal, PhD, Amgen: Dr. Dahal is Senior Principal Scientist at Amgen Inc. Apart from supervising junior scientist, his current role involves representing PKDM (pharmacokinetics and drug metabolism) in multi-disciplinary project teams in discovery to development stages, design in vitro and in vivo studies to characterize/understand PKDM properties of the project compounds, provides recommendations to the teams to design better compounds with minimal metabolic and DDI liabilities. He reviews data, design/monitor studies to understand PKDM related challenges to mitigate the risks. He routinely prepares documents for regulatory filing. Before joining Amgen Upendra worked at Celgene and Pfizer. Upendra has a diverse research interest and has demonstrated a good track record of peer reviewed publications in various research areas.

William Fitch, PhD, Stanford University: Dr. Fitch consults in drug metabolism, bioanalysis and mass spectrometry at Stanford University and for Bay Area pharmaceutical firms. He received his BS at University of Michigan and his PhD at Stanford. He did postdoctoral stints in biosynthetic chemistry at Yale, synthetic chemistry at Syntex and clinical mass spectrometry at Stanford. His 31 year industrial career focused on applications of mass spectrometry in environmental analysis, agricultural chemical discovery, combinatorial chemistry and pharmaceutical discovery. Dr Fitch has published 76 papers, articles and book chapters.

Brijesh Garg, PhD, PepGen: Dr. Garg is working as a Biomarker Lead at PepGen, where he has been instrumental in managing and supporting Biomarker and ADA assays for Duchenne muscular dystrophy and myotonic dystrophy type 1 programs. He holds a PhD in Pharmacology from Northeastern University, Boston, which he completed in 2017. Before joining PepGen, Brijesh spent over 3 years at Takeda, working on various rare disease projects spanning complement systems, neuromuscular diseases, and employing diverse modalities like small molecule, biologics (antibodies, peptides), and gene therapy. During his time at Takeda, he had the privilege of contributing to both clinical and non-clinical programs, supporting biomarker and bioanalytical work for commercial molecules. With an impressive research career of over 15 years, Brijesh's expertise has been honed through his work at esteemed institutions like the Dana Farber Cancer Institute and the Flatley Discovery Lab, focusing on cancer-related protein-protein interactions and Cystic Fibrosis research.

Liang Jin, PhD, AbbVie: Dr. Jin received his Ph.D. in Molecular and Cellular Biology from Stony Brook University. He is currently Senior Scientist II in Quantitative, Translational, and ADME Sciences at AbbVie. Dr. Jin works primarily on data analysis for LC-MS based proteomics and integrating multi-omics data for target nomination and biomarker discovery.

Anette Karle, PhD, Novartis: Dr. Karle is an expert in the immunogenicity field and is well known for her contribution in external collaborations such as the Innovative Medicines Initiative. She acquired her PhD in 2008 on the topic of altered antigen presentation in nitrated pollen allergens, applying the MAPPs technology. Joining Novartis as a lab head in 2009, she spearheaded the establishment of in vitro and in silico immunogenicity potential

assessment technologies (in particular the MAPPs assay) to support candidate design and selection in early drug development and to increase the understanding of factors contributing to immunogenicity. Transitioning into the role of immunogenicity expert at Novartis in early 2021, she is now leading several Novartis-internal immunogenicity-related cross-functional teams and acts as an advisor for Novartis project teams regarding immunogenicity-related aspects, in particular mechanistic investigations of root causes of immunogenicity against biotherapeutics.

Dieter Lang, PhD, Bayer AG: Dr. Lang is a Principal Scientist in drug metabolism at Bayer AG located in Wuppertal, Germany. With almost 30 years' experience, his major interests are the enzymology of drug metabolizing enzyme, especially CYP1A1, and metabolite identification and structure elucidation. He received his Ph.D. in chemistry and toxicology from the University of Erlangen, Germany, and carried out his postdoctoral fellowship with Allan Rettie at the University of Washington, Seattle, USA.

Edward L. LeCluyse, PhD, LifeNet Health: Dr. LeCluyse is a Chief Scientist in the LifeSciences Division, Institute of Regenerative Medicine at LifeNet Health, Research Triangle Park, NC. Dr. LeCluyse received his Ph.D. in Biochemistry at the University of Kansas and did his post-doctoral training in Pharmacology and Toxicology at the University of Kansas Medical School. He has over 35 years of experience in basic and translational research in industry, biotech and academia, and has held prior positions at Merck & Co., Invitrogen, The Hamner Institutes for Health Sciences and UNC's Eshelman School of Pharmacy. Dr. LeCluyse enjoys doing research most on primary liver cells from both normal and diseased tissues and designing new in vitro human hepatic model systems to study mechanisms of liver toxicity and disease. Dr. LeCluyse is the author of over 130 publications, book chapters, and review articles, and has presented numerous lectures and workshops on in vitro liver related model systems and research applications.

Sarah Lloyd, PhD, AbbVie: Dr. Lloyd completed her Bachelor of Science at the University of Wisconsin - La Crosse where she did research exploring the unique cardiovascular properties of hibernating ground squirrels. She then pursued her Ph.D. in Interdisciplinary Biological Sciences at Northwestern University. Her dissertation was centered on understanding the transcriptional mechanisms important for maintaining human skin tissue homeostasis. Now as a postdoctoral fellow at AbbVie, she investigates extracellular matrix biology in lung fibrosis while also working to build more complex human lung models.

Steven W. Louie, MS, Moderna: Steven is an Associate Director in the Drug Metabolism Pharmacokinetic Kinetic (DMPK) Department at Moderna Therapeutics in Cambridge, Massachusetts. His primary role is to support in vitro ADME IND submissions packages for lead molecule candidates. His secondary role is to identify and develop in vitro mechanistic studies to address ADME PB/PK parameters of LNP-mRNA for drug disposition in preclinical discovery and development. In addition to these roles, Steven has taken on Project Representative responsibilities supporting several therapeutic areas working cross functionally within discovery and development teams. Steven has 20+ years preclinical discovery and development experience with Novartis, Amgen, Merck, and GSK in DMPK.

Lori Payne, PhD, Alturas Analytics, Inc.: Dr. Payne is the Executive Director of Business Development at Alturas Analytics, Inc. She earned a BS in Environmental Biochemistry from UC Davis and a Ph.D. in Chemistry from LSU Baton Rouge. She was also a Peace Corps volunteer in Central America.

Dr. Payne has a distinguished career in large pharma in addition to managing and directing growth and development in Contract Research Organizations with bioanalytical, discovery, and analytical groups in animal and human health. She was Vice-President of Bioanalytical, Analytical, and Discovery at BASi (now Inotiv). Because of her leadership, she received numerous awards for her lean and continuous improvement focus.

After joining the Alturas team in 2019, Dr. Payne successfully organized the research committee and contributed to the growth of the company. She acts as liaison between scientific and management staff at Alturas and prospective clients while looking for new business development and research opportunities. She enjoys maintaining business relationships, mentoring, and staying abreast of current developments in the bioanalysis of large and small molecules.

Suresh Peddigari, PhD, Moderna: Dr. Peddigari received his Ph.D. in Molecular Biology from Kumamoto University in Japan and post-doctoral training in Oncology at US institutions. He has 15 years of academic and industry research experience and expertise in various therapeutic modalities such as small molecules, antisense oligonucleotides, CRISPR gene editing technologies for drug discovery and published in peer-reviewed journals.

He previously worked at Bristol Myers-Squibb as Senior Investigator and led early discovery targets validation for immuno-oncology and inflammatory diseases drug discovery. Currently, Suresh serves as Molecular assays lead scientist at Moderna, developing and validating assays for PK assessment of mRNA LNP therapeutics and supporting clinical studies.

Bhagwat Prasad, PhD, Washington State University: Dr. Prasad is an Associate Professor in the Department of Pharmaceutical Sciences, Washington State University (WSU), Spokane, WA. He leads several federally- and industry-funded research programs on characterization of interindividual variability in drug disposition (drug transport and metabolism) and serves as a director of the proteomics-based research in non-cytochrome P450 enzymes (PRINCE). Dr. Prasad has published >110 peer-reviewed articles and >100 conference abstracts and delivered over 85 invited talks at various national and international conferences. Dr. Prasad is the recipient of 2018 ISSX North American New Investigator Award. Dr. Prasad also received the Early Career Faculty Showcase award at the 2018 ASPET meeting. Dr. Prasad also served as Secretary of the Drug Metabolism and Disposition Division of ASPET. Dr. Prasad is a member of the editorial boards of Drug Metabolism and Disposition and Clinical Pharmacology and Therapeutics. Dr. Prasad obtained his MS in 2006 and Ph.D. in 2010 in Pharmaceutical Analysis from NIPER, Mohali, India, and he was a postdoc and assistant professor at the department of Pharmaceutics, University of Washington, Seattle, WA from 2011-2014 and 2014-2019, respectively.

Edward Price, PhD, AbbVie: Dr. Price is a Senior Scientist at AbbVie's Quantitative, Translation & ADME Sciences department, where he drives computational ADME in drug development, emphasizing data analytics and model creation to assist teams in making informed, data-driven decisions. While earning his Ph.D. in chemistry from the University of Central Florida's Nanoscience Technology Center under Dr. Andre Gesquiere, Edward also played an instrumental role at AEgis Technologies. At AEgis, he built PBPK models and extended consulting expertise to both industry and academia. His research, spanning biologics, nanoparticles, and absorption processes, has been published in Science Advances, Scientific Reports, and the Journal of Medicinal Chemistry.

Akshaya Ramesh, PhD, Genentech: Dr. Ramesh is a Principal Bioinformatics Scientist at Genentech, in the department of computational sciences. She primarily works on neuroimmunology projects, with a focus on multiple sclerosis (MS). She is interested in better understanding disease progression in MS, and identifying novel pathways that contribute to the disease using large scale omic datasets. She obtained her PhD in genetics and genomics from Boston University, and did a postdoctoral fellowship in the department on neurology at University of California, San Francisco.

Ramola Sane, PhD, Bristol Myers Squibb: Dr. Sane is a Principal Scientist at Bristol Myers Squibb. She graduated from the University of Minnesota with a PhD in 2012. At BMS, she has worked on a number of modalities as a DMPK scientist since 2014. Her area of interest is PK/PD modeling and simulation as well as human PK and efficacious dose projection for small molecules as well as biologics.

Simone Schadt, PhD, Roche: Dr. Schadt is a Distinguished Scientist at Roche and leads the ADME chapter, which covers ADME optimization and characterization of small molecules, oligonucleotides, and therapeutic proteins from early discovery to post-marketing. Simone has co-authored several publications on the effects and the fate of xenobiotics: reactive metabolite assessment and drug induced liver injury (DILI), metabolites in safety testing (MIST), oligonucleotide and protein biotransformation. She holds a biochemistry degree from the University of Tübingen and a PhD from Technical University of Berlin. Simone began her industry career as a postdoctoral fellow at Boehringer Ingelheim, later becoming a lab head in the drug metabolism group before joining Roche in 2011.

Dhaval K. Shah, PhD, MS, BPharm, Upstream Bio: Prof. Shah is an Associate Professor of Pharmaceutical Sciences at the State University of New York at Buffalo. Prior to becoming the faculty Prof. Shah served as a Principal Scientist in the 'Translational Research-Modeling & Simulation' group at Pfizer Inc. He received his PhD from the Department of Pharmaceutical Sciences at the State University of New York at Buffalo in 2010. His research

focuses on understanding the determinants for the absorption, distribution, metabolism, and elimination (ADME) of protein therapeutics and novel biologics. His lab uses the principles of Pharmacokinetics-Pharmacodynamics (PK-PD) Modeling & Simulation to support the discovery, clinical translation, and late phase development of novel biologics like engineered antibodies, multi-specific proteins, immuno-oncology agents, engineered T cells, antibody-drug conjugates, and gene delivery vectors.

Arkadeep Sinha, PhD, Upstream Bio: Dr. Sinha is the Associate Director of Bioanalytical Sciences at Upstream Bio, where he oversees PK, ADA, and NAb analysis, as well as the generation of critical reagents to support the assays. Following his graduate work on pancreatic cancer metastasis at Michigan State University, Arkadeep has approximately 10 years of experience in the drug development space with experiences across large/mid-size pharma, startups, and CRO. His expertise lies in supporting all aspects of bioanalysis for biologics ranging from mAbs, bispecifics, ADCs, BiTEs, and oligonucleotide-based therapies.

Qin Sun, PhD, FDA: Dr. Sun is the Therapeutic Biologics Program (TBP) biologics lead in the Office of Clinical Pharmacology (OCP), CDER, FDA. Her key job functions include guide and support reviews and policy development for new molecular entity (NME) biologics or biosimilar products. In addition, Qin is PI or co-PI for biologics related research projects. Qin joined FDA in 2016. Before that, she worked at Pharmaceutical Product Development (PPD) from 2015 to 2016, and at Bristol-Myers Squibb from 2008 to 2014. Qin received her PhD from University of Virginia. Her work experience extends from drug discovery to drug development, and finally to regulatory review, focusing on biologics and biosimilars currently.

Yuting Wang, PhD, AbbVie: Dr. Wang is a Sr Scientist in the Quantitative, Translational, and ADME Sciences (QTAS) department at AbbVie where she functions as bioanalytical lead for large-molecule biologics in the non-regulated LCMS team. She supports pre-clinical early discovery/PK/PD/TK studies across a range of therapeutic areas and modalities which include mAb, ADC, and multi-specifics. Yuting received her B.S. in Pharmaceutical Sciences from Nankai University, China. She then obtained her Ph.D. in Chemistry from the University of Florida and completed her postdoctoral training at Washington University in St. Louis in metabolomics.

Jessica Weaver, PhD, LifeNet Health: Dr. Weaver joined the LifeSciences division in the Research and Development department at LifeNet Health in 2018 and then moved to the Product and Development team in 2020. She has more than 10 years of experience in biochemistry studying diabetes and disease related fields. Jessica received her BS in Biological Sciences at the College of William and Mary and her PhD in Chemistry from the University of Colorado, Boulder. She did a post doctoral fellowship at the University of Pennsylvania School of Medicine working on the vaccinia virus. Before moving to LifeNet Health, she worked at Eastern Virginia Medical School pursuing advances in type I and type 2 diabetes in both the lab and the clinic.

Michael Weis, PhD, Kymera Therapeutics: Dr. Weis is the Director of PK/PD Modeling and Simulation at Kymera Therapeutics. Prior experience includes Valo Health, and the drug development advisory firms Rosa and Co. and Entelos. In the latter two roles, he applied modeling and simulation approaches in collaboration with over a dozen small and large biotechnology and pharmaceutical companies, and across a wide variety of therapeutic areas, modalities, and discovery and development phases. He received his BSE, MSE, and PhD degrees in Systems and Control Engineering from Case Western Reserve University, where his academic work focused on mathematical models of signaling pathways and cell cycle control with applications to oncology research.

Joleen T. White, PhD, Bill & Melinda Gates Medical Research Institute: Dr. White is Head of Bioassay Development at Bill & Melinda Gates Medical Research Institute (Gates MRI). She oversees bioassay staff responsible for the development programs across global health including small molecules, monoclonal antibodies, and probiotics. The Gates MRI motto "Our bottom line: lives saved" resonates deeply for her, enabling her to pursue her passion of helping under-served patients in a full-time position.

Joleen earned her Ph.D. in Biochemistry from The Scripps Research Institute. Prior to her position with Gates MRI, she worked across bioanalytical, biomarker, and immunogenicity methodology and interpretation at EMD Serono, Biogen, Bristol-Myers Squibb, and BioMarin Pharmaceutical Inc.

Joleen is active in the international bioanalytical and immunogenicity community, including chairing or moderating 10 conferences and sessions, and representing Gates MRI and previous employers on AAPS and the International IQ Consortium working groups. She also supports data science initiatives with PhUSE and CDISC.

Zhengyin Yan, PhD, Genentech: Dr. Yan received his PhD in biochemistry from Georgetown University. After a brief tenure as a postdoctoral at the National Cancer Institute, he joined Johnson & Johnson Pharmaceutical Research Institute in Spring House, PA, where he used to manage the in vitro DMPK and discovery bio-analytical groups. In 2015, Zhengyin moved to Genentech and currently serves as Distinguished Scientist leading in vitro predictive ADME group to support drug discovery. His research interest has been in the ADME-guided lead optimization which includes developing new in vitro methodologies for novel molecules and designing in vitro and in vivo studies to assess and/or predict drug-like properties such as drug clearance and drug interactions potential. He has published more than 60 peer reviewed articles and co-edited three books in his field.

Hao Yang, PhD, Thermo Fisher Scientific: Dr. Hao Yang is a product application specialist supporting pharma and biopharma applications in Chromatography and Mass Spectrometry Division within Thermo Fisher Scientific. His current focuses are oligonucleotide characterization and bioanalysis using Orbitrap and triple quadruple technologies.

Min Zhao, PhD, Moderna: Dr. Zhao is an experienced immunologist/virologist with over 10 years of experience in viral vaccine development-related research. She specializes in designing and executing virus neutralization assays/studies to assess the efficacy of vaccines and therapeutic candidates.

Dr. Zhao's academic career began at Xiamen University, China, where she earned her ph.D. in Virology/Immunology. She further expanded her research capabilities during her tenure as a Pre-doctoral Visiting Fellow at the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Following this, she served as Postdoctoral Associate at Yale School of Medicine.

Throughout her research experience, Dr. Zhao has made significant contributions including the development of novel cellular assays, monitoring immune responses of mRNA-based SARS-CoV-2 vaccines, developing proprietary neutralizing antibodies against RSV pre-F protein, expressing, purifying, and characterizing VLPs as candidate vaccine antigens for HEV.

Currently, she serves as a Scientist in the Clinical Biomarkers Department at Moderna. In this role, her responsibilities span a wide range, with a primary on the development, qualification, and validation of virus neutralization assays. This crucial part of her job involves conducting clinical sample analysis to support programs across multiple therapeutic areas.

Dr. Zhao is deeply committed to scientific excellence and maintain a meticulous attention to detail in my all undertakings. Her current role enables me to apply my scientific knowledge and expertise in facilitating a better understanding of disease mechanisms and aiding in the development of innovative therapeutic strategies.

POSTER ABSTRACTS

Sensitive Quantitation of Antisense Oligonucleotides Using Capitainer® qDBS Microsampling Device Coupled with Hybridization LC-MS/MS

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PURPOSE

Antisense oligonucleotides (ASOs) are short synthetic oligodeoxynucleotides (15-25 nucleotides) that bind to mRNA, blocking gene expression and reducing disease-related protein synthesis. Their promising therapeutic potential has generated a strong demand for sensitive and selective analytical methods, particularly when using microsampling techniques for low-volume blood collection.

Aligned with recent initiatives for patient-centric healthcare and easing recruitment efforts for vulnerable patient populations and pediatrics in decentralized clinical trials, Capitainer AB recently introduced the quantitative dried blood spot (qDBS) microsampling device, designed to minimize discrepancies associated with conventional DBS approaches by allowing volumetrically accurate and precise capillary blood collection independent of hematocrit. However, the inherent limitation in low microsampling blood volume (e.g., 10 µL) can lead to compromised lower limits of quantitation (LLOQ), particularly for ASOs whose total ion current is disseminated amongst multiple charge states under electrospray ionization conditions.

Recently, hybridization extraction coupled with LC-MS has emerged as a novel oligonucleotide quantitation strategy applied to several matrices, including plasma, serum, cerebrospinal fluid, and many tissue types. However, to date, there have been no reports of ASO quantitation from dried blood microsamples, in part due to the complexity of the extracts, which confound traditional sample preparation approaches. Therefore, using complementary capture probes in a hybridization workflow to increase sensitivity and selectivity, the current research details the successful extraction and quantification of fomivirsen (FME) and its 5′n-1 metabolite from dried blood microsamples, collected using the Capitainer qDBS device.

METHODS

Human blood samples fortified with FME and 5′ n-1 metabolite were absorbed onto the 10 µL Ahlstrom 222 cellulose substrate of the Capitainer qDBS device and allowed to air-dry in the presence of desiccant for a minimum of 24 hr.

Dried blood qDBS microsamples were carefully detached and placed in a 96-well plate, to which a 5/32" stainless steel grinding bead was added, followed by adding the n+2 analogue of FME as internal standard (IS). Dried blood microsamples were extracted using impact-assisted-extraction (IAE) in the presence of surfactant, followed by affinity purification of the ASO-Probe complex using functionalized magnetic beads.

After multiple wash steps, the targeted ASO was released from the capture probe via thermal denaturation. The LC separation was performed on an Acquity Premier BEH C18 column (50 x 2.1mm, 1.7 µm) using a mobile phase containing ion pairing reagents. FME, 5′ n-1 metabolite, and n+ 2 internal standard were detected using a SCIEX 6500+ QQQ operated in negative ESI/MRM mode.

RESULTS

Impact-assisted extraction of the targeted ASOs from the cellulose substrate of the Capitainer qDBS was optimized to overcome a moderate reduction in extractability with increasing blood hematocrit up to a maximally evaluated level of 50%. Notably, the latter phenomenon has been

previously reported and is not a device-dependent phenomenon.

The addition of detergent to extraction solvent was critical to achieve high ASO recovery; however, this was accompanied by the complete desorption of all blood components, thereby complexifying extracts. Parenthetically, the near quantitative recovery of targeted ASOs can be attributed to increased solubility and reduced surface tension, facilitating buffer permeation into the cellulose substrate.

Chromatographically, while reducing flow rate typically enhances ASO sensitivity, an excessively low flow rate (≤0.3 mL/min) can exacerbate the impact of void volume, compromising peak width, separation resolution, and sample throughput. To minimize pre-column void volume, a divert valve was integrated post-injection to mitigate mobile phase gradient delay, in turn leading to reduced peak width concomitant with gains in signal-to-noise ratio. Chromatographic optimization, in combination with the high sensitivity conferred by the SCIEX 6500+ when monitoring the MRM transitions of 741.5→ 95.0 and 703.0→ 95.0 for FME and 5′ n-1 metabolite, respectively, allowed an LLOQ of 0.50 ng/mL to be achieved from only 10 µL of dried blood sample.

Calibration curves spanning 0.50 - 500 ng/mL were established for both FME and 5′n-1 metabolite with precision and accuracy assessments at four QC concentration levels (LOQ, 3x LOQ, geometric mean, and 75% of ULQ), all within acceptance criteria. Specificity and lack of matrix effect bias were confirmed from individual blood donors, maintaining consistent quantitation for each FME and 5′n-1 metabolite. The high level of overall assay specificity may be attributed to the selectivity with which targeted ASOs could be isolated from complex dried blood extracts, facilitated by using the capture probe comprised of a full-length complementary sequence.

CONCLUSION

The research reported herein comprises the first report for ASO quantitation derived from dried blood samples, using FME and its 5′n-1 metabolite as representative analytes for assay development, establishing performance attributes for extraction from the Ahlstrom 222 cellulose substrate incorporated into the Capitainer qDBS microsampling device. The method evaluation met acceptance criteria for specificity, matrix effect, hematocrit bias, and precision and accuracy for a quantifiable range of 0.50 - 500 ng/mL, requiring only 10 µL of the collected blood sample. Our novel workflow consisting of IAE coupled with highly specific and sensitive hybridization LC-MS/MS carries significant implications for the advancement of ASO therapeutic development in that critical patient populations may be more readily accessed for decentralized clinical trials.

Development of Alkaline Phosphatase Prodrug Bioconversion Assays to Support Drug Discovery

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Phosphate prodrug strategies can be used to overcome liabilities such as poor solubility or crystallinity, and excess aggregation of antibody drug conjugate payloads. Phosphate prodrugs require enzymatic conversion to active molecules. Therefore, assessing the likelihood of bioconversion in vivo by in vitro experiments should help prioritize candidate synthesis as well as pharmacokinetic studies. Ultimately, these in vitro assays should enable in vitro in vivo extrapolation and the data generation needed to build models predictive of bioconversion. Using human as well as preclinical species, subcellular fractions, serum and plasma, we have developed in vitro bioconversion assays measuring substrate (pro-drug) depletion corroborated by monitoring or quantifying active drug formation. Assay conditions were optimized towards achieving maximum catalytic activity. For example, EDTA, a common anti-coagulant was found to be inhibitory to plasma phosphatases and subcellular fraction incubations were carried out in Tris-Cl buffer rather than phosphate buffer which may inhibit bioconversion. Bioanalytical conditions were also optimized to enable analysis of prodrug and active drug (with wide difference in polarity) in the same run. Sodium orthovanadate was confirmed as an effective inhibitor of phosphatases upon coincubation and we have used this agent to elucidate the role of phosphatase mediated bioconversion in various biomatrices (Ki value, 9.8 μM; competitive inhibition model). Test compounds as substrates for phosphatase included model prodrugs, fosamprenavir, fosphenytoin and prednisolone phosphate. In general, intestinal S9 exhibited conversion rates higher than liver S9 or serum, and significant catalytic activity was observed only with some model compounds in serum from rodents, but not other species. We anticipate these assays to find utility in candidate nomination and selection by confirming bioconversion and/or prediction extent of conversion in vivo.

Sensitive quantification of the protein targeting chimera (PROTAC) TL 13-112 in rat plasma using an LC-MS/MS workflow

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Purpose

Proteolysis targeting chimeras (PROTACs) are endogenous protein degradation tools, capable of removing specific protein targets using a cell's own disposal machinery. PROTACs have evolved as a therapeutic modality, as several candidates have now moved into clinical trials. Sensitive and selective assays for high-confidence detection and quantification of PROTACs are needed to ensure safety and efficacy in the drug development pipeline and because PROTACs have expressed high potency in nanomolar drug concentrations. In this study, low-pg/mL quantification for the PROTAC, TL 13-112, and its inactive control, TL 13-110, was achieved at a lower limit of quantification (LLOQ) of 10 pg/mL using a highflow LC-MS/MS platform.

Methods

PROTACs were spiked into rat plasma at concentrations ranging from 10 pg/mL to 15000 pg/mL. Following protein precipitation, samples were vortexed and centrifuged at room temperature. The supernatant was transferred to a new Eppendorf tube and dried under nitrogen flow. Dried extracts were reconstituted prior to the analysis.

PROTACs were separated using a Phenomenex Kinetex XB-C18 column (2.1 x 50 mm, 1.7 µm, 100 Å). The LC system was operated at a flow rate of 0.3 mL/min. Analysis was performed on a SCIEX 7500 system in positive mode. Collision energy, source and MS parameters were optimized to achieve sensitive MS/MS quantification.

Results

Calibration curves were constructed across concentrations ranging from 10 pg/mL to 15000 pg/mL. Individual concentrations were run in triplicate. An LLOQ of 10 pg/mL was achieved for TL 13-112 and TL 13-110. No interferences were observed in the matrix blank (rat plasma) for either analyte. Strong linearity was achieved for both analytes with a linear dynamic range (LDR) of 3.2 orders of magnitude.

Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% for the higher concentrations. The %CV of the calculated mean of the concentration should be below 20% at the LLOQ and below 15% for all higher concentrations Accuracy was within ±11% and ±12% of the nominal concentration for TL 13-112 and TL 13-110, respectively. The %CV was <10% for both analytes.

Calculated values for accuracy and %CV were within the acceptance criteria at each concentration level.

Conclusion

Overall, a highly sensitive method for the quantification of PROTACs in rat plasma was developed with excellent accuracy and precision at low-pg/mL levels.

Drug protein binding assessment using rapid and high-throughput magnetized silica beads method

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Purpose

The key parameter in pharmacokinetic modeling of a novel drug is the unbound fraction (fu) which is determined by protein binding assays. Only unbound drug is available to interact with the target, and therefore fu has direct implications on the drug's efficacy, as well as its toxicity, and DDI risk. Contemporary protein binding methods, such as the rapid equilibrium dialysis (RED) device, ultrafiltration (UF), and ultracentrifugation (UC), are time consuming and have methodological challenges assessing the binding of larger molecule drugs—such as oligonucleotide drug.

Methods

A novel method of utilizing human liver microsomes (HLMs) bound to magnetized silica beads may solve some of these issues because HLMs-bound beads can be separated from incubations in seconds. In this study, we validate this method using suspensions of HLM coated magnetized silica beads to measure the protein binding of test compounds for small molecules in a high-throughput system. We developed semi-automate method using the Kingfisher Flex system to increase its throughput. Furthermore, we explore the possibility of using this novel method with larger molecules such as oligonucleotides.

Results

We report concentration dependent protein binding of four 4 small molecules, amitriptyline, clozapine, midazolam, and warfarin. We find strong correlations between our f_u values, and the values reported in a published reference for three of these compounds. The mean correlation coefficient (r) across experimental runs were 0.992 for clozapine, 0.976 for midazolam, and 0.549 for warfarin. Amitriptyline showed binding consistent with in-house historical data measured by RED and UC methods (mean HLM-bead f_u = 35.37% vs mean UC f_u =36.24% vs mean RED f_u = 39.34%).

Conclusion

These results demonstrate that the combination of HLM-coated silica beads and Kingfisher Flex system can assess protein binding of compounds rapidly with automation that increases work efficiency and reduces costs. This method has further been developed to measure the protein binding of antisense or CRISPR types of oligonucleotide drugs.

From MS signal analysis to AI based method: Solutions for metabolism data processing automation

Ismael Zamora, Fabien Fontaine, Tatiana Radchenko, Ramón Adaila, Luca Morettoni, Albert Garriga, Xavier Pascual, Nadia Zara, Axel Rebollo and Vera López

Purpose

The aim of this poster is to show the combination of a traditional peak finding algorithms applied in multiple compound families and an AI solution that collect the result from those algorithms and automatic and continuously learn from the MS expert selection to produce a peak selection that fit the purpose the analysis. Since it is not the same an analysis for discovery Metabolite Identification, where the focus is most of the time to explain the clearance observed under certain experimental condition to find out the metabolic bottle neck to design compounds with better characteristics, than in late discovery where species comparison are performed to understand if metabolism is different across species or when doing metabolite identification to find out the reactive metabolites, different peak selection is typically needed in this case although the initial information is the same LC-MS data format. The expert user drives the peak selection attending to the final purpose, but also the expected throughput and time lines.

Methods

The Mass Spectrometer (MS) is usually the final detector for many Drug Metabolism and Pharmacokinetic measured end points and it is used in in-vitro ADME calculation like clearance, metabolite identification or in-vivo Pharmacokinetic quantification. We are showing in this presentation how signal analysis algorithms are applied for LC-MS data processing for molecules of any modality (small molecule, PROTAC, Macrocyclic Peptide, peptides, Oligonucleotides and Antibodies). The algorithm modifications needed to transition from small to macro molecule is also described an applied to one case per compound modality covering: atom based analysis versus monomer based analysis, peak selection based on monoisotopic m/z versus Most Abundant Mass or Average Mass or the incorporation of Charge deconvolution to enhance sensitivity and avoid complexity. In addition to the signal analysis we are also showing in this presentation the development of Machine Learning models that learns from user peak selection criteria (peak quality, multiple sample kinetic, MS and MSMS spectra quality) and the intended usage of the data processing (soft Spot analysis, Metabolite identification) to automatically select/hide Chromatographic peaks. The Machine learning model is therefore introduced into a self-learning mechanism (AI) where the data for user peak selection is incorporated into the model building and predictions are refined with new data.

Results

The poster will show the application of this feature on 2 different datasets one dedicated for multiple time point analysis (Soft Spot ID) and another one for GSH trapping experiment. Finally, an application of Machine Learning models in the field of MS based analysis of DMPK data will also be introduced for the selection of the m/z transition in a typical MRM experiment by the automatic selection of m/z to be used in a QQQ instrument try to avoid the experimental condition optimization, using the predicted transitions to compute the compound of interest Area that is used in DMPK end points computation like Clearance and Papp determination.

Conclusion

The fit of purpose application of AI in Metabolite Identification degrease the time for peak selection and help in the automation of this task where different models may be needed depending on the experimental intention , the time lines and resource availability.

Development and validation of a quantitative bioanalytical method for obeldesivir and its major metabolite in plasma

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Purpose
Obeldesivir (GS-5245) is a novel oral antiviral drug against SARS-CoV-2 which is hydrolyzed pre-systemically to GS-441524 and subsequently forms intracellularly the same active triphosphate metabolite as remdesivir (Veklury®). The COVID-19 pandemic resulted in widespread SARS-CoV-2 infection with more than 750 million reported cases and almost 7 million deaths globally. Only two antiviral drugs have already been fully approved and marketed to address the still-existing global health crisis. However, due to limitations in the broad applications of these medications, there is a need for improved treatments with a lower pill burden, low drug-drug interaction potential, and a high barrier to resistance such as obeldesivir. To advance the development of obeldesivir, a reliable and sensitive assay is required to measure the parent and its major nucleoside metabolite (GS-441524) in plasma of nonclinical toxicology species. One of the main challenges in the respective assay development and optimization is the rapid conversion of GS-5245 to GS-441524 during plasma sample handling and analysis. In this study, we describe a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for the analysis of obeldesivir and its major metabolite in rat, dog and rabbit plasma that have been fully validated to support the GLP toxicological studies.

Methods
Obeldesivir and its major circulating metabolite, GS-441524, were quantified in the presence of stable isotope-labeled internal standards (ISs:13C3-obeldesivir, GS-1170756 and 13C3-GS-441524, GS-828840) in K2EDTA rat, dog or rabbit plasma treated with 80 mM dichlorvos. The analysis was performed on an LC-MS/MS system consisting of Shimadzu, Prominence, 20 Series HPLC equipped with Phenomenex®, Synergy Fusion-RP (50x2.0 mm) column at a flow rate of 0.5 mL/min. The mobile phases were: A, ammonium formate 5 mM (aq); and B, acetonitrile. The gradient elution started with 10%B and increased to 90%B over 2.3 minutes, then maintained for an additional 0.9 minutes before returning to initial conditions. A solid phase extraction (SPE) of analytes utilized an Oasis MCX 96-well plate. Briefly, the plasma samples (25 µL aliquot) were step-wise loaded, washed and eluted with methanol:ammonium hydroxide (100:5, v:v). Collected extracts were then evaporated to dryness and reconstituted with acetonitrile:water (10:90, v:v). After vortex mixing and centrifugation, the supernatants were analyzed by LC-MS/MS (injection volume: 3-6 µL). Mass spectrometry detection was performed by positive atmospheric pressure chemical ionization (APCI) on a Sciex® API 5500 using the following MRM transitions: m/z 362.1→ 202.1 (obeldesivir), 365.1 → 205.1 (13C3-obeldesivir), 292.0 → 265.2 (GS-441524) and 295.1 → 267.2 (13C3-GS-441524).

Results
The method was developed and optimized in rat, dog, and rabbit plasma. The calibration ranges were: 0.3-300 ng/mL and 50-50,000 ng/mL for obeldesivir and its major circulating metabolite, respectively. The study found dichlorvos treatment and wet ice conditions prevented the conversion of obeldesivir to GS-441524 in whole blood and plasma. In addition, the calibration standards and quality control samples had to be prepared with a pre-chilled treated plasma on wet ice to maintain the stability of GS-5245. The SPE conditions were optimized for consistent recoveries (>75%) for both analytes and ISs. The observed significant matrix effects for the polar nucleoside GS-441524 were mitigated using improved chromatographic separation combined with APCI. The method validation conformed to the applicable regulatory guidance and included: precision and accuracy; matrix selectivity; freeze-thaw stability; processed sample stability and viability; whole blood stability; long term stability; and incurred sample reanalysis.

Conclusion
A reliable, sensitive, and specific LC-MS/MS method was developed and validated for measuring obeldesivir and its major metabolite in rat, dog, and rabbit plasma. Challenges during the plasma sample handling and analysis were identified and successfully overcome. In summary, the

bioanalytical method detailed above was deemed suitable to support GLP toxicological studies required for the regulatory approval of obeldesivir as a novel oral treatment for COVID-19.

Development of a novel neutralizing antibody assay for a multi-domain ligand trap

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Purpose

Neutralizing antibodies (NAbs) can have a significant impact on bioavailability, efficacy, and safety of a therapeutic protein. Thus, it is important that sensitive and robust immunogenicity assays are developed for detection of clinically relevant NAbs. The drug of interest is a multidomain ligand trap designed to bind endogenous ligands that are elevated in the serum of patients.

Methods

We developed a competitive ligand-binding assay for the detection of NAbs to a multidomain ligand trap drug using a Meso Scale Discovery platform. Different assay formats were initially tested. An electroluminescence method, coupled with an acid dissociation and drug depletion methods, was selected for further development because it would allow for optimal sensitivity and cut point to detect NAbs in human serum.

Results

The assay cut point using 54 naïve healthy human serum samples was 20.68% and the sensitivity was 78 ng/mL. Naïve sera spiked with high (1500 ng/mL; HPC) and low (100 ng/mL; LPC) positive controls were all detected above the assay cut point with percentage inhibition consistently higher for HPC compared to LPC, showing an adequate selectivity. Tested levels of sample hemolysis did not affect the specific and precise detection of NAbs in the serum. The assay was able to tolerate up to 11.1, 1.23, and 0.411 µg/mL of free drug at 1500, 200, and 100 ng/mL of the positive control, respectively. The presence of two different soluble endogenous ligands did not significantly interfere with the assay at all tested concentrations.

Conclusion

We successfully developed a competitive ligand-binding NAb assay for a large multidomain ligand trap. The assay has demonstrated acceptable sensitivity, selectivity, drug tolerance and target interference, and will be used for the specific and precise assessment of NAbs in support of clinical studies.

Prostate Cancer Patient Stratification and Monitoring Using LC/MS/MS-Based Targeted Lipidomics and Discovery Proteomics

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Purpose

Prostate cancer (PCa) accounts for approximately 40% of all cancers worldwide, nevertheless both its diagnosis and prognosis remain challenging. Circulating prostate-specific antigen (PSA) concentrations are the most common blood-based biomarkers currently available in clinical practice. However, PSA by itself is not accurate as there is no reliable PSA range that is an explicit signifier for prostate cancer. Combinations of multiple levels of molecular information have the potential to improve biomarker panels and help gain better understanding of disease and patient response to treatment. The aim of this study was to employ LC/MS/MS based targeted lipidomics and discovery proteomics to differentiate prostate cancer patients based on disease severity and identify putative "biomarkers" for disease prognosis or patient monitoring.

Methods

Prostate cancer sera-based samples from 42 individuals were pooled to form 6 phenotypic groups, controls, active surveillance, brachytherapy, hormone therapy, radiotherapy and hormone therapy, prostatectomy. The samples were appropriately extracted and processed for lipidomic and proteomic analysis. Lipidomics was performed using an 8 min targeted HILIC/MS/MS analysis [1] and proteomics was performed in discovery mode using a 15 min microbore (1mm ID) RPLC separation with ion mobility-based accurate mass detection [2].

Results

The quantitative lipid LC/MS/MS assay was validated, according to FDA "Guidance for Industry" on bioanalytical methods 2017, for a total of 436 bioactive lipids, with the potential to measure up to 2,000 lipid species. Following initial screening of the PCa sera samples a targeted assay for 39 endogenous lipids across the Cer, LPE, LPI, PG, PI, and SM classes, which appeared to be key differentiators of sample cohorts, was employed for sample analysis. Lipid calibration lines showed typical R2 values ranging from 0.985 to 0.996 and QC CVs were all below 15%. Multivariate analysis of the results highlighted several significant lipid classes including the SM, Cer, and LPEs, which differentiated the subject groups. For example, LPE (20:3), LPE (P-16:0), and LPE (P-18:1) were key lipids for differentiating between individuals diagnosed with prostate cancer and those undergoing hormone therapy, with the LPE (20:3) concentration in the hormone therapy patient pool found to be 146.5 ng/mL and 119.8 ng/mL in the active surveillance patient pool. Proteomic analysis of the PCa serum samples resulted in the identification of a total of 533 proteins revealing the differential expression of proteins linked to patients receiving hormone-radiotherapy or undergoing surgery. Unsupervised principal components analysis of the proteomics samples showed clear separation of the individual patient groups, with 60% of the variance in the data described by PC1 and PC2. Analysis of the samples revealed that the phenotypic pools for the controls, active surveillance, hormone therapy and brachytherapy, and patients with no cancer clustered closely together but were nevertheless clearly separated. The prostatectomy and hormone-radiotherapy phenotypic pools were also clearly separated from each other as well as the other groups. Partial least squares discriminate analysis of the data revealed that albumin showed significant elevation in the prostatectomy group as did haptoglobin (HPT) an acute inflammatory response marker which was also over expressed. Proteins contributing to the deviation observed for the hormone-radiotherapy group resulted from the downregulation of dynein axonemal heavy chain 5 (DYH5), apolipoprotein A-IV (APOA4), and IGLC3.

The lipidomic and proteomic data were employed, both individually and separately to build predictive models of prostate cancer. The proteomics only data yield a RoC with a predictive score of 0.91 for control vs mild disease, and 0.86 for control vs advanced disease, the lipidomic only data

produced RoC scores of 0.94 and 0.95 respectively for the same two groups. Combining both the proteomics and lipidomics data improved the RoC scores to 0.96 for both the control vs mild disease and control vs advanced disease cohorts.

Conclusion

Targeted LC/MS/MS based lipidomics and rapid discovery proteomics were applied to a human prostate cancer study and shown to differentiate between the individual treatment therapies and identify the lipids and proteins responsible for the statistical separation of the patient groups. Although both approaches showed potential as predictive models of disease vs control the lipidomic methodology was simpler, faster and delivered improved RoC values compared to the proteomics model.

1. Proteome Res. 2022 Nov 4;21(11):2596-2608. doi: 10.1021/acs.jproteome.2c00297. Epub 2022 Oct 20.
2. J Proteome Res. 2021 Mar 5;20(3):1705-1715. doi: 10.1021/acs.jproteome.0c00821. Epub 2021 Feb 10.

Understanding the Pharmacometabodynamics of Gefitinib Using Ion Mobility MS Based Metabolomics and Microsampling

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3. Waters Corporation, Wilmslow, Cheshire, UK

Purpose
The objective of this study was to illustrate how LC/MS based analysis can be used to understand the relationship between drug and metabolite pharmacokinetics and changes in endogenous metabolic processes.

Methods
Gefitinib, an EGFR inhibitor, was dosed intravenously and orally to mice at 10 and 50 mg/kg. Blood samples were collected pre-dose and post dose via tail bleeding. The urine samples were collected and processed in a similar manner. The pharmacokinetics of the drug and major metabolites was determined by reversed-phase LC-MS/MS using a 5-minute gradient in positive ion ESI mode. The exogenous and endogenous metabolites were monitored using UPLC- MS on an ion mobility QTof with either a reversed-phase (RP) or HILIC gradient over 10 min.

Results
IV pharmacokinetics showed a peak plasma concentration of 4.4 µg/ml, 6 min post dose, with a half-life of 2.6 hr. Oral pharmacokinetics showed a peak concentration of 7.0µg/ml 2 hr post dose. Clearance was determined to be 14.4 mL/min/kg, with the volume of distribution at steady-state of 2.8L/kg. Gefitinib was rapidly and extensively metabolised to the metabolite metabolites. The Tmax for the major metabolites, O-demethylated and morpholino carbonyl, was 0.75 hr, with exposures of 917 and 812ng/mL h. Ten circulating plasma metabolites of gefitinib and 15 eliminated in urine were characterized using UHPLC/IM/HRMS, in addition two novel glucuronide and one new sulphate metabolites were identified. Untargeted metabolic phenotyping (metabolomics) using UPLC/IM/MS enabled 4475 urine features to be detected, resulting from the effects of the drug and its metabolites on the endogenous metabolic pathways. A total of 2400 of these endogenous metabolites were identified by database analysis, employing accurate mass of precursor, product ions and CCS values. Changes in endogenous metabolite profiles, both increasing and decreasing in amounts, appeared shortly after dosing and had largely returned to their pre-dose values by 24 hrs. The changes in the amounts of endogenous metabolites excreted in the urine mirrored to some extent the plasma pharmacokinetics of the drug, demonstrating a possible pharmacometabodynamic effect. The changes in endogenous metabolites resulting from gefitinib administration showed increases (e.g., tryptophan, taurocholic acid, and the dipeptide lysyl-arginine) and decreases (e.g., deoxyguanosine, 8-hydroxydeoxyguanosine, and asparaginyl-histidine) relative to control animals and could be mapped to the pharmacokinetic profile of gefitinib. By 8–24 hr, the post-dose concentrations of most metabolites had returned to near control values.

Conclusion
Untargeted metabolic phenotyping of biological samples has identified time-related changes in endogenous metabolites excreted that correlate with the circulating concentrations of the drug and its metabolites. The largest changes in endogenous metabolites coincided with the highest plasma concentrations of gefitinib and returned to control values as the drug concentrations fell. These metabolic responses have the potential to provide opportunities to examine both on and off target effects of drugs (and their metabolites). Such knowledge may enable a better understanding of the mode of drug action.

The Boost of in vitro ADMET, in vivo PK and Bioanalysis for Drug Discovery and Development

Pin Jiang, Dengji Zhang, Cheng Lou, Mimi Wan, and Shuangqing Peng

Purpose

The aim of this poster is to show the multiple research cases of in vitro ADME, in vivo PK. To demonstrate the importance and practical aspects of ADME and PK studies in drug discovery and development research, several case studies are presented in this poster. Case studies emphasizes the importance of ADME, PK and Bioanalysis in early phase studies of new drugs (e.g. oliogonucleotides, PROTACs, ADCs, etc.) to assist in clinical development. The targets of this article were to assess plasma protein binding, liver microsomal metabolic stability, and the pharmacokinetics of different drugs.

Methods

The microsomal stability assay is commonly used to rank compounds according to their metabolic stability, which influences how long the candidate may remain intact while circulating in plasma. In an assay similar to the metabolic stability assay, liver microsomes are used to determine the CYP450 inhibition profile of test compounds by measuring the % metabolism of a known substrate. The hERG channel inhibition assay is a highly sensitive measurement which will identify compounds exhibiting cardiotoxicity related to hERG inhibition in vivo.

Results

This poster highlights impact of ADME and PK studies on clinical and commercial success of biologics, with a particular focus on emerging applications and technologies and linkage with mechanistic PK/PD modeling and biomarker research. This poster will show several cases of ADME and PK studies of different drugs. Characterization of ADME properties help to explore and explain how pharmacokinetic processes happen, so as to provide safety considerations of a new drug on which risk-based assessments can be made. ADME studies are key at each preclinical stage of the drug discovery process, from high throughput screening (HTS), hit identification, lead optimization and finally the selection of a candidate molecule for clinical development.

Conclusion

Driven by the need for improved clinical success and by the competitive landscape in pharmaceutical industry, demands for mechanistic ADME studies of novel biologics continue to increase. Medicilon's DMPK&BA department offers our clients a broad spectrum of high quality services in the areas of in vitro ADME, in vivo pharmacokinetics and bioanalytical services, for both small and large molecule drugs, such as proteins, antibodies, oliogonucleotides, ADC and new modalities. We have available all common laboratory animal species such as non-human primates, canines, minipigs, mice, rats, rabbit and etc.

Overcoming Matrix Interference for an Enzymatic Pharmacokinetic Ligand Binding Assay for Protein X

Matthew Solomon, Dong Hun Lee, Ketal Shah, Deeptak Verma, Benjamin Bell, Linlin Luo

Purpose

The initial development of the pharmacokinetic assay for Protein X was performed by a contract research organization (CRO) but was hindered by the presence of high and various levels of its endogenous counterpart (1000-3000 ng/mL) in human plasma. This led to poor sensitivity as well as inconsistent accuracy and precision data presenting challenges for further development. The enzymatic kit utilized by the CRO was commercially available, comprising of proprietary reagents designed to detect endogenous counterpart. These challenges necessitated a shift in assay strategy, involving the transfer of method development and troubleshooting in-house to address and resolve the issues.

Methods

Standard pharmacokinetic ligand binding assays typically utilizes capture and detector antibodies which bind to the molecule of interest. Meanwhile, this enzymatic assay quantifies the analyte by measuring the level of coated substrate remaining on the plate after analyte incubation. According to the manufacturer's suggestion, standard curve was constructed in reaction buffer to quantitate blank and quality control (QC) samples in pooled plasma. With this method, blank plasma samples appeared to show very high levels of the endogenous counterpart ranging from 1000-3000 ng/mL. Quantifying QCs with or without background subtraction led to inconsistencies showing high percent bias of QC recoveries. A surrogate matrix, MSD Diluent 2, was introduced instead of the reaction buffer to construct calibrators intended to match with plasma matrix to see if the assay performance improves. The issues were not resolved with this modification. Therefore, it was decided to prepare the standards and QCs in plasma and apply background subtraction strategy for quantification. However, this strategy still caused day-to-day inconsistencies in which further optimization efforts were focused on inactivating the endogenous enzyme. To address this issue, calibrators, QCs, and test samples in plasma were diluted in phosphate-buffered saline (PBS, pH 7.4) instead of reaction buffer (~pH 5) before transferring onto a proprietary plate. Using PBS pH 7.4, as sample diluent, we were able to inactivate endogenous enzyme completely. This strategy enabled the elimination of endogenous background improving assay consistency and performance.

Results

The initial development challenge was that the endogenous enzyme would catalyze the immobilized substrate, leading to poor sensitivity and inconsistent accuracy and precision data. Collaborating with Merck's discovery department, it was observed that differing enzymatic activities between Protein X and endogenous enzyme occurred at various pH levels. Using this information, switching sample diluent from the kit's reaction buffer, which has a pH around 5, to PBS at pH 7.4 resulted in the complete inactivation of the endogenous enzyme, while maintaining the enzymatic activity of Protein X. Prior to inactivation of endogenous enzyme, up to 113% bias was observed in accuracy and precision. After inactivation, accuracy and precision profile produced a mean percent bias of the QCs, in six runs, ranging from -10.7% to 2.38%. This modification also led to a significant improvement in assay specificity and dilution linearity.

Conclusion

Understanding the differing enzymatic activities between Protein X and the endogenous counterpart at various pH levels, led to the switching of the sample diluent from proprietary reaction buffer to PBS (pH 7.4). This switch results in the complete inactivation of the endogenous enzyme, eliminating the matrix interference. Subsequently, the method was transferred back to the CRO for qualification.

New Statistical Approach for CP Determination for Data Sets with Multiple Population Distributions – Use of Deconvolution to Retain All Data Points and Avoid Over-Removal of Data As Outliers

Charles Gu, PhD[1]; Liam Heins[1]; Richard Snyder[2]; Michael Brown, PhD[2]; William Duncan, PhD[1]; Stephanie Pasas-Farmer, PhD[1]
[1] BioData Solutions, LLC;
[2] Q2 Solutions

Description

As drug modalities have increased in complexity, so have the challenges that bioanalytical scientists face in immunogenicity assay development as well as statistically setting an appropriate cut point. For many biologics and Cell and Gene Therapy Products (CGTPs), the detection of pre-existing antibodies in drug-naïve populations – due to past treatments or exposures to certain drug delivery components (e.g. viral or lipid nanoparticle delivery) – has increased greatly. The downstream impact is the impeding of setting of an appropriate cut point to determine drug induced immunogenicity. Pre-existing antibodies during cut point assessment can lead to data sets with multiple population distributions, hindering the ability to determine cut point values with the appropriate false positive rates when using conventional statistical approaches.

Purpose

To evaluate a new approach without outlier removal that can identify and calculate cut points values in data sets demonstrating complex distribution and potentially containing more than one clinically relevant population.

Methods

The deconvolution approach utilizing the Expectation-Maximization Algorithm was evaluated through four distinct simulation settings, each comprising 100 iterations of the EM algorithm in order to model the two populations within the dummy data set: drug-naïve and diseased. These settings varied in mixing proportions as well as the mean and standard deviation values of the signal-to-noise ratio (S/N). Specifically, the drug-naïve population exhibited lower S/N mean and standard deviation, while the diseased population demonstrated higher values. To evaluate the classification performance, the Rand index and adjusted Rand index were computed. These indices provide a quantification of the agreement between the true underlying populations and the classifications obtained from the EM algorithm. Furthermore, the screening cut point, determined based on the deconvoluted results, was compared against the known underlying populations to assess the percentage differences.

Additionally, the algorithm was applied to a case study where no preidentified sub-populations were known but suspected by subject matter experts. Cut points were calculated after identifying distinct populations through fitting normal distributions using the Expectation-Maximization algorithm.

Results

This publication presents the findings from simulation studies as well as the analysis of a real dataset obtained from clinical stage biologics. The simulation studies revealed that multiple populations within certain datasets can significantly impact the accuracy of cut point determination. By employing the deconvolution approach, the analysis of the real dataset demonstrated the emergence of distinct populations that were initially treated as a single population. This observation emphasizes the importance of calculating multiple cut points within a single dataset to appropriately account for statistically observed multiple populations. The results from both the simulation studies and the analysis of the real dataset collectively highlight the necessity of considering and addressing the presence of multiple populations for accurate data interpretation.

Conclusion and Novelty

Removal of data through outlier testing can be unable to distinguish between a data set with multiple populations vs outliers resulting in either over removal of data or overestimation of CP values. However, this publication demonstrates that using a devolution approach for cut point assessment can more accurately estimate the cut points for complex clinical populations and is a potential approach to overcome the impact of pre-existing antibodies or interferences within the data set.

Note: We will add one or more clinical datasets to the poster

Analysis of the Monoclonal Antibody Pembrolizumab in Human Blood Collected via Volumetric Adsorptive Microsampling (VAMS) Technology and Utilizing LC-MS/MS Detection

Ramakrishna Reddy Voggu, Eric Thomas, Labcorp Bioanalytical Chemistry

Purpose

There is growing interest in application of microsampling in quantitation of therapeutics and biomarkers in biological matrix in clinical and non-clinical spaces. With advances in analytical sensitivity, required sample volumes for analysis have been reduced. A study assessing the feasibility of performing quantitative analysis of Pembrolizumab in human whole blood collected using Mitra® (Neoteryx) volumetric adsorptive microsampling (VAMS) devices was conducted. Pembrolizumab, a humanized monoclonal IgG4 antibody directed against PD-1 (commonly prescribed for the treatment of various cancer types) was selected to assess the practicality of protein analysis via LC-MS/MS analysis coupled with VAMS technology.

Methods

Aliquots of Pembrolizumab-fortified human whole blood were collected using MitraTM VAMS® device and dried overnight at ambient temperature. The dried Mitra® tips were transferred into a 96-well plate; buffer was added, and the plate was vortex-mixed to solubilize the analyte. Pembrolizumab was digested using a SMART Digest™ Trypsin kit. Following digestion, a stable isotopically-labeled peptide was added as an internal standard. Quantitation of the surrogate peptides was performed by LC-MS/MS (Sciex API5500™) utilizing ESI+.

Results

The LC-MS/MS method demonstrated acceptable accuracy, precision and robustness, supporting the quantification of Pembrolizumab. Accuracy and precision were assessed using QCs prepared at 1.00, 3.00, 12.5, 50.0 and 80.0 µg/mL. The intra-assay precision was ≤5.6875% and accuracy was within ±4.3903% across all the QC levels. Sufficient selectivity and specificity were also demonstrated. Lot-to-lot consistency was demonstrated for Pembrolizumab, demonstrating consistent recovery of the selected Pembrolizumab surrogate peptide and also a lack interference from by-products of the manufacturing process.

Conclusion

This study demonstrates the feasibility of LC-MS/MS detection for biotherapeutic protein quantitation coupled with VAMS technology. These results point towards future flexibility for remote and at-home sampling, simplifying the lives of patients who find themselves on strict drug regimens.

Method Validation of Phospho-Tau217 (pTau-217) Quantitation Using the Quanterix Simoa™ HD-X Platform and Potential Applications

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Purpose

Both blood-based (plasma and serum) and CSF tau phosphorylated at Threonine 217 (pTau-217) have been shown to be able to discriminate early to mild Alzheimer's disease (AD) from non-AD neurodegenerative disorders and healthy controls with high sensitivity and specificity. Here we present the fully validated ultra-sensitive pTau-217 assays for human plasma, serum, and CSF using the Quanterix Simoa HD-X platform. It is a new addition to the established protein biomarker assays (pTau-181, GFAP, and Nf-L) that we have validated in support of clinical trials and the early diagnosis of neurodegenerative diseases.

Methods

pTau-217 Simoa CARe Advantage kit from ALZpath/Quanterix has been used for the method development and validation of pTau-217 assay for human plasma, serum, and CSF. All three validations have followed the 2018 FDA BMV and 2022 ICH M10 guidelines.

Results

Validation of pTau-217 quantitation in human plasma, serum, and CSF has demonstrated that the validated pTau-217 assay has an analytical LLOQ of 0.00977 pg/mL. It is one of the most sensitive assays with a minimal required sample volume of 33.3 µl for plasma and serum, and 5 µl for CSF, and is relatively cost-effective, compared to the current published data (see reference 1-4). The MRD for plasma and serum and CSF were determined. The analyte passed short-term stability tests. It also tolerates moderate hemolytic, and lipemic interferences in plasma and serum. pTau-217 was able to be detected in 100% tested normal plasma, 90.0% normal serum and 92.3% normal CSF samples, and 100% AD plasma and CSF samples. The validated assay was successfully applied in the sample analysis and the data show dramatic differences on the pTau-217 levels between normal subjects and AD patients in both plasma and CSF.

Conclusion

Validation of the pTau-217 Simoa assay in human plasma, serum, and CSF demonstrated that it is a novel approach with ultra sensitivity, high accuracy, and assay robustness. Together with the established sensitive pTau-181, GFAP, and Nf-L assays, these assays are available for application in clinical trials and the early diagnosis of neurodegenerative diseases.

Development of a Novel Milli-fluidic Liver Tissue Platform for Mechanistic Pharmacokinetic (PK) and Drug-Drug Interaction (DDI) studies

Shiny Amala Priya Rajan*, Jason Sherfey, Shivam Ohri, Lauren Nichols, J. Tyler Smith, Paarth Parekh, Emily Geishecker, R. Scott Obach, Murat Cirit1

Purpose

A crucial step in lead selection during drug development is accurate estimation and optimization of hepatic clearance and DDI using in vitro methods. After the FDA Modernization Act 2.0, the need for in vitro alternative technologies has been drastically increased. Human tissue chips, aka organs on chips, provide a platform to develop new alternative methodologies (NAMs). However, current methods are limited by lack of physiological relevance, short culture/incubation times, use of drug absorbing materials, and evaporation during long-term incubation, which have limited utility to develop NAMs for mechanistic pharmacology studies. Here, (i) we developed novel liver tissue chip (LTC) technology to address the limitations of existing technologies, (ii) evaluated its human-relevance with phenotypic characterization including morphology, in vitro and clinical biomarkers and gene expression similarities and (iii) evaluated the platform for PK and DDI mechanistic studies.

Methods

We designed and manufactured a milli-fluidic LTC with continuous recirculation to enable mechanistic pharmacology studies by generating multi-scale data (media- and tissue level). We evaluated primary hepatic tissue model stability & function over 15 days (tissue morphology, albumin & urea production, mRNA gene expression and metabolic activity) and compared these results with clinical values. Then, we tested 16 test compounds to estimate hepatic clearance and assessed rifampicin induced DDI on midazolam.

Results

Phenotypically, the LTC exhibited functional and polarized hepatic culture with sustained metabolic CYP activity for at least 15 days. Both albumin and urea production rates observed in LTC was comparable to clinical rates. Drug clearance studies demonstrated high in vitro in vivo correlation (IVIVC) and accurate clinical exposure predictions with physiologically based pharmacokinetic (PBPK) modeling. We also demonstrated that LTC can be induced by rifampicin and observed a 50.6% and 29.7% decrease in AUC of midazolam depletion and metabolite formation kinetics compared to control.

Conclusion

We used advances in bioengineering to develop a novel purpose-built tissue chip platform to address current challenges in preclinical invitro platform. The preliminary data demonstrated high reproducibility and the utility of this platform to study pharmacokinetic and DDI processes to advance mechanistic understanding how drugs will perform with human physiology.

Comprehensive Characterization of tRNA by Ultra High-Performance Liquid Chromatography High-Resolution Accurate Mass Spectrometry

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Purpose

tRNAs have a mass of ~ 25k Da, range in length for 70-100 nt and contain the greatest density of post transcriptional modifications. These modifications range from simple methylations to more intricate chemistry resulting from extensive enzymatic pathways. The 3' end of tRNA ends in the sequence CCA, with the terminal adenosine carrying the amino acid specific to the tRNA anticodon. Historically, RNase digestion followed by LC-MS/MS analysis has been applied for analysis of tRNAs and their modifications. Here, using UHPLC-HRAM-MS we show the ability to fully characterize a tRNA through deconvolution of an accurate mass isotopic peak envelope, identify single base substitutions, and monitor levels of acceptor stem maturation with monoisotopic mass errors ≤ 5ppm.

Methods

tRNA PHE standard was purchased from Sigma Aldrich. Nucleoside separation was performed on an Accucore C18+ column using a Vanquish™ UHPLC with ammonium acetate mobile phase. Oligonucleotide and intact tRNA chromatography were performed using a Thermo Scientific MabPac™ Reverse Phase column using ion pairing chromatography on a Vanquish™ Horizon UHPLC. Partial oligonucleotide analysis was performed using Thermo Scientific SMART Digest™ RNase T1 kit. Acquisition was performed on a Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer running Xcalibur™ 4.3. Data was processed using Thermo Scientific™ Compound Discoverer™ 3.2, Freestyle™ 1.8 and BioPharma Finder™ 5.1.

Results

Intact analysis was performed on the Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer at 120K resolution using intact protein mode at low pressure. Two hundred nanograms of tRNA yeast PHE standard was injected on column and resulted in a single peak having a charge state envelope between states 13 and 27. Zooming into the highest charge state (-20) showed three distinct isotopically resolved peaks, the main, large peak and two smaller peaks ± center. Known sequences of PHE and its isodecoder, both containing internal modifications, were created in BioPharma Finder 5.1 and an Intact Analysis was performed using the terminal truncation feature available in the software. Deconvolution of observed peaks resulted in the identification of tRNA PHE and its isodecoder, both having truncation at the 3' terminal. Further iterative deconvolution with sequence modifications for acceptor stem CCA nucleotide losses confirmed the identification of both tRNA species having variation in acceptor stem maturation. The most abundant tRNA in both species terminated in CpCp motif with identified mass difference between monoisotopic and theoretical at < 5ppm. Verification of the acceptor stem truncation was performed utilizing a partial digestion strategy followed by UHPLC-HRAM-MS/MS analysis and BioPharma Finder™ 5.1 mapping.

Conclusion

The use of high resolution mass spectrometry allows for full characterization of tRNAs and allows for a quick and easy method for monitoring modification status.

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Mass Analytica Our mission is to develop software to boost drug design and optimization processes. We also do contract research organization on their fields for several pharmaceutical companies abroad.

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