APPLIED PHARMACEUTICAL ANALYSIS

OCTOBER 7-9, 2024

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2024 WORKSHOPS: OCT 7 - Regulated Bioanalysis | OCT 8 - Discovery Bioanalysis & New Technologies | OCT 9 - Mechanistic ADME

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Welcome to the 2024 Applied Pharmaceutical Analysis Conference.

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

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APA 2024 CONFERENCE AGENDA

DAY 1: Monday, Oct. 7 | Regulated Bioanalysis Workshop

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

8:30 - 8:40 AM **Workshop Introduction**
Joseph Tweed, Bicycle Therapeutics

SESSION I: Regulatory and Industry Perspectives on M10 Implementation

8:40 - 8:45 AM **Session Introduction**
Nevena Mollova, Gilead; James Schiller, Merck & Joseph Tweed, Bicycle Therapeutics

8:45 - 9:05 AM **Recent Data Integrity Findings in Bioresearch Monitoring**
Yi-Ying Chen, FDA

9:05 - 9:25 AM **Regulatory inspections from a CRO perspective – balancing guidance, SOPs and sponsor direction**
Amanda Leskovar, Icon

9:25 - 9:45 AM **The Evolving Role of Bioanalytical Scientist in Drug Development Beyond ICH-M10**
Faye Vazvaei, Merck

9:45 - 10:15 AM **PLENARY: *Lessons Learned from Regulatory Submissions Involving Endogenous Therapeutic Analyte: Do You See What I See?***
Chongwoo Yu, FDA

10:15 - 10:35 AM **Panel Discussion**

10:35 - 10:55 AM **Break & Exhibits**

10:50 - 11:10 AM VENDOR PRESENTATION
PCR-Based Assays for Bioanalysis: Challenges and Latest Recommendations
Carrie Vyhlidal, KCAS Bio

SESSION II: Advances in Sampling Strategies and Drug Delivery Innovations

11:10 - 11:15 AM **Session Introduction**
Lori Payne, Alturas Analytics & Jeongsup Shim, Genentech

11:15 - 11:40 AM **Implementation of Low Volume Sampling Technologies for PK and Biomarker Analysis: Technical and Logistical Challenges and Solutions**
Xiaoyun Yang, Genentech

11:40 - 12:05 PM **Accelerator Mass Spectrometry (AMS) Applications for Supporting Clinical Development**
Xiaomin Wang, BMS

12:05 - 12:30 PM **Qualification of Low Energy Accelerator Mass Spectrometer and Opportunities To Increase Industry Utilization**
Stephen English, Pharmaron

12:30 - 1:40 PM **Lunch, Exhibits, and Poster Viewing (1:10 pm - 1:40 pm)**

1:40 - 1:55 PM VENDOR PRESENTATION
Unlocking Bioanalytical Automation: Bridging Development and Practical Application
Tom Zhang, Worldwide Clinical Trials

Session III: Advances in Drug Modalities and Emerging Technologies for Characterization

1:55 - 2:00 PM **Session Introduction**
Ang Liu, J&J; Fumin Li, Unitides Bioanalytical Lab & Darshana Jani, Moderna

2:00 - 2:40 PM **PLENARY: *Application of AI in Drug Development***
Joe Rajaro, Accenture

2:40 - 3:05 PM **A Novel Hybridization LC-MS/MS Methodology for Bioanalysis of siRNA**
Long Yuan, Biogen

3:05 - 3:30 PM **Bioanalytical Method Case Studies Across the mRNA-LNP Therapeutic Portfolio**
Jason DelCarpini, Moderna

3:30 - 3:50 PM **Break & Exhibits**

3:50 - 4:15 PM **Development of Nonclinical Total Anti-Drug Antibody (ADA) Assays to Support The Reproductive Toxicology Studies for Zinpentraxin Alfa**
Audrey Arjomandi, Genentech

4:15 - 4:40 PM **Navigating Challenges in the Development of Neutralizing Antibody Assays: Key Considerations and Strategies**
Nazneen Bano, Merck

Session IV: Innovation Junction: Bridging Science and Solutions in Pharma

4:40 - 4:55 PM **SPONSOR SHOWCASE**
Anthony Genovese, Alturas; Matthew Hutzler, Inotiv; Larry Elvebak, LabIntegrity

Session V: Rapid Fire Poster Presentations

4:55 - 5:00 PM **Session Introduction**
Joseph Tweed, Bicycle Therapeutics

5:00 - 5:20 PM **Poster Presentations**

POSTER PRESENTERS:

1. **Simple and Sensitive Multiplexed HRMS/MS Method Development for Quantitation of PPMO and PMO in Cyno Plasma using Protein Precipitation**
   Abhi Shah, PepGen

2. **Singlicate analysis in ligand binding assays from discovery to regulated clinical studies: Implementation strategies and benefits**
   Danielle Salha, AltaSciences

3. **Antibody-drug conjugate bioanalysis: A case study in stability, matrix effects, and recovery**
   Paige Malec, Labcorp

4. **Accurate and Precise Quantitation of the Pentadecapeptide BPC-157 from Human Blood Collected with the Tasso-M20 Microsampling Device and Analyzed by HPLC-MS/MS**
   Leslie Hvozda, Alturas Analytics

DAY 2: Tuesday, Oct. 8 | Discovery Bioanalysis & New Technologies Workshop

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

8:30 - 8:35 AM **Workshop Introduction**
Hiroshi Sugimoto, Takeda

SESSION I: Emerging Analytical & Bioanalytical Platform Technology

8:35 - 8:40 AM **Session Introduction**
Hiroshi Sugimoto, Takeda & Zack Parsons, BMS

8:40 - 9:20 AM **PLENARY: *Single-Cell Spatial Dissection Of The Pancreatic Tumor Microenvironment***
William Hwang, Harvard Medical School

9:20 - 9:50 AM **Evaluation of Brain Region- and Cell Type-Derived Spatial Knockdown Efficacy of Oligonucleotides Using a Spatial Transcriptomics Platform**
Quishi Liu, Takeda

9:50 - 10:20 AM **Bio-Digital Transformation of Proteomic Analysis**
Youhei Kosugi, Takeda

10:20 - 10:50 AM **Break & Exhibits**

10:50 - 11:20 AM **Basics of Machine Learning and Its Applications in Drug Discovery**
Reilly Eason, Merck

SESSION II: Novel Strategy to Advance Biotherapeutic Development

11:20 - 11:25 AM **Session Introduction**
Yu Tian, AbbVie & Ju Liu, Eli Lilly

11:25 - 11:55 AM **Measuring Endogenous and Transgene-Expressed Protein Levels in Gene Therapy Bioanalytical and Biomarker Studies**
Milan Kothiya, Eli Lilly

11:55 - 12:25 PM **Domain Specificity in the Tabs and Nabs Assay: Never too late for the characterization of ADA for multi-domain therapeutics**
Richard Hughes, Resolian

12:25 - 12:55 PM **A Comparison of bDNA and RT-qPCR Methodologies for Quantitation of LNP Encapsulated mRNA**
Syed Ali, Moderna

12:55 - 2:10 PM **Lunch, Exhibits, and Poster Viewing (1:40 - 2:10 pm)**

2:10 - 2:20 PM **VENDOR PRESENTATION**
Build Resilience and Maintain Unparalleled Sensitivity for Long-Term Bioanalysis
Robert Proos, Sciex

SESSION III: Innovation Junction: Bridging Science and Solutions in Pharma

2:20 - 2:25 PM **Session Introduction**
Hongying Gao, Innovo Bioanalysis LLC & Christopher Kochansky, Exelixis

2:25 - 3:05 PM **Vendor Presentations**
Bill Coode, KCAS Bio; Tom Zhang, WWCT; Peggy Teng, Medicilon; Anahita Keyhani, Altasciences; Tilak Chandrasekaran, Sciex; Chris Bunker, Alamar Bio

SESSION IV: Recent Case Study to Support New Modalities (oligonucleotides/C&GT)/bioanalytical

3:05 - 3:10 PM **Session Introduction**
John Wiliams, Vertex, Jonathan Josephs, Genentech & Jing Tu, GSK

3:10 - 3:40 PM **The role of DMPK in non-viral Gene Therapies**
Nag Chemuturi, Eli Lilly

3:40 - 4:00 PM **Break & Exhibits**

4:00 - 4:30 PM **Innovative Approaches and Case Studies in the Bioanalysis of Oligonucleotide Therapeutics**
Aihua Liu, Resolian

4:30 - 5:00 PM **Characterizing In Vivo Stability of Large Molecule Conjugates Using Complementary Bioanalytical Tools**
Jeff Lin, Genentech

5:00 - 6:15 PM **Reception & Exhibits**

DAY 3: Wednesday, Oct. 9 | Mechanistic ADME Workshop

7:30 - 8:30 AM **Registration, Breakfast, Exhibits**
8:30 - 8:40 AM **Workshop Introduction**
Nagendra Chemuturi, Eli Lilly

SESSION I: Role of NonCYP Enzymes in DDI

8:40 - 8:45 AM **Session Introduction**
Raman Sharma, Pfizer & Nagendra Chemuturi, Eli Lilly

8:45 - 9:10 AM **Uncertainties and Opportunities in Predicting Non-CYP Mediated Drug Metabolism**
Sandhya Subash, WSU

9:10 - 9:35 AM **Mitigation Strategies for Drug-Induced Liver Injury (DILI) - Still an Aspiration?**
Kaushik Mitra, Janssen

9:35 - 10:00 AM **Recent Advancements in Predicting CYP-Independent Metabolism**
Jasleen Sodhi, Septerna

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

10:25 - 10:45 AM **Rapid Fire Poster Presentations**

1. **Overcoming Challenges in LC- High-Resolution Mass Spectrometry (HRMS) Method Development for Quantitation of PPMO and PMO in Human Urine**
Shaoxia Yu, PepGen

2. **Stable Isotope Labeling by Amino Acid in Culture (SILAC) Approach for the Detection of Trapped Reactive Metabolites in Plated Hepatocytes**
Chris Bode, Pharmaron

3. **Closing the Translational Gap in Drug Development: Human Multi-Tissue Chip Platform for Predictive and Mechanistic Preclinical Studies**
Shiny Rajan, Javelin Bio

4. **Metabolism of 14C-retatrutide following subcutaneous administration in healthy male participants**
Ravikanth Veluri, Eli Lilly

SESSION II: PK/PD Challenges of New Modalities: ADCs, in vivo Gene Therapies, and PROTACs

10:45 - 10:50 AM **Session Introduction**
Sara Shum, Orna Therapeutics & Hongbin Yu, Boehringer Ingelheim

10:50 - 11:15 AM **Preclinical To Clinical Translation of ADCs Using PK/PD Modeling**
Minu Pilvankar, Boehringer Ingelheim

11:15 - 11:40 AM **Empirical PK/PD Modeling of Novel In Vivo CRISPR/Cas9 Genome Editing Therapies**
Adam Amaral, Intellia

11:40 - 12:05 PM **Addressing Protac™ ADME Challenges**
Stefanus Steyn, Pfizer

12:05 - 1:20 PM **Lunch, Exhibits, and Poster Viewing (12:35 - 1:00 pm)**

1:20 - 1:25 PM **Plenary Speaker Introduction**

1:25 - 2:05 PM **PLENARY: *Prospects for New Approach Methodologies and Complex In Vitro Models to Reduce, Refine and Replace Animals in Drug Safety Testing***
Terry Van Vleet, AbbVie

SESSION III: The importance of tracking missing metabolites/ new technologies (covalent drugs)

2:05 - 2:10 PM **Session Introduction**
Eric Ballard, Takeda

2:10 - 2:35 PM **A "Missing" Trazpiroben Metabolite Observed as the Most Abundant Metabolite in Human Plasma and Urine**
Sean Zhu, Takeda

2:35 - 3:00 PM **Human ADME Characterization of Linerixibat Reveals Unusual Pharmacokinetic Properties: Implications for Clinical Development**
Maciej Gliszczynski, GSK

3:00 - 3:20 PM **Break & Exhibits**

3:20 - 3:45 PM **Impact of Missing Metabolites During Lead Optimization in Drug Discovery**
Joyce Liu, Genentech

SESSION IV: New Approaches to in Vitro ADME Assays for Improved Drug-Drug-Interaction and Clearance Predictions

3:45 - 3:50 PM **Session Introduction**
Benjamin Johnson, BMS

3:50 - 4:15 PM **Prediction of Aldehyde Oxidase Mediated Clearance: Recent Progress in Quantitative Translation**
Prakash Bolleddula, iTeos Therapeutics

4:15 - 4:40 PM **Predicting Clinical Drug-Drug Interactions of Cytochrome P450 Time-Dependent Inhibitors - How Successful Are We?**
Elaine Tseng, Pfizer

4:40 - 5:05 PM **Hepatocyte Uptake and Loss Assay (HUpLA): Proof-of-Concept of an All-in-One System for Same Day Measuring Hepatic Influx, Egress, and Metabolic Clearance Based on the Extended Clearance Concept**
Julia Pauly, AbbVie

5:05 - 5:10 PM **Conference Closing Remarks**

APA ABSTRACTS

REGULATED BIOANALYSIS WORKSHOP

SESSION I: REGULATORY AND INDUSTRY PERSPECTIVES ON M10 IMPLEMENTATION

Recent Data Integrity Findings in Bioresearch Monitoring

Yi-Ying Chen, FDA

FDA's Bioresearch Monitoring (BIMO) consists of compliance programs that provide instructions to FDA personnel while conducting inspections to evaluate industry compliance with the Federal Food, Drug, and Cosmetic Act and other laws administered by FDA. The Office of Study Integrity and Surveillance (OSIS) is responsible for the In Vivo Bioavailability and Bioequivalence (BA/BE) Studies compliance program and oversees the Good Laboratory Practice (Nonclinical Laboratories) and Inspection of Nonclinical Laboratories Conducting Animal Rule-Specific Studies compliance programs with other FDA Centers.

FDA expects that all data submitted to the Agency, including data from BA/BE and GLP studies submitted in support of INDs, NDAs, BLAs and ANDAs to be accurate, complete, and reliable and that industry maintain data integrity throughout the data lifecycle of the drug products and biologic therapeutics. In recent years, FDA has observed data integrity concerns during the inspection of testing sites and during the assessment of BA/BE and GLP study data submitted in support of applications. Data integrity concerns can impact application acceptance for filing, assessment, regulatory actions, and approval as well as post-approval actions, such as therapeutic equivalence ratings. My presentation will give an overview of how OSIS conducts inspections of testing sites that perform BA/BE studies to verify that the study data are reliable to support a regulatory decision. I will also review some recent data integrity issues observed during inspections and discuss their evaluations based on scientific approaches.

Regulatory inspections from a CRO perspective – balancing guidance, SOPs and sponsor direction

Amanda Leskovar, Icon

An FDA inspection in 2024 of a bioanalytical CRO in support of a BLA and NDA – details of the agency's focus and the CRO's takeaways on sponsor 'direction'.

The Evolving Role of Bioanalytical Scientists in Drug Development

Faye Vazvaei-Smith, Merck

Over two years have passed since the International Council of Harmonization adopted the "Bioanalytical Method Validation and Sample Analysis" in May 2022. This guideline has been the subject of discussion at many meetings since its adoption. While the guideline has been viewed as a positive step in the harmonization and standardization of bioanalytical method validation and sample analysis for drugs and metabolites measurement globally, there are still aspects of the recommendations that remain ambiguous within the bioanalytical community. Consistently, cross-validation is mentioned as one of the most ambiguous recommendations since it removes the need for acceptance criteria from the equation and recommends the measurement of any bias in cases when cross-validation is warranted. This notion is still difficult to grasp as bioanalytical scientists are accustomed to applying rules to the acceptability of the data they produce and report. In this presentation, using case studies, I will demonstrate how such statistical approaches could be implemented and how they can aid the teams in determining the appropriate dosages for patients.

This call for bias measurement for cross-validation is an important step in moving bioanalytical scientists out of their comfort zones and engaging in more dialogue on drug development as contributing scientists offering solutions rather than remaining service providers. A vibrant interaction between scientists, as with so much problem-solving, is necessary for accelerating and enriching both the scientist and the process, revealing possibilities that would be otherwise harder to achieve individually as one step after another in a chain. This is becoming especially relevant for modalities such as macrocyclic peptides, drug conjugates (e.g., antibody-drug conjugates, peptide drug conjugates), pro-antibodies, and multi-functional antibodies, where modality- and technology-agnostic bioanalytical experts are needed to help design appropriate experiments to adequately characterize the pharmacokinetics and immunogenicity of the molecules of interest, for example, the number of PK assays needed to characterize antibody-drug conjugates. We, as bioanalytical scientists, must bring our expertise and insights to the drug development table and push the boundaries to help accelerate the process, bringing safer and more effective medicine to patients in an efficient manner.

PLENARY PRESENTATION

Lessons Learned from Regulatory Submissions Involving Endogenous Therapeutic Analyte Bioanalysis: Do You See What I See?

Chongwoo Yu, FDA

Endogenous therapeutic analytes include hormones, neurotransmitters, vitamins, fatty acids and inorganic elements that are naturally present in the body because either the body produces them or they are present in the normal diet. The accurate measurement of endogenous therapeutic analytes poses a challenge when the administered exogenous therapeutic analyte and its endogenous counterpart cannot be distinguished.

Real case examples with endogenous therapeutic analyte bioanalysis during drug development in support of regulatory submissions will be presented. This presentation will highlight common challenges encountered, lessons learned related to bioanalysis of endogenous therapeutic analytes, and provide practical tips and strategies to consider from a regulatory perspective.

VENDOR PRESENTATION

PCR-Based Assays for Bioanalysis: Challenges and Latest Recommendations

Carrie Vyhlidal, KCASBio

The field of cell and gene therapies continues to see momentum in all stages of development with an estimated >4000 gene, cell or RNA therapies in the development pipeline. PCR-based assays are widely used to support studies of biodistribution, shedding, transgene expression and kinetics of these promising therapies. In spite of broad use, there are limited regulatory guidelines and an overall lack of harmonization for the development and validation of PCR-based molecular assays for bioanalysis. In this presentation, we will discuss current recommendations and consensus from the industry on best practices for qPCR and dPCR assay bioanalysis and data management.

SESSION II: ADVANCES IN SAMPLING STRATEGIES AND DRUG DELIVERY INNOVATIONS

Implementation of low volume sampling technologies for PK and biomarker analysis: technical and logistical challenges and solutions

Xiaoyun Yang, Genentech

While low volume sampling technologies offer numerous advantages over venipuncture, implementation in clinical trials poses technical and logistical challenges. In a proof-of-concept study, we evaluated the pharmacokinetic (PK) profiles of two monoclonal antibody therapeutics, crenezumab and etrolizuamb, using various low volume sampling devices (Mitra, Tasso-M20 and TassoOne Plus) acompared to conventional venipuncture. Additionally, we measure soluble mucosal addressin cell adhesion molecule-1 (sMAdCAM-1) as a PD biomarker for etrolizumab across these devices. For the PK analysis, serum concentrations and different PK paramaters from venipuncture and TassoOne Plus samples were highly comparable for both drugs. After applying a baseline hematocrit value, the dried blood concentrations and PK parameters were comparable to those obtained from venipuncture. For sMAdCAM-1, we observed significantly higher concentration in dried blood samples collected using Mitra and Tasso-M20 compared to serum in some paired samples, which was attributed to interference from the dried blood extraction buffer. To mitigate this interference, we pivoted to a more sensitive assay platform with samples substantially diluted into an appropriate buffer. Both liquid blood collected in TassoOne Plus and dried blood collected using Mitra and Tasso-M20 demonstrated great concordance with venipuncture serum for sMAdCAM-1 measurement. However, a bias was observed in Mitra dried blood samples, presumably due to the different sample collection sites in comparison with venipuncture and Tasso devices. Our study highlights the potential of low volume sampling technologies for PK and biomarker analysis, and underscores the importance of understanding the challenges and limitations of these technologies before integrating them into clinical studies.

Accelerator Mass Spectrometry Applications for Supporting Clinical Development

Xiaomin Wang, BMS

Accelerator Mass Spectrometry (AMS) is a highly sensitive technique used to measure the concentration of isotopes, such as 14C, in biological samples. AMS allows for very low dosing of a labeled drug to study its pharmacokinetics and metabolism of the drug without exposing subjects to significant risks. This technique can measure trace amounts of drug-related material in biological matrices, providing insight into the drug's absorption, distribution, metabolism, and excretion (ADME). Consequently, AMS has found significant applications in mass balance studies, metabolite profiling, pharmacokinetics, and

pharmacodynamics.

With the significant advancements of new-generation instrumentation, AMS is expected to have broader applications in the future drug development. This presentation will highlight two case studies to illustrate the unique applications of AMS:

1. A Phase 1 open-label study in healthy subjects to evaluate the absolute bioavailability and absorption, distribution, metabolism, and excretion (ADME) of AG221.
1. Quantification of azacitidine incorporation into human DNA/RNA as a direct measure of target engagement

Additionally, recent US Food and Drug Administration (FDA) guidances for AMS applications will be reviewed.

Qualification of Low Energy Accelerator Mass Spectrometer and Opportunities to Increase Industry Utilization

Stephen English, Pharmaron

Accelerator Mass Spectrometry (AMS) has been increasingly applied as a technique for the detection of low levels of 14C material in support of drug development (1). Originally, this technique required graphitization as a sample preparation step to render all sample material into elemental carbon prior to AMS detection. This application is still in routine use; however, in recent years, the advancement of an automated gaseous interface has been explored with multiple instrument manufacturers developing further optimized models. The newest such model, the Low Energy AMS (LEAMS), manufactured by IonPlus, has been subjected to a series of qualification activities by Pharmaron (Germantown) Lab Services, Inc. to show efficacy of the instrument to support radioanalysis activities in support of human absorption, metabolism, and excretion (hAME) and human absolute bioavailability (hABA) applications. These qualification experiments represent a thorough comparison between graphite single-stage AMS (SSAMS) and gaseous LEAMS. Dynamic range, co-equivalence with SSAMS, linearity with liquid scintillation counters, accuracy and precision at modern carbon background, accuracy and precision at instrument background, and a thorough assessment of memory effects have been performed. Evaluation of this data positions LEAMS as a suitable option for the detection of low levels of 14C material and widens the opportunities for microtracer applications. In addition to the efficacy of the instrument technically, LEAMS utilizes a 21CFR Part 11 compliant software package allowing for a routine computer system validation in line with other complementary technologies currently supporting hAME and hABA applications. Historically, the decision to use AMS has been primarily motivated by compound specific properties such as long-half-life or low-exposure. However, in recent years it has become more accepted as a valuable analytical tool, supported by the recommendation from industry leaders to introduce a microtracer dose into Phase 1 AME/ABA (2) as well as the issuance of the FDA draft guidance (3). This presentation will review the technical qualification performed and present a justification for LEAMS to support hAME and hABA applications. Additionally, the presentation will evaluate the typical approach for running hAME studies with conventional dose levels (≥20 µCi) and hypothesize whether LEAMS represents an opportunity to shift more such applications towards lower microtracer doses in the future based on turn-around time, throughput, and cost considerations.

VENDOR PRESENTATION

Unlocking Bioanalytical Automation: Bridging Development and Practical Application

Tom Zhang, Worldwide Clinical Trials

Bioanalytical automation has evolved significantly, with key innovations shaping the field. However, its application in bioanalytical labs remains limited compared to other areas, primarily due to programing and regulatory challenges. Here the success stories from implemented automation systems are shared highlighting valuable lessons and best practices.

SESSION III: ADVANCES IN DRUG MODALITIES AND EMERGING TECHNOLOGIES FOR CHARACTERIZATION

PLENARY PRESENTATION

Application of AI in Drug Development

Joe Rajarao, Accenture

Artificial Intelligence (AI) has the potential to transform many aspects of drug discovery and development. The use of AI in target identification, drug screening, clinical trial design and execution, as well as regulatory processes is showing promise. This presentation will explore the use of AI and related domains, such as machine learning, neural networks, and large language models (LLMs) to improve productivity and drive outcomes. The excitement and promise of AI , especially in the regulated bioanalytical space, must be balanced with caution and thoughtful skepticism. The talk will also touch on exploratory efforts to

use LLMs to create bioanalytical method documents to reduce human effort and improve outcomes.

A novel hybridization LC-MS/MS methodology for bioanalysis of siRNA

Long Yuan, Biogen

Small interfering RNAs (siRNAs) are a new class of oligonucleotide therapeutics rapidly growing in drug research and development for the treatment of various diseases. Accurate, sensitive, and reliable quantification of siRNAs in various biological matrices is critical for understanding the pharmacokinetic (PK), toxicokinetic (TK) and biodistribution of siRNA drug candidates. Traditionally, hybridization enzyme linked immunosorbent assay (hELISA) is commonly used for the quantitation of oligonucleotides with its good sensitivity. However, hELISA often cannot differentiate the full-length oligonucleotide analyte from its truncated metabolites (e.g., N-1 metabolites). LC-MS/MS has its unique advantage of high specificity to differentiate from the truncated metabolites, but its sensitivity is often significantly lower compared to hELISA.

Here we present a novel hybridization LC-MS/MS methodology for the quantification of siRNA in biological samples. This methodology utilized a capture probe complementary to the antisense strand of the target siRNA to specifically hybridize and extract the antisense strand from the samples, and therefore, was able to achieve greatly improved sensitivity that is comparable to hELISA. Strategies, challenges, and solutions for developing hybridization LC-MS/MS methods for siRNA quantitation will be described. Different probes (DNA, DAP, PNA, and LNA probes) and key parameters that affect assay performance, including denaturation, hybridization, elution, and column temperatures, were comprehensively evaluated. Based on the results, a practical strategy was proposed and applied for efficient method development for siRNA analytes. A hybridization LC-MS/MS method was successfully developed and qualified for the quantification of a test siRNA over the range of 1.00 - 1000 ng/mL. The developed method has been used to support multiple animal studies.

Bioanalytical Method Case Studies Across the mRNA-LNP Therapeutic Portfolio

Jason DelCarpini, Moderna

Bioanalytical Method Case Studies Across the mRNA-LNP Therapeutic Portfolio will delve into the development and application of robust bioanalytical methods tailored to the unique challenges presented by mRNA-LNP (Lipid Nanoparticle) therapeutics. Through a series of case studies, this presentation will highlight the evolution of assay methods, including the detection and quantification of mRNA-derived proteins and immunogenicity assessments of the mRNA-derived as well as the LNP and it components. By exploring successes and lessons learned from various mRNA therapeutic programs, this talk will offer valuable insights into optimizing bioanalytical methods to support the rapidly growing mRNA-LNP pipeline.

Development of nonclinical total anti-drug antibody (ADA) assays to support the reproductive toxicology studies for zinpentraxin alfa

Audrey Arjomandi, Genentech

Zinpentraxin alfa is a recombinant human pentraxin-2 molecule designed for treating patients with idiopathic pulmonary fibrosis. While already in phase III clinical trials, developmental and reproductive toxicology studies in animal models were initiated to assess the reproductive safety after repeated exposure to the therapeutic. To support these studies, immunogenicity data was required to evaluate drug exposure and safety, necessitating the implementation of anti-drug antibody (ADA) assays for multiple toxicology species. This presentation details the challenges and efforts involved in developing two distinct ADA assays for evaluating immune response to zinpentraxin alfa in rat and rabbit. Difficulties arose from the pentameric structure of the molecule, the formation of different complexes, the presence of an endogenous counterpart, and complications with the standard bridging ELISA format. Additionally, the assay needed high drug tolerance to accommodate the studies involving high drug doses. We adapted a generic assay format to detect total anti-zinpentraxin alfa antibodies in the serum from both species, achieving good sensitivities and drug tolerance. Although some optimizations were required for each species, the overall assay format remained consistent. These assays were successfully used to generate immunogenicity data and assess the impact of ADAs on toxicokinetics. Overall, our work highlights the importance of evaluating alternative assay formats for novel drug modalities.

Navigating Challenges in the Development of Neutralizing Antibody Assays: Key Considerations and Strategies

Nazneen Bano, Merck

The development of a Neutralizing Antibody (NAb) assay poses numerous challenges, as outlined in this abstract. The selection of an appropriate assay format, such as cell-based or non-cell-based competitive ligand binding assays, is critical and depends on several factors. Ensuring the assay's sensitivity and specificity is essential to accurately detect low levels of NAbs and differentiate them from other antibodies

or interfering factors. Determining the cut-point, based on assay variability using samples from treatment-naïve subjects, is crucial for interpreting NAb presence or absence. Matrix interference, caused by components like soluble receptors or endogenous counterparts, must be understood and mitigated to obtain reliable results. Evaluating drug tolerance is necessary to detect NAbs in the presence of therapeutic proteins at various concentrations. This presentation will delve into strategies and considerations for mitigating these factors during assay development.

DISCOVERY BIOANALYSIS & NEW TECHNOLOGIES WORKSHOP

SESSION I: EMERGING ANALYTICAL & BIOANALYTICAL PLATFORM TECHNOLOGY

PLENARY PRESENTATION

Single-cell spatial dissection of the pancreatic tumor microenvironment

William L. Hwang, Harvard Medical School

Despite aggressive treatment, pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal malignancy with a 5-year overall survival of only 13%. Prior work has revealed these tumors are composed of malignant cells with distinct states (e.g. classical, basal-like, and neural-like progenitor) that are associated with different multicellular communities, interactions with non-malignant cell types, treatment sensitivities, and clinical outcomes. While chemotherapy and radiotherapy remain core treatments for PDAC, therapeutic resistance is pervasive and arises from poorly understood cancer cell-intrinsic reprogramming to resistant states as well as extrinsic interactions in the tumor microenvironment (TME) to support growth, proliferation, and metastasis. Herein, I will describe our efforts in developing and applying multimodal spatial proteotranscriptomic (SPT) methods to patient-derived tumors and complementary functional assays in preclinical models to improve our understanding of the interactions among malignant and non-malignant cells and how this may lead to emergent properties such as tumor progression and therapeutic resistance.

Evaluation of brain region- and cell type-derived spatial knockdown efficacy of oligonucleotides using spatial transcriptomics platform

Qiushi Liu, Takeda

The delivery of antisense oligonucleotides (ASOs) via intrathecal (IT) administration has become an increasingly prominent therapeutic approach for central nervous system (CNS) disorders due to the ability to directly administer ASOs into the cerebrospinal fluid space. However, previous studies have indicated that the brain biodistribution of ASOs following IT administration is heterogeneous, which may be significant at the cellular level, necessitating a detailed understanding of knockdown (KD) efficacy within individual cell types. Conventional evaluation methodologies, such as tissue homogenate-based pharmacokinetics/pharmacodynamics analysis, pose challenges in accurately assessing KD efficacy among different cell types. Although several previous studies have evaluated cell type-specific KD efficacy using single-cell RNA sequencing technology, spatial information is often lacking. In this study, we utilized a spatial transcriptomics platform to evaluate the KD efficacy of ASOs in various brain regions and cell types of mice following intracerebroventricular and IT administration. Our results clearly indicated that the region- and cell type-specific KD efficacy of ASOs was highly heterogeneous across the brain. In this presentation, we will also discuss the challenges and future perspectives of spatial KD evaluation using spatial transcriptomics platform.

Bio-digital transformation of proteomic analysis

Youhei Kosugi, Takeda

Proteomics enables the discovery of potential biomarkers for disease diagnosis, prognosis, and treatment monitoring. By comparing proteomic profiles between healthy and diseased states, researchers can identify specific protein signatures associated with various conditions, paving the way for personalized medicine and targeted therapies. Moreover, proteomics plays a key role in drug discovery and development by elucidating drug targets, understanding drug mechanisms of action, and assessing drug efficacy and safety.

One of the challenges in proteomics is the vast amount of data generated during experiments, which requires sophisticated bioinformatics tools and resources for its analysis and interpretation. Prioritizing the most relevant and biologically significant findings from the large datasets is crucial but can be a complex task. The integration of bioinformatics approaches to prioritize proteins of interest based on their biological relevance, abundance, and interactions is essential for extracting meaningful insights from proteomic data. In this

presentation, I will introduce an example of digital transformation that automates bioinformatics analysis by combining multiple tools, thereby reducing the burden and giving the power to rapidly get insights from the proteomics data for researchers.

After identifying biomarker candidates through non-targeted proteomics, a quantitation method of the targeted biomarker is required. While the quantification of proteins using ELISA is common and useful, its sensitivity and selectivity heavily depend on the antibodies employed. In case where quantitative methods utilizing specific antibodies are unavailable, quantification using LC/MS can be particularly advantageous. When establishing a quantification method for a specific protein using LC/MS, the information on its signature peptides used is crucial. Therefore, by tidily compiling the information of peptides detected in non-targeted proteomics into a database, it becomes feasible to smoothly develop the assay for the target protein. The process and content of constructing this database will also be addressed during the presentation.

The digital streamlining of proteomics analysis offers numerous benefits in terms of efficiency, accuracy, and scalability. By leveraging digital tools and technologies, researchers can automate data processing, streamline workflows, and facilitate a comprehensive understanding of biological systems. Overall, digital transformation of omics analysis enhances research productivity and accelerates biomarker discoveries.

Basics of machine learning and its applications in drug discovery

Reilly Eason, Merck

Artificial Intelligence/Machine Learning (AI/ML) has garnered significant attention in the pharmaceutical industry in recent years. Much has been made of its potential to be a transformative technology in the discovery of new drugs. However, the intricacies of machine learning often remain opaque to bench scientists, leading to a perception of it as a 'black box' method. Furthermore, some of the hyperbole surrounding ML has obscured the tangible impact it can have on accelerating the discovery process. This has at times led to a disconnect between the scientists responsible for building ML models and those who stand to benefit from their application. Here we seek to bridge that gap between model builders and consumers by explaining some of the fundamental principles for ML model construction and demonstrating a few real-world applications in the industry. Our objective is to demystify ML and highlight its potential as a valuable tool for bench scientists in their efforts to discover new medicines.

SESSION II: NOVEL STRATEGY TO ADVANCE BIOTHERAPEUTIC DEVELOPMENT

Domain specificity in the Tabs and Nabs Assay: Never too late for the characterization of ADA for multi-domain therapeutics

Richard Hughes, Resolian

Administration of bispecific antibody therapeutics, like many other biopharmaceuticals, can induce unwanted anti-therapeutic antibodies. The development of suitable analytical methods to detect ADA and Nabs is hence necessary, as unwanted immunogenicity against large molecule therapeutics remain one of the root cause of clinical program termination. The generation of ADA/Nabs can impact the pharmacokinetic (PK) exposure, bioavailability of therapeutics and pharmacodynamics (PD) effects depending on their distinct characteristics. The combined functionality of bispecific modalities requires additional considerations in order to develop a fit for purpose assay, because ADA/Nabs against each of protein domain may have different clinical impact. Therefore, the ADA/Nabs assay developed for proteins with multiple specificity should be able to confirm the immuno-reactivity of each individual binding domain. By comparison to developing assays for standard mono-specific antibodies, bi-specifics can present additional complications such as the requirement of higher drug tolerance or the lack of suitable cell-based assay systems that truly reflect the in vivo mechanism of action.

This presentation will cover case-studies arising from the immunogenicity assessments of two bi-specific monoclonal antibodies that target two different tumors. In the first example, the domain characterization was carried out in the confirmatory tier and the presentation will explore the advantages of such a strategy. In a second example, the domain specificity was not developed in the confirmatory analysis but was subsequently developed and validated in the neutralizing assay. This presentation will also highlight how adequate selection of the positive control is crucial from both the ADA and the Nabs assay and the choice of assay format in order to achieve desired assay sensitivity, drug tolerance and target tolerance.

A Comparison of bDNA and RT-qPCR Methodologies for Quantitation of LNP Encapsulated mRN

Syed Ali, Moderna

Background: The development of mRNA-based therapeutics necessitates validated and reliable quantitation methods to ensure the safety and efficacy of these novel treatments. We investigate

and compares the performance of branched DNA (bDNA) and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) methods in quantifying lipid nanoparticle (LNP) encapsulated nascent peptide imaging luciferase (NPI-Luc) mRNA in rat tissues and serum. **Methods:** Following a single intravenous administration of encapsulated NPI-Luc mRNA to rats (1 mg/kg), the mRNA concentration in various tissues and serum was measured using both bDNA and RT-qPCR techniques. Data were analyzed using Bland-Altman, Deming regression, and Passing-Bablok statistical methods to compare the performance and reproducibility of the assays. **Results:** The pharmacokinetic data obtained from both bDNA and RT-qPCR methods were consistent and comparable in several matrices. Both assays demonstrated similar mRNA concentration profiles across different laboratories, indicating high reproducibility. **Conclusion:** Both bDNA and RT-qPCR assays are reliable for mRNA quantitation in biodistribution studies, with each method exhibiting specific advantages. The choice between these methods may depend on the specific requirements of the pharmacokinetic study, such as sensitivity and comprehensive kinetic profiling. The consistent results across different laboratories also suggest that RT-qPCR may offer better transferability for multi-site studies.

VENDOR PRESENTATION

Build resilience and maintain unparalleled sensitivity for long-term bioanalysis

Robert Proos, Sciex

Accurate and precise measurement of pharmaceutical drugs in complex matrices can be challenging due to the presence of matrix contaminants. To ensure the highest levels of accuracy and precision, it is imperative to use highly robust analytical techniques. Triple quadrupole mass spectrometers are widely used in quantitative bioanalysis because of the stable analytical performance over an extended period. Here, exceptional sensitivity and stability were demonstrated to support bioanalytical workflows in high-throughput laboratories.

SESSION IV:
RECENT CASE STUDY TO SUPPORT NEW MODALITIES (OLIGONUCLEOTIDES/C&GT)/BIOANALYTICAL

Innovative Approaches and Case Studies in the Bioanalysis of Oligonucleotide Therapeutics

Aihua Liu, Resolian

Oligonucleotides (ONs) are emerging as a powerful class of therapeutics due to their unique ability to modulate gene expression. However, their clinical potential is hindered by challenges related to delivery and stability in vivo. Poor stability and inadequate cellular uptake have impeded their therapeutic efficacy. To address these issues, chemical modifications have been developed to enhance ON properties. Additionally, innovative delivery systems, such as lipid nanoparticles (LNPs) and N-acetylgalactosamine (GalNAc) conjugates, have been designed to improve ON stability and targeted tissue delivery. These advancements have enabled lower therapeutic dosages, necessitating the development of more sensitive, accurate, and reliable bioanalytical methods for quantifying ONs in various biological matrices.

Given the complexity of ON molecules and their formulations, no single analytical technique is sufficient. Therefore, a multi-platform approach is essential to support the development of ON therapeutics. In our laboratory, we have developed numerous bioanalytical methods using a range of techniques, including hybridization-based liquid chromatography-mass spectrometry (LC-MS), hybridization-based LC-fluorescence detection (LC-FD), LC-high resolution MS (LC-HRAM), LC-tandem mass spectrometry (LC-MS/MS), and quantitative polymerase chain reaction (qPCR). These methodologies support a range of programs, including drug screening, pharmacokinetic and toxicokinetic studies, and clinical trials.

This presentation will explore the latest advancements in the bioanalysis of ON therapeutics, focusing on hybridization-based and chromatographic-based assays. Real-world case studies will illustrate the practical applications, strengths, and limitations. Furthermore, the presentation will also discuss strategies to overcome common analytical challenges, such as matrix effects, the need for ultra-low sensitivity, non-specific binding, and the use of analogue internal standards. In conclusion, the successful bioanalysis of ON therapeutics relies on a comprehensive, multi-platform approach that leverages the strengths of various analytical techniques; to ensure accurate and reliable quantification, supporting the development and clinical application of these promising therapeutics.

Characterizing In Vivo Stability of Large Molecule Conjugates Using Complementary Bioanalytical Tools

Jeff Lin, Genentech

Novel large molecule conjugates are emerging to deliver sophisticated

mechanisms of action aimed at modulating traditionally "undruggable" targets where small molecules and antibodies may fall short. However, in vivo stability liabilities (i.e. biotransformation) in these modalities, including antibody-oligonucleotide conjugates and Fab conjugates, have been observed. Additionally, stability findings under in vitro stress conditions may not fully translate to in vivo scenarios. Here, we report a multi-pronged approach using LC-MS and capillary electrophoresis-based methods to characterize and quantify biotransformation liabilities and the translatability from in vitro/ex vivo to in vivo conditions, including but not limited to linker deconjugation, clipping, and amino acid level modifications.

MECHANISTIC ADME WORKSHOP

SESSION I: ROLE OF NONCYP ENZYMES IN DDI

Uncertainties and opportunities in predicting non-CYP mediated drug metabolism
Sandhya Subash, WSU

Over the past decade, metabolism by aldehyde oxidase (AO) has been gaining importance as drug design has evolved in the direction of making compounds that are more stable to cytochrome P450 (CYP) mediated metabolism. Failure to sufficiently characterize AO mediated metabolism has led to inaccurate predictions of clearance resulting in either toxicity or poor exposure in humans, leading to discontinuation of compounds. In my talk, I will delve into the potential causes behind the underestimation of AO-mediated drug metabolism and propose strategies to mitigate this issue. The integration of protein content and activity differences, consideration of AO activity loss, and the inclusion of extrahepatic clearance and additional pathways to improve the in vitro to in vivo extrapolation (IVIVE) of AO-mediated drug metabolism will be discussed. The application of a proteomics-informed physiologically based pharmacokinetic (PBPK) model to refine IVIVE will be discussed, illustrating these concepts with examples of well-known AO substrates.

Mitigation Strategies for Drug-Induced Liver Injury (DILI) – Still an Aspiration?
Kaushik Mitra, Janssen

Drug-induced liver injury remains a challenge after years of research, strategies and misses. This presentation will cover the last two decades of thinking around DILI-derisking strategies, encompassing chemistry, drug metabolism, multiple omics, immunology and in silico platforms, and will look into where we can go from here.

Recent Advancements in Predicting CYP-Independent Metabolism
Jasleen K. Sodhi, Septerna

As lead optimization efforts have successfully reduced metabolic liabilities due to cytochrome P450 (CYP)-mediated metabolism, there has been an increase in the frequency of involvement of non-CYP enzymes in the metabolism of investigational compounds. Although there have been numerous notable advancements in the characterization of non-CYP enzymes with respect to their localization, reaction mechanisms, species differences, and identification of typical substrates, accurate prediction of non-CYP-mediated clearance, with a particular emphasis with the difficulties in accounting for any extrahepatic contributions, remains a challenge. This presentation will highlight recent advancements in the characterization of drug metabolism and the in vitro to in vivo extrapolation of clearance for non-CYP drug metabolizing enzymes.

SESSION II: PK/PD CHALLENGES OF NEW MODALITIES: ADCS, IN VIVO GENE THERAPIES, AND PROTACS

Preclinical to clinical translation of ADCs using PK/PD modeling
Minu Pilvankar, Boehringer Ingelheim

Antibody-drug conjugates (ADCs) are complex cancer therapeutics composed of an antibody, a cytotoxic payload, and a linker. ADCs have captured the interests of drug developers due to their ability to achieve specificity by targeting tumor-specific antigens and releasing potent cytotoxic drugs inside the tumor. However, safety concerns related to off-target and on-target off-tumor toxicities can arise despite their specificity, thus, leading to the failure of several ADCs in clinic. Therefore, it is crucial to improve the understanding of efficacy/toxicity preclinically with improved confidence in translation to clinic. Pharmacokinetic/pharmacodynamic (PK/PD) modeling provides a quantitative tool to understand the complex processes of drug action that lead to efficacy and toxicity. Such models are particularly beneficial for a complex modality like ADCs allowing integration and comprehension of multiple processes involved in the mechanism of action. This talk will focus on the development and application of PK/PD models to predict clinical outcome, target feasibility, investigate parameter sensitivity, optimize ADC selection and overall improve preclinical to clinical translation for ADC development.

Empirical PK/PD Modeling of Novel In Vivo CRISPR/Cas9 Genome Editing Therapies

Adam Amaral, Intellia

The programmable nature of CRISPR/Cas9 aims to address the underlying mechanism of a variety of human diseases at the genetic level with one editing modality. In animals and in cells, delivery of Cas9 endonuclease with distinct sgRNAs has shown precise and accurate DNA modification at different, targeted genomic locations. This presentation describes the empirical pharmacokinetic (PK)/pharmacodynamic (PD) modeling of preclinical data used to support first-in-human starting dose and dose-escalation selections of two novel investigational in vivo CRISPR/Cas9 genome editing therapies, NTLA-2001 and NTLA-2002 for the treatment of transthyretin amyloidosis and hereditary angioedema, respectively.

Addressing Protac™ ADME Challenges

Stefanus Steyn, Pfizer

This presentation will explore challenges associated with the ADME characterization of Protacs, a cutting-edge therapeutic approach in drug discovery designed to degrade specific proteins via the ubiquitin proteasome system. Despite Protacs potential, complex structures and beyond Rule-of-5 properties pose unique challenges to classical ADME assays when compared to traditional small molecules. Through plasma protein binding case studies, various methodological approaches for enhancing assay performance will be featured. These approaches include compound solubilizing optimization and employing presaturation techniques to combat non-specific binding. In addition, assay modifications unique to plasma protein determination will be discussed. The presentation will conclude with a flow diagram proposing how to channel Protacs towards the most appropriate plasma protein binding approach based on their properties.

PLENARY PRESENTATION

Prospects for New Approach Methodologies and Complex In Vitro Models to reduce, refine and replace animals in drug safety testing

Terry R. Van Vleet, AbbVie

NAMs provide several opportunities to enhance pharmaceutical safety assessment in key areas and lesson the use of animal models, but some significant gaps remain before total replacement is possible. All in vitro human safety packages are successfully supporting FIH regulatory filings for appropriate drug platforms already. These platforms are increasing in many Pharma portfolios, and this is an obvious opportunity for NAMs to get a foothold. Challenges remain for testing small molecule drugs due to their high potential for off-target interactions and propensity for metabolism. Gaps remaining for NAMs to cover this space are significant and include incomplete cell/structure coverage, incomplete tissue coverage, limited connectivity, low confidence in in vitro to in vivo translation, and limited throughput. These are great opportunities for future focus and investment to help these technologies reach their potential.

SESSION III: THE IMPORTANCE OF TRACKING MISSING METABOLITES/ NEW TECHNOLOGIES (COVALENT DRUGS)

A "Missing" Trazpiroben Metabolite Observed as the Most Abundant Metabolite in Human Plasma and Urine

Sean Xiaochun Zhu, Takeda

Trazpiroben (TAK-906) is a dopamine D2/D3 receptor antagonist developed for the chronic treatment of moderate-to-severe gastroparesis. In the [^{14}C]trazpiroben human ADME study, a novel M50 was observed as the most abundant metabolite in both plasma and urine while not detected in feces. M50 represented a mean of 32.4% of total drug related materials in the plasma. M50 accounted for 16.9% of total radioactivity in urine, but this only represented 0.34% of the administered dose. The molecular ion of M50 was not able to be assigned initially from the LC-MS data of the plasma and urine samples. Based on the structure of trazpiroben, the metabolite was proposed to be one of the three compounds, isophthalaldehydric acid, isophthalic acid, and 3-(hydroxylmethyl)benzoic acid. By extracting the m/z of those three compounds, M50 peak was observed in the LC-MS chromatogram acquired at the negative ion mode in the urine sample. M50 was identified as isophthalic acid and confirmed with authentic standard. M50 was not discovered in either rat or dog radiolabeled ADME studies or any in vitro metabolism studies conducted prior to the human ADME study. It was a "missing" metabolite until characterized in the human ADME study. There were three potential reasons making this metabolite undetectable in the prior studies: (1). M50 ionization efficiency was low in both positive and negative ion modes. As a result, M50 might be formed in the prior in vitro or in vivo met ID studies but was not able to be detected; (2). Formation of M50 was a minor metabolic pathway in humans. It only accounted for 0.34% of administered dose and represented ~1% of total observed metabolites in the excreta. It might be a trace metabolite in human in vitro met

ID studies if it was formed but might be missed for identification; (3). M50 was not observed as a major metabolite in any biomatrices in the [^{14}C]trazpiroben rat and dog ADME studies, which may be attributed to species difference in metabolism and disposition between preclinical species and humans. Even if M50 was formed in those species, it might not be characterized as it could be an insignificant metabolite. In summary, a previously missing metabolite M50 was observed and characterized in the [^{14}C]trazpiroben human ADME study.

Human ADME characterization of linerixibat reveals unusual pharmacokinetic properties: implications for clinical development

Maciej Zamek Gliszczynski, GSK

Linerixibat, an oral small-molecule IBAT inhibitor under development for cholestatic pruritus, was designed for minimal absorption from the intestine (site of pharmacological action). This presentation will describe a clinical study of [^{14}C]-linerixibat IV and oral ADME. Oral bioavailability of linerixibat was exceedingly low (0.05%), primarily because of a very low fraction absorbed (0.167%; fraction escaping first-pass gut metabolism~100%), with high hepatic extraction ratio (77.0%) acting as a secondary barrier to systemic exposure. Linerixibat exhibited absorption-limited flip-flop kinetics: longer oral versus intravenous half-life (6-7 h vs. 0.8 h). In vitro studies predicted rapid hepatic clearance via CYP3A4 metabolism, which predicted human hepatic clearance within 1.5-fold. However, linerixibat was minimally metabolized in humans after intravenous administration: 80% elimination via biliary/fecal excretion (>90%-97% as unchanged parent) and ~20% renal elimination by glomerular filtration (>97% as unchanged parent).

Linerixibat unequivocally exhibited minimal GI absorption and oral systemic exposure, with absorption-limited flip-flop kinetics. Linerixibat represents a unique example of high CYP3A4 clearance in vitro but nearly complete excretion as unchanged parent drug via the biliary/fecal route. Implications of these unusual pharmacokinetic properties to the clinical develo pment of linerixibat will be discussed.

Impact of missing metabolites during lead optimization in drug discovery

Joyce Liu, Genentech

During lead optimization, potential drug candidates are refined to improve their efficacy and pharmacokinetic properties. As part of this process, in vitro stability assays with hepatocytes or other liver fractions are often used to assess compound stability and metabolism. However, in some instances, compound clearance and the distribution of metabolites observed in these in vitro assays may substantially differ from what is later observed in preclinical animal species or in human, leading to metabolites that are essentially "missed" in vitro. These missing metabolites can potentially impact drug development and lead to new findings about the pharmacokinetics and pharmacodynamics properties of drug candidates. Here, we explore a few case studies in which protein degraders and small molecules were optimized during drug discovery yet still resulted in newly appearing or significantly underestimated metabolites upon in vivo metabolite profiling. We will also discuss some of the underlying reasons for missing metabolites in these examples, the impact of these missing metabolites, and possible strategies for mitigating missing metabolites during lead optimization in the future.

SESSION IV: NEW APPROACHES TO IN VITRO ADME ASSAYS FOR IMPROVED DRUG-DRUG-INTERACTION AND CLEARANCE PREDICTIONS

Prediction of Aldehyde Oxidase Mediated Clearance: Recent Progress in Quantitative Translation

Jayaprakasam Bolleddula, iTeos Therapeutics

Human aldehyde oxidase (AO) is a cytosolic homodimeric molybdo-flavoenzyme involved in the oxidation of aldehydes and nitrogen-containing aromatic heterocycles, the reduction of N- and S-oxides, and the hydrolysis of amides. Many AO substrates have been terminated from clinical development due to unexpected high clearance, low bioavailability, and toxicity due to metabolites. Under prediction of AO-mediated clearance with IVIVE approaches were implicated for these unanticipated PK properties of AO substrates. Subsequently multiple scaling factors have been proposed to improve IVIVE accuracy. Despite the advancements in tools and approaches, prediction of human pharmacokinetics for substrates of AO remains challenging. In addition to clearance prediction, it is also important to determine the fraction metabolized by AO (fm,AO) for assessment of drug-drug interaction (DDI) potential. Lack of confidence in IVIVE and assessment of fm,AO for human dose and DDI predictions, respectively creates uncertainty in developing clinical pharmacology strategies for AO substrates. This presentation will focus on the recent progress in development of robust empirical scaling factors and physiologically based pharmacokinetic (PBPK) modeling framework for prediction of in vivo clearance and DDIs of AO substrates.

Predicting Clinical Drug-Drug Interactions of Cytochrome P450 Time-Dependent Inhibitors – How successful are we?

Elaine Tseng, Pfizer

Successful prediction of CYP450 drug-drug interactions (DDI) for time-dependent inhibitors (TDI) continues to be studied extensively whether it is from improvement of experimental methods to employment of sophisticated mathematical software. Experimentally, human liver microsomes (HLM) and human hepatocytes (HHEP) are the most common in vitro tools for studying time-dependent inhibition. Many labs have demonstrated successful predictions using either HLM or HHEP, however it is inconclusive that one consistently does a better job over the other. Through our own recent efforts, the use of HLM and HHEPs towards the prediction of CYP3A DDIs for 23 TDIs was evaluated. This evaluation was coupled with a comparison of static mathematical calculations and dynamic software modeling for the prediction of the clinical DDI. Use of mechanistic static and dynamic physiologically-based pharmacokinetic modeling show that HLM, coupled with the estimated average unbound organ exit concentration of the inhibitor, offered the most accurate projections of observed DDI with geometric mean fold errors of 2.0 and 1.7, respectively, without any false negative predictions. Although CYP3A is the predominant metabolizing isoform, few but notable DDIs exist for TDIs of other CYP enzymes. Building on our findings for CYP3A, we shift our focus towards prediction of DDIs caused by TDI of these other enzymes. Initial analysis of 21 drugs suggests that using HLM and estimated average unbound organ exit concentration of the inhibitor is also sufficient for a successful prediction of clinical DDI. This exercise demonstrates the ability to use simple in vitro test systems paired with suitable inhibitor concentration in a static model to provide quick DDI assessments that would be in good agreement with what is clinically observed.

Hepatocyte Uptake and Loss Assay (HUpLA): Proof-of-Concept of an All-in-One System for Same Day Measuring Hepatic Influx, Egress, and Metabolic Clearance Based on the Extended Clearance Concept

Julia A. Schulz Pauly, AbbVie

Hepatic clearance (CLH) prediction is a critical parameter to estimate human dose. However, CLH underpredictions are common, especially for slowly metabolized drugs, and may be attributable to drug properties that pose challenges (high lipophilicity, low solubility, high non-specific binding) for conventional in vitro ADME assays, resulting in non-valid data, which prevents in-vitro-to-in-vivo correlations and CLH predictions. Other processes, including hepatocyte and biliary distribution via transporters, can also play significant roles in CLH. Recent advances in understanding the interplay of metabolism and drug transport for clearance processes have aided in developing the Extended Clearance Model (ECM). Per the ECM, metabolic clearance depends on the unbound intrinsic metabolic clearance and distribution processes. Here, we demonstrate proof-of-concept of a novel two-step assay enabling measurement of multiple kinetic parameters from a single experiment in plated human primary hepatocytes with and without transporter and CYP inhibitors – the Hepatocyte Uptake and Loss Assay (HUpLA). We evaluated nine drugs with a wide range of physicochemical properties. The majority of drugs are considered to be challenging, including 5 bRo5, 4 low CLH (<30% ER), and 6 high plasma protein binding (fu,p<0.01) drugs. HUpLA accurately predicted the CLH of 89% of drugs (n=8 within 2-fold of the observed CLH). In comparison, the conventional suspension hepatocyte stability assay, which is optimized for Ro5 compounds and metabolic clearance only, poorly predicted the CLH. CLH of only 22% of drugs (n=2) were predicted within 2-fold of the observed CLH. These data suggest HUpLA is advantageous by enabling the measurement of enzymatic and transport processes concurrently within the same system, alleviating the need for applying scaling factors independently, which would be the case for data generated in fully independent experiments. Another key benefit is that HUpLA shows promise as a sensitive measure for low-turnover and otherwise challenging drugs in a one-day assay. This is enabled by the plating, preloading of drug into cells and subsequent washout of media containing drug, thereby permitting measurement of intracellular metabolic drug loss only. In turn, this greatly increases assay dynamic range.

APA BIOGRAPHIES

Syed Ali, PhD, Moderna: Dr. Ali is a Senior Scientist with a background in pharmacology and bioanalytics, currently contributing his expertise at Moderna Therapeutics in Cambridge, MA. His work focuses on the bioanalytical method development and qualification for mRNA, significantly impacting preclinical studies and oncology biomarker programs. Dr. Ali has previously worked at Stoke Therapeutics and Pfizer Inc. Before transitioning into the biotechnology industry, Dr. Ali completed his graduate program at the University of Texas Medical Branch, Galveston, and his post-doctoral training at Yale University, New Haven. He has authored several publications focusing on mRNA therapeutics and small molecules.

Adam Amaral, Intellia: Adam is currently a director of bioanalytic and DMPK at Intellia. Since joining Intellia in 2017, Adam has supported the advancement of novel CRISPR/Cas9 gene editing therapies from non-clinical to clinical stages of development. His contributions in the non-clinical PK, PD and safety data analysis, as well as modeling, have been instrumental to the successful first-in-human dosing regimens employed in the first two in vivo CRISPR gene editing programs. Additionally, Adam has served as the non-clinical lead for several development candidates and manages a productive bioanalysis lab to support early-stage platform and research studies. Prior to Intellia, Adam gained valuable experience at Biogen and Novartis. He holds a BA in Biology from Boston University.

Audrey Arjomandi, PhD, Genentech: Dr. Arjomandi is currently a Senior Principal Scientist at Genentech in the Bioanalytical Sciences group. She got her Ph.D. in pharmacology from the University of Strasbourg I in France. She then came to the U.S. to do a post-doctoral fellowship at the University of California, San Francisco. Audrey started her industry experience in a start-up company called POINT Biomedical and then joined Bio-Rad Laboratories where she worked on serologic clinical diagnostic assays to detect autoantibodies to various diseases. At BioCheck, she was responsible for the development and validation of ultra-sensitive assays on the Simoa platform. Finally, now, she leads the evaluation and implementation of bioanalytical assays, including ADA, PK and biomarker methods, to support biopharmaceuticals in the product development pipeline at Genentech.

Nazneen Bano, PhD, Merck: Dr. Bano is a Principal Scientist with extensive expertise in immunogenicity and is currently leading the Nab team within the PDMB Reg BA PK & ADA Bioanalytical Sciences at Merck. Prior to joining Merck, Nazneen held the position of Senior Scientist at Janssen Research & Development in the Bioanalytical Development Sciences group. In this role, she was responsible for the development, qualification, and validation of immunoassays for various biologics, supporting both non-clinical and clinical studies.

Nazneen's academic journey includes post-doctoral training at the Malaria Research Institute in the Molecular Microbiology and Immunology Department of Bloomberg School of Public Health at Johns Hopkins University, as well as at the Viral Hepatitis Laboratory at the University of Washington. She holds an MS and Ph.D. in Microbiology from Aligarh University in India, and she also obtained an MS in Bioscience Regulatory Affairs from Johns Hopkins University.

Outside of work, Nazneen enjoys spending time with her family. She also has a passion for gardening and cooking.

Jayaprakasam (Prakash) Bolleddula, PhD, iTeos Therapeutics: Dr. Bolleddula is a Senior Director of Clinical Pharmacology at iTeos Therapeutics. Dr. Bolleddula is an experienced scientific leader with 20+ years of experience at the intersections of drug metabolism, drug-drug interactions, PBPK modeling, and clinical pharmacology. Before joining iTeos, Dr. Bolleddula worked at EMD Serono, Agios Pharmaceuticals, Takeda-Boston, and Theravance Biopharma. He has published over 50 peer-reviewed research articles and is also a co-inventor of numerous patents. Dr. Bolleddula obtained Ph.D. in organic chemistry from Sri Venkateswara University, Tirupati, India, and completed a postdoctoral fellowship at Michigan State University.

Nagendra Chemuturi, PhD, Eli Lilly: Dr. Chemuturi received his Bachelor Degree in Pharmacy, with Distinction and two gold medals, from Kakatiya University, India. He worked as a pharmaceutical sales representative before pursuing his Ph.D. at the University of Iowa. He was awarded the AAPS Graduate Symposium Award in 2005 for his dissertation work on the role of nasal drug transporters and metabolism in preferential nose-to-brain

uptake of dopamine into brain. He started his career in the US at Vertex Pharmaceuticals in 2005. Since then, he has worked at both big (Alcon-Novartis and Takeda) and mid-size pharma (Seattle Genetics) and is currently with Eli Lilly as Senior Director inADME Department. His expertise lies in the fields of oncology, rare diseases and ophthalmology having served as lead on several small and large molecule projects. He is currently working on viral and non-viral genetic medicines (gene therapies) for treating rare diseases, cancers and other indications. He has given podium presentations at several scientific conferences, and is active in IQ consortium, AAPS and Boston Society-APA. He has co-authored several articles and book chapters.

Yi-Ying Chen, FDA: Yi-Ying is a Chemist in the Division of New Drug Study Integrity (DNDSI) within the Office of Study Integrity and Surveillance (OSIS) at the Center for Drug Evaluation and Research (CDER), US Food and Drug Administration. In her role, she is responsible for conducting inspections and remote regulatory assessments of in-vivo and in-vitro bioavailability and bioequivalence studies submitted to FDA in support of INDs, NDAs, ANDAs, as part of the Agency's Bioresearch Monitoring (BIMO) program. Before joining CDER, Yi-Ying amassed over 14 years of experience in preclinical research, manufacturing processes, and clinical trial development within the pharmaceutical industry. She earned her degree in Chemical Engineering from National Taiwan University and holds two Master of Science degrees: one in Pharmaceutical Sciences from Northeastern University and another in Regulatory Affairs and Health Policy from the Massachusetts College of Pharmacy and Health Sciences.

Jason DelCarpini, Moderna: With more than 20 years in the pharmaceutical and biotech sectors, Jason DelCarpini has been at the forefront of bioanalytical science, enhancing knowledge in areas such as pharmacokinetics, pharmacodynamics, and immunogenicity for complex therapies, including protein, cell and gene, and mRNA-LNP. As Moderna's Director of Bioanalytics, he spearheads the creation of strategies for bioanalysis in clinical trials, maintaining regulatory compliance and engaging with the wider bioanalytical field.

Reilly Eason, PhD, Merck: Dr. Eason obtained his PhD at the University of Missouri studying computer simulations of shock response in energetic materials. He then spent 9 years working as a Polymer Chemist before making a career pivot to the pharmaceutical industry and joined the Cheminformatics group at Merck in 2022. There he has participated in the deployment of AI/ML QSAR models, modernization of virtual screening efforts, and development of informatics analysis for modalities (TPD and ADCs).

Richard Hughes, Resolian: Richard is Scientific Director for Immunoassay at Resolian where he oversees all technical aspects of LBA method development and validation in the GLP/GCP footprint. This includes leading ADA, PK, Biomarker and flow cytometry method development teams within the UK (Fordham) bioanalytical team. Richard also has translation biology experience from time spent at GSK (Stevenage) and clinical respiratory biomarker expertise from Respiratory Clinical Trials, a Phase 1 unit in London.

William L. Hwang, MD, PhD, Harvard Medical School: Dr. Hwang is an Assistant Professor at Harvard Medical School (HMS) and Physician-Scientist at the Center for Systems Biology, Center for Cancer Research, and Department of Radiation Oncology at Massachusetts General Hospital (MGH) where he leads the Laboratory for Spatial and Systems Oncology, cares for patients with gastrointestinal malignancies, and serves as Associate Director of the Radiation Biology & Research Program. Prior to joining the faculty at HMS and MGH, Will trained in the laboratories of Dr. Tyler Jacks at MIT, Dr. Aviv Regev at the Broad Institute, Dr. Xiaowei Zhuang at Harvard, and Dr. Hagan Bayley at the University of Oxford. Dr. Hwang's independent laboratory studies cell state plasticity and tumor-stromal interactions through the development and application of novel techniques in single-cell and spatial biology, advanced microscopy, and genetic engineering to patient-derived specimens, stromal tumoroids, and mouse models. Current project areas include elucidating the mechanisms of (1) therapeutic resistance mediated by cell state plasticity and malignant-stromal interactions in the tumor microenvironment; and (2) tumor-nerve interactions, which are remarkably important in the pathogenesis of many cancers but poorly understood. His work has been honored with a Rhodes Scholarship, ASCO/CCF Young Investigator Award, AACR NextGen Star, Hopper-Belmont Foundation Inspiration Award, PanCAN Career Development Award, Burroughs Wellcome Fund Career Award for Medical Scientists, and 40 under 40 in Cancer Award – Rising Stars and Emerging Leaders.

Yohei Kosugi, PhD, Prevail Therapeutics: Dr. Kosugi is a director at Takeda Pharmaceutical Company Limited, with over 20 years of experience in the field of DMPK. He has wide coverage of DMPK science including drug-drug interaction (DDI) prediction, in vivo animal model for assessing biopharmaceutics, PBPK/PD modeling, LC/MS-based bioanalysis and machine learning (ML) of ADME prediction. He has authored over 30 peer-reviewed articles. Currently, his research is centered on translational research including biomarker quantification, multi-omics and bioinformatics. He is leading DMPK digital transformation (DX) by incorporating AI/ML, which he learned through ADME prediction, into the biomarker research.

Milankumar Kothiya, Prevail Therapeutics: Milankumar Kothiya is a Principal Scientist at Prevail Therapeutics – a wholly-owned subsidiary of Eli Lilly and Company. Before joining Prevail, he was a Research Staff Associate at Columbia University where he supported research on Alzheimer's disease. Currently, he is responsible for immuno-assay development and qualification to support gene therapy studies. He has more than a decade of experience of performing various cell and molecular biology techniques in neuroscience and cardiovascular diseases field. He is a co-author in several research articles published in peer-reviewed journals. Milan holds a Bachelor of Pharmacy degree from India and a Master of Science degree in Biotechnology from NYU Tandon School of Engineering.

Amanda Leskovar, ICON: Amanda Leskovar is the Senior Director of Bioanalytical Services and acting site head for ICON's bioanalytical laboratory in Whitesboro, NY. After nine years of academic research, Amanda joined ICON (then Prevalere) in 2007 as a scientist in the immunoassay laboratory.

During her 17 years at ICON, Amanda's roles include Scientist, Laboratory Manager, Client Relationship Manager, Director of Project Management, and Senior Director of Bioanalytical Services. With experience in bioanalytical methods, project logistics, people leading and sponsor relationships in addition to supporting dozens of sponsor and regulatory audits, Amanda brings a wealth of expertise from the lens of the contracted service provider.

Jeff Lin, Genentech: Jeff is a bioanalytical scientist with a passion for advancing the field of biotherapeutics. He currently serves as a Senior Scientist in the large molecule bioanalytical group at Genentech. His group plays a vital role in supporting preclinical pharmacology analysis, including biotransformation, pharmacokinetics, and pharmacodynamics, using various analytical techniques such as mass spectrometry, capillary electrophoresis, and plate-based immunoassays. His current focus is on oligonucleotide-based modalities, and he also has experience with diverse modalities such as antibodies/bispecifics, enzyme replacement therapies, and Fab conjugates. Jeff is science-driven and has authored multiple publications in top-tier peer-reviewed journals, including the Journal of the American Chemical Society.

Aihua Liu, PhD, Resolian: Dr. Liu is the Executive Director of Bioanalysis at Resolian. She holds a Ph.D. in Analytical Chemistry, an M.S. in Pharmaceutical Chemistry, and a B.S. in Pharmacy. Before joining Resolian, Dr. Liu held senior positions at Alliance Pharma, Dyad Labs, Covance/ LabCorp and Tandem Labs.

Dr. Liu is a seasoned expert in bioanalysis with over two decades of experience, including 12 years in regulated bioanalysis. Her research interests focus on developing and validating innovative bioanalytical methods, as well as sample analysis. She specializes on oligonucleotides, ADCs, proteins, biomarkers, small molecules, and new modalities.

Dr. Liu has contributed significantly to the field, authoring more than 85 conference posters/oral presentations, peer-reviewed publications, and book chapters in the areas of bioanalysis and analytical chemistry.

Joyce Liu, PhD, Genentech: Dr. Liu is a Principal Scientist in the DMPK department at Genentech. Her work currently focuses on biotransformation studies to support discovery and early-stage development projects for both small molecules and new modalities. Joyce received her B.S. in Chemical Engineering from the California Institute of Technology and her Ph.D. in Bioengineering from the University of California, Berkeley. She has also previously worked in the areas of natural product biosynthesis, biocatalysis, and enzyme engineering.

Qiushi Liu, PhD, Takeda: Dr. Liu received a Ph.D. in Science from Osaka University in Japan and is currently an Associate Scientist in the DMPK&M department at Takeda Pharma, with extensive experience in cutting-edge bioanalysis research. His research primarily focuses on evaluating the biodistribution of oligonucleotides, PK/PD relationships, and gene pathway analysis using imaging-based technologies, such as the spatial transcriptomics platform.

Kaushik Mitra, PhD, Janssen: Dr. Mitra is currently a Distinguished Scientist at Johnson & Johnson (JnJ), where he serves as the DMPK lead for the Specialty Ophthalmology therapeutic area. He also is an adjunct professor in the Department of Chemistry at the University of Missouri, Columbia. Prior to joining JnJ, Kaushik spent 17 years at Merck Research Laboratories, firstly as Director of the ADME function within DMPK and later as Director of the Molecular and Investigative Toxicology group. In these capacities, Kaushik has supported the advancement of multiple compounds of different modalities to the clinic across therapeutic areas. His interest lies in the preclinical optimization of drug candidates via early optimization of ADME properties and derisking of potential adverse effects through mechanistic and in silico interventions. Kaushik has co-authored three book chapters on drug risk assessment, published 30+ research papers and is active in speaking engagements in national and international conferences, including the co-leadership of courses on mitigation strategies for genetox and bioactivation liabilities in drug candidates. Kaushik earned his Ph.D. in organic chemistry from the University of Missouri, Columbia and was subsequently a Susan G. Komen post-doctoral fellow in the Department of Biological Engineering at the Massachusetts Institute of Technology.

Minu R. Pilvankar, PhD, Boehringer Ingelheim: Dr. Pilvankar is a Principal Scientist in the Biotherapeutics Discovery department of Boehringer Ingelheim Pharmaceuticals, Inc. at Ridgefield, CT. Dr. Pilvankar holds a Ph.D. in Chemical Engineering, along with a Graduate Certification in Interdisciplinary Toxicology, awarded in May 2019, from Oklahoma State University-Stillwater. Dr. Pilvankar also holds a Master of Science degree in Chemical Engineering awarded in December 2015, from Oklahoma State University-Stillwater.

At Boehringer Ingelheim, Dr. Pilvankar leads Discovery Research projects across multiple therapeutic areas, including Oncology, Cardio-metabolic disorders, and Immuno-oncology, both in the US and globally. She serves as a subject matter expert in cross-functional projects teams and specializes in PK/PD modeling for biotherapeutic proteins. Her expertise spans monoclonal and multi-specific antibodies, ADCs, and other therapeutic modalities. Dr. Pilvankar's work focuses on developing mechanism-based mathematical PK/PD models, integrating chemical kinetics, mathematics, biology, and computational skills to enhance translation from preclinical research to clinical applications. She has authored several scientific publications with most of them focusing on her modeling work.

Robert Proos, Sciex: Robert has been working in Mass Spectrometry for over 20 years in a wide variety of applications. He has been with Sciex since 2014 and has spent the last several years focusing on small molecule analysis and the application of new hardware, software and workflows.

Joe Rajarao, PhD, Accenture: Dr. Rajarao is a research scientist with bio-pharmaceutical experience and academic training that spans genomics, bioinformatics, pharmacology, and translational medicine. His passion for research stems from his mother's battle with cancer, which led to his joining the lab that characterized the first genetic trigger for melanoma. He has discovered and characterized over a dozen novel genes, developed assays to evaluate drug-target interactions, and has published in journals as Molecular Cell, Genome Research, and Neuropeptides. As a Management Consultant at Accenture, he has advised numerous pharmaceutical and contract research labs on how to address their data integrity and compliance needs.

Dr. Rajarao earned a PhD in Pharmacology at the Pennsylvania State University and pursued post-doctoral training in translational bioinformatics at Wyeth Research. He then joined Wyeth as a Senior Scientist and led multiple pre-clinical programs with target identification, assay development, and method validation support. Joe made the move to lab informatics where he implemented bioanalytical lab execution systems in multiple biopharma and CROs. He has been active in the bioanalytical field, having published in Bioanalysis and presenting at numerous industry forums. Joe is an active member of the American Association of Pharmaceutical Scientists (AAPS) and is vice-chair for a working group to explore Robotics, AI, and ML in Regulated Bioanalysis.

Julia Schulz Pauly, PhD, AbbVie: Dr. Schulz Pauly is a Senior Scientist in the Quantitative, Translational, and ADME Sciences (QTAS) department at AbbVie where she functions as a PBPK modeler, supporting small molecule projects with first-in-human dose projections and DDI risk assessments. Previously, Julia was a Postdoctoral Fellow at AbbVie exploring novel methods to predict human hepatic clearance by evaluating transporter-enzyme interplay and plasma protein binding-related artifacts. Julia received her B.S. in Pharmacy from Goethe University Frankfurt/Main, Germany, and a Ph.D. in Pharmaceutical Sciences from the University of Kentucky, where she studied blood-brain barrier efflux transporters.

Jasleen K. Sodhi, PhD, Pfizer: Dr. Sodhi received her undergraduate degree from the University of California Berkeley and her Ph.D. from the University of California San Francisco under the mentorship of Dr. Leslie Benet, where her research focused on developing methodologies to identify clinically relevant transporter involvement in complex drug-drug interactions and on improving IVIVE of hepatic clearance from a theoretical perspective. Jasleen has published approximately 40 peer-reviewed articles or book chapters spanning topics such as IVIVE, methodologies to interpret complex drug-drug interactions, and the novel finding that aldehyde oxidase can mediate amide-hydrolysis reactions. Jasleen is approaching 20 years of experience in the pharmaceutical industry, including with Genentech, Plexxikon, and is currently affiliated with Septerna as an Associate Director of DMPK.

Stefanus J. Steyn, PhD, Pfizer: Dr. Steyn I has a Ph.D. in Pharmaceutical Chemistry and has completed post-doctoral studies in the laboratory of Prof Neal Castagnoli at Virginia Tech. He has over 20 years pharmaceutical industry experience with over forty co-authored publications. He has spent most of his career at Pfizer in various roles within PDM (DMPK), supporting projects ranging from oncology to neuroscience and currently, Inflammation and Immunology (I&I). Stefanus is currently a Research Fellow, and his responsibilities include setting the DMPK research and project strategies within the I&I Research Unit. In addition, his team and he function as Project Representative within I&I while I also have responsibilities as a Research Project Lead for various Discovery programs. His interests include prediction of human ADME as well as exploring physicochemical properties and how they relate to ADME with a focus on absorption. Protacs are of special interest given their unique beyond Rule-of-5 properties and the ADME challenges they present relative to classical small molecules.

Sandhya Subash, PhD, Washington State University: Dr. Subash serves as a post-doctoral research associate within the Department of Pharmaceutical Sciences at Washington State University. She leads the collaborative project "PRINCE" (Proteomics-based Research Initiative for Non-Cytochrome P450 Enzymes), aimed at facilitating the prediction of drug metabolism mediated by non-cytochrome P450 (non-CYP) enzymes. Dr. Subash earned her Ph.D. in Medicinal Chemistry from the University of Mumbai, India, where her research focused on population phenotyping of aldehyde oxidase.

With over 8 years of industrial experience in Drug Metabolism and Pharmacokinetics (DMPK), Dr. Subash previously held the position of Senior Research Investigator at the Biocon-Bristol Myers Squibb R&D Centre (BBRC) in Bangalore, India. Her expertise lies in designing mechanistic in vitro ADME studies to evaluate and address metabolism-related challenges, thereby facilitating the clinical translation of compounds. Dr. Subash has contributed to several peer-reviewed publications and has presented at various international DMPK symposia and conferences. She also serves on the editorial board of Frontiers in Pharmacology and has provided peer reviews for journals such as Pharmacology Research and Perspectives and the European Journal of Drug Metabolism.

Her research interests center around utilizing proteomics to explore differences in non-CYP enzyme expression, aiming to overcome challenges in in vitro to in vivo extrapolation (IVIVE) and prediction of drug clearance and fractional contribution.

Elaine Tseng, Pfizer: Elaine is a Principal Scientist within Pharmacokinetics, Dynamics and Metabolism at Pfizer in Groton, CT. She earned her combined BS/MS degree in Pharmaceutical Sciences from the State University of New York (SUNY) at Buffalo. She has over 20 years of experience as an ADME scientist supporting drug discovery and early drug development in neuroscience and oncology research units. Her research interests have evolved from discovery laboratory support in areas of small molecule drug metabolism to focusing on drug-drug interactions of P450s, including enzyme kinetics, reaction phenotyping, and time-dependent inhibition; more recently focusing on disposition of low clearance compounds.

Terry Van Vleet, PhD, AbbVie: Dr. Van Vleet received a Bachelor's degree in Zoology with a minor in Chemistry from Weber State University and a doctorate in Toxicology/Molecular Biology studying mechanisms of carcinogenesis and metabolic activation of carcinogens, at Utah State University (USU). His postdoctoral training was at the Medical University of South Carolina, studying mechanisms of renal toxicity with an emphasis in mechanisms of mitochondrial dysfunction. Prior to joining AbbVie, he worked at Bristol Myers Squibb in Mt Vernon, Indiana, for 11 years in positions of increasing importance culminating as the Head of the Molecular and In Vitro Toxicology Group. At AbbVie, he is currently Head of the Investigative Toxicology and Pathology (ITP) Department. He is a Diplomate of the American Board of Toxicology (DABT), a Fellow of Academy of Toxicological Sciences (FATS), and a European Registered Toxicologist (ERT). Dr. Van Vleet has more than 60 peer review publications and has served as a past Chair of the Innovation and Quality (IQ) Microphysiological Systems (MPS) Affiliate Consortium.

Faye Vazvaei-Smith, Merck: Faye is currently Executive Director of the Regulated PK & ADA Bioanalysis Group of Merck Research Laboratories, West Point, PA, USA. In this role, she leads the Regulated PK & ADA Bioanalytics group with overall responsibility for strategic and scientific oversight for method development, validation, and implementation of regulated bioanalytical assays to quantitate drugs, metabolites, and anti-drug antibodies supporting small molecules, peptides, and biologics utilizing chromatographic-mass spectrometry and ligand binding assays. Before joining Merck in April 2019, Faye was with Roche for seventeen years. She held several positions rising from Lab Head to Global Head of Bioanalytical Outsourcing and Bioanalytical Oncology Group Head in the Department of Clinical Pharmacology and Bioanalytical R&D. Faye is highly engaged within the global bioanalytical community through AAPS and IQ. She co-chaired the IQ working group on Metabolite Bioanalysis, which was sunsetted recently, and she is currently the co-chair of the IQ working group in Bioanalysis of Drug Conjugates. She recently completed three years of service as the Member at Large on the AAPS Board of Directors. Also, she served on the International Council of Harmonization Bioanalytical Method Validation and Samples Analysis (ICH-M10) Expert Working Group as the BIO organization representative. Faye has a bachelor's degree in chemical engineering from Sharif University of Technology, Tehran, Iran, and a master's degree in biotechnology from Johns Hopkins University.

Carrie Vyhlidal, PhD, KCASBio: Dr. Vyhlidal is the Associate Director of Cell & Gene Therapy at KCASBio at their headquarters in Olathe, Kansas, U.S.A. where she leads a team of scientists that are experts in molecular and cell-based technologies. Dr. Vyhlidal obtained her Ph.D in Biochemistry with additional training in pharmacology, pharmacogenetics and pharmacogenomics. She has extensive experience developing PCR-based bioanalytical assays to detect and quantify pharmacogenetic, pharmacokinetic, and pharmacodynamic biomarkers that predict therapeutic and toxic responses to drug exposures, including cell and gene therapies.

Xiaomin Wang, PhD, BMS: Dr. Wang, Scientific Director (bioanalysis) in therapeutic areas oncology (i.e., hematology and solid tumor) and immunology, joined Celgene/BMS in 2007 (Summit, NJ). Prior to joining Celgene, Xiaomin was Research Scientist at Forest Labs (Farmingdale, NY). He was responsible in supporting Lexapro and Namenda developments. He was Research Scientist at MDS-Sciex (Toronto, Canada). He involved for new mass spectrometer development (i.e., API4000 QTRAP) and conducted global technical sales for promoting LC-MS/MS applications in herbal medicine research.

He contributed 6 new drug applications (NDAs) with successful approvals by FDA, Pomalidomide for multiple myeloma (2013), Apremilast for psoriatic arthritis (2014), Enasidenib for acute myeloid leukemia (2017), Ozanimod for multiple sclerosis (2020), Onureg for Acute Myeloid Leukemia (2020) and Augtyro for NSCLC (ROS1 positive) in Nov 2023.

Xiaomin's original research has been involved from gas phase ion/molecular reactions, analytical methodologies for herbal medicine analysis, biomarker research and clinical pharmacology, and his work has been published in *International J Mass Spectrometry and Ion Processes, Analytical Chemistry, Drug Metabolism and Disposition* and *J Clinical Pharmacology*, and other top-tiered journals (>40 publications).

Xiaomin received his Ph.D. in physical chemistry with Professors Diethard Kurt Bohme and Raymond E. March at York University (Toronto, Canada), also received Industrial Research Fellowship from NSERC Canada and post-doctoral training with Professor Jack Henion at Cornell University (Ithaca, NY, USA).

Xiaoyun Yang, PhD, Genentech: Dr. Yang is a senior principal scientist at Genentech BioAnalytical Sciences. She obtained her Ph.D. in Bioengineering from the University of Utah and has a wealth of industry experience, especially in assay development, which she acquired from her graduate studies through to her professional experiences at BioRad, BD Biosciences, Amgen, and Gilead.

Since joining Genentech in 2021, Xiaoyun has demonstrated exceptional scientific leadership, and portfolio impact through her roles as BAS project lead for different programs. She has also served as our BAS point of contact for all oncology and ophthalmology biomarker assays, many of which she has taken on by overseeing their development and qualification both internally and through collaborations with CROs. Additionally, she has been instrumental in evaluating innovative technologies for our CPET microsampling and Point-of-Care (POC) studies.

Chongwoo Yu, PhD, FDA: Dr. Yu is a Master Clinical Pharmacology Reviewer in the Office of Clinical Pharmacology at the US Food and Drug Administration (FDA). Dr. Yu received his BS in Chemistry and MS in Physical Organic Chemistry from Hanyang University (Korea) and Dr. Yu earned his PhD in Analytical Chemistry with the focus on Drug Metabolism and Mass Spectrometry from the University of Illinois Chicago (Chicago, IL).

Subsequently, Dr. Yu has worked in the Department of Pharmacokinetics, Dynamics, and Metabolism (PDM) at Pfizer (Ann Arbor, MI) and the Drug Metabolism and Pharmacokinetics (DMPK) Department at Schering-Plough (currently, Merck; Kenilworth, NJ) for several years. At both organizations, Dr. Yu has been heavily involved in carrying out various types of drug metabolism, pharmacokinetics, and drug-drug interaction (DDI) studies using mass spectrometry.

Dr. Yu joined the FDA as a Clinical Pharmacology reviewer in 2007. Dr. Yu's work has been focused on the evaluation of: (1) Reproductive and Urologic Drug Products; (2) Bone and General Endocrine Drug Products; (3) Diabetes, Lipid Disorders, and Obesity Drug Products; and (4) Cardiology and Nephrology Drug Products. Dr. Yu served as a member of various FDA guidance working groups including the one for theBioanalytical Method Validation Guidance. Dr. Yu currently serves as the chair of the FDA (internal) Bioanalytical Research (BAR) Scientific Interest Group (SIG).

Long Yuan, PhD, Biogen: Dr. Yuan is currently Director and Head of bioanalytical group in the Department of Drug Metabolism & Pharmacokinetics at Biogen (MA, USA). He is leading a group of scientists providing bioanalytical support for various modalities, e.g., small molecule drugs, oligonucleotides, biologics, and AAV gene therapies, in drug discovery and development. Long received his B.S. degree in Medicinal Chemistry from Fudan University (Shanghai, China), and Ph.D. degree in Medicinal Chemistry from College of Pharmacy, University of Illinois Chicago (IL, USA). Before joining Biogen, he worked in the Department of Bioanalytical Sciences at Bristol Myers Squibb (NJ, USA). His research focuses on developing bioanalytical methodologies for various molecules, with recent interest on oligonucleotides, including ASO, siRNA, and antibody-oligonucleotide conjugates. He is co-lead of AAPS Bioanalytical Community Oligonucleotide Discussion Group and board member of Eastern Analytical Symposium (EAS). He has published over 45 peer-reviewed papers or book chapters.

Maciej J. Zamek-Gliszczynski, PhD, GSK: Dr. Zamek-Gliszczynski has 18 years of pharmaceutical industry (Eli Lilly and GSK) experience in leading DMPK and PK/PD/clinical pharmacology aspects of oncology, endocrine/metabolic, and infectious disease programs at all stages between discovery, clinical development, and post-marketing (lead on linerixibat and gepotidacin NDAs; many clinical candidate selections & INDs). He is an experienced global manager, having led the Quantitative Drug Disposition group responsible for understanding safety and efficacy implications of DDIs and special populations for the entire GSK portfolio. Dr. Zamek-Gliszczynski is a member of GSK's Global Labelling Committee, which oversees all new and revised drug labeling, as well as the Nephrology Safety Panel, which advises clinical teams on renal safety issues.

Maciej Zamek-Gliszczynski received his B.S. in chemistry and Ph.D. in pharmaceutical sciences under the direction of Profs. Kim Brouwer, Gary Pollack, Mary Paine, and K. Sandy Pang at UNC-Chapel Hill. Dr. Zamek-Gliszczynski's research is focused on PK/PD and DDI implications of drug and metabolite transport. He is the author of >100 manuscripts and presentations on this subject (>7,000 cites, h-index = 39). He serves on the editorial boards of *Drug Metabolism and Disposition* and formerly *Pharmaceutical Research*. Dr. Zamek-Gliszczynski is a steering committee member of the International Transport Consortium (ITC), was past chair of AAPS PK/PD/Drug Metabolism (PPDM) section, served as GSK management representative

on IQ Translational ADME Leadership Group (TALG), and served in ASCPT leadership. He has been active in organizing DMPK/clinical pharmacology meetings with ITC, ASCPT, and AAPS. As adjunct professor at UNC, Dr. Zamek-Gliszczynski lectures in graduate-level PK/PD courses and serves as external committee advisor. He enjoys developing scientists and has an established mentorship record at the associate scientist, junior and peer Ph.D., as well as graduate student and post-doc levels.

Tom Zhang, PhD, Worldwide Clinical Trials: Our large molecule bioanalytical lab is headed by Dr. Tom Zhang, industry recognized large molecule bioanalysis expert. Dr. Zhang is responsible for evaluating and recommending analytical platforms in support of quantitative large molecule work; actively promoting technical development programs to keep Worldwide at the forefront of technology in support of large molecule bioanalysis; and maintaining state-of-the-art knowledge pertaining to large molecule bioanalytical methodologies.

Sean Xiaochun Zhu, PhD, Takeda: Dr. Sean Xiaochun Zhu is a scientific director at Takeda Pharmaceuticals. He is the head of Global Biotransformation and Isotope Chemistry Group and also serves as a DMPK project representative in the cross-discipline project team. He has extensive experience in biotransformation, DDI, PK, and ADME sciences to support discovery and development of small molecules, peptides, and large molecules. Sean has published thirty articles in peer-reviewed journals.

POSTER ABSTRACTS

A Simple, Rapid Method for Simultaneously Quantitative Analysis of Multiple Bile Acids in Biofluid and Tissue Samples

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INTRODUCTION

Bile acids (BAs) are metabolites of cholesterol and are the major constituents of bile. Primary bile acids are synthesized by the hepatocyte and conjugated with glycine/taurine. Secondary bile acids are formed by gut microbiota, which include dihydroxylation, dehydrogenation, and epimerization1. Bile acids facilitate absorption of lipids, fat soluble vitamins and cholesterol elimination in intestine. They signal and regulate receptors in multiple metabolic pathways, including FXR3, TGR54, and S1PR25. Irregular level of BAs is not only highly toxic to liver and other organs, but also an indicator of hepatobiliary disease and drug-induced liver injury (DILI). Moreover, quantitative difference in the BA levels and composition between preclinical species and human may pose a major challenge for the accurate prediction of DILI. It is thus necessary to tightly and comprehensively monitor BA concentrations in blood and various organs.

METHOD

In this work, we present a simple and rapid LC-MS/MS method which analyzes 18 BAs simultaneously with the potential of additional hydroxy bile acid analysis. BAs were detected using negative MRM mode in SCIEX 6500+ triple quad instrument couples with Infinity UHPLC. Mobile phase A 0.1% formic acid in water and mobile phase B, methanol: acetonitrile (95:5 v/v) were used to separate the BAs on Agilent Poroshell 120 EC-C18 column.

RESULT & CONCLUSION

High interspecies BA levels were observed in plasma and tissues (liver, kidney). Total BA profile is highly variable across species, guinea pig has highest BA level while dog has the lowest level. Taurine conjugation was predominant in mouse, rat and dog while NZ rabbit have very low levels of taurine conjugated BA. On the other hand, high glycine conjugation was seen in guinea pig, human and rat while dogs were found with very low levels of glycine conjugated BA. There was a huge inter species difference in ratio of primary to secondar BA, minipig and WH rat have higher pri/sec BA ratio while guinea pig and human have lowest pri/sec BA ratio.

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Simple and Sensitive Multiplexed HRMS/MS Method Development for Quantitation of PPMO and PMO in Cyno Plasma using Protein Precipitation

Abhi Shah, Pallavi Lonkar, Shaoxia Yu

PURPOSE

PGN-EDODM1 is a peptide conjugated phosphorodiamidate morpholino oligomer (PPMO) investigational drug currently being developed at PepGen for treatment of Myotonic Dystrophy Type 1 (DM1), a neuromuscular disease. Liquid Chromatography (LC)-Triple-quadrupole mass spectrometer (QqQ) combined with protein precipitation sample preparation remain the workhorse especially for small molecular bioanalysis. However, there are challenges including interference with endogenous compounds, more severe matrix effects, and lack of selectivity for oligonucleotides analysis in plasma. Additionally, liquid-liquid extractions, solid-phase extractions, and hybridization assays require more time and resources. Using intact masses on high-resolution mass spectrometry (HRMS) has demonstrated the ability of quantitation with high selectivity, yet suffers from insufficient sensitivity. Parallel Reaction Monitoring (PRM) mode on the QE+ not only has comparable sensitivity compared to QqQs using S/MRM, but also a superior selectivity than QqQ. Utilizing the C-trap and multiplexing functionality on QE+ in PRM mode, we have overcome the above problems and developed a simple protein precipitation extraction procedure for the cyno monkey bioanalytical PK assay with lower limit of detection of 10ng/mL.

METHODS

A 30uL of cyno plasma was protein precipitated with 220uL of 1:1 MeOH/MeCN+2% formic acid. Following centrifugation and drying down of supernatant, 150uL of 0.1% TFA and 0.2% BSA in H2O was reconstituted and 20uL was injected for LC-HRMS/MS analysis. The LC-HRMS/MS method has been developed using multiplexing PRM of 4 different charge states and summing signal intensity resulting in 4 fragments post-acquisition. Multiplexing PRM with multiply charged species was utilized for highly selective and sensitive LC-MS quantitation with a good linear range.

RESULTS

Dynamic linear range from 10 to 20000 ng/mL with linear curve using 1/x2 weighting for bioanalytical quantitation of PPMO-DM1 and PMO-DM1 in cyno plasma has been developed with excellent inter and intra-day accuracy and precision using simple protein precipitation.

CONCLUSION

A sensitive, selective, and robust bioanalytical method in cyno plasma has been developed in-house with lower limit of quantification of 10 ng/mL using multiplexed PRM mode on QE+. The LLOQ could be further lowered if needed. The simple protein precipitation coupled with multiplexing charge states in PRM scans is an attractive strategy for development of plasma PK assays for PPMO and PMO oligos due to similar fragmentation and optimization of duty cycle. This method can be applied to multiple species for discovery and development support.

The Challenges in LC-APCI-MS/MS Method Development and Optimization for Pocapavir, a Poorly Ionized Capsid Inhibitor, in C57BL/6 Mouse Plasma

Ashley Davie, Renmeng Liu, Elaine To, and Yurong Lai

PURPOSE

Pocapavir is a capsid inhibitor that blocks the picornavirus uncoating and the intracellular release of viral RNA after infection. Pocapavir presents positive poliovirus treatment results in vitro and in vivo by decreasing the viral shedding duration and increased survival. However, as an investigation drug, the preclinical pharmacokinetic (PK) profile must fully be characterized, specifically the understanding of the terminal phase elimination. Unfortunately, it has its own challenges as the compound is poorly ionized using electrospray ionization (ESI) resulting in limits of quantification sensitivity and an accurate PK characterization.

We developed a sensitive and robust bioanalytical method using atmospheric pressure chemical ionization (ACPI) coupled with LC-MS/MS to overcome the limitations for quantifying pocapavir in plasma samples. The method was validated in the spirit of Good Laboratory Practice (GLP), meeting all acceptance criteria. The method was applied to accurately determine the pharmacokinetic parameters of pocapavir following a single oral dose at 300 mg/kg in C57BL/6 mice.

METHODS

Pocapavir and the isotope labeled internal standard, pocapavir-d3, were sourced from MedChem Express and Gilead Sciences medicinal chemistry department, respectively. Mouse plasma samples were subject to protein precipitation by addition of acetonitrile to final concentrations of 91% containing pocapavir-d3. The calibration standards range 20-20,000 ng/mL and are prepared in mouse plasma. Sciex 7500 triple quadrupole mass spectrometer is used to quantify pocapavir using multiple reaction monitoring (MRM) channel 420.9→264.0 under negative ion mode. Ion source parameters are curtain gas at 40 psi, gas 1 at 50 psi, gas 2 at 70 psi, and spray voltage at 4500V. Mobile phase A is water and mobile phase B is acetonitrile. A linear gradient from 50-98% of B over the course of 1.5 minutes is applied to achieve separation between pocapavir and matrix interference using an Aglient SB-C18 column.

RESULTS

A method validation in compliance with internal standard operating procedures (SOPs) adapted from the ICH M10 was conducted to evaluate the robustness of the developed bioanalytical method. The quality control (QC) sample accuracy and precision for the lower limit of quantification (LLOQ, 20 ng/mL), low QC (LQC, 60 ng/mL), medium QC (MQC, 1000 ng/mL) and high QC (HQC, 15,000 ng/mL) levels all meet the acceptance criteria of within 15% (20% for LLOQ) for within- and between-runs (n=3). Dilution integrity is established at a 10-fold sample dilution. Hemolysis and matrix effects do not affect the performance of the assay. No carryover is observed. Pocapavir presents 92.6%, 77.5%, and 66.5% recovery at LQC, MQC, and HQC levels, respectively. Furthermore, Pocapavir is stable for 34 days in the stock solution prepared in DMSO and stored at -80 °C, and remains stable for up to 2 hours at room temperature in mouse whole blood. In mouse plasma, pocapavir is stable for 96 hours at room temperature. Pocapavir may undergo up to 4 freeze-thaw cycles in mouse plasma. After extraction, the samples are stable for up to 20 hours at 4°C.

Once establishing the robustness of the assay, we apply the validated method in a pharmacokinetic study where four C57BL/6 mice are orally dosed with pocapavir at 300 mg/kg. Mouse plasma samples are collected at 0.5, 1, 3, 6, 12 and 24 hours post-dose before storage at -80°C. Noncompartmental analysis (NCA) is used to calculate the PK parameters of pocapavir from the calculated plasma sample concentrations. Each animal's terminal phase is successfully captured, resulting in the average terminal half-life estimation of 3.15 ± 1.56 hours.

CONCLUSION

In spirit of GLP, we validated a robust bioanalytical method to quantify the concentration of pocapavir in C57BL/6 mouse plasma using APCI-LC-MS/MS. We established freeze-thaw, long term, and bench top stability. We have also demonstrated appropriate separation of interference due

to potential matrix effects or plasma hemolysis. The developed bionanalytical method is robust and suitable for quantifying pocapavir in mouse plasma, where we can capture the terminal phase at 300 mg/kg within 24 hours post-dose to fully characterize its PK properties.

Nonclinical in vitro and in vivo data demonstrated a low immunogenicity risk and favorable potential of PGN-EDO51 for the treatment of DMD

Brijesh Garg, Ashling Holland, Jeffrey Foy, Shaoxia Yu, Jason Flavin, Pallavi Lonkar

PURPOSE

PepGen's Enhanced Delivery Oligonucleotide (EDO) cell-penetrating peptide technology is engineered to optimize tissue delivery and nuclear uptake of therapeutic oligonucleotides. PGN-EDO51, a peptide-conjugated oligonucleotide, is PepGen's investigational candidate for the treatment of people with Duchenne muscular dystrophy (DMD) amenable to exon 51 skipping and is in Phase 2 clinical trials. As part of the nonclinical development program of PGN-EDO51, its immunogenicity potential was evaluated in vitro and in vivo.

METHODS

The immunogenicity potential of PGN-EDO51 was evaluated in vitro using an EpiScreen™ time course CD4+ T-cell assay in human peripheral blood mononuclear cells (PBMCs). PBMCs from fifty healthy donors were incubated with PGN-EDO51 or the PMO or peptide (fewer than 17 amino acids) components of PGN-EDO51. T-cell responses were measured using an uptake proliferation assay and compared to relevant positive and negative controls. In vivo, the potential for an anti-drug antibody (ADA) response was characterized in non-human primates (NHP) administered with PGN-EDO51 at 45 mg/kg once every 4 weeks (11 doses total) via intravenous infusion of PGN-EDO51.

RESULTS

Proliferation data analysis of the frequency and magnitude of the CD4+ T-cell responses confirmed that PGN-EDO51, and the PMO and peptide portions of the molecule, were non-cytotoxic and considered to have a low risk of immunogenicity in the clinic. Additionally, using a validated ADA assay, repeat dosing of PGN-EDO51 did not demonstrate any ADA response in the confirmatory assay.

CONCLUSION

Combined, these nonclinical data suggest PGN-EDO51 has no/low potential of eliciting immunogenicity responses in repeat dose clinical studies. These data informed the design of the ongoing PGN-EDO51 Phase 2, multiple-ascending dose studies, CONNECT1-EDO51 and CONNECT2-EDO51. ADA analysis will be performed on Phase 2 clinical samples on a cohort-by-cohort basis and data will be reported at a future date.

Singlicate analysis in ligand binding assays from discovery to regulated clinical studies: Implementation strategies and benefits

Osseman Quentin, Vasken Parsekhian, Danielle Salha

PURPOSE

Oligonucleotide, protein therapeutics and biomarkers represent a diverse array of biopharmaceutical entities with distinct molecular structure and functionalities. In the realm of pharmaceutical research and development, the precision and reliability of bioanalytical data are paramount for ensuring safety, efficacy, and quality of the therapeutic drugs under investigation. Singlicate sample analysis, which involves the analysis of individual samples rather than replicates, offers several pertinent advantages in the assessment of these specialized compounds. Singlicate analysis offers similar sensitivity and specificity to replicate analysis in various biofluids (matrices) while adding flexibility to test various parameters simultaneously in early method development and increasing the number of samples analyzed in regulated studies. Furthermore, singlicate analysis optimizes resource use and enhances workflow efficiency in the laboratory. It also allows to make informed decisions early in drug discovery stage when large set of data are required. By focusing bioanalytical efforts on individual samples, we can streamline the use of precious study samples (volume, rare sample), reduce consumable costs, and accelerate data generation for large amount of study sample without compromising robustness and quality.

In GLP and non-GLP studies, adherence to rigorous regulatory standards and scientific principles is fundamental to ensure the integrity and credibility of the bioanalytical data collected. The same principle should be used to ensure that singlicate analysis will provide comparable results to the standard replicate analysis. The ICH M10 guideline supports singlicate analysis of PK samples – as per paragraph 4.2 (i.e., microtiter plates are used for LBAs and study samples can be analyzed using an assay format of one or more wells per sample) and paragraph 4.3 (i.e., the study samples, QCs and calibration standards should be processed in accordance with the validated analytical method).

METHODS

For discovery studies, 5 biomarker evaluation of kidney injury were evaluated using rat Urine. This was completed using colorimetric and MSD ELISA kits including a multiplex assay. Kits were used as per supplier instruction by different analysts on different days. We evaluated in all these assays the precision (%CV) and accuracy (%bias) of STD and QCs to assess the pertinence of singlicate analysis.

A bioanalytical method was developed and qualified to measure an oligonucleotide drug to support a non-GLP TK study in mouse tissues. The bioanalytical method determines concentrations of the oligonucleotide in 4 different tissues by using standard and QCs prepared in one type of tissue. Precision and accuracy runs were performed to determine robustness of the assay and whether singlicate analysis was possible.

A bioanalytical method aimed at measuring the enzymatic activity of the drug in human plasma was assessed for the ability to perform singlicate analysis during validation by evaluating intra and inter-assay precision (%CV) in both singlicates and duplicates. Following successful precision results, remaining validation evaluations and sample analysis were performed in singlicates as well as Incurred sample reanalysis.

RESULTS

For kidney biomarkers: two colorimetric methods showed a mean overall maximum %bias and %CV at 0.4% and 8.3% respectively for STD and at 4.4% and 3.3% respectively for QCs. MSD kits including singleplex and multiplex assay showed overall maximum %bias and %CV at 2.7% and 4.0% respectively for STD and at –9.3% and 4.8% respectively for QCs for biomarkers analyzed. With a limited amount of split and or volume, coordination of sample with concomitant analysis performed reduced the number of freeze thaw cycles for study sample. This impacted the turnaround time as well for sample analysis. All runs met acceptance criteria and a few samples were re-assayed with a suitable dilution.

Evaluation of oligonucleotide concentrations through eight precision and accuracy runs in singlicate analysis showed QC performance with inter

%CV at ≤13.9 and inter %bias within 11.3. Standard samples from precision and accuracy runs showed a maximum %CV of 5.3% and a maximum %bias of 7.3%. The use of STDs and QCs spiked in one tissue to measure the concentration in a few different tissues allows to combine bioanalysis of multiple tissues in the same plate which speeds up data availability. For one specific tissue, as target %CV of less than 15% was not reached, the analysis of those samples was maintained with the standard duplicate approach.

For the validation of the enzymatic assay in human serum all precision and accuracy evaluations were performed in duplicate, and since the intra-run precision for Surrogate and Matrix QCs provided a precision (%CV) below 15% at each QC level, the remainder of the validation evaluations were conducted in singlicate. In sample analysis, at least 10% of the total analyzable study samples were re-assayed and compared to the original values. At least 2/3 (79.4%) of the total number of quantifiable samples tested for ISR evaluation have met the percent difference criteria of within ±30% between original and reassayed concentrations.

CONCLUSIONS

The pertinence of singlicate sample analysis in GLP and non-GLP studies with various matrices and drug entities or biomarkers stems from its ability to maintain data reliability, regulatory compliance, and improve operational efficiencies. In addition to assessing %CV and %bias from replicates, a thorough examination of assay performance is essential for robustness evaluation in single-sample analysis. Typically, this involves comparing the replicate method with the single-sample method in separate plates to assess precision and accuracy across multiple runs. Subsequent statistical analysis can help identify significant differences between the two approaches. By adopting this bioanalytical strategy and ensuring the reliability of generated data, the overall process of bioanalytical method development, validation, and sample analysis can be expedited. Furthermore, it enables the exploration and assessment of biomarkers for secondary endpoints in limited and rare samples.

Synthesis of N-glucuronides of pyrazole moieties in drugs arising from UGT-1A4 mediated glucuronidation in humans

Julia Shanu-Wilson, Liam Evans, Emily Hopkins, Lisbet Kvaerno, Ravi Manohar, Richard Phipps, Frank Scheffler, Jonathan Steele

PURPOSE

Glucuronidation is the most common phase II reaction observed in the metabolism of drugs in humans, involving conjugation of small molecules to glucuronic acid by UDP-glucuronosyltransferases (UGTs). N-glucuronides can be formed as a result of aliphatic and aromatic conjugations with pyrazole, pyridine, pyridazine, pyrrolidine, pyrimidine, imidazole, triazole, and tetrazole moieties all being susceptible ring structures. This poster will show methods for the synthesis and elucidation of such glucuronides.

METHODS

This poster illustrates the synthesis of two human N-glucuronides of pyrazole-containing drugs in clinical development, using both microbial biotransformation and late-stage chemical glucuronidation techniques via two case studies.

RESULTS

Both glucuronides were produced in sufficient amounts to allow structural elucidation and regulatory studies.

CONCLUSION

Here late-stage chemical glucuronidation and microbial biotransformation provided solutions for producing scalable quantities of UGT-1A4 mediated pyrazole-linked N-glucuronides of two clinical stage drugs for definitive structure elucidation by NMR and testing of the metabolites.

Application of the HEPATOPAC® Model for Determination of In-vitro Intrinsic Clearance and Metabolite Profiling

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PURPOSE

HEPATOPAC is a co-culture model in which cultured hepatocytes maintain many differentiated functions, including the expression of drug-metabolizing enzymes, for a duration of several weeks. This enables the accurate determination of in-vitro intrinsic clearance, even for low-clearance drugs, which is not feasible with conventional models in which hepatocytes are cultured alone. An accurate estimate of intrinsic clearance is necessary to establish the optimal dosing regimen for new chemical entities. In this study, one hypothesis was that, with strategic selection of sampling times, clearance can also be calculated for moderate- and high-clearance drugs. A second hypothesis was that quantitative analysis (for determination of clearance) and qualitative analysis (for metabolite profiling) can be achieved in a single injection.

METHODS

A series of model drugs, including risperidone, tolbutamide, and warfarin, was incubated with human hepatocytes in HEPATOPAC, and samples were collected at predetermined time points (0, 4, 24, 48, 96, and 168 hours). The initial drug concentration was 0.1 µM, with 64 µL of culture medium per well of each 96-well plate. At each sampling time, 64 µL of acetonitrile containing a stable-label internal standard was added to one well for each model drug and the bottom of the well was scraped to lyse the cells. The cell lysate was collected, stored at -80°C until analysis, and replaced with cell culture medium to help maintain consistent humidity across the plate over the seven-day culture period.

RESULTS

An aliquot of each model drug sample was analyzed (i) by LC-MS/MS (SCIEX Triple Quad 5500+) for quantitative measurement of the remaining parent compound, and (ii) by LC-HRMS (Thermo Orbitrap Fusion Lumos ETD) for qualitative (and relative semi-quantitative) metabolite profiling. For metabolic clearance, the acquired data was processed using Analyst (SCIEX). For metabolite profiling, the acquired data from the Orbitrap was processed using Compound Discoverer and Xcalibur software (Thermo).

CONCLUSIONS

Hepatic clearance calculated from the HEPATOPAC data was within a factor of 2 of the literature value for risperidone, tolbutamide, and warfarin. In addition, metabolite profiles were obtained for all model drugs. Our results demonstrate the utility of the HEPATOPAC model for metabolite identification and prediction of in-vivo hepatic clearance.

Stable Isotope Labeling by Amino Acid in Culture (SILAC) Approach for the Detection of Trapped Reactive Metabolites in Plated Hepatocytes

Igor Mezine, Guru Valicherla, Chris Bode, Ragu Ramanathan, Sid Bhoopathy

PURPOSE

To perform detection of trapped reactive metabolites (TRM) in parallel with CYP induction experiments.

METHOD

To facilitate the detection of TRM, we investigated the SILAC approach using SIL-glycine (SIL-Gly). We plated CYP induction certified human, rat, and dog hepatocytes using vendor-provided seeding medium supplemented with SIL-Gly (1 mM). The plated hepatocytes (PH) were then incubated with the model compound clozapine (CLZ, 30 μM), for 24 hours. The medium was removed, attached cells were extracted with 50% MeCN, and after centrifugation, cell extract supernatants and medium were analyzed using liquid chromatography coupled with high-resolution accurate mass spectrometry (LC-HRAMS) implemented on an Orbitrap Fusion Lumos instrument. The acquired data were processed using Compound Discoverer (CD) and Xcalibur software.

RESULTS

Data processing resulted in the detection of several known GSH conjugates of CLZ in medium only, and the monoisotopic patterns (MIP) of all GSH conjugates showed incorporation of SIL-Gly in GSH. Interestingly, MIP ratios, defined as the percentage of A0 (SIL-GSH)/A0 (GSH), were both conjugate-specific as well as species-dependent, ranging from 20% to 90%, implying complex interplay between the kinetics of model compound metabolism, uptake of SIL-Gly, and GSH biosynthesis and efflux. Therefore, we used CLZ as a positive control compound to establish MIP search acceptance criteria in CD software.

CONCLUSION

Evaluation of reactive metabolites (RM) formation is an integral part of the modern drug development process. The most widely used in vitro systems to generate and trap RM are liver microsomes and/or S9 fractions. Detection of TRM, quite often present at a low level, is greatly facilitated by applying a mixture of GSH and SIL-GSH, enabling automatic filtering of peaks of interest via a search for the specific MIP. Since such subcellular systems lack a complete set of enzymatic machinery involved in drug metabolism, hepatocytes are often used instead of or in parallel to subcellular fractions to study drug metabolism. However, the detection of low-level TRM in hepatocytes is greatly hindered compared to subcellular models. The developed SILAC approach can be multiplexed with CYP induction studies to perform additional metabolite profiling with facilitated detection of TRM.

The Application of Kirchhoff's Laws to Saturable, Michaelis-Menten, Pharmacokinetics

Jasleen K. Sodhi and Leslie Z. Benet

PURPOSE

Since the recognition of chemical rates of reaction over 150 years ago, all derivations of overall rate constants in chemistry and biochemistry, as well as total clearance values in pharmacokinetics and clinical medicine, are based on solving differential equations. This approach leads to consistent operational results for linear systems but introduces numerous ambiguities for Michaelis-Menten nonlinear systems.

METHODS

Rates of drug elimination (mass/time) are defined by multiplying either drug concentrations or drug amounts by the relevant coefficient of proportionality, a clearance for measured concentrations and a rate constant for measured amounts. We previously demonstrated that coefficients of proportionality for linear kinetic processes could be derived independent of differential equations using Kirchhoff's Laws, by adding rate-defining parallel processes to give the relevant total clearance or rate constants or by adding the inverse of rate-defining processes in series to give the inverse of the relevant total clearance or rate constant. A rate-defining elimination process is one that by itself limits the rate of loss, i.e., the total clearance or total rate constant experimentally may equal this parameter alone (e.g., liver blood flow).

RESULTS

Here we demonstrate that the adaptation of Kirchhoff's Laws (from physics) to parallel and in series kinetic processes enables a simple derivation for both linear and Michaelis-Menten nonlinear systems, without relying on differential equations. Previously, we have shown that it is possible to simply derive the linear hepatic clearance (CL_H) parameter for what was previously regarded as the well-stirred model by considering the rate-defining in series clearance processes, hepatic blood flow (Q_H) and fraction of drug unbound in blood (f_{uB}) multiplied by intrinsic hepatic clearance (CL_{int}), when hepatic basolateral transporters are not relevant.

$$\frac{1}{CL_H} = \frac{1}{f_{uB} \bullet CL_{int}} + \frac{1}{Q_H}$$

(1)

Similarly, the clearance parameter may be solved for the in series saturable metabolism relationship

$$\frac{1}{CL_{H,saturable}} = \frac{1}{\frac{V_{max}}{K_M}} + \frac{1}{\frac{V_{max}}{[S]}} + \frac{1}{Q_H}$$

(2)

where the units of V_{max} are mass/time, versus the Michaelis-Menten V_{max} units of concentration/time.

To our knowledge, the dampening effect of Q_H on $CL_{H,saturable}$ has not been considered previously and can have a significant impact on predicting in vivo nonlinear kinetics. For example, for ethanol at a substrate concentration [S] of 0.1 g/L, where V_{max} = 10 g/hr and K_M = 0.08 g/L, calculated CL_H is 34.4 vs 55.6 L/hr neglecting Q_H. Even at the drunk-driving limit (0.8 g/L = 10xK_M), the effect of Q_H is not negligible (13% difference)

$$\frac{1}{CL_{H,saturable}} = \frac{1}{\frac{10}{0.08}} + \frac{1}{\frac{10}{0.8}} + \frac{1}{90} = \frac{1}{10.1\ L/h}$$

(3)

When Q_H is very large compared to the other two denominator terms, the usual case for most nonlinear metabolic processes, then solution of Eq. 2 gives the familiar Michaelis-Menten relationship, but with V_{max} units of mass/time,

$$CL_{H,saturable} = \frac{V_{max}}{K_M + [S]} \quad (4)$$

where when K_M >> [S], then linear elimination is observed and $CL_H = f_{uB} \bullet CL_{int} = \frac{V_{max}}{K_M}$.

When [S] >> K_M, then $CL_{H,saturable} = \frac{V_{max}}{[S]}$.

CONCLUSIONS

We maintain that for saturable kinetic processes following Eqs. 2-4, the in vitro V_{max} and K_M values may not be the same as the in vivo values; these parameters should be determined experimentally in vivo for different steady-state [S] conditions. This is because the in vitro Michaelis-Menten parameters are determined in the same volume of fluid, serving as the basis for the Briggs-Haldane derivation, so that the difference between using units of amounts vs concentrations introduces no error. However, in vivo certainly the volume of distribution for lipophilic drugs will not be the same as the metabolic enzymes, nor will the volume of distribution for the enzyme-substrate complex be the same as the substrate. We further maintain that because of these volume differences, enzyme-substrate on and off rates in vitro may not be predictive in vivo. The Kirchhoff's Laws derivation, however, gives the appropriate relationship, Eq. 2, independent of these differences.

Development of a Hybrid Immunoaffinity LC-MS/MS Assay for the Quantitation of Total Fc-Fusion ligand Trap Drug in Human Serum

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PURPOSE

To characterize the pharmacokinetic profile of a Fc-fusion ligand trap drug, a hybrid immunoaffinity LC-MS/MS assay was developed. This assay employed an anti- ligand trap drug ECD domain antibody for analyte enrichment, enabling the quantitation of the total Fc-fusion ligand trap drug in human serum.

METHODS

A custom monoclonal anti-ECD domain antibody was generated using rabbit single B cell sorting technology and selected based on its binding affinity and resistance to ligand interference. An immunoaffinity approach utilizing magnetic beads coated with the anti-ECD domain antibody was used to enrich the Fc-fusion ligand trap from human serum. Following bead washing, the captured ligand trap drug was denatured with RapiGest, reduced with DTT, alkylated with iodoacetamide, and digested with trypsin. The resulting extract was analyzed via HPLC and MS/MS detection using positive ion electrospray.

RESULTS

The assay exhibited a calibration range from 0.05 µg/mL to 25 µg/mL, with a sample analysis range from 0.05 µg /mL to 250 µg/mL, achieving acceptable precision and accuracy. Both inter- and intra- assay accuracy and precision were within ± 15%. The assay selectivity, ligand interference and the patient matrix interference were also evaluated, all meeting the acceptance criteria.

CONCLUSION

The LC-MS/MS method accurately quantified total concentrations of the Fc-fusion ligand trap drug. This assay is suitable for the quantitative analysis of the total drug concentration in human serum.

Accurate and Precise Quantitation of the Pentadecapeptide BPC-157 from Human Blood Collected with the Tasso-M20 Microsampling Device and Analyzed by HPLC-MS/MS

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PURPOSE

Collection of samples for bioanalysis is typically conducted by a trained phlebotomist at a clinic or hospital and requires a painful blood draw and large volumes of blood. This makes pediatric blood sampling difficult and can be burdensome for the elderly or critically ill. The Tasso-M20 device enables at-home, automated self-collection of samples. The device automatically collects 17.5 microliter samples in minutes with very little pain or blood loss. BPC-157 is a pentadecapeptide gastric peptide that possesses free radical scavenging activity and has been shown to reduce inflammation by blocking the production of pro-inflammatory mediators (nitric oxide, prostaglandins, and leukotrienes). BPC-157 has been shown to have healing properties for gastrointestinal fistulas, intestinal lesions, and liver lesions and can be used for the treatment of inflammatory bowel disease and congestive heart failure. BPC-157 has also been shown to accelerate the healing of damaged tendons and ligaments. In order to accurately quantify the peptide in blood collected on the Tasso device an extraction and HPLC-MS/MS method was developed with a linear range of 1.00-2000 ng/mL.

METHODS

A method was developed to extract BPC-157 from blood collected using a Tasso-M20 device followed by HPLC-MS/MS analysis. For standard preparation BPC-157 was spiked into blood and 17.5 microliters was pipetted onto exposed blank Tasso-M20 tips. The tips were dried at ambient and placed into a DWP 96 and extracted using a methanol/acetonitrile precipitation. The supernatant was dried and then reconstituted with 100 µL water/acetonitrile containing 0.1% formic acid 4/1 . The sample was then analyzed by HPLC-MS/MS on an API-7500 (Sciex) mass spectrometer operating in positive ESI mode. Separation was achieved using an Agilent Pursuit Diphenyl column (10 cm x 2.1 mm, 5 µm). Mobile phase A consisted of water with 0.1% formic acid. Mobile phase B was prepared in acetonitrile containing 0.1% formic acid.

RESULTS

The data indicates that with a simple precipitation extraction and HPLC-MS/MS analysis BPC-157 can be accurately and precisely quantified from human blood collected with the Tasso-M20 device. A linear calibration curve can be generated from 1.00 to 2000 ng/mL.

CONCLUSION

A simple extraction and HPLC-MS/MS method has been developed to accurately and precisely quantify BP-157 from human blood collected using the Tasso-M20 device.

Antibody-drug conjugate bioanalysis: A case study in stability, matrix effects, and recovery

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PURPOSE

Antibody-drug conjugates (ADCs) are an increasingly popular approach to selectively deliver a drug, the "payload", to target cells or tissues with minimal off-target effects. However, their complexity creates unique challenges in regulated bioanalysis. In this work, the drug of interest is a proprietary ADC with a self-immolative linker activated by β-glucuronidase. Upon release, the cytotoxic payload undergoes further conversion to the active form of the drug. Three bioanalytical assays in non-human primate (NHP) plasma were desired: total antibody, free payload, and conjugated payload.

METHODS

All three assays were developed using liquid chromatography and triple quadrupole mass spectrometry detection. The free payload is extracted from stabilized NHP plasma using protein precipitation (PPT). Total antibody quantitation was performed using PPT followed by digestion with soluble SMART Digest™ trypsin. SILu™MAB was used to provide a stable isotope labeled signature peptide for the internal standard. For quantitation of the conjugated payload, the intact ADC was isolated via PPT and the payload was released though incubation with β-glucuronidase followed by supported liquid extraction (SLE).

RESULTS

Plasma samples required treatment to prevent conversion of the free payload to the active form. Dichlorvos provided acceptable stability but hemolysis failures were observed that were attributed to hemolysis-related instability. Further screening identified ascorbic acid as an effective stabilizer, even in hemolyzed plasma. For total antibody quantitation, PPT prior to trypsin digestion was found to increase the yield by roughly 20-fold relative to direct plasma digestion. Several signature peptides were screened to provide sufficient selectivity from the native NHP proteome. Multiple approaches were tested to isolate the intact ADC from the free payload. Immunoaffinity enrichment provided the best selectivity against the free payload, but the conjugated payload could not be efficiency released by β-glucuronidase hydrolysis. Size exclusion gave mid-range recovery, but poor selectivity and non-linearity was observed at low ADC concentrations. PPT was less selective for the intact ADC than immunoaffinity enrichment but performed best for recovery and sensitivity in combination with SLE.

CONCLUSION

Three LC-MS/MS assays were developed and validated for the analysis of a novel ADC in NHP plasma. Immunoaffinity enrichment is the gold standard for hybrid LC-MS quantitation of the conjugated payloads of ADCs, but for this ADC, insufficient sensitivity was observed due to suspected enzyme inhibition. Protein precipitation and size exclusion filtration were characterized as alternative ADC isolation approaches. Ultimately, protein precipitation followed by β-glucuronidase hydrolysis and SLE provided the best combination of sensitivity and ruggedness. Despite the complex methodology and lack of internal standard tracking through most of the extraction, the method was successfully validated according to regulatory guidelines. While each target was analyzed separately in this study, further optimization may allow for the combination of assays and the quantitation of multiple ADC forms from a single aliquot.

Metabolism of 14C-retatrutide following subcutaneous administration in healthy male participants

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ABSTRACT

Retatrutide is a novel synthetic molecule, which is an agonist of the glucose-dependent insulinotropic polypeptide receptor (GIPR), glucagon-like peptide-1 receptor (GLP-1R), and glucagon receptor (GCGR), Retatrutide is under development globally for the treatment of obesity as an adjunct to a healthy dietary pattern and physical activity, and type 2 diabetes as an adjunct to diet and exercise. In the current study, a single subcutaneous dose of 14C radiolabeled retatrutide (1.81 – 1.84 mg/participant, approximately 100 µCi) was administered to seven male human subjects. The overall interpolated mean recovery of radioactivity in urine and feces samples was 85.2% of the dose over the 1512-hour study. An interpolated mean of 76.7% of the dose was excreted in urine, and 8.45% was excreted in feces through the last collection interval (1512 hours post-dose; Day 64). Most of the recovered radioactivity (mean of 68.4%) was. excreted within 504 hours post-dose. Metabolite profiling from selected plasma, urine, and feces samples were carried out using offline radioprofiling and LC/HR-MS/MS analysis. Hamilton pooled plasma (0-1008 hr) samples showed retatrutide and six minor circulating metabolites. Urine samples pooled across subjects at different time intervals and each subject time-point pools revealed no parent drug but three prominent metabolites along with four minor metabolites. Feces samples pooled across subjects at different time intervals showed no parent drug. In feces, majority of the radioactivity was not retained by reverse phase HPLC indicating fecal metabolites were more polar than urinary or plasma metabolites. Identification of the polar metabolites was not achieved due to lack of chromatographic retention, likely matrix suppression and low concentration. Overall, six minor metabolites were identified in plasma resulting from proteolytic cleavage of the amino acid backbone, each individually accounting for less than 4% of total circulating radioactivity. Retatrutide was cleared by metabolism with no intact parent drug observed in urine or feces. The primary pathways that contributed to the metabolism of retatrutide were proteolytic cleavage of the amino acid backbone with β-oxidation, oxidation, and decarboxylation of the C20 fatty acid chain and amide hydrolysis in the linker region.

Enhance productivity with smart algorithms for streamlining high-resolution data reduction in bioanalytical workflows

Robert Proos and Rahul Baghla

SCIEX

PURPOSE

Conventional workflows for quantitative bioanalyses of peptides and small molecules are increasingly adapted to LC-MS/MS platforms such as triple quadrupole mass spectrometers, due to their great quantitative performance and excellent sensitivity. More recently, suggestions to employ accurate mass spectrometry for quantitative bioanalysis have emerged because of increased selectivity. Systems such as time-of-flight (TOF) mass spectrometers can produce highly complex datasets, which can be challenging, especially in demanding schedules in discovery bioanalysis. Handling such large and information-intensive datasets requires the right tools to streamline the time-consuming data review process in drug discovery.

METHODS

Bioanalytical analysis of several small molecules and peptides was performed in plasma matrix. Peptides and small molecules were analyzed in separate LC-MS assays. High-flow LC-based separation was performed on a C18 column, and analysis was performed using a ZenoTOF 7600 system with the MRMHR mode applied. The MRMHR method included collecting data from a set range of fragment ions per precursor ion evaluated. Source and gas conditions and MRMHR method parameters were optimized for each compound.

RESULTS

The fragment ions were then evaluated using intelligent software to select the best choices and sum for sensitivity, selectivity and linear dynamic range. The final report out consisted of the quantitative performance parameters such as accuracy, coefficient of variation and also linear regression from the calibration curve. Results were compared to the bioanalytical guidelines and were found to meet the bioanalytical criteria.

CONCLUSION

Overall, the software solutions that support streamlined workflows for quantitation were demonstrated with data acquisition from a high-resolution mass spectrometer, with the aim of accelerating drug discovery turnaround times.

Overcoming Challenges in LC- High-Resolution Mass Spectrometry (HRMS) Method Development for Quantitation of PPMO and PMO in Human Urine

Abhi Shah, Pallavi Lonkar, Shaoxia Yu

PURPOSE

PGN-EDO51 is a peptide conjugated phosphorodiamidate morpholino oligomer (PPMO) investigational drug currently being developed at PepGen for treatment of Duchenne muscular dystrophy (DMD), a neuromuscular disease. Bioanalytical method development in human urine by LC/MS (Liquid Chromatography/Mass Spectrometry) is always a challenge due to the severe nonspecific binding and matrix effect. This is caused by differences in pH, ionic strength and/or protein content that vary in individual samples. Additional challenges are developing bioanalytical methods for novel therapeutic PPMO in human urine due to the neutral PMO backbone coupled with charges from the peptide, as well as the lack of relevant publications for method validation in the bioanalytical community. This work will describe how we have overcome these challenges and successfully developed a method for the first time, using HRMS in conjunction with UPLC (Ultra-Performance Liquid Chromatography) for the bioanalysis of human urine samples. This method has been validated.

METHODS

A 30uL of human urine was used and extracted by SPE using µelution HLB oasis plates. A LC-HRMS method has been developed. Multiplexing PRM with multiply charged species was utilized for highly selective and sensitive LC-MS quantitation with a good linear range.

RESULTS

Linear range in 50 to 25000 ng/mL for bioanalytical quantitation of oligonucleotide drugs has been developed with excellent accuracy and precision. This method has been successfully validated. Despite the most challenging matrix effect, 11 out of 12 lots in low QC's and 12 out of 12 lots in high QC's have all passed validation criteria.

CONCLUSION

A reliable and robust bioanalytical method in human urine has been developed and validated for the support of human sample analysis.

Closing the Translational Gap in Drug Development: Human Multi-Tissue Chip Platform for Predictive and Mechanistic Preclinical Studies

Shiny Amala Priya Rajan*, Shivam Ohri, Lauren Nichols, Paarth Parekh, Murat Cirit

PURPOSE

Current paradigm of drug development faces significant challenges with translational gap between preclinical findings and human clinical outcomes. Extrapolating pharmacokinetic (PK) parameters from existing preclinical models including in vitro cell culture and nonclinical animals, often proves unreliable due to inherent limitations to humans. This challenge is further amplified with the emergence of new modality drugs beyond small and large molecules. Species-specific pathophysiology differences in animals lead to inconsistencies between preclinical and clinical results. Additionally, limited complexity of conventional in vitro cultures is not suitable to study complex biological interactions relevant to drug absorption, distribution, metabolism, and excretion (ADME), urging the need for more sophisticated models.

METHODS

Javelin's tissue chip combines human-specificity, and long-term metabolically functional culture to evaluate human PK parameters. While the LTC maintains long-term metabolically functional hepatic culture, the LTC+ integrates this liver MPS and polarized intestinal culture (RepliGut® planar – jejunum) under continuous media recirculation providing constant MPS interaction. Here, we utilized Javelin's tissue chip (Liver chip, LTC; Entero-Hepatic chip, LTC+) to (i) accurately predict clinical ADME parameters such as hepatic clearance, (ii) assess physiological relevance of multiple donor and perform long-term Drug-Drug interactions (DDI) studies and (iii) demonstrate the feasibility of using an integrated Liver-Gut system for oral bioavailability studies.

RESULTS

Low turnover drug clearance studies on hepatic platform demonstrated high IVIVC and accurate clinical exposure predictions (1.1 average absolute fold error). Long-term PK based DDI studies conducted using victim drugs, midazolam and alprazolam, in the presence of perpetrator drug, rifampicin showed a 43% and 22% decrease in AUC of substrate depletion curve respectively leading to a 2- and 2.6-fold increase in intrinsic clearance. To demonstrate feasibility of oral bioavailability studies, we characterized the functional activity of LTC+ and compared it to hepatic only culture.

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. This translational data can also be incorporated into PBPK modeling to predict human drug exposure and first in human dose estimation.

Determining DMPK & Changes in Plasma and Liver Lipidome Following Repeat Oral Administration of Methapyrilene Over 5 Days To Male Wistar Rats Using HRMS Targeted HILIC-MS/MS & Imaging Mass Spectrometry

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PURPOSE

Omics-based biomarker technologies including metabolic profiling (metabolomics /metabonomics) and lipidomics are making a significant impact on disease understanding, drug development, and translational research. A wide range of pathophysiological processes involve lipids and monitoring changes in lipid concentration can give valuable insights into drug toxicity and off target pharmacology. Methapyrilene, an antihistamine and anticholinergic, developed in the 1950's for the treatment of insomnia was removed from the market in 1970 as it was demonstrated to cause cancer in rats following chronic administration. Here we report the metabolism and lipid dysregulation detected by HRMS targeted HILIC-MS/MS and DESI MS Imaging in the plasma and liver tissue lipid profiles of male Wistar rats following the repeat oral administration of methapyrilene over 5 days at 0, 50 and 150 mg/kg.

METHODS

Male Wistar rats were oral dosed with methapyrilene at 0, 50, 150 mg/kg/day over 5 days. Blood was collected via vena cava at 24, 72 and 120 hours. Drug metabolism was determined using reversed – phase UHPLC-HRMS and lysis of plasma and urine samples. For lipid analysis a total of 435 unique lipids were quantitatively measured using an 8 min HILIC method coupled to a tandem quadrupole mass spectrometer operating in positive and negative ion MRM mode. Batch QC samples were created by pooling 10 µL of plasma from each sample, the batch QC was processed in an identical manner to the study samples and analysed regularly throughout the batch to monitor assay performance. Lipid concentration was determined by isotope dilution using the EquiSplash mix internal standard in each sample. The non-polar triglycerides and cholesterol esters eluted at the beginning of the HILIC separation followed by the PC, PE, PG, SM, Cer, lipids with the LPE and LPC lipids eluting at the end. The peak intensities were determined using Skyline (MacCoss lab) and exported to MetaboAnalyst 5.0 for statistical analysis. A total of 253 and 240 lipids were detected in positive and negative ion modes, respectively. The dysregulated lipids and drug metabolites identified in HILIC-MS/MS were monitored in the liver samples of rats. Two animals per dose group were sacrificed 24 h post dose on days 1, 3 and 5, liver samples were frozen, sectioned and analysed by MS imaging using DESI connected to the same tandem quadrupole MS operating in MRM mode.

RESULTS

Methapyrilene was extensively metabolized to 40 major and numerous minor metabolites, the major route of metabolism was deamination, desmethylation, oxidation and loss of thiophene moiety followed by conjugation via sulphation, glucuronidation and glutathione. Principal components analysis (PCA) of the positive and negative ion QC sample data showed that there was no observable time related drift during the analysis. Positive ion data showed that the vehicle samples D1, 3 & 5 clustered together with the D1 50 and 150 mg/kg/day samples being positioned adjacent to them. There was strong evidence of a time related trajectory D1 -> D3 -> D5 for both 50 and 150 mg/kg samples. The 150 mg/kg/day samples showed the greatest change with the D3 150 mg/kg/day samples being clearly separated from the vehicle / D1 / D3 50 mg/kg/day group with the D5 50 and 150 mg/kg samples being furthest separated. The negative ion data showed a similar pattern to the positive ion analysis, there was clear time related trajectory D1 -> D3 -> D5 for the 150 mg/kg/day samples. The 50 mg/kg/day samples showed a slightly different trajectory with D1 and D3 clustering together and D5 mapping more closely to the 150 mg/kg samples. Heatmap analysis (+ve and -ve ion) using features consistent with the top 50 T-test/ANOVA values showed that the D3 and 5 150 mg/kg/day samples had the greatest variation with the bile acids, PEs, LPEs, PIs, GlcCer, Cer, SM and LPCs signal response increasing in the while response PCs decreased. The liver samples from D1, D3 and D5 were analysed in positive ion and negative ion mode. The DESI MS imaging raw data was subject to Principal Components Analysis (PCA) and

confirmed that the bile acids, PEs, LPEs, PIs, GlcCer, Cer, SM and LPCs lipids showed increased dysregulated in the liver samples with the PC signals reduced. The extent of lipid dysregulation was greater in the 150 mg/kg dose group than the 50 mg/kg dose group on all sampling occasions. For the 150 mg/kg dose group there was a clear dose related time trajectory. The D1 samples showed only a minor change between the vehicle group and the dosed sample groups. The tissue imaging data revealed that the major extent of lipid dysregulation occurred in the lobes of the liver, characterized by hepatocellular necrosis, bile duct proliferation, and inflammatory cell infiltration, which is consistent with the histological analysis of the samples with haematoxylin and eosin staining. Accurate mass DESI MS confirmed the presence of the major functionalization and conjugation metabolites of methapyrilene.

CONCLUSIONS

Targeted LC-MS/MS and DESI MS/MS was applied to the analysis of plasma and liver tissue samples following the repeat administration of methapyrilene to male rats at 50 and 150 mg/kg. Analysis of the data showed that bile acids, PEs, LPEs, PIs, GlcCer, Cer, SM and LPCs signal response increased following dosing, while the response of PCs and FFA decreased. There were observable temporal and dose related changes following repeat administration.

Determination of the Pharmacokinetics and Biotransformations of a PROTACs Tyrosine Kinase Inhibitor (gefitinib-PROTACs 3) Following Subcutaneous Administration to Male Rats

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PURPOSE

Proteolysis Targeting Chimeras (PROTACs) are an emerging class of drug molecules which work by mobilizing the ubiquitin–proteasome system to achieve proteasome-mediated degradation of the target protein. PROTACs consist of three components i) target binding TBM), ii) linker and iii) ubiquitin E3 ligase binding moieties (LBM). PROTACs eliminate all functions of the protein, providing differentiated pharmacology and do not require binding moieties that inhibit protein function. They also significantly increase the number of "druggable" proteins opening up the possibility for new safer medicines. PROTACs are "large small molecules" >800g/mol and understanding their DMPK properties presents a new challenges in monitoring fate of and quantifying both the TBM and LBM. Here we report the determination of the pharmacokinetics and metabolic biotransformation of gefitinib and gefitinib-PROTACs 3 in male Wistar rats.

METHODS

Male Wistar rats were dosed subcutaneously with either gefitinib, gefitinib-PROTACs 3 or drug free vehicle at 10 mg/kg. Blood was collected via tail bleed using a microsampling device at regular intervals over the during the following 24 hours. Derived plasma (10 µL) was subjected to protein precipitation with acetonitrile (containing d6 gefitinib as internal standard, 50ng/mL), vortex mixed and centrifuged prior to analysis. Gefitinib-PROTACs 3 pharmacokinetics was obtained via quantitative analysis using a 2 min reversed - phase UHPLC-MS/MS method coupled to a tandem quadrupole mass spectrometer operating in positive ion MRM mode. Metabolite identification was performed on a 10 min reversed – phase UHPLC method coupled to a Multi Reflecting Time-of-Flight mass spectrometer operating in positive mode.

RESULTS

The derived plasma and urine samples were analysed by UHPLC-MS/MS and UHPLC-HRMS to determine the pharmacokinetics, elimination and metabolic fate of the dosed compounds. Pharmacokinetic analysis showed peak plasma concentrations of 106 and 62 ng/mL, at, Tmax = 8 and 6 h, with AUCs = 818 and 824 ng.h/mL for gefitinib and gefitinib PROTACs 3 respectively. A total of nine major metabolites of the gefitinib-PROTACs 3 were detected in the circulatory system. The gefitinib-PROTACs 3 molecule was subject to cleavage of the linker to produce the E3 ligase and the gefitinib target binding moiety (TBM). Two ligase and two TBM metabolites were identified, which differed by the length of remaining linker chain. The TBM cleavage metabolites were further conjugated to form two sulphate and two glucuronide metabolites. The gefitinib-PROTACs was detected at low concentrations in the urine samples, with the cleavage and conjugate metabolites concentrations peaking in the 1-3hr samples. Urine gefitinib PROTACs - 3 concentrations steadily declined over the next 20h but these analytes were still present in the 8-24h samples. The biotransformation of gefitinib resulted in O-demethylation, metabolism of the morpholine ring and defluorination, some of these were further conjugated to form sulphate and glucuronide metabolites.

CONCLUSIONS

The pharmacokinetics and drug metabolism of the tyrosine kinase inhibitor gefitinib and the PROTACs analogue were evaluate in the male Wistar rat over a 24 h period following a single subcutaneous dose. The PROTACs analogue showed a peak plasma concentration of 62 ng/mL occurring 8 h post dose, compared to a peak plasma concentration of 106 ng/mL and a Tmax of 6h for gefitinib. The PROTACs compound was extensively metabolized via cleavage of the ligase TBM linker followed by oxidation and conjugation of the gefitinib like TBM.

Quantitation of Complement Factors in Human Serum using MicroVue Multiplex Assay

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PURPOSE

The complement system, a key player in the immune response, has emerged as a significant focus in vaccine research due to its multifaceted role in both innate and adaptive immunity. Vaccine-induced activation of the complement system can modulate the inflammatory response, which is necessary for an effective vaccine response but must be carefully regulated to avoid adverse effects. Understanding the role of complement in vaccine-induced inflammation is critical for the development of vaccines. To determine if vaccine infusion has any adverse effect, we quantitated complement levels in human sera using MicroVue multiplex complement assay, which is a customizable platform and measures the levels of individual complement proteins in human plasma or serum, providing a comprehensive snapshot of complement system homeostasis and activation.

METHODS

Qualification of four complement proteins (Bb, C3a, C5a, and C5b-9) was performed using 10-plex MicroVue Complement assay (Quidel Ortho). Human sera were collected from healthy individuals and those with diseases known for higher complement activation (rheumatoid arthritis and multiple myeloma). Normal human sera, activated with zymosan, were used as positive controls. Upper Limit of Quantification (ULOQ), high/medium/low QCs, and Lower Limit of Quantification (LLOQ) were prepared using either endogenous samples directly or spiking matrix with respective recombinant proteins. Sensitivity was evaluated using the precision panel and parallelism data. The QC and sample concentrations were evaluated by dilution to the minimum required dilution (MRD) in assay buffer. Concentrations of the analytes in human sera were back-calculated from respective 5-PL standard curves and adjusted for the MRD. The qualification followed ICH M10 Bioanalytical Method Validation guidelines, assessing parameters such as accuracy, precision, functional sensitivity, parallelism, linearity, and stability.

RESULTS

MicroVue multiplex complement assay was successfully developed and qualified to quantitate Bb, C3a, C5a, and C5b-9 levels in human serum. The assay showed a dynamic range of 0.0029 - 0.39 µg/mL, 0.33 – 144.57 ng/mL, 0.0061 - 2.92 ng/mL, and 1.54 – 713.98 ng/mL for Bb, C3a, C5a, and C5b-9, respectively. The intermediate precision and repeatability of the assay ranged between 6% and 18% (acceptance criteria ≤25% CV). For parallelism, serum samples with high endogenous analyte concentrations, near the upper end of the standard curve, were serially diluted with assay buffer. The CVs of parallelism samples were <25%. Dilution linearity was performed by spiking recombinant proteins into serum with very low endogenous levels of the analytes and serially diluting in C3-depleted serum, however, no linear relationship between the dilutions was observed. Therefore, parallelism and precision results were used to define ULOQ and LLOQ of the assay. The serum samples demonstrated acceptable stability up to five freeze-thaw cycles presenting recovery compared to baseline within 88% to 112% range. Although the qualification was successfully completed, one of the limitations was lower sensitivity of the assay.

CONCLUSION

MicroVue complement assay was successfully developed and qualified to accurately quantitate Bb, C3a, C5a, and C5b-9 complement factors in human serum within the assay range. This method enables the characterization of complement profile in reaction to vaccine infusion. Use of serum matrix could be one of the possibilities of lower sensitivity and quantitation of complements in plasma may improve the sensitivity of the assay. A future study is planned with paired plasma and serum samples to address this question.

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