



OCTOBER 6-8, 2025

SANOFI | Cambridge, MA

2025 WORKSHOPS: OCT 6 - Regulated Bioanalysis | OCT 7 - Discovery Bioanalysis & New Technologies | OCT 8 - Mechanistic ADME

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Welcome to the 2025 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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Stresser, AbbVie; Hongbin Yu, Boehringer Ingelheim

APA 2025 CONFERENCE AGENDA

DAY 1: Monday, Oct. 6

Regulated Bioanalysis Workshop

7:30 - 8:30 AM **Registration, Breakfast, Exhibits**
8:30 - 8:40 AM **Workshop Introduction**
Nevena Mollova, Gilead

10:55 - 11:20 AM **Identification of Critical Reagents for Novel Biotherapeutic Modalities: Considerations and Case Studies**
Krisna Duong-Ly, Merck

SESSION I: Precision Strategies in Regulated BA & Biomarker Validation

8:40 - 8:45 AM **Session Introduction**
Darshana Jani, Moderna; Ang Liu, Johnson & Johnson; Nevena Mollova, Gilead

8:45 - 9:05 AM **PK Cross Validation: Past, Present, Future**
Patrick Breslin, J & J

9:05 - 9:25 AM **Metabolite Bioanalysis in Drug Development: Recommendations From the IQ Consortium Metabolite Bioanalysis Working Group**
Wenkui Li, Novartis

9:25 - 9:45 AM **Oversight of Bioavailability and Bioequivalence Studies in Support of New and Generic Drugs and Therapeutic Biologics**
Sean Kassim, FDA

9:45 - 10:05 AM **Biomarker Validation in Clinical Drug Development 2025**
Lindsay King, Pfizer

10:05 - 10:20 AM **Panel Discussion**
10:20 - 10:40 AM **Break & Exhibits**

10:40 - 10:50 AM **SPONSOR PRESENTATION**
Application of LC-MS/MS Methods for Characterization of Low-abundance Peptides, Proteins, and Biomarkers in Biological Samples
Jason Hamilton, Worldwide Clinical Trials 


SESSION II: Analytical Innovations in Emerging Modalities & Nucleic Acid-Based Therapies

10:50 - 10:55 AM **Session Introduction**
Seema Kumar, Flagship & Jennifer Cunliffe, Novartis

11:20 - 11:45 AM **Characterization of Critical Reagents with Different New Modalities**
Rosmary Lawrence-Henderson, Pfizer

11:45 - 12:10 PM **Unraveling xRNA Immunogenicity: Insights and Future Horizons**
Maria Jadhav, Novartis

12:10 - 1:20 PM **Lunch, Exhibits, and Poster Viewing (1:10 pm - 1:30 pm)**

1:20 - 1:30 PM **SPONSOR PRESENTATION**
Leveraging Orthogonal Platforms to Address the Bioanalysis Needs of New Modalities in Drug R&D
Benjamin Wei, Medicilon 

Session III: Immunogenicity Matters: Advances in ADA Assay Development and Monitoring

1:30 - 1:35 PM **Session Introduction**
Fumin Li, Unitides Bioanalytical Lab & James Schiller, Merck

1:35 - 2:00 PM **Signal-to-Noise Ratio: A Streamlined Alternative to Titer for Anti-Drug Antibody Assessment**
Amy Li, Genentech

2:00 - 2:25 PM **Bioanalytical Immunogenicity Strategy for the Clesrovimab Program: A Case Study**
Brad Roadcap, Merck

2:25 - 2:50 PM **When Singlicate and Duplicate Diverge: Evaluating Risk, Performance, and Feasibility in Ligand Binding Assays**
Douglas Donaldson, Moderna

2:50 - 3:15 PM **Clinical Immunogenicity Monitoring of an Fc Fusion Protein Drug with Two Endogenous Counterparts: From Screening to Domain Specificity and Cross-Reactivity**
Jinsong Yang, Keros

3:15 - 3:35 PM **Break & Exhibits**

5:25 - 6:25 PM

Sciex's User Meeting & Reception

Unlocking Sensitivity and Selectivity: ZenoTOF 8600 for BioA, DMPK, and Beyond

Tatjana Talamantes, Senior Product Manager, HR
Accurate Mass

Session IV: Beyond Buzzwords: Real-World AI and ML Applications in Bioanalysis



- 3:35 - 3:40 PM **Session Introduction**
Yongjun Xue, BMS & Erik Burns, Alturas Analytics
- 3:40 - 4:00 PM **Machine Learning Guided Selection of Chiral Supercritical Fluid Chromatography Conditions**
Kiyoto Tanemura, BMS
- 4:00 - 4:20 PM **Leveraging AI in Bioanalysis: Real-World Applications of Large Language Models**
Stephanie Pisas-Farmer, BioData Solutions
- 4:20 - 4:40 PM **Exploration of AI and ML-Powered Models for Predicting Immunogenicity in Preclinical Tox Studies**
Afsana Trini, BMS
- 4:40 - 5:05 PM **Intelligent BioAnalysis (iBA) System: Pioneering the Future of Bioanalysis**
Andreas Luippold, Boehringer Ingelheim

Session V: Rapid Fire Poster Presentations

- 5:05 - 5:10 PM **Session Introduction**
Ying Zhang, Bicycle Therapeutics & Brendan Tierney, Pfizer
- 5:10 - 5:25 PM **Poster Presentations**

POSTER PRESENTERS:

- 1. Development & Implementation of a 6-plex Hybrid LC-MS/MS Method for Quantitative BA of CDH3 MSLN T cell engager (TCE)**
Margarita Semis, Amgen
- 2. Rapid and Sensitive Analytical Method for Amylin Analogs in Blood Sugar Regulation Therapeutics**
Tilak Chandrasekaran, Sciex
- 3. Evaluation of the MSD S-Plex Neuropanel 1 Multiplex Assay for Neurological Biomarkers**
Sarah Hwang, Sanofi

DAY 2: Tuesday, Oct. 7

Discovery Bioanalysis & New Technologies Workshop

7:30 - 8:30 AM **Registration, Breakfast, Exhibits**
8:30 - 8:35 AM **Workshop Introduction**
Yu Tian, AbbVie

11:55 - 12:20 PM **In Silico Immunogenicity Risk Assessments: AI-Enhanced Prediction of Clinical Immunogenicity Outcomes with EpiVax's ISPRI Toolkit**
Aimee Mattei, EpiVax

SESSION I: Bioanalytical Challenges and Approaches for Oligonucleotides in Therapeutic Development

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

8:35 - 8:40 AM **Session Introduction**
Jing Tu, GSK; Linlin Dong, Takeda; Hongying Gao, Innovo Bioanalysis

1:35 - 1:45 PM **SPONSOR PRESENTATION**
Build Resilience and Maintain Unparalleled Sensitivity for Long-term Bioanalysis
Matthew Stone, Sciex



8:40 - 9:05 AM **LC-MS Bioanalytical Support for Oligonucleotide PK/PD Studies**
John Chen, NovaBio Assays

SESSION III: AI & Machine Learning in Bioanalysis and Drug Discovery

9:05 - 9:30 AM **Bridging HELISA and LC-MS for Quantification of siRNA and Its Metabolite in Preclinical Studies**
Yunlin Fu, Novartis

1:45 - 1:50 PM **Session Introduction**
Yu Tian, AbbVie & Zachary Parsons, BMS

9:30 - 9:55 AM **From Brain to Biofluids: Translational Readouts of Target Engagement for Tau-Lowering C16-siRNA**
Diana Cha, Alnylam

1:50 - 2:30 PM **PLENARY TALK**
AI/ML - From Hype to Practical Integration: How to Deliver Impact
Gary Jenkins, AbbVie

9:55 - 10:05 AM **SPONSOR PRESENTATION**
Integrating New Approaches Methods into Drug Discovery and Development
Paige Vinson, Southern Research



2:30 - 2:55 PM **Basics of Machine Learning and Its Applications in Drug Discovery**
Reilly Eason, Merck

10:05 - 10:35 AM **Break & Exhibits**

2:55 - 3:20 PM **Leveraging AI in Computational Toxicology**
Wei Liang, AbbVie

3:20 - 3:40 PM **Break & Exhibits**

SESSION II: Immunogenicity & ADA Assessment

SESSION IV: Complex Biologics & Bioanalytical Strategies & Biomarker Analysis and Applications in Drug Development

10:35 - 10:40 AM **Session Introduction**
John Williams, Vertex & Ju Liu, Eli Lilly

10:40 - 11:05 AM **MHC-associated Peptide Proteomics (MAPPS) Assay for Characterization of Presented Peptide Epitopes**
Emilee Knowlton, PeplImmune

3:40 - 3:45 PM **Session Introduction**
Jonathan Joseph & Paddy Eangoor, Sanofi

11:05 - 11:30 AM **Integrated Immunogenicity Risk Assessment in Protein-based Biotherapeutic Drug Development**
Xiaobin Zhang, Takeda

3:45 - 4:10 PM **A Two-Cycle Immunoaffinity Enrichment Strategy with Acid Treatment to Enhance Biotherapeutics Assay Sensitivity in Tissues**
Yipei Zhang, Takeda

11:30 - 11:55 AM **Overcoming Challenges Associated with Anti-drug Antibody (ADA) Assay Development for Antisense Oligonucleotides (ASOs)**
Brittany Youngs, Stoke Therapeutics

4:10 - 4:35 PM **Strategic Bioanalytical Approaches for Complex Biologics: Tools and Case Studies**
Paddy Eangoor, Sanofi

4:35 - 4:55 PM **Rapid Fire Poster Presentations**

POSTER PRESENTERS:

1. Development of Multiplex RT-qPCR Assay for Highly Similar mRNA

Sequences

Marissa Mitola, Moderna

2. Advanced LC-MS/MS Assay for the Quantitation of Novel Therapeutic

Oligonucleotides in Human Plasma: Case Study

Aihua Liu, Resolian

3. Bioanalytical Strategies for Developing Surrogate Matrix to Quantify CNS

Lu Tan, Alnylam

4. Proteomic Signatures of Gaucher Disease Type 3 Reveal Response to

Venglustat and Imiglucerase Combination Therapy Using Olink Explore

HT

Pavithra Krishnaswami, Sanofi

4:55 - 6:10 PM **Reception & Exhibits**

DAY 3: Wednesday, Oct. 8

Mechanistic ADME Workshop

7:30 - 8:30 AM **Registration, Breakfast, Exhibits**
8:30 - 8:40 AM **Workshop Introduction**
Donglu Zhang, Genentech

8:40 - 9:20 AM **PLENARY: Human Mass Balance and Metabolite Profiling Studies: Regulatory Guidance, Technical Approaches and Clinical Implications**
Chandra Prakash, Agios

SESSION I: PROTACs and Molecular Glues with Respect to ADME Challenges and Human PK/Dose Projection; Early Dose Prediction Strategies

9:20 - 9:25 AM **Session Introduction**
Raman Sharma, Pfizer; Sara Shum, City Therapeutics & Hongbin Yu, Boehringer-Ingelheim

9:25 - 9:50 AM **Bridging Traditional and Novel Compounds in Discovery: Permeability and ADME Challenges with Protacs**
Mark Niosi, Pfizer

9:50 - 10:15 AM **ADME Profiling of Clinical Stage PROTACs**
Jesse Yu, Relay Therapeutics

10:15 - 10:40 AM **Mathematical Modeling for Human Dose Prediction of Degradors**
Jan Elias, Boehringer-Ingelheim

10:40 - 11:05 AM **Break & Exhibits**

11:05 - 11:25 AM **Rapid Fire Poster Presentations**

1. **Scalable In Vitro Methods for Accessing Major Human Metabolites of Structurally Complex Drugs using PolyCYPs and PolyUGT Enzymes**
Frank Scheffler, Hypha Discovery

2. **An Optimized Approach for Simultaneous Quantification of Total Antibody-Conjugated Payload Using Immunocapture LC-MS/MS**
Shengsheng Xu, J&J

3. **Development of Sensitive Intact Assays for Evaluating Molecular Stability and Pharmacokinetics of Protein Oligonucleotide Conjugate**
Yinzhen Guan, Sanofi

4. **In Vitro Reaction Phenotyping of Carboxylesterase Isoforms Using Supersomes: Method Development and Optimization to Assess Enzyme Contributions to Metabolic Clearance**
Taeseok (Sammy) Oh, Resolian

SESSION II: Use of AI & ML for Early Dose Prediction and ADME Properties

11:25 - 11:30 AM **Session Introduction**
Christopher Kochansky, Exelixis; Donglu Zhang, Genentech & April Chen, AstraZeneca

11:30 - 11:55 AM **Human Dose Projection at the Point of Design**
Kaushik Mitra, J & J

11:55 - 12:20 PM **Scoring Success in Molecule Making - From Idea Generation to PK Candidate Selection**
Fabio Broccatelli, Altos Labs

12:20 - 12:45 PM **AI-aided Drug Design from Drug Metabolism Perspectives**
Lionel Cheruzel, Genentech

12:45 - 2:00 PM **Lunch, Exhibits, and Poster Viewing (1:30 - 2:00pm)**

SESSION III: New Insights on the Discovery and Characterization of Covalent Inhibitors

2:00 - 2:05 PM **Session Introduction**
Benjamin Johnson, BMS & David Stresser, AbbVie

2:05 - 2:30 PM **DMPK Considerations for the Development of Targeted Covalent Inhibitor Drugs: An IQ Consortium Assessment**
Martin Dowty, AbbVie

2:30 - 3:05 PM **Analytical Strategies for Covalent Drug Discovery - Targeting Residues Beyond Cysteine**
Lyn Jones, Harvard University

3:05 - 3:25 PM **Break & Exhibits**

3:25 - 3:50 PM **GSH Conjugation in a Covalent Drug Discovery Paradigm: The Untold GST Story**
Cody Fullendwider, Vividion Therapeutics

SESSION IV: Recent Progress on Understanding Mechanisms of Hepatotoxicity

- 3:50 - 3:55 PM **Session Introduction**
James Driscoll; Nagendra Chemuturi, Eli Lilly &
Eric Ballard, Takeda
- 3:55 - 4:20 PM **Covalent Protein Modifications By Reactive
Metabolites: Evidence For Drug-Induced Liver Toxicity**
Qinying Yu, Genentech
- 4:20 - 4:45 PM **Covalent Binding & TAK-994 Drug-Induced Liver Injury**
Matthew Wagoner, Takeda
- 4:45 - 4:50 PM **Conference Closing Remarks**

APA ABSTRACTS

REGULATED BIOANALYSIS WORKSHOP

SESSION I: PRECISION STRATEGIES IN REGULATED BA & BIOMARKER VALIDATION

PK Cross Validation: Past, Present, Future

Patrick Breslin, Johnson and Johnson

Over the course of drug development, the bioanalytical assay may need to be transferred and validated at multiple bioanalytical laboratories to support large or global clinical studies. Additionally, bioanalytical assays may undergo revision, and new assay format may be implemented in study support as the program progresses. Typically, the correlation between different assays or across different laboratories needs to be demonstrated by conducting assay cross-validation, such that data across clinical studies can be objectively compared and pooled. With the implementation of ICH M10, this presentation will provide a comprehensive overview of the past and current approaches to cross-validation for PK assays. The historical use of incurred sample reanalysis acceptance criteria will be briefly reviewed and the new methodology outlined in the updated harmonized guidance will be discussed using case studies. The situations when a cross-validation is needed are well established, however, there is no guidance on the design of statistical approaches and the interpretation of cross-validation data. In this presentation, a novel process developed in collaboration with our Clinical Pharmacology and Biostatistician colleagues, aimed at producing a thorough comparison of two methods, will be discussed. This comparison will consider factors such as sample selection (incurred vs spiked), sample number, and method variability while utilizing Bland-Altman and Deming regression for the analysis. In addition, a transformed version of Deming integrated within the Bland-Altman will be explored. Through case studies, we will pose a question: Can bioanalytical methods ever completely abandon the use of percent difference? And ultimately, we will address a key question on everyone's mind—can a method ever fail a cross-validation?

Metabolite Bioanalysis in Drug Development: Recommendations from the IQ Consortium Metabolite Bioanalysis Working Group

Wenkui Li, Novartis

The intent of this presentation is to share the recommendations of the International Consortium for Innovation and Quality in Pharmaceutical

Development Metabolite Bioanalysis Working Group on the fit-for-purpose metabolite bioanalysis in support of drug development and registration. This report summarizes the considerations for the trigger, timing, and rigor of bioanalysis in the various assessments to address unique challenges due to metabolites, with respect to efficacy and safety, which may arise during drug development from investigational new drug (IND) enabling studies, and phase I, phase II, and phase III clinical trials to regulatory submission. The recommended approaches ensure that important drug metabolites are identified in a timely manner and properly characterized for efficient drug development.

Oversight of Bioavailability and Bioequivalence Studies in Support of New and Generic Drugs and Therapeutic Biologics

Sean Kassim, FDA

The FDA's Office of Study Integrity and Surveillance (OSIS), located within the Center for Drugs' Office of Translational Sciences, oversees four compliance programs under the FDA's Bioresearch Monitoring program. OSIS conducts inspections and remote regulatory assessments of sites performing GLP, Animal Rule, and bioavailability/bioequivalence studies for new and generic drug applications, as well as therapeutic biologics. For analytical BA/BE programs, the office reviews method validations and bioanalytical analyses to assess compliance with federal regulations and make data quality and integrity recommendations. These reviews provide critical insights about data reliability for the primary assessors making final application decisions, ensuring that the medicines essential for the protection of the public health are supported by high-quality studies, conducted under robust quality management systems, using clear processes, at the direction of committed applicants.

Biomarker Validation in Clinical Drug Development 2025

Lindsay King, Pfizer

In drug development biomarker assay validation has and continues to struggle to differentiate itself from pharmacokinetic (PK) assay validation from a regulatory guidance perspective. Traditional single plex ligand binding and mass spectrometry assays remains a staple for pharmacodynamic biomarker assays, but the range of technologies now used to measure biomarkers is enormous. Context of use has redefined fit for purpose, but the term assay qualification has spread. However, the 2 key differentiating factors and associated challenges for biomarker assay validation relative to PK remains. First biomarker calibrators are rarely true reference standards. Second endogenous

analyte biology may not be well understood so both parallelism and endogenous analyte stability are needed. This talk will briefly review the evolution of biomarker assay validation in drug development, where we are today and review with brief examples biomarker calibrators and parallelism validation experiments that continue to cause challenges and confusion.

SPONSOR PRESENTATION

Application of LC-MS/MS Methods for Characterization of Low-Abundance Peptides, Proteins, and Biomarkers in Biological Samples

Jason Hamilton, Worldwide Clinical Trials

- Challenges associated with analysis of low-abundance compounds
- Approaches to optimize analytical methods of low-abundance compounds
- Application of optimized analytical methods

SESSION II:

ANALYTICAL INNOVATIONS IN EMERGING MODALITIES & NUCLEIC ACID-BASED THERAPIES

Identification of critical reagents for novel biotherapeutic modalities: considerations and case studies

Krisna C. Duong-Ly, Merck

A wide range of innovative biotherapeutic modalities has emerged, encompassing bispecific and multi-specific antibodies, antibody-drug and peptide-drug conjugates, cyclic peptides, and many more. While these novel platforms have the potential to offer significant benefits to patients, they also pose challenges for bioanalytical scientists developing assays to assess their pharmacokinetics (PK) and immunogenicity. Often, the traditional clinical bioanalytical assay suite of one drug concentration, one anti-drug antibody, and one-neutralizing antibody assay per drug candidate is not adequate for these modalities. Multiple drug PK assays can be required when drug candidates can be present in more than one form. For multi-specificity biologics, additional assays may be required to elucidate the contributions of individual domains to immunogenicity.

After the analytes and assays of interest have been identified, critical reagents may become the next hurdle. Critical reagents are the essential building blocks of bioanalytical assays as their attributes

can directly impact analyte measurements. Improper critical reagent selection can negatively impact dose selections as well as assessments of PK/PD correlations and immunogenicity risks. Defining the purpose of the bioanalytical assay, outlining the attributes required of critical reagents, and having processes in place to confirm that potential reagents meet these criteria ensures the quality of the bioanalytical assay.

In this talk, strategies for critical reagent identification for novel biotherapeutic modalities will be presented. These strategies take into consideration the required bioanalytical assays and the specificity characteristics of potential critical reagents. Case studies that integrate these strategies with computer-based assessments and biophysical characterization of potential critical reagents will also be presented.

Characterization of Critical Reagents for Novel Biotherapeutic Modalities

Rosemary Lawrence-Henderson, Pfizer

In biopharmaceutical development, ligand binding assays are utilized to assess pharmacokinetic (PK) parameters and detect immunogenicity against the drug. Critical reagents are the foundation of these assays and ensure a high degree of specificity and sensitivity are obtained. Large molecule biotherapeutics have evolved significantly over the past 40 years, transitioning from mouse monoclonal antibodies to antibody drug conjugates, fusion proteins, multidomain targeting antibodies and gene therapies. Correspondingly, the complexity of bioanalytical assays has increased, necessitating highly characterized critical reagents for accurate, robust analysis.

Our paradigm to develop the high quality reagents needed to build these complex PK and immunogenicity assays is a three-pronged characterization approach: 1) high specificity to enable reagent binding to its intended target; 2) appropriate kinetics, aligning the reagent's affinity parameters with its intended function in the assay, and 3) the reagent must have a positive biophysical profile to guarantee a consistent, long-term supply. This presentation will review two case studies highlighting critical reagent development for multidomain biotherapeutics, detailing the process from campaign initiation to reagent selection.

Unraveling xRNA Immunogenicity: Insights and Future Horizons

Maria Jadhav, Novartis

xRNA-based therapeutics represent a transformative class of molecules in modern drug development. However, their immunogenicity impact

remains incompletely characterized, posing unique challenges for both preclinical and clinical development. In this presentation, we will highlight two major hurdles in the immunogenicity assessment of xRNAs and share our strategies to address them.

First, we will examine the difficulty in generating a reliable positive control for anti-drug antibody (ADA) monitoring—a critical component for immunogenicity assessment throughout development. We will present case study examples that illustrate practical solutions and lessons learned.

Second, we will explore how xRNAs compel a broader immunological assessment framework that includes not only adaptive but also innate immune responses.

This session will offer scientific insights and forward-looking perspectives to support the evolving landscape of xRNA therapeutic development.

SPONSOR PRESENTATION

Leveraging Orthogonal Platforms to Address the Bioanalysis Needs of New Modalities in Drug R&D

Benjamin Wei, Medicilon

The rapid emergence of novel modalities in drug research and development—such as gene therapies/editing, cell-based treatments, ADCs, AOCs, PROTACs, ASOs, biologics, and vaccines—presents unique challenges in bioanalysis. Traditional bioanalytical methods often fall short when addressing the complex and diverse nature of these innovative therapies. This presentation explores the application of orthogonal platforms—combinations of complementary techniques designed to provide more comprehensive and reliable analysis of these new modalities. By leveraging the strengths of various analytical approaches, including LC-MS/MS, HRMS, MSD/ELISA, qPCR/ddPCR, scRNA-seq, flow cytometry, and cell-based assays, we aim to enhance sensitivity, specificity, and throughput in bioanalytical workflows, ensuring more accurate assessments of pharmacokinetics, immunogenicity, and efficacy. Key examples of orthogonal strategies and their impact on overcoming challenges posed by these new modalities will be presented, along with best practices for their implementation in drug development pipelines.

SESSION III:

IMMUNOGENICITY MATTERS: ADVANCES IN ADA ASSAY DEVELOPMENT AND MONITORING

Signal-to-Noise Ratio: A Streamlined Alternative to Titer for Anti-Drug Antibody Assessment

Amy Li, Genentech

Antibody therapeutics can elicit an immune response in the host organisms leading to the formation of anti-drug antibodies (ADA). These ADAs can significantly impact therapeutic efficacy, pharmacokinetics (PK), and safety in both pre-clinical and clinical studies. Therefore, ADA assessment is a critical component in the development of biotherapeutics.

Immunogenicity assessment typically involves a tiered based approach where samples are first screened to identify potential positive samples, followed by a confirmatory assay to validate true positives. Traditionally, the magnitude of ADA response has been measured using titer assays which offer a semi-quantitative readout. However, titer assays can be resource-intensive, requiring additional sample volume, reagents and analyst time.

Recent investigations have demonstrated that signal-to-noise (S/N) ratio is a viable alternative for evaluating ADA magnitude. However, adopting S/N as a surrogate metric requires the use of an immunoassay platform with a broad dynamic range, to avoid saturating the assay signal at high ADA response.

Our study showcases a generic total ADA assay on the Gyrolab platform for monoclonal antibody therapeutics in cynomolgus monkey preclinical studies. This Gyrolab assay demonstrated a significantly wider dynamic range (approx. 5-log) and increased drug tolerance compared to the assay performed using colorimetric ELISA format. Notably, the S/N from the Gyrolab assay showed an excellent correlation with ADA titer values (Spearman's $r > 0.98$) across a broad ADA concentration range.

This strong correlation, coupled with the assay's broad dynamic range, supports using S/N ratios as a reliable and efficient readout for ADA responses, eliminating the need for separate titer assays in preclinical settings. Our findings align with broader industry trends where S/N has shown strong correlation with titer and comparable outcomes in assessing ADA impact on PK and pharmacodynamics, even in clinical studies. Adopting S/N ratio reporting offers substantial benefits, including reduced time, cost, sample volume requirement, and

resources, thereby streamlining immunogenicity assessments. This approach, particularly with platforms like Gyrolab that provide wide dynamic ranges, represents a significant advancement in efficiency and quality of immunogenicity assessment for monoclonal antibodies.

Bioanalytical Immunogenicity Strategy for the Clesrovimab Program: A Case Study Brad Roadcap, Merck

Respiratory syncytial virus (RSV) represents a significant unmet clinical need, being the leading cause of bronchiolitis and lower respiratory infections (LRIs) in infants. It is estimated to account for 28% of acute LRIs and contributes to 13% to 22% of deaths from these conditions in children under five years of age, with annual mortality rates ranging from 94,600 to 149,400 globally. In response to this critical health challenge, MK-1654 (clesrovimab) has been developed and designed to prevent infant RSV infections. Clesrovimab, a fully human neutralizing monoclonal antibody, binds to a highly conserved site on the RSV F protein, demonstrating equipotent activity against both RSV A and B strains, the primary causes of RSV infection. Its unique YTE mutation in the Fc region extends its half-life, allowing for a single-season dose that protects throughout the RSV season.

The immunogenicity strategy for the clesrovimab development program was built around a robust bioanalytical framework focusing on three primary assays. The pharmacokinetic (PK) drug assay utilized LC-MS/MS for quantitative measurement, employing protein precipitation and trypsin pellet digestion. A serum neutralization assay (SNA), a cell-based assay that measures any neutralizing activity that blocks the cells' RSV infection, was included as a functional PK assessment. Additionally, an anti-drug antibody (ADA) evaluation was implemented using a standard three-tier testing strategy, including screening, confirmatory, titer assessments, along with a fourth tier of testing for domain mapping. Given the molecule's low-risk profile for immunogenicity, a neutralizing antibody (NAb) assay was not planned for Phase 1. An integrated PK/PD ADA data assessment strategy, aligned with the 2019 FDA immunogenicity guidance, justified omitting NAb testing for the late-stage program. The FDA granted a NAb waiver for MK-1654 phase 3 studies, marking the first published instance of waived NAb testing. As trials progressed, additional fit-for-purpose assays were introduced to investigate a high-titer ADA signal observed in late-stage infant samples. This presentation will cover the dynamic immunogenicity strategy that led to the FDA's successful approval of the drug for RSV prevention in infants.

When Singlicate and Duplicate Diverge: Evaluating Risk, Performance, and Feasibility in Ligand Binding Assays Douglas Donaldson, Moderna

Singlicate testing has gained momentum in ligand binding assays (LBAs) as a viable alternative to traditional duplicate analysis, driven by regulatory flexibility (ICH M10, FDA 2019, EMA 2017), improved assay precision, and operational efficiency. While the scientific community increasingly supports singlicate approaches for well-developed assays, questions remain regarding the impact of diverging results between singlicate and duplicate formats—particularly around cut point estimation, sensitivity, and low-titer sample classification.

This presentation combines internal case study data with published evidence to explore the practical implications of transitioning to singlicate testing in regulated immunogenicity workflows. Variance component analyses from internal method validation show that well-to-well variability contributed <10% of total assay variance, supporting the feasibility of singlicate use. Sensitivity curves and t-test comparisons of cut points revealed statistically significant differences between singlicate and duplicate conditions; however, these differences did not materially impact assay performance or decision thresholds.

External case studies further support this finding: recent evaluations across multiple platforms and assay tiers demonstrated ≥95% agreement between singlicate and duplicate data, with discrepancies generally limited to samples near the screening cut point or in positive control assessments. Together, these results confirm that singlicate testing—when guided by robust validation and risk assessment—can maintain scientific rigor while improving throughput, reducing costs, and supporting patient-centric sampling strategies.

SESSION IV: BEYOND BUZZWORDS: REAL-WORLD AI AND ML APPLICATIONS IN BIOANALYSIS

Machine Learning Guided Selection of Chiral Supercritical Fluid Chromatography Conditions Kiyoto Tanemura, BMS

Enantiomers can exhibit different pharmacological properties, leading to different therapeutic or toxicological effects. Therefore, the separation of enantiomers through chiral chromatography is a critical component of bioanalysis in bioanalytical study support. However, the chromatographic conditions required to achieve chiral

resolution, are not known a priori and are difficult to predict due to the multiple variables and complex interactions involved in the separation process. Achieving chiral resolution typically involves trial and error or comprehensive screening during method development, which can be time consuming. To streamline the selection of chiral supercritical fluid chromatographic conditions, we trained a predictive model using historical comprehensive screening data. We applied a graph convolutional net to molecular structures to extract predictive substructures and provide a ranking of chromatography conditions expected to achieve chiral resolution. Our results highlight potential time savings using the predictive model, as well as chemical insights gained from model interpretation.

Leveraging AI in Bioanalysis: Real-World Applications of Large Language Models

Stephanie Pajas-Farmer, BioData Solutions

Data interpretation, reporting, and submission are critical components of bioanalysis, involving the processing, synthesis and dissemination of complex data to regulatory agencies, collaborators, and internal stakeholders. The integration of Large Language Models (LLMs) in bioanalysis presents a transformative opportunity to enhance efficiency, accuracy, and compliance in data reporting and regulatory submissions. LLMs such as Co-Scientist and BERT are revolutionizing how bioanalytical professionals generate, interpret, and communicate data by automating literature reviews, report generation, and drafting regulatory submissions. Other LLM tools enable data parsing and summarization for critical decision making. By leveraging AI-powered tools, organizations can reduce manual workloads, ensure regulatory adherence, and accelerate time-to-market for pharmaceutical innovations.

This presentation will delve into real-life applications of LLMs in bioanalytical drug development. Attendees will learn how LLMs support scientists in conducting comprehensive document reviews, generating consistent and accurate reports, and preparing regulatory submissions that align with frameworks such as those from the FDA and EMA. The session will also cover AI-driven summarization tools that help synthesize large datasets into actionable insights, enhancing decision-making in pharmaceutical research. Additionally, the presentation will highlight interactive AI-powered platforms that facilitate knowledge sharing and collaboration among multidisciplinary teams, driving innovation and improving bioanalytical workflows.

While the potential of LLMs is immense, the presentation will briefly address common hesitations and the evolving regulatory landscape, providing a balanced view of the opportunities and challenges. By the

end of the session, attendees will have a comprehensive understanding of how LLMs can be effectively integrated into bioanalytical processes to improve efficiency, accuracy, and compliance.

Exploration of AI and ML-Powered Models for Predicting Immunogenicity in Preclinical Studies

Afsana Trini, BMS

Introduction: Immunogenicity (IMG) remains a critical challenge in the development of biotherapeutics, as immune responses to therapeutic proteins can compromise both safety and efficacy. Although preclinical immunogenicity assessments are not directly predictive of human outcomes, they are essential in IND-enabling toxicology studies to interpret atypical pharmacokinetic (PK) and pharmacodynamic (PD) profiles and to inform risk management strategies. However, these assessments can be resource intensive.

Purpose: This study aims to advance the prediction of immunogenicity in preclinical studies by integrating artificial intelligence (AI) approaches, specifically neural ordinary differential equations (ODEs), to model PK profiles and infer anti-drug antibody (ADA) onset. By leveraging data-driven methodologies, we seek to enhance immunogenicity risk assessment and streamline preclinical workflows for low IMG-risk biotherapeutics.

Methods: Comprehensive preclinical PK and ADA datasets spanning multiple modalities were systematically curated, cleaned, and structured for analysis. We implemented the neural ODE algorithm as described by Lu et al. to predict PK concentrations for subsequent dosing intervals using PK concentration with time for first dose. Neural ODEs were selected for their capacity to model irregular and sparse time series, which are characteristic of PK and ADA sampling in preclinical studies. Deviations between predicted and observed PK profiles will be analyzed to identify potential ADA onset events.

Results: An automated data pipeline was established, integrating PK concentrations, dosing regimens, ADA measurements, species, and other relevant variables. Exploratory data visualization revealed diverse relationships between PK and ADA responses, with some cases demonstrating clear ADA-mediated PK alterations. Initial application of Lu's neural ODE framework to our pilot dataset highlighted challenges related to data sparsity and inter-individual variability, resulting in suboptimal predictive performance.

Conclusion: While initial results using Lu's neural ODE algorithm were limited by data heterogeneity and sampling irregularities, our findings demonstrate the feasibility of applying advanced AI models

to preclinical immunogenicity prediction. Ongoing optimization of model architecture and training strategies, tailored to the unique characteristics of preclinical datasets, is expected to substantially improve predictive accuracy. This approach holds significant promises for enabling early detection of ADA onset, refining immunogenicity risk assessment, and ultimately reducing the resource burden of preclinical IMG analysis for biotherapeutic development.

Intelligent BioAnalysis (iBA) System: Pioneering the Future of Bioanalysis

Andreas H. Luippold, Boehringer Ingelheim

ESI-MS-based bioanalysis is a crucial component of drug discovery, delivering high-quality data from biological matrices. However, the analysis of diverse samples with varying matrices and analytes can be labor-intensive and time-consuming, requiring complex optimization of analytical parameters. To address this issue, we introduced the iBA (Intelligent BioAnalysis), a unique, fully automated bioanalysis platform. It simplifies the bioanalytical process, enhancing safety, data quality, efficiency, and productivity, setting the stage for the future of intelligent bioanalysis labs.

The iBA system manages more than 2000 in vivo studies with different analytes and matrices each year. The system incorporates all necessary tools for a fully automated sample processing workflow, including a liquid handling workstation, acoustic liquid dispenser and cooled storage, all connected to a fully automated Rapid-Fire™ system. The iBA hardware is integrated within a modular software platform (AIMS) that interfaces with process databases and other software components. This setup enables the iBA to manage the entire workflow within a single system.

The iBA workflow uses standardized and optimized processes, eliminating human error and ensuring high process safety and data quality with comprehensive process documentation. The introduction of the iBA system offers considerable potential for enhancing efficiency in bioanalysis labs, delivering faster results to clients, and significantly impacting drug discovery projects.

To further reduce manual intervention and establish an intelligent and even higher grade of automation, a smart freezer system combined to a mobile robot as well as smart data evaluation and decision-making processes are currently being developed.

SCIEX USER MEETING & RECEPTION

Unlocking Sensitivity and Selectivity: ZenoTOF 8600 for BioA, DMPK, and Beyond

Tatjana Talamantes, Sciex

In the fast-paced world of pharmaceutical and biopharmaceutical development, precision and throughput are everything. The ZenoTOF 8600 system introduces a new era of high-resolution accurate mass spectrometry, purpose-built to elevate workflows with unmatched sensitivity, dynamic range, and speed without compromising data quality.

This talk will highlight how ZenoTOF 8600 empowers scientists to push the boundaries of quantitation and characterization, even in complex matrices. We'll explore real-world applications in BioA and DMPK, and touch on their potential in MetID workflows, showcasing how ZenoTOF 8600 delivers deeper insights, faster decisions, and greater confidence in every data point.

Whether you're tackling low-level quantitation or navigating challenging biotransformation, ZenoTOF 8600 is designed to meet the demands of modern pharma and biopharma labs.

DISCOVERY BIOANALYSIS & NEW TECHNOLOGIES WORKSHOP

SESSION I:

BIOANALYTICAL CHALLENGES AND APPROACHES FOR OLIGONUCLEOTIDES IN THERAPEUTIC DEVELOPMENT

LC-MS Bioanalytical support for oligonucleotide PK/PD studies

John Chen, NovaBio Assays

Oligonucleotides (ON) represent a new therapeutic modality that can modulate gene expression via a range of processes. During oligonucleotide research and development process, sensitive bioanalysis is an essential tool to characterize their pharmacokinetic (PK) and pharmacodynamic (PD) properties. This presentation will discuss strategies to optimize different ON sample extraction methods based on their mass, hydrophobicity, and conjugation. Furthermore, the presentation will also discuss oligo metabolite identification, Ab and oligo analysis in antibody-oligo-conjugates, protein biomarker LC-MS analysis.

Bridging HELISA and LC-MS for Quantification of siRNA and Its Metabolite in Preclinical Studies

Yunlin Fu, Novartis

Small interfering RNAs (siRNAs), also called short interfering or silencing RNAs, have been rapidly advancing in drug discovery and development for the treatment of various diseases. A reliable method for quantitative analysis of siRNAs in biological samples such as plasma and tissues is crucial in the evaluation of their toxicokinetic (TK), pharmacokinetic (PK), and pharmacodynamic (PD) properties to support respective development programs. Common bioanalytical platforms for siRNA quantification include hybridization-ELSA (HELISA), liquid chromatography (LC) based methods (e.g., LC-FLD and LC-MS), and quantitative polymerase chain reaction (qPCR) methods. Given the high sensitivity and throughput provided by HELISA, it is widely used in drug discovery and early nonclinical development. However, HELISA lacks the needed selectivity for differentiation the parent siRNA from its truncated metabolite(s) such as N-1 and N-2. This major limitation restricts its use in accurate measurement of siRNAs and their associated truncated metabolites in regulated studies. In contrast, LC based method, especially LC-MS has gained recognition in recent years as the most selective platform for quantifying siRNA and its truncated metabolites to support drug development.

NVS001, a siRNA drug candidate being developed at Novartis, consists of a 23-mer antisense strand and a 21-mer sense strand conjugated with a lipid. HELISA has been initially employed to measure NVS001 concentrations in various PK and TK studies. Later, assay platform was switched to LC-MS to provide simultaneous quantification of NVS001 and its AS3'N-1 metabolite, which was estimated to be 30-50% of the total exposure in rats and monkeys. To fully utilize the available HELISA data for understanding PK-PD and other aspects of NVS001, plasma exposure measured by two bioanalytical methods (HELISA and LC-MS) was bridged in vitro and in vivo in rat and monkey plasma.

This presentation provides comprehensive details on the experimental designs, acceptance criteria, and the outcomes of the bridging. The results demonstrated successful bridging between HELISA and LC-MS in quantification of in vitro and in vivo samples. Finally, extrapolation factors were established by using the exposure ratio of NVS001 to the total of NVS001 and AS3'N-1 metabolite. These factors were applied to the studies supported by HELISA for more accurate assessments.

From Brain to Biofluids: Translational Readouts of Target Engagement for Tau-Lowering C16-siRNA

Diana Cha, Alnylam Pharmaceuticals

Tau-lowering C16-siRNA conjugates demonstrate robust brain target knockdown in preclinical species; however, translating target engagement (TE) into human-relevant biofluids remains challenging. We assessed candidate TE biomarkers in non-human primate (NHP) CSF and plasma using ultrasensitive ligand-binding assays (S-PLEX MSD, Simoa), focusing on total Tau and brain-derived (BD) Tau isoforms. Biomarker responsiveness, robustness, and specificity were benchmarked against brain parenchymal Tau knockdown. Ligand-binding and molecular (qPCR) platforms were applied to derive pharmacodynamic (PD) measures, which were integrated with tissue PK and time-matched CSF/plasma assessments to establish PK/PD/TE relationships in NHP studies. We highlight key challenges in preclinical bioanalytical support for TE biomarker development—including assay sensitivity, matrix effects, compartmental lag, and inter-animal variability—and propose practical solutions in study design, fit-for-purpose assay controls, and optimized sampling strategies. Together, these approaches aim to improve the precision, reliability, and translatability of TE biomarkers for C16-siRNA therapeutics.

SPONSOR PRESENTATION

Integrating New Approach Methods into Drug Discovery and Development

Paige Vinson, Southern Research

A paradigm shift is underway in drug discovery and development (DDD), moving from conventional methods toward computational approaches and advanced in vitro models. Both strategies require substantial investment and carry inherent risks, and rarely can an organization adopt new approach methods (NAMs) across all DDD stages at once. Key challenges include recruiting specialized talent, investing in new technologies, and adapting to unfamiliar processes. To bridge these gaps, many organizations pursue partnerships that complement their capabilities. For smaller, resource-limited companies or high-risk, early-stage programs, such collaborations allow NAMs to be vetted and validated before full adoption. At Southern Research, we have developed NAM platforms, such as virtual screening validation and 3D human tissue models, that connect computational discovery with experimental validation. While these models hold promise for accelerating milestones in DDD, their success depends on robust assays to validate in silico hits and to measure phenotypes in biological

models. Efficient execution of these assays is critical to realizing the potential of NAMs.

This presentation will highlight examples of how Southern Research has incorporated NAMs into the DDD process through virtual screening validation and 3D tissue models to reduce risk, accelerate timelines, and strengthen discovery. We will also discuss the assays and technologies that support these platforms and enable their effective integration into modern drug discovery.

SESSION II: IMMUNOGENICITY & ADA ASSESSMENT

MHC-associated Peptide Proteomics (MAPPS) Assay for Characterization of Presented Peptide Epitopes

Emilee Knowlton, Prolmmune, Inc.

MAPPS assays are a highly sensitive and robust in vitro approach that has been used to identify naturally processed and presented epitopes from a wide range of input material. MAPPS assays have demonstrated success in several research areas and are a key tool in immunogenicity risk assessment to characterize potentially immunogenic regions of a therapeutic. Prolmmune offers the ProPresent® Antigen presentation assay in which monocyte-derived dendritic cells are loaded with antigen and MHC-peptide complexes are immunoprecipitated to allow for peptide epitope sequencing via LC-MS/MS. Prolmmune will share case studies in which ProPresent has been used in a number of applications and in combination with functional T cell assays such as the ProMap PBMC assay.

Integrated Immunogenicity Risk assessment in Protein-based Biotherapeutic Drug Development

Xiaobin Zhang, Takeda

Immunogenicity of biotherapeutics poses a significant concern that may impact the efficacy or safety of drug in patients. It is crucial to select candidates with low immunogenicity risk or de-immunize the candidates with high risk at an early stage, thereby avoiding resource expenditure on candidates with high inherent immunogenicity risk. In this presentation, I will introduce an integrated immunogenicity risk assessment workflow for evaluating and selecting drug candidates at the preclinical stage.

- In silico prediction to assess the immunogenicity risk, rank the candidates, and/or identify the immune hotspots in molecule for de-

immunization in early-stage development.

- Cell-based assay with human PBMC to evaluate the impact of selected candidates on immune cells.
- Humoral immunity assessment in the in vivo studies to interpret the effects of anti-drug antibodies (ADA) on pharmacokinetics (PK) or safety findings in tox studies.

In summary, the integrated immunogenicity assessment of the drug candidates with these in silico, in vitro, and in vivo assay platforms will enhance the success ratio in drug development.

Overcoming Challenges Associated with Anti-drug Antibody (ADA) Assay Development for Antisense Oligonucleotides (ASOs)

Brittany Youngs, Stoke Therapeutics

Oligonucleotide therapeutics (ONTs) have a significant potential to address diseases that have been challenging to treat with small molecules or biologics. Several ONTs like antisense oligonucleotides (ASOs) and short interfering ribonucleic acids (siRNAs) have been approved for human use, in the last two decades. Though the potential risk from immunogenicity of ASOs and siRNAs is low, anti-drug antibody assessment (ADA) against these modalities is routinely conducted in non-clinical and clinical studies to determine potential impact of ADAs on pharmacokinetics/pharmacodynamics (PK/PD) and safety of these ONTs. The challenges for development of ADA assays for these modalities have been underappreciated. The generation of ADA positive control by immunization of animals is critical for assay development. This is not a trivial process as the ONTs are chemically modified to minimize the risk of activating the innate immune system, therefore, as a result, ONTs have poor immunogenic properties. In addition, affinity purification of the potential ADA positive control may sometimes be challenging due to low titers and potential loss of binding activity during purification. These amongst other factors can lead to poor assay sensitivity and overall assay performance, that does not meet the regulatory requirements.

This talk will focus on considerations for ADA assay development for ONT based programs. Strategies for overcoming challenges associated with generation of positive control for ADA assay development will be discussed. Two case studies including successful development and validation of ADA methods STK-001 and STK-002 (ASOs being developed by Stoke Therapeutics), will be presented.

In Silico Immunogenicity Risk Assessments: AI- Enhanced Prediction of Clinical Immunogenicity Outcomes with EpiVax's ISPRI Toolkit

Aimee Mattei, EpiVax, Inc.

The development of biologic therapeutics necessitates comprehensive strategies to assess immunogenicity, a key factor influencing drug efficacy and safety. This presentation will focus on the innovative use of in silico immunogenicity risk assessment tools, particularly the AI-enhanced ISPRI (Interactive Screening and Protein Reengineering Interface) toolkit from EpiVax. ISPRI's computational methods identify effector and regulatory T cell epitopes and predict anti-drug antibody (ADA) responses, offering insights into minimizing risks during the drug development process.

As biologic formats increase in complexity, assessing the immunogenic potential of various constructs becomes critical. The adaptation of established immunogenicity analysis approaches to evaluate CD4+ T cell epitope content in multifaceted biologic formats, including non-antibody scaffolds and bispecific antibodies, will be discussed. This adaptation offers valuable insights into the distinct characteristics of individual components that may influence immunogenic responses. In a retrospective analysis of monoclonal and bispecific antibodies, ISPRI predictions provided consistent results in a fraction of the time and cost compared to published immunogenicity risk assessments made by combining observations from three in vitro assays and public in silico tools. In addition, nearly all (92%) promiscuous T cell epitopes predicted by ISPRI aligned with peptides identified in MHC-Associated Peptide Proteomics (MAPPS) assays, considerably outperforming traditional public T cell epitope prediction tools.

AI and machine learning (ML) have recently been integrated into ISPRI, significantly enhancing its predictive accuracy by a six-fold increase in the correlation between predicted and observed ADA rates, as well as an 85% reduction in false negatives. The integration of AI into in silico immunogenicity assessments represents a significant leap forward in the biopharmaceutical development landscape. By enhancing the predictive capabilities of existing tools like ISPRI, researchers can make informed decisions early in drug development, ultimately leading to safer and more effective therapeutic options.

SPONSOR PRESENTATION

Build Resilience and Maintain Unparalleled Sensitivity for Long-term Bioanalysis

Matt Stone, 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 III: AI & MACHINE LEARNING IN BIOANALYSIS AND DRUG DISCOVERY

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 IV:
COMPLEX BIOLOGICS & BIOANALYTICAL STRATEGIES
& BIOMARKER ANALYSIS AND APPLICATIONS IN DRUG
DEVELOPMENT**

Two-cycle Immunoaffinity Enrichment Strategy with Acid Treatment to Enhance Biotherapeutics Assay Sensitivity in Tissues

Yipei Zhang, Takeda

Accurate bioanalysis of therapeutic proteins is critical for reliable PK and TK assessments. Immunoaffinity (IA) LC/MS/MS offers high specificity with fewer reagent requirements than ligand-binding assays but can suffer from high background in tissue matrices due to nonspecific binding.

A two-cycle IA LC-MS/MS approach was developed to address this challenge. The additional enrichment cycle effectively reduced background interference, improving sensitivity in tumor and liver tissues while maintaining assay precision and accuracy. This simple, high-throughput method provides a valuable tool for enhancing tissue bioanalysis and studying therapeutic protein distribution.

Strategic Bioanalytical Approaches for Complex Biologics: Tools and Case Studies

Padmanabhan Eangoor, Sanofi

The evolving landscape of complex biologics—such as fusion constructs, bispecifics, and antibody-drug conjugates (ADCs)—is significantly influencing the field of bioanalysis. Engineering complex biologics with features like tissue targeting, multi-specificity, half-life extension etc., require an expanded bioanalytical toolkit to capture a complete pharmacokinetic profile. There have been numerous advancements in both ligand binding assay (LBA) and LC-MS technologies to address this growing need. These multi-dimensional analyses are helping project teams make informed decisions regarding dose selection and downstream clinical success. In this presentation the focus will be on strategically selecting the bioanalytical tools from constantly expanding toolkit to meet non-clinical program timelines. A few case studies from complex biologics programs, including peptide fusion constructs and ASO fusion constructs, will be presented, highlighting challenges and successes using traditional LBA, hybridization LBA, signature peptide-based LC-MS/MS and hybridization LC-MS approaches.

MECHANISTIC ADME WORKSHOP

PLENARY PRESENTATION

Human Mass Balance and Metabolite Profiling Studies: Regulatory Guidance, Technical Approaches and Clinical Implications

Chandra Prakash, Agios

Human absorption, distribution, metabolism, and excretion (ADME) study (also referred to as mass balance or radiolabel study) is a key study in the Clinical Pharmacology package of new drug application. It is typically conducted by administration of a single dose of drug containing a radioactive nuclide (typically ¹⁴C) followed by collection of excreta and blood samples. Samples are analyzed for both total radioactivity and the profile of drug-related material in urine, feces, and plasma. The data gathered from this study provides a quantitative and comprehensive overall picture of the disposition of a drug, including excretion pattern and metabolite profiles in circulation and excreta and are highly informative for developing a cohesive strategy for clinical pharmacology studies, drug-drug interaction, organ impairment, monitoring of metabolite(s), and to obtain a waiver for the bioequivalence study. Recently, US Food and Drug Administration (FDA) issued a guidance describing the recommendations regarding clinical pharmacology considerations for conducting human radiolabeled mass balance studies of investigational drug. This presentation will describe the highlights of this regulatory guidance, technical approaches and clinical implications of the human radiolabeled ADME studies.

SESSION I:

PROTACS AND MOLECULAR GLUES WITH RESPECT TO ADME CHALLENGES AND HUMAN PK/DOSE PROJECTION; EARLY DOSE PREDICTION STRATEGIES

Bridging Traditional and Novel Compounds in Discovery: Permeability and ADME Challenges with Protacs

Mark Niosi, Pfizer

A significant challenge with oral bioavailability for large and complex molecules like PROTACs, lies in their propensity to experience poor intestinal absorption. Factors such as low membrane permeability, instability within the intestinal environment, and susceptibility to enzymatic degradation, all can hinder their ability to be orally bioavailable.

Learning objectives: How useful are ADME properties for design and optimization of PROTACs, Opportunities of different analytical and in silico tools to advance drug design, Identify and resolve the challenges within in vitro ADME assays in drug discovery (DDI, PPB, Metabolic stability etc).

ADME Profiling of Clinical Stage PROTACs

Jesse Yu, Relay Therapeutics

PROTACs (Proteolysis Targeting Chimera) are large, bi-functional molecules that represent a new modality through which specific disease pathways can be inhibited. By degrading target proteins, PROTACs may confer a pharmacologic benefit over traditional small molecule inhibitors. Unfortunately, PROTACs are very difficult to characterize with conventional ADME assays due to the beyond rule of 5 chemical space they inhabit. Due to their heterobifunctional nature, PROTACs have a high MW, low permeability and often low solubility. Despite these limitations, there are a handful of PROTACs that have entered the clinic with promising results. As more clinical data become available for PROTACs, there is a growing interest in understanding if conventional relationships used to predict human pharmacokinetics apply to this new class of compounds. In this work, publicly disclosed human PK data for clinical PROTACs will be compiled. Internal ADME profiling will be conducted and paired with rat and dog PK studies to determine in vitro to in vivo correlation. Additionally, solubility and permeability will be assessed to determine any correlations to oral bioavailability. These results are an early attempt to understand the translational relevance of conventional ADME data sets for PROTACs and may help to identify further areas of improvement.

Mathematical Modeling for Human Dose Prediction of Degraders

Jan Elias, Boehringer Ingelheim

Human dose prediction for therapeutic modalities such as PROTACs and molecular glues is a critical step in drug development. These novel therapeutic agents present unique challenges due to their mechanisms of action, which necessitate incorporating target engagement dynamics and protein degradation kinetics into predictive models. To address these complexities, the integration of preclinical data and mathematical modeling is essential to bridge the gap between animal studies and human pharmacokinetics. This talk will review some approaches to dose prediction, including mechanistic modeling, scaling methods, and translational strategies.

SESSION II: USE OF AI & ML FOR EARLY DOSE PREDICTION AND ADME PROPERTIES

Scoring Success in Molecule Making – From Idea Generation to PK Candidate Selection

Fabio Broccatelli, Altos Labs

Optimizing small molecules in drug discovery is a complex, multifactorial problem. During optimization campaigns, drug hunters are tasked with distilling vast amounts of information from numerous in silico, in vitro, and in vivo endpoints to quantify compound quality and progression.

This presentation explores novel approaches that leverage physiologically based pharmacokinetic (PBPK) modeling, machine learning, and physicochemical properties to inform compound scoring. The talk will describe a workflow that uses these methodologies to objectively prioritize compounds for synthesis and in vivo testing.

The audience will learn how this approach has been successfully applied to drug discovery projects, significantly reducing the reliance on animal testing and accelerating decision-making.

SESSION III: NEW INSIGHTS ON THE DISCOVERY AND CHARACTERIZATION OF COVALENT INHIBITORS

DMPK Considerations for the Development of Targeted Covalent Inhibitor Drugs: An IQ Consortium Assessment

Martin Dowty, AbbVie

Targeted covalent inhibitors (TCIs) represent a promising modality of small molecule drugs that use a reactive warhead to inactivate target proteins of interest potentially increasing pharmacodynamic effect and target efficiency and selectivity. In 2023 the IQ consortium DMPK Guidelines for Targeted Covalent Drugs Workgroup surveyed IQ member companies to explore the knowledge around exploitation of this compound class. Several areas deserving special consideration were highlighted for TCIs. Characterization of target biology (target turnover rates) and application of appropriate modelling and simulation methods (inhibitory potency and inactivation rate) to predict efficacy and dose were identified as a central feature of many TCI development programs. The prediction of PK and in particular clearance in humans using traditional methodology required additional prudence potentially

requiring alternate modeling approaches based on understanding of CL pathways. Respondents highlighted the importance of balancing sufficient warhead reactivity, while avoiding excessive off-target binding. Simple TCI reactivity assays, for instance reactivity with glutathione (GSH), were leveraged by many drug discovery programs. TCI drug binding to off-target proteins while important in the assessment of TCI safety may not necessarily be a safety concern. And despite an apprehensiveness towards TCI safety, a comparison of clinical drug safety profiles for TCI and non-TCI did not reveal any increased safety risk for TCI drugs. Although it was acknowledged that prediction of idiosyncratic reaction by its nature is difficult to assess by currently available tools. Collectively, TCIs have shown value in the toolbox of medicinal chemistry and with some additional design considerations can become effective medicines to treat unmet patient needs.

Analytical Strategies for Covalent Drug Discovery - Targeting Residues Beyond Cysteine

Lyn Jones, Harvard University

Covalent modulation of proteins using electrophilic small molecules provides an opportunity to drug challenging therapeutic targets. The standard paradigm relies on the development of acrylamide-containing inhibitors that covalently engage cysteine residues in protein binding sites. However, the cysteine thiol is the most nucleophilic amino acid residue which complicates the development of selective drugs and chemical probes. Moreover, cysteine is the rarest amino acid and is often not available for targeting. As a result, we have developed new ways to rationally modify tyrosine, lysine and histidine residues in diverse proteins in a site-specific manner, to deliver a variety of pharmacologies, including inhibitors, correctors, and molecular glue degraders. This talk will provide a background to next-generation covalent drug discovery and describe analytical techniques that are essential to the property-based design and optimization of selective covalent modulators.

SESSION IV:

RECENT PROGRESS ON UNDERSTANDING MECHANISMS OF HEPATOTOXICITY

Covalent Protein Modifications by Reactive Metabolites: Evidence for Drug-induced Liver Toxicity

Qinying Yu, Genentech

Drug-induced liver injury is the major cause of late-stage drug development failures and often leads to safety-related drug

withdrawals. Lapatinib, an EGFR/HER2 antagonist, has been commonly used to treat breast cancers for the last 15 years. Despite promising therapeutic outcomes, idiosyncratic hepatotoxicity of lapatinib has also been observed in some patients. A black box warning of lapatinib led to limited uses in clinic and the mechanisms of the lapatinib hepatotoxicity remain unknown. In this work, utilizing lapatinib as tool molecule, we discovered a new class of reactive quinone methides resulting from immolation, previously unknown lapatinib metabolites. To better understand drug-induced hepatotoxicity, we utilized proteomics approaches to identify specific protein targets that are modified by quinone methide in human hepatocytes. Identified proteins with quinone methide adducts all play essential roles in key cellular processes including mitochondrial energy production, neutralization of reactive oxygen species and glutathione antioxidant defense. Disturbance of the protein functions have been reported in many liver pathological conditions and the combined risk factors may collectively contribute to overall hepatotoxicity. In addition, we elucidated structure and biological activities of these reactive metabolites. Specifically, formation of quinone methide from F-positional isomers correlated with the cytotoxicity in primary human hepatocytes especially from a genotyped P450 3A5*3/*1 heterozygous donor. To the best of our knowledge, this work is the first investigation to demonstrate that quinone methide formation through bioactivation followed by simultaneous covalent modifications on key functional proteins. Simultaneous disruption of multiple cellular processes can rationalize lapatinib-induced hepatotoxicity.

Covalent Binding & TAK-994 Drug-Induced Liver Injury

Matt Wagoner, Takeda

The frequency of drug-induced liver injury (DILI) in clinical trials remains a challenge for drug developers despite advances in human hepatotoxicity models and improvements in reducing liver-related attrition in preclinical species. TAK-994, an oral orexin receptor 2 agonist, was withdrawn from phase II clinical trials due to the appearance of severe DILI. Here, we investigate the likely mechanism of TAK-994 DILI in hepatic cell culture systems examined cytotoxicity, mitochondrial toxicity, impact on drug transporter proteins, and covalent binding. A potential covalent binding liability was uncovered with TAK-994 following hepatic metabolism consistent with idiosyncratic DILI and the delayed-onset clinical toxicity. Although idiosyncratic DILI is challenging to detect preclinically, reductions in total daily dose and covalent binding can reduce the covalent body binding burden and, subsequently, the clinical incidence of idiosyncratic DILI.

APA BIOGRAPHIES

Patrick Breslin, Johnson & Johnson: Patrick Breslin is a Senior Manager in the Bioanalysis group of Preclinical Sciences and Translational Safety (PSTS) where he leads the Clinical PK Development and Bioanalysis teams in Spring House, PA. He has over 25 years of experience in the pharmaceutical industry developing and validating GLP and Clinical bioanalytical methods supporting different modalities across multiple therapeutic areas. He received his Biochemistry and Molecular Biology degree from Penn State University with a follow-up Immunology and Microbial Pathology M.S. degree from Jefferson University in Philadelphia.

Fabio Broccatelli, PhD, Altos Labs: Dr. Broccatelli is the current head of the DMPK and Computational Chemistry groups at Altos Labs. His PhD and postdoctoral work, conducted at the University of Perugia, UCSF, and the Institute of Cancer Research in London, focused on ML predictions of ADME properties, virtual screening, and in vitro to in vivo PK correlations (IVIVc).

Previously, Dr. Broccatelli built the Computational ADME team within the DMPK department at Genentech and led the San Diego pre-clinical DMPK group at BMS, where he supported the Oncogenesis portfolio. His main research interests include ML/AI, PBPK, PKPD, and IVIVc across multiple modalities. He has authored over 37 peer-reviewed articles, patents, and book chapters, and serves as a reviewer for more than 15 scientific journals. He also initiated and co-led the in silico ADME IQ group.

Diana J. Cha, PhD, Alnylam Pharmaceuticals: Dr. Cha is Associate Director of Bioanalytical Sciences at Alnylam Pharmaceuticals, where she leads a cross-functional team supporting molecular and ligand-binding assays for internal PK and PD analyses, across multiple programs to advance RNAi therapeutics. With over a decade of experience in the RNA world, her area of expertise are in biomarker development and translational bioanalysis. Prior to joining Alnylam, she contributed to biomarker discovery in Alzheimer's disease at Amgen and Brigham & Women's Hospital/Harvard Medical School, following her PhD research at Vanderbilt University on extracellular RNAs.

John Chen, PhD, NovaBioAssays: Dr. Chen serves as Senior Vice President at NovaBioAssays, one of the Bioanalytical and Analytical Chemistry CRO in Great Boston Area. He helped establish and expand NovaBioAssays bioanalysis business during the last nine years. Before NovaBioAssays, John had worked in multiple biotech and CRO companies, including Agilux, Synta Pharma, NeuGenesis, and Celgene. John's research interests focus on bioanalysis and DMPK studies on drug and biomarkers of small molecules, oligonucleotide, peptide, protein, ADC, etc.

Lionel Cheruzel, PhD, Genentech: Dr. Cheruzel earned his Ph.D. in bioinorganic chemistry at the University of Louisville, KY. After a postdoctoral appointment in the laboratory of Prof. Harry B. Gray at the California Institute of Technology, he began his independent career in the Department of Chemistry at San Jose State University. His research focused on developing light-driven P450 biocatalysts using Ru(II)-based photosensitizers. He is currently a Senior Principal Scientist leading the Discovery Biotransformation group at Genentech, investigating the metabolism of small molecules and new therapeutics.

Douglas Donaldson, Moderna: Douglas Donaldson is a Senior Scientist in the Biomolecular Assay Group at Moderna, where he specializes in the development and validation of ligand binding and immunogenicity assays to support clinical and non-clinical programs. With expertise in a range of bioanalytical platforms—including ELISA, MSD, and multiplex ligand binding technologies—he plays a key role in designing and executing regulated assays under GLP and GcLP environments. Douglas is also actively involved in establishing internal GcLP capabilities and advancing method development aligned with Moderna's mRNA therapeutic pipeline. He holds a Master of Science degree and brings deep scientific rigor to assay development, troubleshooting, and strategic program support.

Martin Dowty, PhD, AbbVie: Dr Dowty received his Ph.D. from the University of Wisconsin-Madison in pharmaceutical sciences and completed post-doctoral studies at the Waismen Center Research Institute. Martin has over 30 years of pharmaceutical industry experience (P&G, Pfizer, and most recently AbbVie) and over 70 publications in the areas of drug discovery and development, small and large molecules, translational sciences, ADME, active metabolites, reversible and covalent inhibitors, DDI, endogenous biomarkers, MIST characterization, and PBPK modeling. Martin is currently a

scientific director in the department of development biological sciences at AbbVie representing nonclinical safety and DMPK on both small molecule and biologics project teams.

Krisna Duong-Ly, PhD, Merck: Dr. Duong-Ly is currently a Principal Scientist at Merck in the Regulated Bioanalytics group. In this role, she supports critical reagent generation and characterization in support of pharmacokinetic and immunogenicity assay development. In addition, she has served as a bioanalytical development lead for biologics programs in the preclinical and clinical space. Prior to joining Merck, Dr. Duong-Ly developed extensive experience in the fields of protein biochemistry and assay development. She received a Ph.D. in Molecular Biophysics from Johns Hopkins School of Medicine and completed a postdoctoral fellowship at the Fox Chase Cancer Center. Dr. Duong-Ly then transitioned to supporting early discovery programs at Janssen R&D where she conducted biochemical, cell-based, and biophysical characterization assays.

Padmanabhan (Paddy) Eangoor, PhD, Sanofi: Dr. Eangoor is a Senior Principal Scientist in the Translational Medicine Unit at Sanofi. In this role, he leads a team of scientists focused on designing and executing non-clinical bioanalytical strategies to support drug discovery and development.

Dr. Eangoor brings extensive industry experience, having held roles of increasing responsibility at Merck, Moderna, and Mirai Bio. A recognized leader in bioanalysis, he is deeply committed to advancing therapeutic development through strategic, efficient, and practical bioanalytical solutions. His expertise spans multiple therapeutic areas and diverse modalities, establishing him as a subject matter expert in the field.

He earned his Ph.D. in Pharmaceutical Sciences from Mercer University and holds an Executive Certificate in Management and Leadership from the MIT Sloan School of Management.

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).

Jan Elias, PhD, Boehringer Ingelheim: Dr. Elias is a Principal Scientist at Boehringer Ingelheim, specializing in PK/PD modeling within the NCE research division in Vienna, Austria. His work focuses on developing and applying quantitative modeling approaches for novel therapeutic modalities in oncology, as well as other therapeutic areas, including degraders such as PROTACs and molecular glues. He holds a PhD in Applied Mathematics from Pierre and Marie Curie University in Paris, France. Before joining Boehringer Ingelheim in 2021, he worked in academia, gaining experience in applying mathematical principles to address complex biological problems.

Yunlin Fu, Novartis: Yunlin Fu is a Senior Principal Scientist of Bioanalytics in the Department of Pharmacokinetic Sciences (PKS) at Novartis Biomedical Research. She scientifically oversees the LC-MS bioanalysis and toxicokinetic assessment of drug candidates at the third parties. In her scientific capacity, Yunlin focuses on the evaluation and implementation of new technologies in LC-MS bioanalysis of new modalities, including small/large molecule conjugates, radioligand molecules and oligonucleotides/siRNAs. Yunlin received her MS degree from the Rutgers University, New Brunswick, NJ, USA. She has published 20 research articles and made more than 10 presentations (oral and poster) in the scientific workshops or conferences. She is the co-editor of Wiley book on Sample Preparation in LC-MS Bioanalysis (John Wiley and Sons, 2019).

Cody Fullenwider, Vividion Therapeutics: Cody Fullenwider has been a DMPK scientist leading cutting edge advancements in the field of drug discovery and development for the past 20 years across multiple organizations, including Boehringer Ingelheim, AbbVie (Pharmacocyclics), Takeda and most recently Vividion Therapeutics (Bayer). Cody is considered an expert in CYP inhibition, induction and transport. He has published multiple papers highlighting novel or cutting-edge methods to assess time dependent inhibition and CYP induction. His work on cytochrome P450 time dependent inhibition has been cited within the M12 Drug Interaction Studies Guidelines. Cody currently leads the In Vitro DMPK team at Vividion. His current research focuses on optimizing ADME screening cascades for covalent compounds and building a greater understanding of Glutathione-S-transferases and their impact on covalent drug discovery and development.

Jason Hamilton, PhD, Worldwide Clinical Trials: Dr. Hamilton, Associate Director, Method Validation at Worldwide Clinical Trials, has nearly 20 years of laboratory experience spanning clinical, research & development, and analytical testing laboratories, along with five years of experience managing laboratory teams.

Maria Jadhav, PhD, Novartis: Dr. Jadhav is regulated bioanalysis and biomarker development expert with over a decade of experience spanning discovery through late-stage development. She currently serves as Director and leads the GCP Laboratory Facility at Novartis, BioMedical Research in Cambridge, MA.

She holds a PhD Swiss Federal Institute of Technology (ETH) Zürich, and is contributor to scientific publications and regulatory documentation.

Her career spans matrix and team leadership roles at Novartis, Roche and biotech and includes oversight of key regulatory bioanalytical deliverables, immunogenicity, and translational biomarker integration. Dr. Jadhav has also represented Novartis in industry consortia such as the European Immunogenicity Platform (EIP) and the European Bioanalysis Forum (EBF) and American Associations of Pharmaceutical Sciences (AAPS), contributing to cross-industry standards in immunogenicity and bioanalysis.

Gary Jenkins, PhD, AbbVie: Dr. Jenkins has spent most of his career with Abbott/AbbVie, serving in diverse scientific and managerial roles within the Quantitation, Translational, and ADME Sciences (QTAS) department (formerly DMPK-BA). Prior to returning to school, he was an associate at TSRL/PORT Systems and a Mass Spectrometrist/GLP Quality Control analyst at Parke-Davis/Pfizer in Ann Arbor. Dr. Jenkins earned his PhD in Pharmacology from the University of Michigan Medical School, studying neuronal Nitric Oxide Synthase and cellular triage signals, including investigation of proteasomal degradation mechanisms. Joining Abbott/AbbVie in 2006, he led the automation and stability assay team, spearheaded Electronic Laboratory Notebook implementation, and provided support from Drug-Drug Interaction (DDI) and enzymology to Bioanalysis (BA) for in vitro and in vivo preclinical studies.

Dr. Jenkins was a DMPK-BA representative on multiple pre-clinical and clinical programs, including Venetoclax, contributing to numerous IND filings. He expanded his team's capabilities to include cellular characterization and large molecule quantitation using Mass Spectrometry, and leadership of project representatives for AbbVie's late-stage oncology portfolio. Today, Gary leads teams in Drug Metabolism and Disposition (including MS imaging), non-regulated BA, In Vitro ADME Sciences, Biologics Bioanalysis, Characterization and Disposition (Endogenous and Therapeutic, LC-MS based), and Computational ADME/data sciences (AI/ML). He has co-authored >50 articles on a wide range of scientific topics and is an active participant in multiple industry and scientific consortia.

Lyn Jones, PhD, Harvard University: Dr. Jones completed his PhD studies in synthetic organic chemistry at the University of Nottingham, and his postdoctoral research at The Scripps Research Institute, California in chemical biology. He joined Pfizer Sandwich (UK) as a medicinal chemistry team leader, eventually becoming Head of Chemical Biology and Lead Discovery Technologies. He transferred to Pfizer Cambridge (USA) to become Head of Rare Disease Chemistry, and Head of Chemical Biology. He then helped establish Jnana Therapeutics as Head of Chemistry and Chemical Biology, before moving to Dana-Farber Cancer Institute in Boston as Chief Scientist of the Center for Protein Degradation. He is currently Principal Investigator and Faculty Member of the Chemical Biology Program at DFCI, and his lab focusses on the development of next-generation covalent chemical biology with the objective of expanding the druggable proteome.

Sean Y. Kassim, PhD, FDA: Dr. Kassim is the director of the Office of Study Integrity and Surveillance (OSIS) in the Office of Translational Sciences (OTS) in FDA's Center for Drugs (CDER). OSIS oversees bioavailability and bioequivalence (BA/BE) and Good Laboratory Practice (GLP) studies in support of pharmaceutical development, as part of FDA's Bioresearch Monitoring (BIMO) program.

Previously, Sean served as the director of the Office of Scientific Investigations (OSI), in CDER's Office of Compliance, overseeing compliance programs and enforcement for pharmaceutical BIMO (GCP, IRB) and post-market reporting (PADE, REMS, PMR) activities. He started at FDA as a reviewer for the BE/GLP compliance program in OSI's predecessor, the Division of Scientific Investigations.

Before coming to FDA, Sean worked at the University of Washington in Seattle. Sean received his doctorate from Washington University in St. Louis and his undergraduate degree from the University of Maryland Baltimore County.

Lindsay King, PhD, Pfizer: Dr. King is currently Executive Director and Head of Clinical and Translational Biomarkers at Pfizer. He leads a team responsible for the end-to-end clinical translational biomarker and assay strategies as well as execution for Internal medicine, Inflammation and Immunology, and Anti-Infective clinical programs. He has held multiple roles at Pfizer over the last 17 years in clinical and preclinical departments including in GLP Bioanalytics, as ADME department project representative for Antibacterials, Neuroscience and Oncology BioTx programs, as Biotherapeutics PK and immunogenicity Lab head focused on ADCs, as well as developing novel flow/imaging cytometry biomeasures methods to support Modeling and Simulation for candidate selection and first in human dose predictions. He has served as chair of the Ligand Binding Assay Bioanalytical Focus Group within American Association of Pharmaceutical Scientists and continues to be active in this and other external scientific communities. Lindsay received his Ph.D. in Zoology from the University of Toronto, Canada.

Emilee Knowlton, PhD, Prolmmune, Inc.: Dr. Knowlton gained her PhD in Infectious Diseases and Microbiology from the University of Pittsburgh under the direction of Prof. Charles Rinaldo, identifying immune responses to lytic infection with Human Herpes Virus-8 and the role of cytokines in the pathogenesis of Kaposi's Sarcoma. She joined Prolmmune in 2013 after completing her Post Doc in Rinaldo's lab. She works on the Prolmmune team providing innovative solutions for clients that radically improve our understanding of both desired and unwanted immune responses.

Rosemary Lawrence-Henderson, Pfizer: Rosemary Lawrence-Henderson is a Principal Scientist in the Pharmacokinetics, Dynamics and Metabolism group at Pfizer inc. She holds a Bachelor of Science in Clinical Laboratory Science-Medical Technology and spent her earlier career as a Senior Medical Technologist in the Clinical Immunology-Molecular Diagnostic laboratory of Lahey Clinic Medical Center. She has 18 years' experience in the Biopharmaceutical industry in both regulated and non-regulated large molecule bioanalysis. Currently she leads the Critical Reagent group at Pfizer which is responsible for antibody generation to large molecules and peptides, scientific oversight of CRO's, protein purification and modification using innovative coupling chemistries and strategies, a multitude of characterization techniques, along with lifecycle management of critical reagents in a centralized inventory.

Amy Hao Li, PhD, Genentech: Dr. Li is a Principal Scientist in the BioAnalytical Sciences department at Genentech. She earned her Ph.D. in Chemical and Systems Biology from Stanford University and her B.Sc. with Honors in Biochemistry from the University of Wisconsin-Madison. At Genentech, she is part of the Immunoassay team, focusing on developing innovative bioanalytical strategies and methods to support the progression of novel biotherapeutics from preclinical research through clinical application. Her expertise encompasses bioanalytical assay development, enzyme biochemistry, and drug discovery for both small and large molecules. Her recent work on the plug-and-play anti-drug antibody assays on the Gyrolab immunoassay platform has resulted in significant streamlining of preclinical immunogenicity studies.

Wenkui Li, PhD, Novartis: Dr. Li serves as Director of Bioanalytics in the Department of Pharmacokinetic Sciences (PKS) at Novartis Biomedical Research (NBR) in East Hanover, NJ, USA. His expertise encompasses LC-MS bioanalysis for toxicokinetic and pharmacokinetic evaluations of drug candidates and their metabolites, supporting both preclinical and clinical drug development at Novartis. Dr. Li completed his doctorate at Peking Union Medical College and the Chinese Academy of Medical Science in Beijing, China. He has published over 130 research articles and delivered more than 60 presentations at scientific workshops, symposia, and conferences. Dr. Li is also the lead editor of three books with John Wiley & Sons: (1) Sample Preparation in LC-MS Bioanalysis (2019), (2) Dried Blood Spots: Applications and Techniques (2014), and (3) Handbook of LC-MS Bioanalysis: Best Practices, Experimental Protocols and Regulations (2013). He was co-chair of the AAPS Bioanalytical Community from 2019 to 2020 and the IQ Metabolite Bioanalysis Working Group from 2020 to 2024. He is currently serving as co-chair of the IQ Bioanalysis of Drug Conjugates Working Group (2024-present).

Andreas Luippold, PhD, Boehringer Ingelheim: Dr. Luippold studied chemistry with focus on analytical chemistry and biochemistry at Reutlingen University and holds a PhD from Technical University of Berlin. In 1996 he joined Boehringer Ingelheim's Department of Pharmacokinetics and Metabolism.

In his current position he is Director of Discovery Sciences Technologies, a research group supporting Boehringer Ingelheim's global research with

many platform technologies for histopathology, AAVs, Mass Spectrometry based biomarker discovery and quantitation, Metabolite Identification and Bioanalysis.

His passion is driving innovation by means of cutting-edge science and technology. The implementation of his innovative approaches have been presented at several ASMS, SLAS and early APA conferences.

He is member of the American Society for Mass Spectrometry (ASMS) and SLAS where he was a member of the scientific committee for several years.

Aimee Mattei, EpiVax: Aimee Mattei is the Director of Immunoinformatics at EpiVax. Her team provides in silico immunogenicity risk assessments supporting research, collaborative, and commercial projects. She leads the development of novel in silico methods for assessing the immunogenic risk of biotherapeutics and their impurities. She holds an M.S. in Pharmaceutical Chemistry and has previous experience in the manufacturing of peptides supporting research programs from concept assessment through lead optimization in the endocrinology and oncology therapeutic areas.

Kaushik Mitra, PhD, Johnson & Johnson: Dr. Mitra is a Distinguished Scientist at Innovative Medicine, Johnson & Johnson (JnJ) and 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. His interest lies in the preclinical optimization of drug candidates via early optimization of ADME properties and derisking of potential adverse effects through computation tools and mechanistic interventions. During his career in the pharmaceutical industry, Kaushik has supported the advancement of multiple compounds of different modalities to the clinic across various therapeutic areas. 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. 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.

Mark Niosi, Pfizer: Mark Niosi is a distinguished scientist with over 24 years of expertise in drug metabolism and pharmacokinetics (3yr at Purdue Pharma, and +21yrs at Pfizer). Studied Toxicology at Saint John's University and graduate studies in Forensic Toxicology. He has been deeply engaged in the drug discovery and development efforts for degraders at Pfizer, spanning from early concept to late-stage clinical aspects. He contributes to troubleshooting and profiling ADME properties and performing DDI risk assessments across a broad spectrum of projects; from small molecules and those beyond Ro5, with a pioneering focus on PROTACs.

He has been at the forefront of innovation, integrating new technologies like LC/MS platforms, ex vivo absorption techniques, GastroPlus PBPK modeling, ACD simulations, and inhouse insilico modeling - pushing the boundaries in discovery and development phases in research. Mark also serves as a lab head, and mentors our next generation of scientists, driving the development and optimization of enzymology in vitro and in vivo assays. As a recognized leader in the field, a frequent collaborator on scientific publications and has been invited to speak at various conferences. He is passionate to advance the field of pharmaceutical sciences, while fostering collaboration and nurturing the future of medicine.

When he's not hard at work finding a cure, he enjoys spending time with his family and experiencing the natural wonders of national parks have to offer across the country.

Stephanie Pisas-Farmer, PhD, BioData Solutions: Dr. Pisas-Farmer is a bioanalytical expert with over 20 years of experience in pharmaceutical, biologics, and hybrid technologies. She founded BioData Solutions® in 2015 to support bioanalytical drug development through specialized consulting and software offerings. Previously, she established. Stephanie has advanced early-stage molecules into potential new drug candidates with bioanalysis support and regulatory compliance planning as well as led teams in global bioanalytical labs, focusing on large molecule and antibody-drug conjugate programs. She holds a PhD and MSc in pharmaceutical chemistry from the University of Kansas and a BS in chemistry from St. Mary's College of Notre Dame.

Chandra Prakash, PhD, Agios: Dr. Prakash is a Principal Research Fellow in DMPK/Clinical Pharmacology department at Agios, Cambridge, MA. He obtained his Ph.D. in synthetic organic chemistry. He held several academic appointments prior to joining the pharmaceutical industry. He worked

for sixteen years at Pfizer and seven years at Biogen. He joined Agios in 2015.

For the last >30 years, Dr. Prakash has been involved in the drug metabolism and clinical pharmacology studies to support drug discovery, development, and registration. His research is primarily focused on the Design and conduct Clinical pharmacology (FIH, SAD, MAD, Food Effect, Bioavailability and DDI) studies in humans and on the development and utilization of novel approaches and techniques which include in vitro methods using human and animal hepatic cellular and subcellular systems, recombinant human drug metabolizing enzymes, sensitive analytical technologies and in silico computational models to assess the metabolism, drug-drug interactions and toxicological aspects of the new chemical entities. He is the author of more than 290 manuscripts, book chapters, presentations, and patents. He also coedited five volumes of Handbook of Metabolic Pathways of Xenobiotics. He served as the editor-in-chief of the Journals "Current Drug Metabolism" and "Drug Metabolism Letters" and editorial board member of several journals. He is also chair of Boston chapter of PBSS international. He organized/chaired several national and international scientific meetings/workshops related to drug metabolism, clinical pharmacology and toxicity. He served as the chair of ISSX publication committee and as a member of ISSX financial committee.

Brad Roadcap, Merck: With over 26 years of dedicated service at Merck, Brad has established himself as a leader in the field of pharmacokinetics. Currently, he serves as the Director of Pharmacokinetics within the Department of Regulated Bioanalytics, where he leads one of the bioanalytical groups focused on therapeutic regulated bioanalytical support.

Brad's career at Merck began at the bench level, where he honed his technical skills and knowledge in bioanalytical chemistry. Through hard work and commitment, he progressed through various roles, ultimately reaching his current leadership position.

For the past eight years, Brad has been the development lead for the clesrovimab program, overseeing critical aspects of its bioanalytical development and ensuring the program's success in clinical trials.

Matthew Stone, PhD, Agios: Dr. Stone currently is a Sr. Advanced Workflow Specialist at SCIEX focusing on business development and collaborations within life sciences and pharma-related research. He received a BA in chemistry at Carleton College in Northfield, MN and later earned a Ph.D. in biochemistry at the University of Minnesota where he developed protein derivatives of factor VIIa with enhanced activity as potential enzyme replacement therapies. He did post-doctoral research at the National Institute on Aging and the University of Minnesota Center for Mass Spectrometry and Proteomics core facility focusing on mass spectrometry-based functional proteomics and participating in various collaborative proteomics-based research projects.

Tatjana Talamantes, PhD, Sciex: Dr. Talamantes, is a Senior Product Manager at SCIEX, where she leads strategy and development for high-resolution accurate mass spectrometry platforms, including the ZenoTOF 8600 system. With a strong background in biomedical sciences and over a decade of experience in LC-MS innovation, Tatjana has worked across R&D, commercialization, and customer engagement at companies like MOBILion Systems and Thermo Fisher Scientific. She's passionate about translating complex analytical challenges into practical solutions that support pharmaceutical research and development. Tatjana enjoys collaborating with scientists and industry partners to advance technologies that improve drug discovery, development, and regulatory workflows.

Kiyoto Aramis Tanemura, BMS: Kiyoto Aramis Tanemura is a Research Investigator in the Data Science group within the Quantitative Pharmacology and Data Analytics organization of Bristol Myers Squibb. He initially joined the company to develop a quantitative systems pharmacology (QSP) cardiovascular platform model and is now building the machine learning capability through method development within the organization. He is also the author of "Python for Chemists" (American Chemical Society) which aims to help chemists leverage computational and algorithmic methods through Python in their research.

Afsana Trini, PhD, BMS: Dr. Trini is a Principal Scientist in Precision Medicine, Bioanalytical & Translational Sciences (PMBATS) at Bristol Myers Squibb (BMS). She earned her Ph.D. in Pharmaceutics and Drug Design from Saint Joseph University in 2019 and holds an M.Pharm in Clinical Pharmacology from the University of Dhaka.

At BMS, Dr. Trini serves as the bioanalytical lead for biotherapeutics across preclinical and clinical development, supporting DMPK (Drug Metabolism and Pharmacokinetics) and immunogenicity functions. She supported bioanalytical efforts for multi-domain antibody therapeutics, antibody-drug conjugates (ADCs), and cell therapy.

Prior to joining BMS, Dr. Trini was a Senior Scientist at PPD Inc., where she served as Principal Investigator for multiple bioanalytical GLP and non-GLP studies.

Dr. Trini is an active member of the American Association of Pharmaceutical Scientists (AAPS) and currently serves as co-lead of the Early Career Bioanalytical Scientist (ECBS) community at AAPS in 2024.

Paige Vinson, PhD, Southern Research: Dr. Vinson leads the High-Throughput Screening Center at Southern Research, where her team uses advanced robotic equipment, cell-based assays, and biochemical assays to screen hundreds of thousands of compounds for potential treatments against viruses, bacterial infections, and diseases such as diabetes, cancer, and cystic fibrosis. Her expertise supports both hit identification and downstream drug discovery efforts.

Previously, Paige held leadership roles in laboratory automation at Thermo Fisher Scientific and spent twelve years at Vanderbilt University's Warren Center for Neuroscience Drug Discovery, directing HTS and contributing to molecular pharmacology projects. She has collaborated extensively with academic and biopharmaceutical partners, with a focus on GPCR targets in CNS disorders. Paige earned her bachelor's in chemistry from the University of South Alabama, a Ph.D. in analytical chemistry from Emory University, and completed postdoctoral training there studying monoamine oxidases A and B.

Matt Wagoner, PhD, Takeda: Dr. Wagoner leads the Global Investigatory Toxicology team at Takeda Pharmaceuticals, where their team applies complex in vitro models and in silico approaches to help make safer, and more effective, medicines. Before Takeda, Matt led the mRNA safety strategy for AstraZeneca Pharmaceuticals, and worked to develop and deploy in vitro and in vivo models in support of oncology and cardiovascular drug discovery projects.

Matt collaborates with NASA to help solve the human health challenges facing astronauts in space, and is a founding member of Space Biotech Connect, and has served on the Translational Research in Space Health Institute Scientific Advisory Board.

In the academic arena, Matt co-taught a drug discovery course at Simmons College in Boston. He received his PhD in Molecular and Cellular Pharmacology from the University of Wisconsin-Madison and bachelors in biochemistry from the University of Illinois Urbana Champaign.

In lieu of hobbies, Matt has four kids that love to go on outdoor adventures.

Benjamin Wei, PhD, BMS: Dr. Wei is the Vice President of DMPK & Bioanalysis at Medicilon USA Corp in Lexington, MA. With over 20 years of leadership experience in bioanalysis, pharmacokinetics/pharmacodynamics, toxicology, and translational research, he has worked across CROs, biotech companies, and large pharmaceutical organizations. Most recently, he served as the CEO and Co-founder of Bluebrim Bioscience and has held senior positions at JOINN Laboratories, Biomere, Hopewell Therapeutics, and Pfizer. Dr. Wei earned his PhD from Peking Union Medical College in Beijing, China, and completed his postdoctoral training in the Department of Biochemistry at Purdue University.

Jinsong Yang, PhD, Keros Therapeutics: Dr. Yang is Director of Bioanalytical at Keros Therapeutics, where he leads a team of scientists and oversees the assay development, validation and sample analysis for PK, ADA and biomarker on LBA and LC-MS/MS platforms across Phase 1-3 studies. He has more than a decade of experience in regulated bioanalytical sciences across therapeutic modalities, including antibodies, proteins, mRNA, and siRNA, along with leadership in CRO partnerships and critical reagent management, and service as a bioanalytical SME for regulatory submissions including INDs, EOP2 and NDA support. Previously, he held Bioanalytical roles at Dicerna, Moderna, and Shire. Jinsong earned his Ph.D. in Biochemistry & Molecular Biology from Shanghai Second Medical University and completed post-doctoral training at UT Southwestern Medical Center in Dallas.

Brittany Youngs, Stoke Therapeutics: Brittany Youngs is currently a Senior Scientist in the Translational DMPK and Clinical Pharmacology department at Stoke Therapeutics, focused on immunogenicity and bioanalytical methods to determine PK, PD, and to detect anti-drug antibodies to support development of oligonucleotide therapeutics for rare diseases. Prior to joining Stoke, Brittany was at Affinivax working in the Analytical Development department focusing on LBAs for immunogenicity to support vaccine development. Brittany has a combined 9 years of experience in developing ligand binding assays to support preclinical and clinical vaccine development and development of oligonucleotide therapeutics. She received her Master's degree in Molecular Biology and Biotechnology from Clarkson University.

Jesse Yu, Relay Therapeutics: Jesse is a principal scientist at Relay Therapeutics. His primary roles at Relay are to serve as a project representative in DMPK and a PBPK modeler in Clinical Pharmacology. Prior to his time at Relay, Jesse was a Senior Scientist at Genentech also working as a DMPK representative/PBPK modeler. Jesse has a particular interest in understanding the translation of in vitro ADME data through a PBPK framework.

Qinying Yu, PhD, Genentech: Dr. Yu obtained her Ph.D. from the University of Wisconsin-Madison. Since joining Genentech in October 2021, she focused on developing and optimizing ADME assays for new modalities, improving efficiency and capacity. Qinying utilized proteomics strategies to quantify drug metabolizing enzymes, offering valuable insights beyond traditional readouts with potential to impact DDI prediction. Furthermore, her research into reactive drug metabolites has provided mechanistic insights into DILI by identifying mitochondrial protein targets.

Xiaobin Zhang, PhD, Takeda: Dr. Zhang is an associate director in the Translational Bioanalysis and Biomarkers group at Takeda Pharmaceuticals, where he primarily supports the immunogenicity assessment of large molecular therapeutics. His expertise encompassed various modalities, including peptide, antibody, antibody-drug conjugates, multidomain proteins, and oligonucleotide-based therapeutics for oncology, neuroscience, and gastrointestinal disease. Prior to joining Takeda, Xiaobin held positions as staff scientist and lead scientist at Covance, where he focused on the method development and validation of PK, ADA, and NAB assays. Additionally, he served as a senior scientist responsible for the cell-based bioassay method development and validation in the GMP lab at PPD. Dr. Zhang earned his Ph.D. from Peking University Health Science Center and completed postdoctoral training at University of Nebraska Medical Center and University of Wisconsin-Madison.

Yipei Zhang, PhD, Takeda: Dr. Zhang, Scientist II - Senior Scientist at Takeda Pharmaceuticals since 2022, working within the Bioanalytical Science & Immunogenicity group in the DMPK&M department. Previously, served as a Scientist - Senior Scientist at Kala Pharmaceuticals from 2018 to 2022. Holds a Ph.D. in Analytical Chemistry from the University of Massachusetts Lowell in 2018. At Takeda, specializes in developing LC/MS-based bioanalytical assays to characterize small and large molecule drugs in biological matrices to support PK and TK studies.

POSTER ABSTRACTS

Advanced LC-MS/MS Assays for the Quantitation of Novel Therapeutic Oligonucleotides in Human Plasma: Case Study

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PURPOSE

BB-031 is a first-in-class RNA aptamer designed to target von Willebrand Factor (vWF), a key structural component in the blood clotting cascade involved in thrombus formation, stabilization, and expansion. Preclinical studies have demonstrated that BB-031 outperforms recombinant tissue plasminogen activator (rTPA) in ischemic stroke models by reopening blocked arteries with enhanced safety and reduced bleeding risks. Importantly, BB-031's thrombolytic activity can be neutralized by BB-025, which binds to BB-031 to form a stable complex. Accurate quantification of BB-031, BB-025, and the BB-031/BB-025 complex is essential for understanding their pharmacodynamics and therapeutic potential. However, analytical challenges include efficient extraction, chromatographic separation of all three oligonucleotides, and ensuring specificity and stability during analysis.

This study aimed to develop high-throughput, selective, and robust LC-MS/MS assays to quantify BB-031, BB-025, and the BB-031/BB-025 complex in human plasma.

METHODS

Two methods (Method One and Method Two) have been developed for the analysis of BB-031, BB-025, and the BB-031/BB-025 complex in human plasma.

Method One: Quantification of BB-031 and BB-025

An LC-MS/MS method was developed using solid-phase extraction (SPE) with a mixture of ammonium hydroxide, ammonium bicarbonate, tetrahydrofuran, acetonitrile (MeCN), and water as the elution solvent. The eluted sample was dried under nitrogen and reconstituted in a solution containing hexafluoroisopropanol (HFIP), diisopropylethylamine (DIPEA), EDTA, and water.

Samples were injected into a Thermo DNAPac RP column, with mobile phases composed of HFIP, DIPEA, EDTA, MeCN, and water. Detection was performed using a SCIEX Triple Quad 6500+ MS/MS system in negative electrospray ionization mode.

Method Two: Quantification of BB-031/BB-025 Complex via Immunocapture

Human plasma samples (20 μ L) were processed using immunocapture probes targeting either BB-025 or BB-031. Two workflows were evaluated:

- BB-025-Targeting Probes (PNA/DNA): Designed to remove free BB-025, followed by quantification of the remaining complex. These probes showed poor performance in removing free BB-025, leading to overestimation of complex levels.
- BB-031-Targeting Probe (DNA-Based): Successfully captured the BB-031/BB-025 complex and enabled direct quantification of BB-025 during the immunocapture step.

Samples were processed using streptavidin-coated magnetic beads on a KingFisher automation system. After multiple wash steps, the complex was eluted by heating to 90 °C for 10 minutes. Eluates were analyzed using a SCIEX Triple Quad 6500+ mass spectrometer coupled with a Shimadzu HPLC system, achieving a 5-minute runtime per sample.

RESULTS

Method One:

Early development faced challenges including non-specific binding (NSB), internal standard selection, and short column lifespan. A standardized Method Development Protocol (MDP) with nine troubleshooting steps was applied to address issues such as NSB, low recovery, solubility, and stability.

- NSB Mitigation: BB-025 and BB-031 are highly hydrophilic and prone to NSB. Additives like Triton X-100, CHAPS, EDTA, and NaCl were evaluated; saline was most effective.
- IS Selection: A synthesized SIL-IS (BB-031-d27) showed impurities and interference. Screening 10 candidates from an oligonucleotide IS library led to the selection of a suitable IS with comparable retention and chromatographic properties.
- Column Optimization: A third pump was added for column backflushing, significantly extending column life and maintaining performance over thousands of injections.
- SPE Optimization: A water/MeCN (60/40, v/v) elution solvent effectively removed salts and interferences.
- Extraction Efficiency: Centrifugation replaced traditional positive pressure manifolds, reducing extraction time to ~30 minutes.

After thorough method development, the method was validated over a concentration range of 50.0 to 25,000 ng/mL, with a dilution quality control (DQC) set at 300,000 ng/mL. Inter-assay accuracy ranged from -2.7% to 7.5%, and inter-assay precision ranged from 5.4% to 10.9%. Specificity, selectivity, matrix effects, and stability were evaluated according to M10 guidance and met the recommended criteria.

Method Two:

- Probe Evaluation: Three probes were tested: two targeting BB-025 (PNA and DNA) and one targeting BB-031 (DNA). Only the BB-031 targeting probe effectively captured the BB-025/BB-031 complex. Probes designed to target free BB-025, whether PNA or DNA, struggled to capture free BB-025, resulting in inadequate removal and overestimation of complex concentrations.
- Binding Condition: Systematic evaluation of incubation conditions revealed their critical role in probe-complex binding and the stability of the BB-031/BB-025 complex. The BB-031 targeting probe demonstrated the best performance and was chosen for the method. Initial poor binding was attributed to secondary structures formed by the molecules. Increasing the incubation temperature significantly enhanced binding efficiency and enabled linear calibration curves. An optimal temperature of 50 °C was identified, ensuring efficient binding while maintaining complex stability. Additionally, buffer salt content, detergent concentration, and pH were systematically evaluated to achieve robust and specific binding.
- Excess BB-025 in Plasma: BB-025 in plasma samples posed a challenge by potentially nonspecifically interacting with the complex during extraction. To address this, wash conditions were systematically optimized by testing high salt concentrations, varying pH levels, detergent amounts, temperature, and buffer types to effectively remove excess BB-025 and minimize nonspecific binding. Furthermore, blocking oligos were added during the sample extraction to further reduce nonspecific binding.
- Specificity and Accuracy: The final assay using the BB-031-targeting probe accurately quantified BB-025 in the presence of up to 100 µg/mL excess BB-025 in plasma, successfully passing the specificity tests. The method demonstrated a quantification range from 50.0 to 25,000 ng/mL, making it suitable for pharmacokinetic and toxicology studies. The accuracy and precision of the assay were confirmed in multiple runs, demonstrating its robustness for quantifying the BB-031/BB-025 complex in human plasma.

CONCLUSIONS

We successfully developed and validated two robust LC-MS/MS assays for quantifying BB-031, BB-025, and their complex in human plasma. Method One addressed challenges in NSB, recovery, and column longevity, while Method Two introduced a novel immunocapture approach to selectively quantify the BB-031/BB-025 complex.

These are the first published assays for BB-031, BB-025 and BB-031/BB-025 complex in human plasma. The immunocapture LC-MS/MS method represents a significant advancement in RNA therapeutic quantification, offering high specificity, sensitivity, and throughput. These assays provide essential tools to support the clinical development of BB-031, a promising thrombolytic therapy with superior safety and efficacy compared to current standards.

Development of an HRMS-HILIC Based Bioanalytical Platform for Antisense Oligonucleotide Metabolite Identification in Human Plasma

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PURPOSE

To develop a HRMS-HILIC based bioanalytical platform for monitoring metabolic stability and identifying metabolites of antisense oligonucleotides (ASO) in human plasma. ASO are short synthetic nucleotide sequences designed to target specific genes and regulate their expression through mechanisms such as gene silencing or splicing. A major analytical challenge in ASO metabolism studies is the formation of shorter oligonucleotide fragments (shortmers), which may retain biological activity and therefore require careful characterization.

METHODS

Fomivirsen and nusinersen were selected to develop and optimize a HILIC-based bioanalytical method in human plasma using the Sciex ZenoTOF 7600 mass spectrometer. Separation of ASO and their metabolites was achieved on an ACQUITY Premier BEH Amide Column (1.7 μ m, 2.1 \times 50 mm). Mass spectrometry data were processed with Biological Explorer 7.0 using the Nucleotide Intact Mapping template. Metabolic stability and metabolite formation were evaluated in vitro by incubation with human plasma at 37 °C for up to 24 hours. SPE was employed for metabolite recovery, and metabolite identification was confirmed using Biological Explorer 7.0.

RESULTS

The optimized method provided baseline separation of fomivirsen, nusinersen, and their metabolites in human plasma. SPE enabled efficient extraction of metabolites, which were subsequently identified through deconvolution of the mass spectrometry data corresponding to individual peaks.

CONCLUSION

This HRMS-HILIC bioanalytical platform allowed reliable assessment of the metabolic stability and metabolite profiles of fomivirsen and nusinersen in human plasma. The chromatographic method was optimized to improve baseline separation and peak shapes. At least two shortmer metabolites were identified for each ASO using the Biological Explorer 7.0 software, highlighting the platform's utility for MetID in ASO bioanalysis.

Quantitation of Succinic Acid and Alpha-Ketoglutarate in biological samples on a high-resolution mass spectrometer

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PURPOSE

Small organic acids are of paramount importance as biomarkers for various biological processes. Due to their small size and high hydrophilicity detection via traditional reverse-phase LC-MS/MS methods is difficult. To overcome the inherent challenges with these molecules a derivatization using 4-bromo-N-methylbenzylamine (4-BNMA) with EDC was used to increase the hydrophobicity of small organic acids. In this study we present a fully qualified non-GLP method for the quantification of succinic acid (SA) and Alpha-Ketoglutarate (aKG) across in mouse plasma on a high resolution mass spectrometer.

METHODS

SA and aKG were extracted from biological and surrogate matrices using protein precipitation with acetonitrile. Standards, batch acceptance, and blanks were prepared in the surrogate matrix of 2% BSA and subjected to the same extraction procedure. The supernatant was subjected to an EDC coupling to 4-BNMA at 60 °C for approximately 1 hour. The post derivatization extract dried down under a stream of nitrogen and reconstituted in 50:50 (v:v) water:acetonitrile. The reconstituted extracts were injected into a ZenoTOF 7600 system with a Shimadzu Nexera 40 LC front controlled by SCIEX OS software. Data was integrated and processed in SCIEX OS software.

A ZENO MRMHR method was used to quantify both SA and aKG. The positive mode precursor ion was set to the exact mass observed for the derivatized analytes in a TOF-MS scan. The TOF start and stop masses were set to scan from 80 to 200 da to capture the BNMA 169 and 171 fragments.

RESULTS

For SA and aKG batch acceptance QCs and the standard curve in surrogate matrix were found to meet acceptance criteria of 20% Bias and ± 20 %CV. Additionally, qualification parameters of IS to analyte interference, analyte to analyte interference, carryover, linearity of dilution, among others, were assessed.

CONCLUSION

The method is qualified for HRMS-based quantitation of aKG and SA in mouse plasma.

A quantitative systems pharmacology (QSP) model for predicting the impact of repeated blood draws on hemoglobin levels in cynomolgus monkey

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PURPOSE

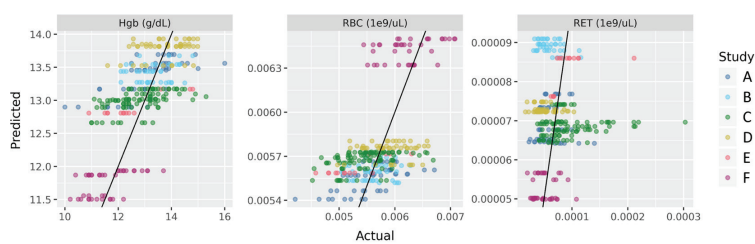
The effect of drug candidates on erythropoiesis is typically determined with a standard set of endpoints in repeat dose toxicity studies. These assessments include measurement of red blood cells (RBCs), reticulocytes (RETs) and hemoglobin (Hgb), among other parameters in blood. Reduced RBC and Hgb levels after test article administration can be an indication of drug-induced effects on erythropoiesis. However, erythropoiesis is a tightly controlled process which is sensitive to a variety of stimuli including blood loss. In addition to assessing the impact of drugs on animals, exposure to drugs is also determined typically by several blood collections clustered around the time of test article administration. These study-related activities have the potential to result in decreased red blood cell mass parameters causing uncertainty in whether the change in blood parameters is caused by the test article, the blood draws themselves, or a combination of both. A QSP model of erythropoiesis and its response to blood draws has been developed to aid in the differentiation of the possible causes for reduced red blood cell mass and to assist with designing blood draw schedules in cynomolgus monkey toxicity studies. At this point, the model is drug agnostic only simulating the effect of blood draws and does not include administration of any test articles.

METHODS

A QSP model for erythropoiesis in cynomolgus monkeys was developed that tracks the RBC maturation from long term hematopoietic stem cells in the bone marrow through RBCs in blood. In addition to the available erythroid proliferation parameters in human, hematology data from control animals in six different cynomolgus monkey repeat-dose GLP toxicity studies with varying blood draw schedules were utilized for estimating model parameters. Five cell types were specifically included in the model: colony forming unit (CFU), short term hematopoietic stem cells (STHSC), and long-term hematopoietic stem cells (LTHSC) in the bone marrow, as well as RBCs and reticulocytes (RET) in blood. Red blood cells and RET levels in the model are directly affected by blood draws, whereas CFU cells are indirectly affected by inhibitory feedback from RBCs and RETs. Inhibitory feedback was modeled through an exponential feedback term on the amplification of CFU cells. This feedback captures the RET rebound observed in experimental data, as well as CFU accumulation observed in literature.

RESULTS

The developed model was able to successfully capture hematology observations (Hgb, RBC, and RET counts) for control cynomolgus monkeys from the modeled toxicity studies:



Moreover, to assist with future study design, the model can predict the effect of different blood draw schedules on Hgb levels, as well as the length of time required for hematology parameters to return to baseline following a certain blood draw scheme.

CONCLUSION

A mathematical kinetic model of blood proliferation and maturation in cynomolgus monkey was developed to inform how to minimize the impact of blood draws on hematology parameters during toxicity studies. Model simulations quantitatively demonstrate the tradeoff between the number of blood draws per day, number of days blood samples are collected, and the volume of each draw. This model can assist with identifying feasible study designs for toxicity studies in cynomolgus monkeys that can accommodate the desired number of assays per day without causing confounding changes to the hematology parameters.

REFERENCE MATERIAL CONCEPT TO HARMONIZE IMMUNOASSAY DIFFERENCES

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PURPOSE

Many industry stakeholders, through direct one-on-one interviews and at a 20-participant roundtable held at USP in Rockville, MD, have identified a need for immunoassay reference materials that could be used to investigate lot-to-lot variations, understand supplier or platform differences, and help bridge datasets from interlaboratory studies. Participants generally agreed that the cases of highest need were assay control and alignment of lot-to-lot variability and multisite analyses. This study evaluates the feasibility of using a common reference standard to improve consistency of immunoassay results across different platforms and kits. While individual labs develop in-house reference material to support bridging across kits and lots, a well-characterized, publicly available reference material that could also support bridging across multiple platforms and laboratories is needed. This feasibility study shows how such a reference material could be applied, with results spanning three platforms and kits from eight vendors for six individual cytokines.

METHODS

Six widely used cytokine markers (IL-1 β , IL-2, IL-6, IL-10, IFN- γ , and TNF- α) were used in this proof-of-concept study. For each cytokine, we tested multiple immunoassay platforms and kits, including nine ELISA plates (kits from three vendors run in triplicate), 12 Luminex plates (multiplex kits from four vendors run in triplicate), and six MSD (Meso Scale Discovery) plates (multiplex kits from one vendor, kits run in triplicate at two separate locations). Each of the runs included the kit calibrant provided by the vendor and two external standards prepared by separately combining recombinant proteins for these six cytokine markers from two separate vendors. As unknowns, 12 plasma samples were spiked with either of the two external standards at various cytokine levels (in the low, mid, and high range of the assay kits). Each kit was tested according to manufacturer's instructions and data was analyzed using both platform specific software and either Belysa Immunoassay or SoftMax Pro software. We employed various approaches to compare the results:

1. Parallelism analysis between the kit and the external standard curves was performed in SoftMax Pro software. Demonstration of parallelism is a prerequisite for the successful use of external standards to harmonize results across a wide concentration range in different kits.
2. A single midrange spiked reference sample was used to adjust the values obtained from the kit calibration curves. This enabled the calculation of sample recovery percentage before and after external reference adjustment to investigate the variability across the kits.
3. Additional analyses, such as Bland-Altman and mountain plots, were also used to visually represent differences and examine the bias and sample distribution between these kits.

RESULTS

Key findings from this proof-of-concept study are:

1. Absolute concentration values between the vendors and platforms varied up to 10-fold compared to nominal assigned values of the recombinant proteins. However, this is not reflective of individual kit performance since both the intra- and inter-assay variations were within the kit specifications. Rather, the wide range reflects the value assignment of various recombinant protein preparations and purity of the calibrants, or immunoreactivity of antibodies within each kit.
2. By running a full standard curve of external reference material in the kits, we were able to determine degree of parallelism of these materials compared to kit calibrants, both visually and by comparing the B-parameter of the Hill's slope in the 4PL and 5PL equations. As an example of the six investigated cytokines, IL-2 B-parameter values across the ELISA and Luminex kits were within a narrow 10% range, confirming the visually observed parallelism.
3. Using an external reference sample, we were able to harmonize the nominal concentration values (recovery) close to the assigned recombinant protein value (range 70-130%) across the multiple vendors and platforms in this study. Every cytokine had a somewhat different range of noncorrected and corrected values depending on the methodology and kits. Directionally all six studied markers showed the positive effect of

external reference samples for value harmonization. Because results demonstrated good parallelism for both external standards against the kit calibrant, value correction was possible by using a single “mid-level” spiked sample (vs. requiring multiple external standards or the entire external standard curve) making this approach very practical. Improvement by using the external standard was clearly demonstrated through Bland-Altman pairwise analyses (for example ELISA vendor 1 vs. ELISA vendor 2). Mountain plots also showed the effect of external standards as a visual display of multiple vendors simultaneously.

Certain commercial equipment, instruments or materials may be identified in this presentation to specify adequately the experimental procedure. Such identification does not imply approval, endorsement, or certification by USP of a particular brand or product, nor does it imply that the equipment, instrument, or material is necessarily the best available for the purpose or that any other brand or product was judged to be unsatisfactory or inadequate.

CONCLUSION

This proof-of-concept study shows the use of external reference materials during immunoassay testing and assay development can correct even large differences in assigned concentration values observed between vendors and platforms and can be spiked into a relevant matrix for use as routine assay controls. When these materials are tested and show parallelism to kit standards, only a single concentration point is needed for accurate correction. Our future work will expand into more detailed study of endogenous samples, matrix effects, and expansion of this work beyond cytokine biomarkers.

Rapid and sensitive analytical method for amylin analogs in blood sugar regulation therapeutics

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PURPOSE

Pramlintide was approved by the FDA in 2005 for the treatment of type 1 and type 2 diabetes. The structure of pramlintide is comprised of a 37-amino acid amylin analogue based on the rat amylin sequence. Typically, pramlintide is applied as an adjunct to insulin therapy for diabetic patients who are using mealtime insulin. Amylin structures such as pramlintide display rapid absorption and are administered in low dosage, therefore require a highly sensitive analytical method to accurately quantify during the evaluation of efficacy and safety in humans.

METHODS

Pramlintide was spiked into 100 μ L of human serum at concentrations ranging from 10 to 100000 pg/mL. Protein precipitation was performed using 0.3 mL chilled 1% formic acid in methanol, followed by vortexing (5 min) and centrifugation (1204 rcf, 5 min). The supernatant was processed via Strata X-CW SPE, conditioned with methanol and 0.1% formic acid in water. After washing with water and 20% acetonitrile, elution was done using 100 μ L of 1% TFA in 80:20 methanol:water. Samples were transferred to autosampler vials for LC-MS/MS analysis. Chromatographic separation was achieved on an ExionLC AE system using a Luna Omega Polar PS C18 column (100 \times 2.1 mm, 1.6 μ m) at 0.6 mL/min, with mobile phases of 0.1% formic acid in water (A) and acetonitrile (B), at 75°C. Injection volume was 10 μ L.

RESULTS

A lower limit of quantitation (LLOQ) of 10 pg/mL was achieved using 100 μ L of human serum and a streamlined SPE method. The assay demonstrated a 6-minute run time with linearity across 10–100,000 pg/mL (4 orders of magnitude). Good quantitative performance was demonstrated with accurate and highly reproducible (%CV <11) results. An average recovery of 68.1% with a %CV <5.75 was demonstrated for pramlintide analysis in human serum.

CONCLUSIONS

A sensitive method for the quantitation of pramlintide in human serum is demonstrated. A LLOQ of 10 pg/mL (100 fg on column) was achieved with a fast runtime of 6 min.

Scalable In Vitro Methods for Accessing Major Human Metabolites of Structurally Complex Drugs using PolyCYPs and PolyUGT Enzymes

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PURPOSE

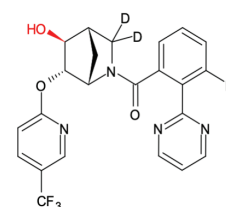
Scalable methods are needed for producing synthetically intractable major human metabolites of drugs in sufficient quantity for further biological testing, and for use as standards in bioanalytical studies. Ideally the method used should eliminate use of animal liver fractions on a larger scale but yet retain the ability to produce the target human metabolite(s).¹ This poster discusses the application of PolyCYPs and PolyUGT enzymes in making clinically relevant metabolites of drugs.

METHODS

PolyCYPs are promiscuous cytochrome P450 enzymes that can produce oxidized metabolites of common human CYPs involved in drug metabolism. PolyUGTs mimic human UGTs in making O-, acyl and some N-glucuronides of drugs. Both enzyme types have been cloned from actinomycete bacteria and expressed in *E. coli* together with the necessary co-factors. PolyUGTs were further purified by affinity chromatography. Whole cell biotransformation methods using the Streptomyces clones containing the enzyme, or the source strain itself, enabled mg to gram quantities of human metabolites of JNJ-61393215 and camonsertib to be produced.

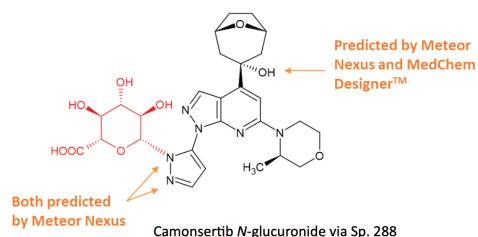
RESULTS

PolyCYPs were used to generate sufficient material for structure identification, and for scaling up the production of M54, a major human hydroxylated metabolite of the orexin-1 receptor antagonist JNJ-61393215.² Screening with PolyCYPs revealed PolyCYP isoform 152 was the most proficient at producing M54, and a subsequent small scale up provided sufficient quantities of M54 for structure elucidation by NMR spectroscopy. A whole cell biotransformation method using a *Streptomyces* strain from which PolyCYP 152 was cloned, provided 7.5 grams of purified M54, revealing stereoselective hydroxylation on the deuterated 2-aza-[2.2.1]-bicycle core structure and providing material for ongoing studies. This biotransformation would have been challenging and resource-intensive to try and achieve via chemical synthesis.



JNJ-61393215 M54 via PolyCYP 152

A major N-glucuronide of the ATR kinase inhibitor camonsertib was first observed in human hepatocyte incubations and material was subsequently needed to determine the structure, evaluate its stability as part of bioanalytical method development, and for use as a standard for estimating its concentration in Phase I clinical samples. The position of attachment was challenging to pinpoint by LC-MS/MS and prediction software was not useful in this case.



Camonsertib N-glucuronide via Sp. 288

The UGT1A4-derived N-glucuronide was scaled-up and 25.1 mg was purified from a bacterial biotransformation, which matched exactly with the human hepatocyte product. NMR spectroscopy revealed the specific point of attachment of the glucuronic acid through NOE data and the gradient HMBC experiment.³

Subsequent to this work, camonsertib was screened against PolyUGTs. The N-glucuronide was specifically produced by PolyUGT 179 at a conversion of 12.4%. The source strain of PolyUGT 179 is the same microbe (Sp. 288) that was originally used to scale-up the N-glucuronide. Use of a PolyUGTs scale-up kit would have provided a quick way to generate sufficient material for structure elucidation by cryoprobe NMR spectroscopy, with a whole cell biotransformation subsequently required for generation of more metabolite for analytical studies.

CONCLUSIONS

- Application of PolyCYPs and PolyUGT enzymes provided a scalable route to access CYP- and UGT-derived synthetically challenging human metabolites of JNJ-61393215 and camonsertib, respectively.
- The biocatalytic platforms bridge the gap between early MetID needs and large-scale metabolite supply, reducing reliance on animal-derived systems and circumventing the need for complex chemical synthesis.

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Enhancing Assay Sensitivity of Existing LBA Assay Methods Using a Nanocarrier-Based Strategy

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PURPOSE

Highly sensitive and accurate bioanalytical methods are essential to support pharmacokinetic (PK) and toxicokinetic (TK) studies. Historically, the enzyme-linked immunosorbent assay (ELISA) has been routinely used for the quantitation of large molecules due to its sensitivity, specificity, and scalability. However, a key challenge in the preclinical space is the limited availability of analyte-specific critical reagents, such as the capture and detection reagents, required for ligand binding assay development. These reagents play a pivotal role in assay performance, and acquiring alternative reagents is not always feasible. As a result, there is a need to improve assay performance without altering these critical components. To address this issue, we evaluated an oligonucleotide nanoparticle carrier which integrates existing ELISA workflows with the exponential amplification capability of PCR to improve assay sensitivity.

METHODS

A 96-well MSD high-bind plate was coated with the target-specific antigen and blocked to prevent non-specific binding. Standards and quality control samples containing a therapeutic monoclonal antibody as the target analyte were added, enabling interaction with the immobilized antigen. After washing, a biotinylated detection antibody was introduced to bind the analyte. Unbound detection antibodies were removed through additional washes, followed by incubation with the nanoprobe, a streptavidin-coated oligonucleotide carrier. Non-bound nanoprobe were eliminated with a stringent wash cycle. To release the antigen-protein complex, the plate was incubated at 70°C for 10 minutes. The eluted product was transferred to a 96-well qPCR plate, mixed with primers and qPCR Master Mix, and subjected to amplification using a real-time qPCR instrument. Cycle threshold (CT) values were collected and analyzed to quantify the target analyte concentration in each sample.

RESULTS

In this study, we utilized a nanoparticle-based technology that integrates the robustness of ligand binding assays with the exponential amplification power of PCR. This approach allowed us to significantly improve assay performance using existing reagents. Using a humanized monoclonal antibody as the target analyte, we achieved a 25-fold improvement in sensitivity, with the LLOQ enhanced from 0.16 µg/mL (ELISA) to 0.006 µg/mL (Immuno-PCR). Furthermore, the assay's dynamic range was extended from 3 to 5 logs, broadening its application and utility. Calibration curves and quality control evaluations demonstrate the precision, accuracy, and reproducibility of the assay. The comparative analysis of PK study plasma samples demonstrated a correlation between concentration profiles from both methods, with a Spearman's correlation coefficient of 0.9499 and a Bland-Altman ratio of 1.25. Notably, Immuno-PCR enabled quantification of low-dosed PK samples that previously fell below the level of quantification (BQL) for the ELISA assay, providing a more accurate PK profile.

CONCLUSIONS

This study demonstrates that the nanoparticle-based technology can overcome limitations imposed by critical reagent availability, offering an effective solution for maximizing assay sensitivity and performance with existing resources.

ACKNOWLEDGEMENTS, DISCLAIMERS, FUNDING, AND OTHER DISCLOSURE OR CONFLICT OF INTEREST STATEMENTS:

This study was sponsored and funded by Takeda Development Center Americas, Inc. I.L., L.D. and M.G.Q. are employees of Takeda Development Center Americas, Inc, Cambridge, MA, USA; whereas W.C., R.I., are employees of Somru BioScience, Inc., Charlottetown, Prince Edward Island, Canada.

Optimization of Engineered Cyclic Peptide Permeability in Caco-2, 2D Organoid and MDCKII Cell Assays

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PURPOSE

Engineered cyclic peptides represent a unique therapeutic modality that combines the advantages of traditional small molecules with the high target binding affinity and specificity usually seen in biologics. However, their development is often challenged by limited permeability, creating challenges for oral peptides and those targeting intracellular mechanisms. With multiple approaches currently available to assess the permeability of engineered cyclic peptides, a comprehensive assessment was undertaken to identify the most robust in vitro approach.

METHOD

Initial efforts to characterize the permeability of a subset of proprietary and commercially available engineered cyclic peptides with a wide range of physicochemical properties using a 2D-organoid assay coupled to a highly sensitive mass spectrometry analytical method demonstrated a weak correlation with in vivo exposures following oral administration. As such, efforts were expanded to evaluate Caco-2 and MDCKII cell assays across an expanded set of peptides aimed at establishing the structure-activity relationships driving peptide permeability. Manual and automated cellular assays were optimized to compare permeability values across Caco-2, 2D organoids and MDCKII cells, assays commonly used to assess drug permeability. Finally, a number of various bioanalytical approaches including high resolution, triple quadrupole and matrix-assisted laser desorption ionization (MALDI) mass spectrometry platforms were evaluated for sensitivity, reproducibility and throughput. Automated liquid handling platforms were incorporated to aid in sample processing.

RESULTS

We have successfully developed a highly sensitive high-throughput bioanalytical LC-MS/MS assay to evaluate the permeability (P_{app}) of 21 engineered cyclic peptides with or without the permeation enhancer sodium caprate across multiple in vitro assays. Multiple physical chemistry properties have been evaluated against the permeability data suggesting limited ability to incorporate structural design into the optimization of peptide permeability. All in vitro assays supported an increase in permeability of engineered cyclic peptides in the presence of sodium caprate.

CONCLUSION

The data from all assays suggests an increased permeability in the presence of the permeation enhancer sodium caprate, consistent with its use as a strategy to increase oral bioavailability. Further evaluation suggests no clear correlation between permeability and physicochemical properties, suggesting optimization of permeability may have limited value in identifying engineered cyclic peptides with improved oral bioavailability. The findings will ultimately offer significant insights and guidance for peptide development across various therapeutic areas.

Enhancing Immunogenicity Risk Assessment: Leveraging ISPRI for Bispecific Antibody Analysis

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PURPOSE

Bispecific antibodies are recombinant molecules designed to target two different epitopes simultaneously. Several engineering techniques have been developed to facilitate their production, including knob-into-holes Fc and Fab mutations. Wen-Ting et al. explore the impact these mutations pose on immunogenicity risk by employing both computational and experimental methods intended to model various steps in the cellular pathway leading to the production of anti-drug antibodies. They select a DC internalization assay to capture the uptake of the protein therapeutic by antigen presenting cells, both an in silico prediction tool and an MHC-associated peptide proteomics assay to model the presentation of the processed peptides by MHC-II, and finally, T cell assays to assess the activation and proliferation of naïve T cells. An immunogenicity risk was assigned to each bispecific antibody and monospecific counterpart based on the results of these individual methods. Lastly, they assigned an overall immunogenicity risk considering the collective results from all methods.

We performed a retrospective immunogenicity analysis of the same panel of bispecific antibodies using the in silico prediction tools hosted on ISPRI (Interactive Screening and Protein Reengineering Interface). ISPRI is a web-based platform for large-scale immunogenicity risk assessments and protein engineering. It uses various tools including EpiMatrix for epitope mapping, ClustiMer for identifying epitope clusters, and JanusMatrix for evaluating regulatory T cell responses which may minimize anti-drug antibody formation. We expected the immunogenicity risk assessment results of our in silico analysis to align with the results outlined by Wen-Tin et. al.

METHODS

To perform this retrospective analysis, we began with a global immunogenicity assessment of each bispecific antibody and monospecific counterpart. Using our proprietary EpiMatrix algorithm, we screened each sequence for Class II (HLA-DR) restricted HLA ligands and putative T cell epitopes. The EpiMatrix scores of the individual T cell epitopes were aggregated and normalized to produce global immunogenicity scores for each antibody. Antibodies and antibody-like molecules frequently contain known regulatory epitopes (aka Tregitopes). The presence of regulatory epitopes can suppress anti-drug immune responses. We discounted the scores of known Tregitopes to yield improved measures of global immunogenic potentials. Finally, we plotted the Tregitope-adjusted EpiMatrix Scores on the antibody immunogenicity scale along with a selection of well-known benchmarks for comparison.

Peptide epitopes that are frequently found within the human proteome (other than Tregitopes) are less likely to drive anti-therapeutic immune responses. Discounting the EpiMatrix Scores of peptide epitopes frequently found in the human proteome can also improve the quality of our assessment of global immunogenic potentials. The JanusMatrix algorithm scores proteins based on their human-like content. To complete our global analysis, we screened all epitopes identified by EpiMatrix against the human proteome using the JanusMatrix algorithm to calculate global JanusMatrix scores.

Next, we proceeded with a regional immunogenicity analysis of each antibody. Using the ClustiMer algorithm, we identified epitope clusters (short segments of amino acids that contain unusually high numbers of putative T cell epitopes) contained within each sequence. Finally, we evaluated each epitope cluster using both the EpiMatrix and JanusMatrix algorithms. Lastly, based on a collective analysis using ISPRI's suite of tools, we assigned an immunogenicity risk to each monospecific and bispecific antibody to compare to the risk categories assigned by Wen-Ting et al. to confirm alignment between the two analysis approaches (Image 1).

Using ISPRI, we generated epitope maps providing a visual of all epitope content and clusters contained within each of the sequences. We compared these to figures produced by Wen-Ting et al. highlighting all MAPPs peptide spectrum matches as well as the immunogenic peptides detected using NetMHCII-pan-4.0 and produced figures overlaying the epitope content identified using ISPRI with their results (Image 2).

RESULTS

After reviewing the collective results from ISPRI and assigning risk categories to each antibody, we found that our *in silico* immunogenicity assessment aligned with the risk categories assigned by Wen-Ting et al. after their comprehensive *in silico* and *in vitro* multi-fold approach to immunogenicity risk assessment (Image 1). Additionally, based on the retrospective immunogenicity analysis, EpiMatrix aligned more closely with the results of the MAPPs assay as compared to NetMHCIIpan-4.0. Of the immunogenic peptides identified using ISPRI, 92% overlap with the peptides detected by the MAPPs assay compared to 59% overlap of the peptides identified using NetMHCIIpan-4.0.

CONCLUSIONS

The results of this retrospective analysis demonstrate ISPRI's utility as a reliable and efficient method to assess immunogenicity risk. ISPRI produced comparable results to those of a multi-tiered *in silico* and *in vitro* approach and provided superior results to those of an alternative *in silico* prediction tool. *In silico* immunogenicity analysis using the ISPRI platform can be used to supplement experimental methods which can often be extremely time-consuming and expensive. Most significantly, this analysis highlights the importance of performing an *in silico* immunogenicity assessment early on in the preclinical development process to mediate loss of clinical efficacy and avoid potential off-target toxicities.

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Bioanalytical Strategies for Developing Surrogate Matrix to Quantify CNS Disease Biomarker Using S-Trap Platform and High-Resolution LC-MS

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PURPOSE

Recent developments have expanded RNAi therapeutics to extrahepatic tissues using lipophilic conjugates. ALN-APP (Mivelsiran), the first conjugate of 2'-O-hexadecyl (C16) to siRNA is designed for the central nervous system (CNS) delivery and is currently in clinical trials. Accurate quantification of target protein levels in cerebrospinal (CSF) and brain regions is essential for evaluating pharmacodynamics (PD) and siRNA potency.

This study describes the development of an LC-MS protein assay to quantify a CNS disease biomarker in biological matrices, supporting PD assessments for an intrathecally dosed RNAi therapeutic targeting a CNS gene. It addresses the bioanalytical challenges in optimizing surrogate matrices for CSF and various monkey brain tissue due to the lack of suitable blank matrices. The study also highlights the potential applicability of the method to universal CNS protein biomarkers, investigates the correlation between brain tissue and CSF and evaluates the feasibility of developing a biomarker assay for use in human CSF.

METHODS

Sample preparation was conducted using the S-Trap method with slight modifications, including reduction, alkylation and trypsin digestion. CSF samples were processed directly, while monkey brain samples were pulverized and homogenized in Clarity OTX Lysis-Loading buffer before S-Trap processing.

Surrogate matrices were utilized to construct standard curves and quality control samples, ensuring parallelism between endogenous analytes and surrogate matrix standard curves, in compliance with the M10 regulatory guidelines.

Eluates were analyzed on a Thermo Q Exactive Orbitrap mass spectrometer coupled with a Dionex UltiMate 3000 HPLC system and Accela Open Autosampler. Parallel reaction monitoring (PRM) in positive acquisition mode was applied for peptide quantification.

Signature peptides were selected by the software Skyline, and the sequences were verified through uniprot.org.

RESULTS

Surrogate canine matrices were used to eliminate interference from endogenous protein levels in humans and monkeys in the protein quantification assay. This approach relied on signature peptide sequences that differ between canine and monkey or human proteins. Different concentrations of surrogate matrix plasma were used in place of matched matrices, including 0.1 % canine plasma for cerebrospinal fluid (CSF) and 10% canine plasma for brain samples, due to the unavailability of the blank canine brain tissue.

Protein digestion efficiency in monkey CSF was evaluated using S-Trap, achieving a recovery rate of over 85%. The precision (coefficient of variation (CV%)) ranged from 2.0% to 18.3%, and the bias ranged from -4.3% to 8.8% for all quality control (QC) concentrations. In the monkey brain, the precision (coefficient of variation (CV%)) ranged from 2.1% to 17.4%, and the bias ranged from -15.8% to 3.5% for all quality control (QC) concentrations.

In addition to the primary quantifier peptide used for protein quantification, a second peptide was employed to qualify the assay. A strong correlation was observed between the protein levels calculated using the quantifier and qualifier peptides in both monkey CSF ($R^2=0.9916$, $n=78$)

and monkey brain samples ($R^2=0.9293$, $n=108$), confirming the robustness of the quantification method. Furthermore, a strong correlation between the monkey CSF and high siRNA potency brain region tissues were observed.

CONCLUSION

A robust protein quantitation assay was developed across different matrices using high-resolution LC-MS and S-Trap quantitative proteomics platform. Bioanalytical methods were successfully applied with a surrogate matrix to ensure precision and accuracy, in compliance with the M10 regulatory guidelines. The method was used to quantify the protein levels of a CNS disease biomarker in CSF and brain following a single IT dose of C16 conjugate siRNA in monkeys for preclinical PD assessment. Additionally, the assay was assessed in human CSF using human specific peptides for a potential clinical biomarker assay.

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Development and implementation of a 6-plex hybrid LC-MS/MS method for quantitative bioanalysis of CDH3 MSLN T cell engager (TCE)

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PURPOSE

CDH3 MSLN T cell engager (TCE) is a dual targeting T-cell engager molecule with selective activity for solid tumors that co-express CDH3 and MSLN. Given the multi-domain molecular structure of TCE, a confirmatory LC-MS/MS bioanalytical method was developed that enabled the simultaneous quantification of multiple TCE domains in a single assay for comparison with the primary ligand binding assay (LBA) method.

METHODS

Selection of surrogate peptides: TCE drug product was digested with trypsin and analyzed on Q-Exactive Orbitrap HRMS operating in DDA mode. The resulting MS/MS data was submitted to Protein Pilot™ software for peptide identification and mapping. Six surrogate peptides representing different domains of TCE (CDH3, CD3, CD3-linker, Fc, MSLN, CD3-ending) were selected based on their selectivity, molecular stability and MS signal intensity.

Method qualification and clinical analysis: TCE was enriched by immunocapture and then digested into surrogate peptides with Trypsin. The quantitation range was from 25 to 5000 ng/mL for CDH3, CD3, Fc and CD3-ending peptides and 250 - 5000 ng/mL for the remaining CD3-linker and MSLN peptides. The method met acceptance criteria as a qualified bioanalytical method and showed robust precision and accuracy performance and matrix stability. The qualified method was used to generate bioanalytical data for comparison with the validated LBA approach in select Phase 1 clinical samples.

RESULTS

A robust 6-plex hybrid LC-MS method for quantitation of TCE in human serum was successfully developed, qualified and implemented for PK sample analysis.

Pre-defined acceptance criteria for accuracy and precision for Quality Controls were met for CDH3, CD3, CD3-linker, Fc, MSLN, CD3-ending peptides with %Bias range -11.0 to 7.6, -4.5 to 3.9, -0.5 to 3.1, -2.8 to 4.8, -3.8 to 2.9, and -4.0 to 5.6, respectively; and Max. Inter-run %CV of 13.7, 4.5, 11.0, 6.2, 18.6, and 6.1, respectively.

Stability in matrix was established for CDH3, CD3, Fc, MSLN, CD3-ending peptides with %Bias range -3.5 to 19.6, -2.8 to 8.7, -1.5 to 4.8, 2.0 to 12.0, and -0.3 to 8.5, respectively; and Max. Inter-run %CV of 11.2, 11.5, 5.0, 4.3, and 13.2, respectively. CD3-linker did not pass in-matrix stability with %Bias range 12.0 to 36.0 and Max %CV of 16.5.

CONCLUSION

A qualified hybrid LC-MS method for TCE which monitored multiple domains was used to assess validity of the primary LBA and confirm in vivo stability of TCE in an ongoing Phase 1 clinical study.

Development of a Multiplex RT-qPCR Assay for Highly Similar mRNA Sequences

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PURPOSE

The rapid evolution of gene and cell therapy has underscored the need for robust bioanalytical techniques to accurately quantify and characterize complex mRNA therapeutics. Among these, reverse transcription quantitative polymerase chain reaction (RT-qPCR) remains a gold standard due to its sensitivity and specificity. However, its application to similar mRNA molecules—particularly those encapsulated in lipid nanoparticles (LNPs)—poses challenges in amplification efficiency, reproducibility, and quantification.

METHODS

We developed and optimized an RT-qPCR assay to quantify highly similar mRNA species in cynomolgus monkey serum. Key aspects of the method include primer-probe design refinement, enhanced RNA extraction strategies, and mitigation of matrix effects to improve assay performance.

RESULTS

Our data will highlight RT-qPCR method development strategies to improve assay robustness, accuracy, and reproducibility for quantification of highly similar mRNA. Additionally, specificity enhancements, and multiplexing strategies were explored to streamline RT-qPCR workflows. Consequently, two duplex assays were developed and cross-reactivity between the highly similar mRNAs were minimized.

CONCLUSIONS

By implementing unique primer-probe design strategy, we aim to develop robust bioanalytical RT-qPCR method for measurement of LNP-encapsulated mRNA in cynomolgus monkey. Our findings might have significant implications for regulatory compliance, assay standardization across laboratories, and the broader success of gene and cell therapy programs.

An Isotope-Tracing Assay Using HILIC-HRMS to Measure Endogenous Metabolites in Drug-Targeting (Activated) Neutrophils

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PURPOSE

Neutrophils are the most abundant immune cells in circulation. Overactivated neutrophils are associated with many autoimmune and inflammatory diseases. The complex engagement of neutrophils among different diseases makes it an exciting and challenging therapeutic target. Uncovering key drivers of pathogenesis as well as translatability of preclinical models are of critical importance for therapeutic development targeting neutrophils. Metabolism plays a key role in neutrophil activation to fulfill high demand for energy and ROS production. In this work, we developed a sensitive isotope-tracing assay to measure endogenous metabolites and the flux in glycolysis and pentose phosphate pathway (PPP) in a human neutrophil model.

METHODS

Primary neutrophils were treated with PMA (positive control, activation stimulant through NOX2) or PMA+DPI (negative control, inhibition of NOX2), and NA11 (PFKL activator that partially reduces neutrophil activation). Neutrophils were cultured with [1,2]-¹³C-glucose to trace metabolic flux in glycolysis and pentose phosphate pathway. Cells were harvested by rinsing off media with PBS and immediately quenched by cold extraction buffer containing 2:2:1 Acetonitrile:methanol:water and internal standards. Cell extracts undergo three freeze-thaw cycles and centrifugation to obtain pellet-free supernatant for LC-MS analysis. Samples were analyzed by a Shimadzu HPLC system coupled to a Thermo QExactive Orbitrap Mass Spectrometer equipped with a HESI source. To separate key metabolites including glycolytic and pentose phosphate pathway intermediates, a HILIC method using a zwitterionic HILIC column (iHILIC) and basic mobile phases was developed. Top 5 data-dependent acquisition was implemented at a MS resolution of 60k. Metabolite ID was achieved by matching with an in-house library. Data analysis was performed by using Skyline software and R scripts for peak integration and natural abundance correction.

RESULTS

Three HILIC columns (Phenomenex Luna-NH₂, Water BEH Amide, and HILICON iHILIC zwitterionic) were assessed by injecting individual standards and mixtures of 17 glycolytic, PPP, redox and energy metabolites. The zwitterionic column outperformed the other two columns that had the best coverage with good peak shapes and low baseline. Baseline separation of two pairs of structural isomers G6P/F6P, and GAP/DHAP was achieved. Baseline separation of four redox metabolites NAD⁺, NADH, NADP⁺, NADPH was also achieved. However, neither of the methods can achieve separation of other isomeric metabolites including 2PG/3PG, and F16BP/F26BP. Using the HILIC-HRMS method, ¹³C isotopologues (series of the same metabolite containing different number of ¹³C atoms) of lactate, ribose-5-phosphate, fructose-6-phosphate were integrated and natural abundance corrected in human neutrophils under different conditions. Lactate ¹³C enrichment was calculated as a metric to represent relative extent of the fructose cycle upon neutrophil activation. Compared with untreated control, a drastic decrease in lactate ¹³C enrichment and increase in [M+1] R5P was observed in human neutrophils upon PMA activation, suggesting a dominating metabolic flux towards oxidative PPP for NADPH production. Upon DPI inhibition, lactate ¹³C enrichment increased along with a drastic decrease in [M+1] R5P, indicating a suppressed oxidative PPP pathway with limited NADPH production. When neutrophils were incubated with PMA and NA11, a partial recovery of lactate ¹³C enrichment and decreased [M+1] R5P was observed, suggesting a compromised flux rebalancing in oxidative PPP by PFKL activation. The result confirms the route of suppression by activating PFKL on neutrophil oxidative burst. Same assay was also performed on neutrophils of other preclinical species to evaluate the translatability to this human neutrophil model.

CONCLUSION

In this study, we developed an isotope-tracing assay to measure ¹³C distributions of endogenous metabolites in glycolysis, PPP, and redox metabolism. We applied the assay to study the metabolic alterations upon neutrophil activation and uncover the metabolic insights of pharmacological activation of PFKL in suppressing neutrophil activation.

Assay Design WebApp: An in-house tool for free/total target and PK assay modeling designed to assist in bioanalytical assay development and optimization

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PURPOSE

A concept was developed at Pfizer regarding target assays aimed at predicting theoretical free target levels and monitoring their changes over time, factoring in affinities and binding dynamics. This initiative led to a collaboration between Pfizer scientists and experts from RES Group Inc, an MIT spin-off renowned for expertise in QSP modeling. The partnership resulted in the creation of the Assay Design WebApp—an intuitive in-house platform designed to provide analytical feedback to modeling scientists. The tool's primary objective is to offer an interface accessible to both modelers and analysts, enabling the assessment of critical parameters in protein target and BioTx PK assay development.

METHODS

This tool is designed as an initial resource for assay development, aiming to reduce the analytical time required for selecting bioanalytical assay starting conditions. It leverages mathematical models for each assay step, such as sample preparation, incubation with capture antibody, and detection antibody application. The Web App has been utilized across multiple projects for the optimization and modelling of predominantly free target and pharmacokinetic (PK) assays. In this context, we demonstrate the tool's impact on optimizing a free target assay within a biotherapeutic monoclonal antibody program*.

RESULTS

We investigated how varying the quantity of capture antibody influences both target levels and drug concentrations and then optimized the assay using the WebApp to ensure complete (100%) capture of the free target. Furthermore, we utilized this tool to assess the uncertainty associated with the binding affinity between the target, the drug, and the capture antibody.

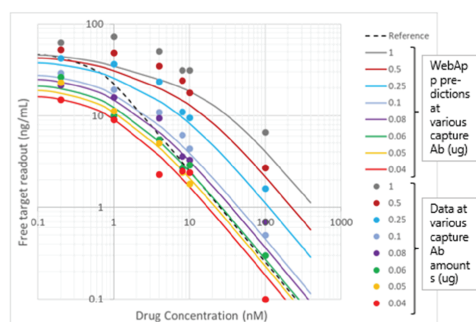


Figure 1. Measured free target readout vs. drug concentration against modeled readouts shows a strong correlation across various capture Ab and drug levels.

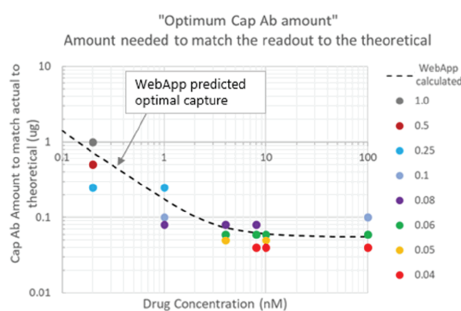


Figure 2. The WebApp predicted the optimal capture antibody amount that was closest to the theoretical antibody amount over a wide range of drug levels

This example of a biotherapeutic drug target illustrates that the WebApp can facilitate target assay development in situations where binding parameters and affinities are not yet established or remain uncertain.

CONCLUSION

The Assay Design WebApp helps optimize PK and target assays by modeling performance ranges and drug capture efficiency while minimizing disruption of target/drug complexes. Even when all assay parameters are not fully known, it enables users to assess how uncertainties affect results and identify which factors need further characterization. Using the WebApp for method evaluation empowers project teams to make informed decisions based on realistic expectations and characterize assay caveats.

* More detailed information on the biotherapeutic drug will be provided on the poster.

Proteomic Signatures of Gaucher Disease Type 3 Reveal Response to Venglustat and Imiglucerase Combination Therapy Using Olink® Explore HT

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PURPOSE

Gaucher disease type 3 (GD3) is a chronic, neuronopathic lysosomal storage disorder caused by mutations in the GBA1 gene, resulting in deficient glucocerebrosidase activity and subsequent accumulation of glycolipids such as glucosylceramide in systemic and neural tissues. This buildup contributes to both multisystem and neurological dysfunction. Venglustat is an investigational, brain-penetrant glucosylceramide synthase inhibitor designed to reduce substrate accumulation and potentially modify disease progression by rebalancing glycolipid homeostasis. The Phase 2, open-label LEAP trial (NCT02843035) evaluated the safety and pharmacodynamic effects of once-daily oral venglustat (15 mg) in combination with maintenance dose of imiglucerase enzyme replacement therapy (ERT) in adults with GD3.

METHODS

To characterize disease-related proteomic alterations and identify biomarkers of treatment response, we performed high-throughput proteomic profiling of cerebrospinal fluid (CSF) and plasma samples using the Olink® Explore HT platform. Samples from GD3 patients (n=9) were collected at three timepoints: pre-venglustat (on stable imiglucerase therapy), Week 26, and Week 52 after initiation of venglustat. These were compared to samples from age-matched healthy controls (plasma, n=29; CSF, n=12).

RESULTS

Proteomic analysis revealed distinct signatures differentiating GD3 patients from healthy individuals, with enrichment in inflammatory and neurological pathways. Several biomarkers demonstrated a shift toward healthy control levels with venglustat treatment, suggesting their potential utility as pharmacodynamic indicators. Trends observed in both CSF and plasma highlight the potential of these biomarkers for monitoring CNS disease activity.

CONCLUSION

These findings underscore the utility of deep proteomic profiling in uncovering disease mechanisms and evaluating therapeutic response in neuronopathic Gaucher disease. The identified biomarkers offer promising avenues for future research and clinical monitoring in GD3.

Evaluation of the MSD S-Plex Neuropanel 1 Multiplex Assay for Neurological Biomarkers

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Sanofi

PURPOSE

The purpose of this study is to evaluate the feasibility of the MSD S-Plex Neuropanel 1 assay in detecting three critical neurological biomarkers—Glial Fibrillary Acidic Protein (GFAP), Neurofilament Light (NFL), and Tau—simultaneously. This evaluation aims to determine the assay's bioanalytical robustness and operational value, specifically in terms of increased data efficiency, cost and time savings, and reduced sample handling. By leveraging the multiplex capability of the assay, this study seeks to extract rich information from a single sample, thereby enhancing the overall efficiency and accuracy of neurological biomarker detection.

METHODS

The MSD S-PLEX Neurology Panel 1 kit is MSD's ultrasensitive multiplex assay that uses proprietary TURBO-TAG and TURBO-BOOST reagents for improved sensitivity. The neurology panel 1 captures GFAP, NFL, and Tau simultaneously in a multi-spot microplate, with each capture antibody coated in a different spot. The MSD platform uses electrochemiluminescence (ECL) technology for signal readout. ECL technology works by using electrochemiluminescent labels that emit light when electricity is applied. In the S-PLEX MSD assay, capture antibodies are immobilized on specific spots of a multi-spot microplate. When a sample containing the target analytes is introduced, these analytes bind to the capture antibodies. After the targets are captured, detection antibodies, labeled with TURBO-BOOST tags, are then added, and bind to the captured analytes, forming an immunocomplex. Subsequently enhancer and TURBO-TAG is added. Finally, after addition of Read Buffer, an electrical current is applied and the immunocomplexes with labels emit light, which is measured to quantify the analytes.

RESULTS

The evaluation parameters included calibration curves, parallelism in plasma and CSF, accuracy and precision, dilution linearity and hook effect, lot-to-lot variation, and cross-reactivity between analytes/reagents. All three analyte calibration curves were fitted with 4PL curve fitting and 1/Y² weighting, achieving an $R^2 > 0.9900$, and back-calculated calibrator % recovery (%RE) within $\pm 20\%$. Four commercial endogenous plasma and CSF samples were titrated and tested multiple times for parallelism. Plasma samples were diluted from neat to 32X, and at the experimentally determined minimum required dilution (MRD) of 4X and subsequent dilutions, the % recovery ranged from 94% to 108%. CSF samples were diluted from neat to 80X, and at the MRD of 10X and subsequent dilutions, the % RE ranged from 86% to 112%. Internal quality control (QC) samples were tested for accuracy and precision. Buffer-spiked samples at the upper limit of quantitation (ULOQ), the high end (HQC), middle of the calibration range (MQC), low end (LQC), and lower limit of quantitation (LLOQ) were tested over 16 separate runs. For all accepted runs, the %CV was $\leq 20\%$ for HQC, MQC, and LQC, and $\leq 25\%$ for ULOQ and LLOQ. The %RE to nominal concentrations was $\leq 25\%$ for HQC, MQC, and LQC, and $\leq 30\%$ for ULOQ and LLOQ. During the evaluation of accuracy and precision, some failed runs showed high recovery for GFAP and NFL and low recovery for Tau. Further investigation and troubleshooting led to a modification in the protocol for the last manual wash step, requiring the plate to be rotated 180 degrees between wash steps, which improved accuracy for all analytes. Additionally, minimizing sample transfers between vials helped better recover Tau levels in the QC samples. The assay did not present a prozone effect, and the titration of the spiked buffer samples demonstrated good linearity. Three different lots of the S-PLEX Neuropanel kit were tested using 17 plasma and 21 CSF samples. Lot-to-lot variability was $<20\%$ for plasma and $<25\%$ for CSF for all analytes. Lastly, cross-reactivity was evaluated by adding a single analyte or a single detector antibody in the well. Clinically relevant concentrations of these 3 analytes were tested to demonstrate potential analyte cross-reactivity in real patient samples. When only GFAP was added, there was 6% cross-reactivity with NFL and 0.2% with Tau. Similarly, NFL showed cross-reactivity of 0.6% with GFAP and 0.07% with Tau. Tau protein showed almost zero cross-reactivity with both GFAP and NFL. No significant cross-reactivity was observed when only a single detector antibody was added to the well.

CONCLUSIONS

The MSD S-Plex Neuropanel for GFAP, NFL, and Tau was evaluated in-house, demonstrating promising results. The assay quantitatively measures three biomarkers in a single well, exhibiting good parallelism, accuracy and precision, and lot-to-lot variability. Technical considerations, such as rotating the plate 180 degrees during last manual wash step and minimizing sample transfers between vials, contributes to better accuracy. However, at high concentrations of GFAP and NFL, analyte cross-reactivity between GFAP and NFL was observed, ranging from 0.6% to 6%.

An optimized approach for simultaneously quantification of total antibody and antibody-conjugated payload using immunocapture LC-MS/MS

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PURPOSE

Antibody drug conjugate (ADC) has emerged as a popular class of anti-tumor therapeutics which increase the target specificity compared with traditional chemotherapy. Fast and effective bioanalytical assays are needed to support different stages of ADC discovery and development. A duplex LC-MS/MS based assay platform has been established previously to quantify total antibody and conjugated payload of ADC simultaneously. That assay utilizes commercially available MS-compatible detergents to partially denature the protein during enzymatic digestion, avoiding traditional reduction and alkylation procedures. Acid hydrolysis was required to precipitate the detergent to minimize its impact on MS, but its efficiency is sample-dependent. Possible incomplete decomposition may lead to high concentration of detergent in the injection solution, which affects MS signal. Here we developed an optimized and efficient LC-MS/MS-based duplex assay incorporating reduction and alkylation steps without the use of detergent. This assay was successfully applied to support bioanalysis of preclinical studies of ADCs with various cleavable linkers.

METHODS

Mouse plasma was used as the matrix to prepare calibration standard and quality control (QC) samples. The calibration range was 10 – 10,000 ng/mL using a sample aliquot of 10 μ L. Immunoaffinity capture was conducted using antigen immobilized on streptavidin magnetic beads. The conjugate payload was firstly cleaved from the ADC by incubation with papain and TCEP, followed by alkylation with iodoacetamide and trypsin digestion. Human IgG peptide, ALPAPIEK, was selected as surrogate peptide for total antibody. The duplex assay was tested for sensitivity, linearity, accuracy and precision.

RESULTS

Previously established duplex LC-MS/MS based assays overcomes the challenges of coupling tryptic digestion of antibody with papain catalyzed ADC linker cleavage to enable one pot reaction. That method omits the reduction/alkylation step, the reason of which is because iodoacetamide (IAA) used for alkylation may react with the cysteine in the enzyme active center of papain. However, the detergent used in that assay needs acid hydrolysis-induced decomposition prior to injection. The efficiency of decomposition was noticed to be highly variable, and the difference may be related to the concentrations of protein/peptide. The un-decomposed detergent remaining in the solution could suppress MS signal, lowering the assay sensitivity. In current workflow, we optimized the digestion procedure and made it compatible with reduction and alkylation, eliminating the use of detergent. The order of adding trypsin and papain were evaluated and optimized for complete and efficient digestion for both total antibody and conjugated payload assays. Tryptic peptide ALPAPIEK peptide from Fc region that tolerates papain digestion was selected as a generic surrogate peptide for total antibody assay for preclinical rodent studies. Sensitivity, linearity, accuracy and precision were evaluated and demonstrated for this optimized duplex assay.

CONCLUSIONS

An efficient LC-MS/MS based duplex assay for total antibody and conjugated payload was developed to support discovery ADC bioanalysis.

Overcoming the Antibody Drug Discovery Bottleneck with Off-the-Shelf Ligand Binding Immunoassays

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BACKGROUND

Antibody based biologics represents a major class of therapeutics, yet their discovery and development remain hindered by time-consuming processes, particularly during early pharmacokinetic (PK) evaluations. Rapid generation of high-quality PK data is critical for lead optimization, but traditional ligand binding immunoassay (LBA) development is often slowed by the need for custom or highly specific reagents. To address this bottleneck, we developed two separate off-shelf fit-for-purpose generic LBAs, for the rapid quantification of human IgG-based biologics in rodent and non-human primate matrices, respectively, enabling faster PK assessment during early-stage drug discovery.

METHODS

Meadowhawk Biolabs developed generic assays to quantify a broad spectrum of biologics that contain the human IgG Fc (fragment crystallizable) domain, including monoclonal, bi-specific, tri-specific antibodies, fusion proteins, multi-domain constructs, and antibody-drug/nucleotide conjugates. Our assays utilize reagents that recognize epitopes in the human IgG fragment crystallizable (Fc) region. These assays are compatible with multiple platforms, including ELISA and the Meso Scale Discovery (MSD) electrochemiluminescence format.

RESULTS

Both assays demonstrated robust lower limits of quantitation (LLOQ), 50 ng/mL using ELISA and 3 ng/mL using MSD across multiple species, (including mice, rat, and non-human primates) and in multiple matrices (such as plasma, serum, and various tissue homogenates). Furthermore, when tested in PK studies, these assays successfully generated pharmacokinetic profiles for various dosing routes using ultra-low sample volumes (as low as 2 μ L per assay).

CONCLUSION

Our generic, ready-to-use immunoassays enable rapid and reliable PK data generation using ultra-low sample volumes, for human IgG-based therapeutics, eliminating the need for lengthy custom assay development. This platform accelerates early-stage biologics discovery by streamlining a critical step in the drug development pipeline.

In Vitro Reaction Phenotyping of Carboxylesterase Isoforms Using Supersomes: Method Development and Optimization to Assess Enzyme Contributions to Metabolic Clearance

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PURPOSE

Reaction phenotyping is a cornerstone of drug metabolism studies, routinely applied to cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes, to identify the main enzymes responsible for the metabolism of a new drug. According to the ICH M12 Guideline on Drug Interaction Studies, if a drug is not metabolized by the major CYP enzymes, other enzymes can be investigated. Carboxylesterases (CES)—particularly CES1b, CES1c, and CES2—play a critical role in the biotransformation of ester- and amide-containing compounds. Their involvement is essential for the metabolic fate of such drug molecules, especially when CYP-mediated metabolism is minimal or absent. In this study, we present comprehensive reaction phenotyping data for CES1b, CES1c, and CES2 using recombinant human supersomes.

METHODS

Due to limited commercially standardized protocols for CES enzymes, we developed and optimized a method to selectively assess CES-mediated metabolism. The conversion of p-nitrophenyl acetate (p-NPA) to p-nitrophenol (p-NP) is a common reaction used to assess esterase activity, including that of CES. We developed a 2-in-1 LC-MS/MS method using p-NPA as a probe substrate with individually expressed CES isoforms to detect both p-NPA depletion and p-NP formation. The clearance of p-NPA and formation of p-NP were measured in CES1b, CES1c, and CES2 during a 1-hour incubation.

RESULTS

The half-lives of p-NPA were 0.280, 0.373, and 0.239 hours in CES1b, CES1c, and CES2, respectively, while buffer control showed 7.24 hours of half-life. Our data reveal differential substrate specificity and activity across CES isoforms, offering key insights into CES-driven drug metabolism. These findings support the inclusion of CES phenotyping in early drug development, particularly for ester-containing compounds, and aid in improving in vitro–in vivo extrapolation (IVIVE) models.

CONCLUSION

This work highlights the importance of expanding phenotyping efforts beyond CYPs and UGTs to capture a broader spectrum of drug-metabolizing enzymes relevant to pharmacokinetics, drug-drug interactions, and inter-individual variability.

A Robust, Highly Sensitive LC-MS/MS Method for Quantification of Orforglipron (a small molecule, non-peptide GLP-1RA) in Human Plasma

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PURPOSE

Small-molecule, non-peptide glucagon-like peptide-1 receptor agonists (GLP-1RAs) have demonstrated significant clinical benefits, including glycemic control, weight reduction, and improvements in cardiovascular and mental health, making them valuable therapeutics for type 2 diabetes mellitus (T2DM) and obesity. Orforglipron is one such novel oral small-molecule GLP-1RA that has shown efficacy comparable to peptide-based agents such as semaglutide, liraglutide, and tirzepatide in Phase I/II clinical trials. To support clinical development, particularly low-dose and special population studies, highly sensitive LC-MS/MS methods are required to accurately quantify Orforglipron at picogram-per-milliliter (pg/mL) levels in human plasma. However, the compound's physicochemical characteristics—including high hydrophobicity, strong hydrogen bonding, and aromatic interactions—pose challenges such as non-specific binding and reduced assay specificity. While recent LC-MS/MS methods for Orforglipron have been reported, they lack detailed evaluation of matrix effects and offer only a lower limit of quantification (LLOQ) of 0.100 ng/mL. Here, we developed a robust, and high-sensitivity LC-MS/MS method quantifying Orforglipron down to 20.0 pg/mL (LLOQ). In addition, we have evaluated matrix effects at two quality control (QC) levels (low and high) using six different lots of blank plasma from T2DM donors to ensure method efficiency and robustness across such clinically relevant populations.

METHODS

Working calibrators and working QCs were freshly prepared from 0.500 mg/mL stock solutions of Orforglipron in DMSO. Working solutions were prepared in 1% (w/v) bovine serum albumin (BSA) in water (w/v) to avoid non-specific binding. Using working calibrators and working QCs, calibration standards at a range of 20.0 – 20,000 pg/mL and QCs with concentrations of 20.0, 60.0, 6,000 and 15,000 pg/mL were prepared in human plasma K2EDTA under wet ice conditions. 20.0 μ L of calibration standards, QC plasma samples, blanks and double blanks were prepared using serial dilution and transferred to a protein lo-bind 96-well plate. Tolbutamide (20 μ L) was added as the internal standard to all wells except double blanks. Protein precipitation was performed using 300 μ L of ice-cold acetonitrile, followed by vortexing and centrifugation. A 260 μ L aliquot of the resulting supernatant was transferred to a clean plate and evaporated at 40 °C. Dried samples were reconstituted using 125 μ L of 0.1% (v/v) formic acid in 50:50 Methanol: Water, (v/v). The plate was vortexed and centrifuged, and 10 μ L was injected onto a ACQUITY UPLC BEH C18 Column, 130Å (2.1 mm X 50 mm, 1.7 μ m), achieving chromatographic separation within 5 minutes by gradient elution with 2mM ammonium acetate with 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). Orforglipron is quantified using a SCIEX 7500+ QQQ with ESI under a multiple reaction monitoring (MRM) scan mode enabling sensitive quantification. Matrix effects were assessed at Low and High QC levels using six individual lots of blank plasma from T2DM donors.

RESULTS

Orforglipron is robustly quantified at 20.0 pg/mL in human plasma K2EDTA, with an acceptable precision ($\leq 10\%$) and mean accuracy ($\pm 12\%$) across the nominal concentration range of 20.0 – 20,000 pg/mL meeting bioanalytical standards. This method demonstrated good selectivity and specificity with a reproducible linearity, and acceptable inter and intra-day accuracy and precision. In addition, matrix effect results at low and high QC levels in six different T2DM plasma lots were within acceptable accuracy and precision criteria further confirming the robustness of the method performance.

CONCLUSION

We developed a robust, highly sensitive LC-MS/MS method for Orforglipron quantification, demonstrating consistent performance in both normal and diabetic plasma. The extended lower quantification range makes this Orforglipron LC-MS/MS method well-suited for pharmacokinetic studies involving low-dose regimens in the treatment of T2DM and obesity or for any future clinical studies.

Multiplexed Free payload, Total Antibody-Drug Conjugate, and Total Antibody Bioanalysis of Brentuximab Vedotin from a Single Rat Plasma Aliquot

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PURPOSE

To demonstrate a novel method which combines immunoprecipitation and protein precipitation sample processing of a single rat plasma aliquot to quantify the free payload, total antibody-drug conjugate (ADC) and total antibody of brentuximab vedotin.

METHODS

A single set of standards and quality controls (QC) containing both brentuximab vedotin and MMAE in rat plasma will be plated and undergo sample processing. The samples will undergo immunoprecipitation to separate the total ADC and total antibody from the free payload. The post-immunoprecipitation rat plasma aliquot will undergo protein precipitation to allow for the analysis of the free payload, MMAE. Brentuximab vedotin captured during immunoprecipitation will undergo both enzymatic payload release and tryptic digestion for the quantitative analysis of the total ADC and total antibody, respectively.

RESULTS

The multiplexed method shows performance that meets the acceptance criteria in terms of accuracy, precision, and dilution integrity that would indicate such a technique could be used for regulated bioanalysis as per ICH m10. With total ADC and total antibody having an acceptance criteria for RE and CV of $\leq 20\%$ ($\leq 25\%$ for LLOQ standards and QCs) and free payload having an acceptance criteria for RE and CV of $\leq 15\%$ ($\leq 20\%$ for LLOQ standards and QCs).

CONCLUSION

Quantitation of Free payload, total ADC, and total antibody can be performed on a single rat plasma aliquot using a combination of combines immunoprecipitation and protein precipitation. Such a multiplexed bioanalysis technique allows for more efficient bioanalysis during clinical by requiring small volumes of plasma, fewer aliquot, and reduces the number of sample preparations needed.

Development of total ASO method in mouse plasma and tissues using LC-FD and LC-MS platforms

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Note: This work was funded by Sanofi

PURPOSE

To develop a methodology for quantifying total anti-sense oligonucleotides (ASO) in Protein- antisense oligonucleotide conjugates (POCs) that could be universally applied across similar POCs. The study compared mass spectrometry and fluorescence detection platforms to determine optimal sensitivity, selectivity, and adaptability for ASO quantification.

METHODS

The study evaluated two mass spectrometry platforms- high resolution mass spectrometry (HRMS) and triple quadrupole mass spectrometry (QQQ or MS/MS) and fluorescence detection for two POCs, coupled with liquid chromatography (LC). For LC-HRMS analysis, the analyte was extracted using Phenomenex Clarity solid phase extraction followed by separation by reverse phase-ion pairing (RP-IP) and detection on Thermo Orbital Trap QExactive mass spectrometry. For LC-MS/MS analysis, the analyte was extracted by ASO hybridization with peptide nucleic acid (PNA) or locked nucleic acid (LNA) probes followed by RP-IP based separation and detection on AB Sciex 7500 mass spectrometry. For fluorescence detection, the method incorporated proteinase K digestion, PNA probe hybridization, and strong anionic exchange column separation with a salt gradient. A potassium chloride precipitation step was added to reduce background interference.

RESULTS

The LC-MS/MS assay using LNA probe demonstrated effectiveness across both plasma and tissue samples with a range of 0.146-145 nM. The LC-MS/MS provided at least 10-fold higher sensitivity compared to LC-HRMS. The fluorescence detection method achieved ranges of 0.582-116 nM for plasma and 5.82-1,160 nmol/kg for tissue samples. The %CV and %bias for quality control samples and back-calculated standard curve in both the methods were within the acceptable range of $\leq 20\%$ in the tissues tested, showing good precision and accuracy.

CONCLUSION

The triple quadrupole platform provided optimal sensitivity, while the orbital trap offered superior selectivity. Fluorescence detection proved the most straightforward to develop. Two of the three platforms were successfully developed and used for pharmacokinetic analysis, which can be minimally optimized and adapted for other similar constructs. Further optimization of the orbital trap method could potentially improve detection limits and enable comprehensive POC-related compound analysis. Additional development using immunocapture approaches could enhance sample cleanup through a dual hybridization process.

Protein Corona Profiling of mRNA Lipid Nanoparticles Unveil Therapeutic Potentials Beyond COVID-19

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PURPOSE

The success of both BN162b2 and mRNA-1273 pandemic vaccines showcases the impact of mRNA lipid nanoparticles (LNP) in the development of novel mRNA therapeutics. Recent advancements in LNP technology have unlocked the possibility for delivering tissue-specific payloads, expanding the platform's application for therapy. This is evidenced by a growing number of clinical trials harnessing this platform for oncology therapeutics, focusing on cancer vaccines targeting multiple neoantigens or direct intratumoral injections of mRNA-LNPs encoding pro-inflammatory cytokines (1-5). Yet, the precise mechanism of how LNPs are partitioned from the site of administration to their subsequent target tissues remains largely unknown. It is now speculated that when LNPs interact with biological fluids, different proteins are recruited and form distinctive biomolecular coronas that are trafficked to different tissues. Despite increasing efforts in the field, studying LNP protein coronas remains particularly challenging due to the detection of low abundance proteins and the large amounts of materials required.

METHODS

In this study, we report a fast, robust, and highly reproducible workflow to provide high-resolution profiling of protein coronas on LNPs. We demonstrate that novel formulations using Sanofi's cationic lipids show distinctive protein coronas when compared to LNPs formulated using commercially available lipids.

RESULTS

Our result also indicates that the composition of LNPs can attract different corona proteins, leading to differences in tissue tropism.

CONCLUSION

This work provides insights on how this platform is crucial in the development of next generation treatments.

This work was funded by Sanofi.

All authors are Sanofi employees and may hold shares and/or stock options in the company.

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Development of Sensitive Intact Assays for Evaluating Molecular Stability and Pharmacokinetics of Protein Oligonucleotide Conjugate

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BACKGROUND

Protein-antisense oligonucleotide (ASO) conjugates represent a promising therapeutic approach for neuromuscular and genetic disorders, combining protein-targeting specificity with gene-silencing capabilities. However, assessing their pharmacokinetic profiles presents unique bioanalytical challenges, particularly regarding in vivo conjugate integrity—critical for efficacy and safety.

METHODS

Two intact assays were developed in mouse plasma using mesoscale discovery (MSD) platform to assess pharmacokinetic profiles. The generic phosphorothioate (PS) assay utilizes a biotinylated anti-phosphorothioate antibody capturing the ASO backbone, with detection via anti-protein antibody labeled with sulfo-tag. The ASO-specific hybridization assay employs a biotin-labeled PNA probe designed to hybridize specifically to the ASO sequence, followed by immobilization on streptavidin MSD plates and detection through sulfo-tagged anti-protein antibody.

RESULTS

Key parameters underwent systematic optimization including buffer composition, probe concentration, hybridization temperature, plate type, reaction volume, incubation conditions, and minimum required dilutions. Both assays demonstrated robust performance across multiple protein-ASO constructs with a dynamic range from picomolar to nanomolar concentrations in plasma. Fit-for-purpose qualification showed %CV and %bias of back-calculated calibrators and quality controls $\leq 20\%$, with minimal matrix effects and no hook effect up to measured concentrations.

CONCLUSION

We established two orthogonal assays for intact protein-ASO conjugate measurement in mouse plasma. The generic PS-based MSD assay and the ASO-specific hybridization assay provide insights into construct stability and pharmacokinetics behavior. These orthogonal assays offer valuable tools for preclinical development, enabling informed optimization of linker design and delivery strategies in protein-oligonucleotide therapeutics.

An Integrated Preclinical Platform for Comprehensive Evaluation of Immunotherapies

Southern Research

ABSTRACT

Southern Research (SR) has developed an integrated preclinical platform that combines immune profiling, mechanistic studies, and efficacy modeling to support the development of novel immunotherapies. This platform leverages high-parameter flow cytometry to characterize immune cell subsets, multiplex cytokine assays to quantify immune activation, and syngeneic and humanized mouse tumor models to evaluate therapeutic efficacy and tumor microenvironment modulation.

Key components of the platform include in vivo tumor immune profiling to assess central memory and exhausted T cell states, intratumoral macrophage polarization analysis to track immunosuppressive versus pro-inflammatory phenotypes, and systemic cytokine profiling following immune stimulation to detect early safety signals. These complementary datasets provide a comprehensive view of drug activity and potential immune-related toxicities.

By enabling early identification of efficacy markers and risk indicators, this approach supports biomarker-driven decision-making, de-risks candidate selection, and accelerates translation of immune-oncology agents toward IND-enabling studies and clinical development.

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