APPLIED PHARMACEUTICAL TOXICOLOGY

VIRTUAL EVENT

May 10-11, 2021

DISCOVERY
TOXICOLOGY WORKSHOP

May 18-19, 2021

DEVELOPMENT
TOXICOLOGY WORKSHOP

PRESENTED BY OUR SPONSORS:

emulate

cyprotex AN EVOTEC COMPANY

STEMCELL™ TECHNOLOGIES

abbvie

MERCK

Pfizer

Boehringer Ingelheim

NOVARTIS

Organized by: The Boston Society

www.bostonsociety.org

2021 APT SPONSORS

PLATINUM SPONSORS:

emulate

cyprotex AN EVOTEC COMPANY

GOLD SPONSOR:

STEMCELL™ TECHNOLOGIES

PHARMACEUTICAL SPONSORS:

abbvie

Pfizer

MERCK

Boehringer Ingelheim

NOVARTIS

ORGANIZERS' WELCOME

Welcome to the 2021 Applied Pharmaceutical Toxicology Conference.

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

ORGANIZING COMMITTEES

Presiding Chair

Chair: Jonathan Heyen, Pfizer

DISCOVERY Toxicology Workshop Organizers

Chair: Jonathan Heyen, Pfizer

Committee:

Zoe Zhong, Genentech
Jodi Goodwin, Takeda
Christine Karbowski, Amgen
Bruce Leroy, AbbVie
Jon Maher, Theravance Biopharma
Darcey Clark, Johnson & Johnson
Rama Pai, Merck
Yoav Timsit, Blueprint Medicines

DEVELOPMENT Toxicology Workshop Organizers

Chair: Rebecca Erickson, Denali Therapeutics

Committee:

Joe Cichocki, Vertex
Paul Cornwell, Eli Lilly
Ed Dere, Genentech
Heather Dowty, Pfizer
Lise Loberg, AbbVie
Florence Lorget, Sparing Vision
Lauren Mihalcik, Aclairo
Eunice Musvasva, Roche
Michel Santostefano, Merck
Jairo Nunes, Takeda
Nardos Tassew, Genentech

APT 2021 CONFERENCE AGENDA

MONDAY, MAY 10 (TIME SLOTS LISTED ARE EDT)

11:00 - 11:10 **Conference Opening**
Jonathan Heyen, Pfizer

DISCOVERY TOXICOLOGY WORKSHOP

SESSION I: IO in Discovery Toxicology Space

Chairs: Rama Pai, Merck and Jonathan Heyen, Pfizer

11:10 - 11:40 **KEYNOTE LECTURE:**
Navigating Through Uncharted Territory at the Frontlines of Immuno-Oncology Innovation as a Nonclinical Safety Scientist
Jacintha Shenton, Janssen

11:40 - 11:45 **Q & A**

11:45 - 12:05 **CAR T Neurotoxicity: Translational Insights**
Juliane Gust, Seattle Children's Hospital

12:05 - 12:10 **Q & A**

12:10 - 12:30 **Experience with Nonhuman Primate Models in CAR-T Therapy Nonclinical Safety Assessment**
Kavita Raman, Amgen

12:30 - 12:35 **Q & A**

12:35 - 1:20 **Break**

1:20 - 1:40 **Nonclinical Evaluation of CD3 Bispecific Antibodies**
Changhua Ji, Pfizer

1:40 - 1:45 **Q & A**

1:45 - 2:05 **Preclinical Safety for a Small Molecule Sting Agonist Immunotherapy**
Christopher Brynczka, Merck

2:05 - 2:10 **Q & A**

2:10 - 2:30 **Poster Session**

SESSION II: Microphysiological Systems (MPS)

Chairs: Jodi Goodwin, Takeda & Zoe Zhong, Genetech

2:30 - 2:35 **Session Introduction**

2:35 - 2:55 **VENDOR PRESENTATION:**
The Human Duodenum Intestine-Chip for Applications in Safety, Absorption, Transport and Metabolism
Sushma Jadalannagari, Emulate

2:55 - 3:00 **Q & A**

3:00 - 3:20 **Surviving the Technology Hype Cycle: Where MPS are Having an Impact Today, and Where They're Needed Next**
Matt Wagoner, Takeda

3:20 - 3:25 **Q & A**

3:25 - 3:45 **3D Renal Proximal Tubule-on-a-Chip for Assessing Drug-Induced Nephrotoxicity**
Tomomi Kiyota, Genentech

3:45 - 3:50 **Q & A**

TUESDAY, MAY 11 (TIME SLOTS LISTED ARE EDT)

DISCOVERY TOXICOLOGY WORKSHOP

SESSION III: Novel Approaches to Identify Off-target Drivers of Toxicity

Chairs: Christine Karbowski, Amgen and Bruce Leroy, AbbVie

11:00 - 11:05 **Session Introduction**

11:05 - 11:25 **Integrating a Big Toxicity Data Framework to Better Evaluate Drug Safety**
Andy Vo, AbbVie

11:25 - 11:30 **Q & A**

11:30 - 11:50 **CiPA: Practical Applications in Discovery**
Scott Mittelstadt, Abbvie

11:50 - 11:55 **Q & A**

11:55 - 12:15 **The Impact of Toxicokinetics on the Design and Interpretation of Toxicology Studies**
Suman Mukherjee, Merck

12:15 - 12:20 **Q & A**

12:20 - 12:50 **PLENARY SPEAKER:**
Reimagining Druggability using Chemoproteomic Platforms
Daniel Nomura, UC Berkeley

12:50 - 12:55 **Q & A**

12:55 - 1:40 **Break**

1:40 - 1:50 **VENDOR PRESENTATION:**
Utility of Human Pluripotent Stem-Cell Derived Cardiomyocytes in Cardiotoxicity Assays Using the Maestro MEA System and STEMdiff Cardiomyocytes Products
Nathan Moerke, STEMCELL Technologies

1:50 - 1:55 **Q & A**

SESSION IV: Case Studies in Investigative Toxicology

Chairs: Yoav Timsit, Blueprint Medicines and Jon Maher, Theravance Biopharma

1:55 - 2:00 **Session Introduction**

2:00 - 2:20 **Early Assessment of Bone Marrow Toxicity in Immunodeficient Mice**
Kim Maratea, AstraZeneca

2:20 - 2:25 **Q & A**

2:25 - 2:45 **Role of Drug Exposure Quantification for Safety and Efficacy Evaluation in Non Clinical and Clinical Trials**
Jonathan Stauber, IMA Biotech

2:45 - 2:50 **Q & A**

2:50 - 3:10 **Spectral Imaging/Multispectral Imaging Approaches to Understand Mechanism of Toxicity**
Chandra Saravanan, Novartis

3:10 - 3:15 **Q & A**

3:15 - 3:35 **Poster Session**

TUESDAY, MAY 18 (TIME SLOTS LISTED ARE EDT)

11:00 - 11:10 **Conference Opening**
Rebecca Erickson, Denali Therapeutics

DEVELOPMENT TOXICOLOGY WORKSHOP

SESSION I: COVID19: Development of Vaccines and Therapeutics

Chairs: Joe Cichocki, Vertex and Michel Santostefano, Merck

11:10 - 11:30 **Rapid Development of REGN-COV2, an Anti-Spike Antibody Cocktail for Treatment and Prevention of COVID-19**
Matt Liu, Regeneron

11:30 - 11:50 **Nonclinical Safety Assessment Strategies for COVID19 Mabs: Expediting Clinical Trials and Emergency Use Authorization**
John Vahle, Eli Lilly

11:50 - 12:10 **COVID19 Vaccine AstraZeneca: Mission Impossible EUA**
Richard Stebbings, AstraZeneca

12:10 - 12:25 **Panel Discussion - Session Speakers**

12:25 - 1:10 **Break**

1:10 - 1:15 **Keynote Speaker Introduction**
Michael Santostefano, Merck

1:15 - 1:45 PLENARY SPEAKER:
Discovery and Development of Vaccines and Worldwide Regulatory Experience
Lisa Plitnick, Merck

1:45 - 1:50 **Q & A**

1:50 - 2:10 VENDOR PRESENTATION:
Transforming DILI Prediction using Transcriptomics and AI
Christopher Strock, Cyprotex

cyprotex AN EVOTEC COMPANY

2:10 - 2:15 **Q & A**

SESSION II: Developing Products for Rare Diseases and Conditions

Chairs: Rebecca Erickson, Denali Therapeutics and Jairo Nunes, Takeda

2:15 - 2:20 **Session Introduction**

2:20 - 2:40 **Regulatory Challenges and Considerations in Rare Disease Drug Development**
Kerry Jo Lee, FDA

2:40 - 3:00 **Safety Considerations in the Development of Pharmacologically Active Proteins for Rare Diseases**
John McNulty, Takeda

3:00 - 3:20 **Risdiplam - Fast Development to Turn the First Small Molecule mRNA Splice Modifier into an Approved Medicine**
Lutz Mueller, F. Hoffman-La Roche Ltd.

3:20 - 3:40 **The Nonclinical Safety Profile of ONPATTRO® (patisiran), a First-in Class RNAi Therapeutic for the Treatment of Polyneuropathy of Hereditary Transthyretin-mediated Amyloidosis (hATTR amyloidosis)**
Jessica Sutherland, Alnylam

3:40 - 4:00 **Panel Discussion - Session Speakers**

WEDNESDAY, MAY 19 (TIME SLOTS LISTED ARE EDT)

DEVELOPMENT TOXICOLOGY WORKSHOP

SESSION III: Gene Therapy: Non-clinical to Clinical Translation

Chairs: Lise Loberg, AbbVie and Eunice Musvasva, Roche

11:00 - 11:05	**Session Introduction**
11:05 - 11:25	**Adapting Traditional Tox Strategy to Patients with High Risk, Genetic Disorders: Do Regulations Speak to the Heart of the Issue?** Lauren Black, Charles River Laboratories
11:25 - 11:30	**Q & A**
11:30 - 11:50	**Things to Consider When Translating Gene Therapy Nonclinical Data to Support Clinical Trials** Laurence Whiteley, Pfizer
11:50 - 11:55	**Q & A**
11:55 - 12:15	**MicroRNA-mediated Inhibition of Transgene Expression** Juliette Hordeaux, UPenn
12:15 - 12:20	**Q & A**
12:20 - 12:45	**Poster Session**
12:45 - 1:30	**Break**
1:30 - 1:35	**Keynote Speaker Introduction** Lauren Mihalcik, Alcairo
1:35 - 2:05	PLENARY SPEAKER: **Engaging CBER on Preclinical Gene Therapy Projects - A Consultant's Perspective** Laura Dill Morton, Aclairo Pharmaceutical Development Group
2:05 - 2:10	**Q & A**
2:10 - 2:30	**Poster Session**

SESSION IV: Peripheral Neuropathy

Chairs: Heather Dowty, Pfizer; Paul Cornwell, Eli Lilly, and Ed Dere, Genentech

2:30 - 2:35	**Session Introduction** Heather Dowty, Pfizer; Ed Dere, Genentech; Paul Cornwell, Eli Lilly
2:35 - 2:55	**Toxic Peripheral Neuropathies: Clinical Perspective** Ahmet Hoke, Johns Hopkins School of Medicine

2:55 - 3:15	**Intra-epidermal Nerve Fiber Analysis: a Sensitive, Clinically-Relevant Tool for Assessing Sensory Nerve Damage in Animal Models** Lisa Magnus, Johns Hopkins School of Medicine
3:15 - 3:35	**Toxicologic Evaluation of the Peripheral Nervous System in Animal Adm. A Large Molecule NME** Chris Aluise, Eli Lilly and Company
3:35 - 3:55	**Improving Preclinical to Clinical Translation of Drug-induced Peripheral Neuropathy: A Case Example with MMAE Containing Antibody Drug Conjugates** Nicola Stagg, Genentech
3:55 - 4:10	**Panel Discussion - Session Speakers**
4:10 - 4:15	**Webinar Closing Remarks**

ABSTRACTS

DISCOVERY TOXICOLOGY WORKSHOP

SESSION I

CAR T Neurotoxicity: Translational Insights
Jule Gust, Seattle Children's Hospital

Neurologic toxicity proved to be an unexpected setback` in the development of CD19-targeting CAR T therapies. In the past five years, the field has matured to FDA approval of multiple CD19-CAR T products, and new CAR T and T cell engaging therapies are being tested at a rapid pace. How can we use the story of CD19 CAR T neurotoxicity to apply lessons learned to the development of new therapies? First, I will describe the clinical manifestations of CD19 CAR T neurotoxicity, and discuss whether the concept of Immune Effector Cell Associated Neurologic Toxicity (ICANS) is able to capture the multitude of toxicities that are being described with other products such as bispecific T cell engagers, BCMA CAR T cells, and others. I will then review the reasons why neurotoxicity was not anticipated in preclinical testing, and what progress we have made in our understanding` of this toxicity. Finally, I will discuss some strategies that could be pursued toward greater predictability of immune-system engaging therapies.

Nonclinical Evaluation of CD3 Bispecific Antibodies
Changhua Ji, Pfizer

Variety of CD3 bispecific antibodies are in preclinical and clinical development for the treatment of liquid or solid tumors. These molecules bind to CD3 on the surface of T-cells and to a tumor-associated antigen (TAA) on the tumor cell surface, and induce cytotoxic activity against tumor cells. The key safety concerns with CD3 bispecifics are cytokine release syndrome (CRS), target organ toxicity, and, in some instances, neurotoxicity. Nonclinical safety assessment is key to understanding the overall safety risk of the target and molecule, which generally includes extensive target expression evaluation, in vitro cytotoxicity and cytokine production in the presence of target and non-target cells to demonstrate specificity, and in vivo toxicology studies in pharmacologically relevant species (usually limited to NHP). To mitigate CRS, step dosing and use of inhibitors of cytokine response (eg. Dexamethasone (Dex) and tocilizumab (Toci)) have showed good promise. Dex, toci, and JAK inhibitor were evaluated in cynomolgus monkeys for their ability to mitigate CRS risk. Only Dex was shown to be able to decrease cytokine release without decreasing the target tissue cytotoxicity (surrogate of anti-tumor activity). Efforts have also been made to optimize molecule design and binding affinities to TAA and CD3 to improve CRS and tissue toxicity profile. Another key aspect of nonclinical activity is to determine an appropriate human starting dose based on MABEL. Human starting doses for many of the earlier CD3 bispecifics have been undesirably low that required multiple dose cohorts to reach clinical benefit. For this reason, FDA now accepts starting doses that target 10-30% pharmacologic activity.

VENDOR PRESENTATION

The Human Duodenum Intestine-Chip for Applications in Safety, Absorption, Transport and Metabolism
Sushma Jadalannagari, Emulate

Traditional in vitro systems fail to replicate the three-dimensional cytoarchitecture and functional complexity of the small intestine, which is an important site for drug and nutrient absorption, digestion, secretion and elimination along with hosting the intestinal microbiome. The poor accuracy of` in vitro models represents a significant challenge in the drug development pipeline. To overcome this, we developed a human Duodenum Intestine-Chip that combines healthy intestinal organoids on a microfluidic chip with tissue-specific human intestinal microvascular endothelial cells cultured in a parallel microchannel under flow and cyclic deformation. The physiological microenvironment established on the Duodenum Intestine-Chip supports the formation of villi-like projections lined by polarized epithelial cells with mature tight junctional networks and that undergo multi-lineage differentiation into the major epithelial cell subtypes such as absorptive enterocytes, enteroendocrine, Paneth, and goblet cells. Transcriptomic analysis reveals the Intestine-Chip more closely aligns with human duodenum tissue in vivo than 3D organoids. This correlates with physiological expression of functional intestinal drug transporters such as MDR1, BCRP and PEPT1 including drug mediated induction of CYP3A4. Further, the Duodenum Intestine-Chip demonstrates drug induced toxicity and barrier disruption, after treatment with the tool compounds indomethacin

and 5-fluorouracil. In addition, we also show that this Duodenum Intestine-Chip can support long-term probiotic bacterial co-culture with lactobacillus rhamnosus GG. Thus, our human Duodenum Intestine-Chip can be used as a model for preclinical drug assessment in a more human relevant model.

SESSION II

3D Renal Proximal Tubule-on-a-Chip for Assessing Drug-Induced Nephrotoxicity

Tomomi Kiyota, Genentech

Nephrotoxicity is a serious safety concern during drug development; and represents a major liability in certain drug classes, even among marketed products. Nephrotoxicants target specific regions (e.g., renal proximal tubules as the primary target site for drug clearance) of nephrons and induce damages by a variety of intracellular stresses. Over the past decade several "kidney-on-a-chip" microphysiological systems (MPSs) have been developed by culturing renal proximal tubular epithelial cells to generate physiologically relevant models with long-term (> weeks) viability, polarized expression of transporters, albumin uptake, and glucose reabsorption. We have been testing one such proximal tubule MPS model to evaluate its capability to capture drug-induced nephrotoxicity using a set of classic nephrotoxicants. Nephrotoxicity is assessed by informative readouts such as cell viability, production of biomarkers, and intracellular stresses. Additionally, considerable effort has been made to implement this model to predict nephrotoxicity of select molecules within our internal portfolio. This talk will highlight our recent experiences in the use of Kidney MPS, including the benefits and challenges in implementing the proximal tubule model for drug safety assessment.

SESSION III

Integrating a Big Toxicity Data Framework to Better Evaluate Drug Safety

Andy Vo, AbbVie

Computational toxicology is a new research field that integrates chemistry, biology, pharmacology, and toxicology with computer science methodologies to predict safety-related in vivo outcomes. Pharmaceutical organizations have begun to exploit new technologies to generate large-scale datasets to better understand both in vitro and in vivo properties of compounds. These individual datasets are often used to make decisions during specific processes in the pipeline on a per compound basis. Together, these datasets provide a big data framework to evaluate drug safety but are often not connected due to challenges related to data interoperability and lack of integration. The complexity and diversity of these datatypes present an enormous challenge as traditional methods may not be sufficient to analyze them. Thus, we look to integrate these internal toxicity datasets into a big data framework and introduce artificial intelligence and machine learning methods to analyze it within the context of our internal chemical space.

In this work, we have connected existing in vitro data from three independent screening panels (CEREP, Kinome, and Bioprofiling) to preclinical toxicity data across AbbVie compounds. Given the complexity of toxicity data (e.g., species, dosing, and assay diversity), we have incorporated domain knowledge within preclinical safety to assist in the curation and interpretation of toxicity data to create a working dataset. To better evaluate this integrated data framework, we have developed a computational pipeline to process these data and apply in silico methods to further increase our understanding of in vitro data relationships and in vivo outcomes for drug safety evaluation. As proof of concept, we have applied this strategy to identify some potential off-targets associated with adverse hematological findings.

CiPA: Practical Applications in Discovery

Scott Mittelstadt, AbbVie

The CIPA initiative began in 2013 with the purpose of developing an in vitro screening paradigm that would allow drug discovery scientists to differentiate between compounds that had high, intermediate and low risk for inducing Torsade de Point. Over the last year, data has been released that allows us to begin understanding the strengths and weaknesses of data obtained from induced pluripotent stem cell-derived cardiomyocytes and in vitro multicurrent analysis. In addition, data will be presented on the translation of the results from CIPA compounds assessed in anesthetized dogs. A potential screening paradigm, combining in vivo data with in vitro analysis, will be presented allowing Drug Discovery teams to make early business decisions regarding the risk of moving compounds forward to later stage discovery and IND enabling work.

The Impact of Toxicokinetics (TK) on the Design and Interpretation of Toxicology Studies

Suman Mukherjee, Merck

TK variability in preclinical toxicology studies needs to be assessed in the context of expected toxicology findings and safety margins. TK variability may lead to overlap in systemic exposures between dose groups, which can lead to a failure to clearly define a dose-exposure relationship and can preclude the establishment of adequate safety margins in toxicology studies. The presentation will include a definition of 'normal' TK variability in preclinical toxicity studies. The impact of route of administration, formulation, physicochemical properties of drug development candidates, dose levels and species on TK variability will be presented. For relatively safe compounds with acceptable safety margins that exhibit TK variability, doses should be spaced as wide apart as possible to avoid overlap of systemic exposure between doses. However, for compounds where TK variability is identified in early de-risking studies and where toxicity is observed at low safety margins, it would be prudent to better understand the physicochemical properties and their potential impact on absorption, to optimize the FaSSIF solubility and to minimize pH dependence on absorption.

PLENARY

Reimagining Druggability using Chemoproteomic Platforms

Daniel Nomura, UC Berkeley

The Nomura Research Group is focused on reimagining druggability using chemoproteomic platforms to develop transformative medicines. One of the greatest challenges that we face in discovering new disease therapies is that most proteins are considered "undruggable," in that most proteins do not possess known binding pockets or "ligandable hotspots" that small-molecules can bind to modulate protein function. Our research group addresses this challenge by advancing and applying chemoproteomic platforms to discover and pharmacologically target unique and novel ligandable hotspots for disease therapy. We currently have three major research directions. Our first major focus is on developing and applying chemoproteomics-enabled covalent ligand discovery approaches to rapidly discover small-molecule therapeutic leads that target unique and novel ligandable hotspots for undruggable protein targets and pathways. Our second research area focuses on using chemoproteomic platforms to expand the scope of targeted protein degradation technologies. Our third research area focuses on using chemoproteomics-enabled covalent ligand discovery platforms to develop new induced proximity-based therapeutic modalities. Collectively, our lab is focused on developing next-generation transformative medicines through pioneering innovative chemical technologies to overcome challenges in drug discovery.

VENDOR PRESENTATION

Utility of Human Pluripotent Stem-Cell Derived Cardiomyocytes in Cardiotoxicity Assays Using the Maestro™ MEA System and STEMdiff™ Cardiomyocyte Media

Nathan Moerke, STEMCELL Technologies

Cardiotoxicity is a major source of failure during preclinical drug development. The establishment of robust in vitro cardiotoxicity screening assays at an early stage of development is critical for de-risking candidate drugs and mitigating the risk of late-stage failure. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are an attractive model to study drug-induced cardiac arrhythmias and cardiotoxicity. The STEMdiff™ Cardiomyocyte system is a suite of products that supports a complete workflow to generate hPSC-derived ventricular or atrial cardiomyocytes and for dissociation, replating, and cryopreservation of these cells. hPSC-CMs produced and processed using this system can be readily assayed for parameters of excitability using the Maestro™ MEA system. Using this assay system, the profiles of compounds known to cause drug-induced arrhythmias and modify excitability can be consistently measured using multiple hPSC-derived cardiomyocyte lines. The combination of STEMdiff™ cardiomyocyte products and the Maestro™ MEA system provides a robust in vitro system for preclinical drug discovery, cardiotoxicity assessment, and medium- to high-throughput phenotypic screening of drug candidates.

DEVELOPMENT TOXICOLOGY WORKSHOP

SESSION I

Rapid Development of REGN-COV2, an Anti-spike Antibody Cocktail for Treatment and Prevention of COVID-19

Matt Liu, Regeneron

An urgent global quest for effective therapies to prevent and treat COVID-19 disease is still ongoing. Regeneron took parallel efforts using both humanized mice and serum from convalescent patients to generate antibodies against SARS-CoV-2 spike protein. From a large collection of fully-human antibodies, we selected two potent, neutralizing antibodies targeting distinct noncompeting epitopes on the receptor binding domain (RBD) of the SARS-CoV-2 Spike (S) protein. Regeneron quickly moved the two antibody cocktail, REGN-COV2 (casirivimab and imdevimab), from preclinical phase to clinical testing, later receiving Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA).` This presentation will cover the area of the preclinical characterization of the two antibodies, clinical efficacy of REGN-COV2, and/or rapid interactions with Agency.

Nonclinical Safety Assessment Strategies for COVID 19 Mabs: Expediting Clinical Trials and Emergency Use Authorizations

John Vahle, Eli Lilly

While ICH S6(R1) provides guidance regarding the nonclinical safety strategies for the development of monoclonal antibodies targeting non-mammalian epitopes (e.g. viral or bacterial targets), the emergence of the COVID 19 pandemic led biopharmaceutical developers, regulators, and academic collaborators to consider safety assessment approaches that would expedite not only clinical trials but ultimately the use of these therapies in medical practice, all the while providing reasonable assurance of safety. This presentation will focus on nonclinical safety and regulatory strategies for monoclonal antibodies directed against the spike protein of SARS-CoV-2. The primary focus will be on those activities starting with the concept of a monoclonal antibody derived from a convalescent patient to entry into clinical rials in 94 days. Beyond the first-in-human strategy the talk will cover considerations for latter stages of development including support of emergency use authorizations and potential registration activities.

PLENARY

Discovery and Development of Vaccines and Worldwide Regulatory Experience

Lisa Plitnick, Merck

A systematic and regulated approach to the nonclinical safety assessment of vaccines is a relatively recent phenomenon compared to small molecule drugs even though the first vaccine was developed hundreds of years ago. When vaccines were initially developed, they were considered inherently safe and only limited nonclinical testing was required. However, as more complex vaccines and adjuvants were developed for use mainly in healthy populations including infants, more stringent nonclinical testing guidelines were introduced. While the WHO guidelines for nonclinical testing represent the gold standard for testing recommendations, individual countries also have country-specific guidelines that in some cases lead to inconsistent guidance. A brief history of vaccination, the importance of the immune system and vaccination, and the routine study designs and considerations for the nonclinical assessment of vaccine safety will be discussed.

VENDOR PRESENTATION

Transforming DILI Prediction using Transcriptomics and AI

Christopher Strock, Cyprotex

The presentation will cover cutting-edge research demonstrating how transcriptomics combined with machine learning and AI can transform the prediction of drug-induced liver injury (DILI). A large database of over 1500 compounds is currently being assessed in multiple 2D and 3D cell-based models. High throughput RNA-Seq is being used to identify transcriptomic fingerprints. These fingerprints provide a mechanistic insight into hepatotoxicity and can be implemented in future screening for DILI risk. Promising preliminary results from the project will be presented.

SESSION II

Safety Considerations in the Development of Pharmacologically Active Proteins for Rare Diseases

John McNulty, Takeda

Lysosomal storage diseases (LSD) are genetic diseases that are

caused by in-born metabolic errors and result in accumulation of substrates in the lysosomes ultimately leading to cellular damage. There are ~ 50-70 different types LSD, which affect 1 in 5000 live births collectively, but when considered individually are considered rare diseases. These diseases typically are first observed in infant or childhood and have a host of symptoms including progressive neurodegenerative effects. The cause of these clinical findings is generally attributed to functional deficits in lysosomal enzymes or lysosomal membrane proteins. As the lysosome is involved in variety of cellular processes (e.g. catabolism, autophagy, cell signaling), impairment of those pathways leads to deleterious effects in a multitude of cell types. However, as full understanding of the role the lysosome plays is not completely understood, and the etiology and mechanism of LSD are still under investigation further advances, could lead to new experimental treatments. In this presentation, highlights of LSDs and current exploratory treatment approaches will be discussed.

Risdiplam: Fast Development to Turn the First Small Molecule mRNA Splice Modifier into an Approved Medicine

Lutz Mueller, F. Hoffmann-La Roche

Spinal muscular athrophy is a rare disease caused by a mutation in the survival motor neuron gene SMN1. A human rescue gene, SMN2, exists but its output is hampered by the fact that the mRNA splicing machinery kicks out Exon 7 of the transcript, which makes the resulting protein instable. A therapeutic approach was to design a small molecule, which penetrates the CNS well upon oral dosing and could convince the splicing machinery to keep Exon 7 in to generate proper levels of functiona protein. However, as alternative mRNA splicing is a fundamental biological process to generate diversity of the genetic code on the DNA, the generation of a small molecule with high specificity for SMN was almost an unsurmountable task. On the basis of a screening fundament from PTC, a small New Jersey company, Roche and the SMA foundation embarked on a race to generate the first safe small molecule mRNA splice modifier to treat SMA. The molecule, risdiplam, was developed in record time and is in the meantime approved under the name “Evrysdi”. This example is exemplifying the possibilities of small molecule research to treat human diseases. It is also the first time in history that this race to develop medicines has resulted in three different modality options for SMA patients, a gene therapy with Zolgensma delivered intravenously, an antisense molceule, nusinersen, delivered intrathecally and risdiplam, a small molecule. All three medicines are breakthrough examples of modern biomedical research.

The Nonclinical Safety Profile of ONPATTRO® (patisiran), a First-in Class RNAi Therapeutic for the Treatment of Polyneuropathy of Hereditary Transthyretin-mediated Amyloidosis (hATTR amyloidosis)

Jessica Sutherland, Alnylam

Hereditary transthyretin-mediated amyloidosis is a rare, life-threatening autosomal dominant multi-systemic disease caused by mutations in the transthyretin (TTR) gene. These mutations destabilize the tetrameric TTR protein and lead to misfolding and accumulation of amyloid fibrils and plaques in the peripheral nervous system, heart, and gastrointestinal tract. The cardinal manifestations of hATTR amyloidosis are polyneuropathy and cardiomyopathy. Patisiran is a double-stranded small interfering RNA (siRNA) encapsulated in a lipid nanoparticle to enable delivery to hepatocytes. Patisiran binds to a specific genetically-conserved sequence in the 3’untranslated region (UTR) of wild type and mutant transthyretin (TTR) mRNA causing its degradation (via RNA interference). This results in reductions in circulating TTR protein levels and tissue TTR amyloid deposits. In 2018, patisiran was approved by the FDA for the treatment of polyneuropathy of hATTR amyloidosis and by the EMA for the treatment of hATTR with stage 1 or 2 polyneuropathy. This presentation will describe the strategic approaches and results from a comprehensive program of nonclinical studies (ie, safety pharmacology, general toxicology, developmental and reproductive toxicology, and carcinogenicity) that was conducted in mice, rats, rabbits, or monkeys to establish the nonclinical safety profile that supported the approval of patisiran.

SESSION III

Things to Consider when Translating Gene Therapy Nonclinical Data to Support Clinical Trials

Laurence Whiteley, Pfizer

This talk will focus on AAV gene therapy vectors and factors to consider in extrapolating nonclinical dose-related effects to support clinical trial design. AAV vector tissue tropism is driven by the vectors protein capsid and its interaction with multiple cell surface receptors. There can be differences in tropism and biologic response between species and these differences need to be considered in nonclinical study design as well as extrapolating the therapeutic dose and safety margin to clinical trial design. Several examples of species differences, including comparisons between human and animals, will be discussed and impact on nonclinical development strategy.

SESSION IV

Toxic Peripheral Neuropathies: Clinical Perspective

Ahmet Hoke, Johns Hopkins School of Medicine

Peripheral neuropathy remains a significant side effect of many drugs, especially many of the classical chemotherapy agents. Although the symptoms of toxic peripheral neuropathies are similar to other types of peripheral neuropathies, there are several unique features that set them apart. Often the underlying agent dictates the time course and extent of symptoms and although many patients improve upon cessation of the offending agent, more than half of patient who develop toxic peripheral neuropathy remain symptomatic for the rest of their lives. Recognition of neuropathy symptoms and prompt evaluation is critical to decreasing the morbidity associated with neurotoxic drugs. Biomarkers and identification of risk factors are critical to lessen the impact of toxic peripheral neuropathies.

Intra-epidermal Nerve Fiber Analysis: A Sensitive, Clinically-Relevant Tool for Assessing Sensory Nerve Damage in Animal Models

Lisa Mangus, Johns Hopkins School of Medicine

In the past two decades, analysis of intraepidermal nerve fibers (IENF) in skin biopsy samples has become a standard clinical tool for diagnosing small fiber sensory neuropathies in human patients. The techniques developed for quantitative IENF analysis in humans have been adapted for large and small animal models and successfully used in research studies of diabetic neuropathy, chemotherapy-induced peripheral neuropathy (CIPN), and HIV-associated sensory neuropathy, among others. Although IENF analysis has yet to become a routine PNS outcome measure in the regulatory setting, it has potential to serve as a highly relevant marker of sensory nerve fiber status in neurotoxicity studies, as well as development of novel neuroprotective and neuroregenerative therapies. This presentation will provide an overview of IENF analysis in common animal models. Methods for achieving quantitative IENF measurements in different species, including specific protocol adaptations, technical challenges, quantitative approaches, and study design considerations will be discussed.

Improving Preclinical to Clinical Translation of Drug-induced Peripheral Neuropathy: A Case Example with MMAE Containing Antibody Drug Conjugates

Nicola Stagg, Genentech

The valine citrulline monomethyl auristatin E (vcMMAE) ADC platform has shown promising clinical activity in several cancers, but peripheral neuropathy (PN) is a frequent adverse event with conventional linker-drug vcMMAE ADCs, resulting in dose reduction or treatment discontinuation. This was not predicted based on nonclinical toxicology studies in monkeys or rats treated with vcMMAE ADCs. We evaluated possible hypotheses for the lack of translatability of peripheral neuropathy. The result of this hypothesis-based approach identified several challenges with trying to recapitulate MMAE ADC-induced peripheral neuropathy in nonclinical toxicology studies. However, it also enabled us to more systematically develop in vitro and in vivo tools to improve translation. For in vitro testing, we evaluated neuronal toxicity of MMAE across species, nonspecific uptake of ADCs in neurons and molecular mechanisms underlying MMAE and MMAE containing ADC effects on microtubules. We also developed a rat model with a dose and schedule based on clinical exposure-response modeling, an expanded neurohistopathology assessment and measurements of MMAE in peripheral nerves over time. Our goal is to be able to use these tools to screen for risk of drug-induced peripheral neuropathy, and the outcome from testing with MMAE containing ADCs as a case example will be presented.

SPEAKER BIOGRAPHIES

Jule Gust, Seattle Children's Hospital Dr Gust is a pediatric neurologist and physician scientist at Seattle Children's. Her research centers on the effects of systemic illness on the developing brain, with a focus on neurotoxicity of new cancer immunotherapies. She is active in clinical research in pediatric CAR T cell therapy, both for leukemia/lymphoma and brain tumors. In the lab, she currently focuses on solving the mechanisms of CD19-CAR T cell toxicity in an effort to understand where injury occurs at the interface of the brain and the immune system.

Ahmet Hoke, MD, PhD, Johns Hopkins School of Medicine Dr. Hoke is Professor of Neurology and Neuroscience and Director of the Daniel B. Drachman Division of Neuromuscular Diseases at Johns Hopkins University School of Medicine. Dr. Hoke's clinical and research interest focuses on peripheral nerve diseases and nerve regeneration. He is the recipient of several awards including Derek Denny Brown Young Neurological Scholar Award (2005) and Wolfe Neuropathy Research prize (2018) given by the American Neurological Association, Myung Memorial Lecture Award (2017) by the Korean Neurological Association and Nejat Eczacibasi Medical Scientist Award (2019) by the Eczacibasi Foundation, Turkey. He is on the Board of Directors of American Neurological Association and Peripheral Nerve Society and Vice-President of the Toxic Neuropathy Consortium. He serves on several editorial boards and is the Editor-in-Chief of Experimental Neurology and Associate Editor for Annals of Clinical and Translational Neurology.

Dr. Hoke's research interest includes studies on biology of peripheral axons and Schwann cells and disorders affecting the peripheral nervous system. He uses in vitro and in vivo models of peripheral neuropathies (chemotherapy induced peripheral neuropathy, HIV-associated sensory neuropathy, and diabetic neuropathy) to study the mechanism of axonal damage and identify therapeutic targets for drug development. In addition, he has a research interest on mechanisms of axonal regeneration, specifically on chronic nerve injury models that recapitulate the challenges of human nerve regeneration.

Juliette Hordeaux, DVM, PhD, DECVP, UPenn Dr. Hordeaux has 10 years of experience in AAV-mediated gene therapy and translational research. She has a French Veterinary Medical Degree, has been European-board certified in Veterinary Pathology since 2011, and received her PhD from the University of Nantes. She has been working in Jim's Wilson Gene Therapy Program for 5 years and currently leads a team of Research Directors, Investigators, and Specialists developing cutting-edge AAV gene therapies and working with internal Core facilities and external Industry partners to collect quality and focused preclinical data. She led or co-led three programs through successful IND applications. Her team also investigates AAV-mediated toxicity, and ways to develop safer gene therapy modalities. She oversees the GTP Histopathology Core that supports Research and Discovery activities as well as GLP-compliant toxicology studies. Juliette authored or co-authored 20 peer-reviewed articles, is co-inventor on several patent applications, and is a regular invited speaker in events discussing AAV-mediated toxicity. She serves as a peer-reviewer for several journals such as Human Molecular Genetics, Human Gene Therapy, the Journal of Veterinary Internal Medicine, and Plos One.

Sushma Jadalannagari, PhD, Emulate Dr. Jadalannagari is a Senior Scientist in the Applied BioSciences Team at Emulate, Inc. She leads a team responsible for the design and execution of studies to evaluate safety and efficacy of pharmaceutical compounds on Emulate's flagship Organ-Chip models. Sushma also worked on the development and launch of Proximal Tubule Kidney Chip for applications in investigative toxicology.

Prior to Joining Emulate, Sushma worked as a Lead Scientist in a start-up company designing and developing novel antibacterial hydrogels to promote infection free wound healing. She was instrumental in developing the company's first product and securing

several million-dollar grants from NIH and DoD. Sushma is a talented scientist with expertise in Tissue Engineering, Regenerative Medicine, Biomaterials and Nanomedicine. She received her PhD in Bioengineering from University of Kansas and has been a primary author on several research articles and patents.

Tomomi Kiyota, PhD, Genentech Dr. Tomomi (Tomo) Kiyota is a Senior Scientist in Investigative Toxicology, Safety Assessment at Genentech, where he has been developing novel in vitro approaches and conducting hypothesis-driven mechanistic investigation to de-risk safety concerns of various modalities for supporting portfolios across therapeutic areas at the discovery and development stages. Tomo has an adjunct appointment in the Department of Pharmacology and Experimental Neuroscience at University of Nebraska Medical Center (UNMC), where he was an Instructor and studied mechanisms of neurodegenerative diseases and viral vector-mediated gene therapy. Tomo received his Ph.D. degree from Kwansei Gakuin University, Japan, and completed his postdoctoral training at Florida State University and UNMC.

Lisa Mangus, DVM, PhD, DACVP, Johns Hopkins School of Medicine Lisa Mangus is a board-certified veterinary anatomic pathologist and assistant professor in the Department of Molecular and Comparative Pathobiology in the Johns Hopkins University School of Medicine. She graduated from the Ohio State University College of Veterinary Medicine in 2009, after which she completed an internship in small animal surgery and medicine at the VCA Veterinary Referral Associates in Gaithersburg, MD. Dr. Mangus did her comparative anatomic pathology residency in the Department of Molecular and Comparative Pathobiology (MCP) at Johns Hopkins University and joined the American College of Veterinary Pathologists as a diplomate in 2014. Concurrently with her anatomic pathology training, Dr. Mangus studied neuroimmunology and HIV-associated sensory neuropathy in the SIV-macaque model under the mentorship of Dr. Joseph Mankowski. Since completing her PhD, she has continued to work on projects investigating the effects of HIV infection and antiretroviral therapy on the peripheral nervous system, with an emphasis on correlating functional, molecular, and morphologic markers of nerve damage, including intra-epidermal nerve fiber loss. In addition to HIV research, Dr. Mangus has a special interest in exotic and aquatic veterinary pathology.

John McNulty, PhD, DABT, Takeda Dr. John McNulty is the Drug Safety Therapeutic Lead for Rare Disease at Takeda in Cambridge, Ma. In this role, he overlooks the Rare Disease portfolio which encompasses multiple modalities, such as gene therapy, enzyme replacement therapies, monoclonal antibodies, and small molecules, these modalities span from target evaluation through registration and expansion into global markets. He has been at Takeda since 2019, and was employed at Shire and AstraZeneca employed as a Project Toxicologist on programs supporting a multitude of indications and prior to that he started his industry career at Schering Plough as GLP Study Director. He received his Ph.D. at the University of Connecticut and following that he conducted Post-Doctoral research at Yale School of Medicine.

Scott W. Mittelstadt, PhD, AbbVie Dr. Mittelstadt has been at Abbott/AbbVie since 2007. In his present role, he leads the Investigative Toxicology and Safety Pharmacology Departments at AbbVie. He served as a board member of the Safety Pharmacology Society and as the society's president in 2002. He has served on a number of other industry working groups including the PhRMA DruSafe QT Interval Task Force and the ILSI/HESI Cardiovascular Safety Subcommittee.

Dr. Mittelstadt began his career with Procter & Gamble Pharmaceuticals in 1994 with the company's Drug Safety Assessment Group. During his 12-year tenure with P&G, he worked as a Principal Scientist and managed the Safety Pharmacology Group, supporting both discovery and development. He was a Postdoctoral Fellow at the Medical College of Wisconsin and graduated from the University of Missouri with a Doctorate in Physiology.

Nathan Moerke, PhD, STEMCELL Technologies Dr. Moerke holds a B.Sc. in Biochemistry from the University of Minnesota, and a Ph.D. in Biological Chemistry and Molecular Pharmacology from Harvard University. Previously he worked in the areas of cancer drug discovery and systems pharmacology at Harvard Medical School, and neuroscience drug development at Denali Therapeutics. Currently, Nathan is a scientist in the Contract Assay Services group at STEMCELL Technologies.

Lutz Mueller, PhD, F Hoffman-La Roche Dr. Mueller serves as a toxicology project leader since 17 years at F. Hoffmann La Roche in Basel, Switzerland. His expertise includes small and large molecules, antisense moeties and AAV gene therapy projects. Prior to Roche, he spent a few years at Novartis in Basel. His career originated in the BfArM, the German equivalent of the FDA in Berlin, Germany where he headed a section on genotoxicity and carcinogenicity. Since 1991, he served on several groups drafting ICH guidelines, including the mutagenicity, carcinogenicity and mutagenic impuritiy guidelines. With now 37 years in toxicology, he has almost seen it all. Most recently, he was a major driving force behind the non-clinical development of the first mRNA splice modifier, risdiplam, which is now approved under the band name "Evrysdi" for treatment of spinal muscular atrophy, SMA.

Suman K. Mukherjee, PhD, Merck Dr. Mukherjee is a Director in the Safety Assessment and Laboratory Animal Resources (SALAR) organization at Merck in West Point PA. Suman received his Ph.D. in Molecular Pharmacology and Toxicology from the School of Pharmacy at University of Southern California and then was a Post-doctoral Fellow in Hematology and Oncology at Children's Hospital Los Angeles. After five years as a faculty member at the College of Pharmacy at South Dakota State University, he joined Merck in 2005. He has been the SALAR representative on a number of drug discovery and development teams and has contributed to several successful filings and registrations. Suman currently leads the Biochemical Toxicology and Toxicokinetics group that is responsible for generating, interpreting and reporting toxicokinetic data for parent molecules and metabolites in GLP and non-GLP toxicity studies with small and large molecules. The group is also responsible for conduct of all the PK studies that support drug discovery programs across all therapeutic areas.

Dan Nomura, PhD, Univ of California at Berkeley Dr. Nomura is a Professor of Chemical Biology in the Department of Chemistry at the University of California, Berkeley and an Adjunct Professor in the Department of Pharmaceutical Chemistry at UCSF. Since 2017, he has also been the Director of the Novartis-Berkeley Center for Proteomics and Chemistry Technologies focused on using chemoproteomic platforms to tackle the undruggable proteome. He is also Co-Founder and Head of the Scientific Advisory Board of Frontier Medicines. Since 2018, he has also been an Associate Editor for Cell Chemical Biology. He earned his B.A. in Molecular and Cell Biology and Ph.D. in Molecular Toxicology at UC Berkeley with Professor John Casida and was a postdoctoral fellow at Scripps Research with Professor Ben Cravatt before returning to Berkeley as a faculty member in 2011. Among his honors are selection as a Searle Scholar, American Cancer Society Research Scholar Award, the Department of Defense Breakthroughs Award, Eicosanoid Research Foundation Young Investigator Award, and the Mark Foundation for Cancer Research ASPIRE award.

Lisa Plitnick, PhD, Merck Dr. Lisa Plitnick is a Distinguished Scientist in the Department of Safety Assessment and Laboratory Animal Resources in Merck Research Laboratories, Merck & Co., Inc. Lisa joined MRL in 2002 and currently serves as the Therapeutic Area Lead for Vaccines and a Nonclinical Safety Lead on vaccine and biologic development teams. Lisa is also the Scientific and Regulatory Advisor for the in Vivo Biologics Release Testing Group in the Merck Manufacturing Division. Her Therapeutic Area Lead responsibilities include oversight of more than 20 programs including traditional and novel vaccine platforms from early nonclinical development through post-marketing. As a Nonclinical Safety Lead, Lisa has been responsible for small molecule, biologic and vaccine programs in various therapeutic areas including infectious diseases, atherosclerosis, diabetes, asthma, osteoporosis and obesity. Lisa has extensive experience preparing and reviewing regulatory submissions (agency interactions and documents supporting early clinical studies through marketing). Of note, Lisa was the nonclinical lead

for the successful NDA/BLA filings for a monoclonal antibody, a biosimilar and a vaccine. Lisa is co-editor of a book entitled Nonclinical Development of Novel Biologics, Biosimilars, Vaccines and Specialty Biologics and has written and/or contributed to book chapters on biologics and vaccines and peer-reviewed journal articles. Lisa is also a member of the BioSafe Specialty Biologics Expert working group for vaccines and the HESI ILSI Immunotoxicology Technical Committee. Lisa received a B.A in Biology from The State University of New York at Oswego and an M.S. and a Ph.D. in Immunology from the Albany Medical College. Following her graduate work she did a postdoctoral fellowship with a focus on Immunotoxicology in the Curriculum in Toxicology at the University of North Carolina at Chapel Hill which was part of a Cooperative Research and Development Agreement with the US EPA, Dow Chemical and DuPont.

Kavita Raman, PhD, Amgen Dr. Raman obtained her PhD in Microbiology and Immunology from the University of Arizona, followed by post-doctoral training at the University of Michigan and San Francisco Veterans Affairs Medical Center (SF VAMC)/ Univ. of California, San Francisco (UCSF). She started her career in the pharmaceutical industry at Medivation (now part of Pfizer), and went on to join Abbvie. She joined Amgen in South San Francisco in 2019 as part of the Nonclinical Safety Sciences group in Translational Biology and Bioanalytical Sciences. She is a project toxicologist for multiple programs including antibodies, T cell engagers and engineered T cells. She also serves as Amgen representative on consortia such as IQ DruSafe, HESI and the CAR-T Consortium.

Jacintha Shenton, PhD, Janssen Dr. Shenton is a Senior Scientific Director at Janssen R&D where she is the Immuno-Oncology Therapeutic Area Lead for Nonclinical Safety (NCS). This includes advising NCS Leads working on both discovery and development stage projects and an understanding of diverse modalities including CD3 bispecific and CAR-T cell therapies. Previously, Jacintha held Project Toxicologist roles at Novartis, MedImmune (AstraZeneca), and Bristol-Myers Squibb where she worked on biologics across several therapeutic areas including immuno-oncology. Jacintha has published several papers and book chapters in the areas of nonclinical safety and immunotoxicology and participates in external organizations including the BioSafe Leadership Committee and the HESI Immunotoxicology Technical Committee (ITC). In 2018, Jacintha co-chaired a HESI-ITC/FDA workshop on 'Preclinical and Translational Safety Assessment of CD3 Bispecifics'. Jacintha earned a Ph.D. in Pharmaceutical Sciences at the University of Toronto and completed a postdoctoral fellowship at Health Canada.

Nicola Stagg, PhD, DABT, Genentech Dr. Stagg is a DABT Senior Scientist/Toxicologist and Toxicology Oncology Therapeutic Area Lead in Safety Assessment at Genentech. In this role, she provides functional oversite of the preclinical safety strategies and outcomes for the oncology portfolio, manages the toxicology analyst group and a Senior Scientist/Toxicologist, serves as a pharmacology subteam lead for a cancer immunotherapy biotherapeutic and lead toxicologist for several ADCs, biotherapeutics and small molecules from discovery to clinical development in different therapeutic areas. Prior to joining Genentech, Dr. Stagg worked as a Manager of Toxicology at Agensys, LLC, the oncology division of Astellas Pharma on ADCs and other biotherapeutics and as a Senior Toxicologist at Dow Agrosciences on pesticides and biotechnology derived proteins for genetically engineered crops. Dr. Stagg received her doctorate in the field of Pharmacology and Toxicology from the University of Arizona with a focus on peripheral neuropathy. Dr. Stagg is a diplomate of the American Board of Toxicology (DABT) and has ~14 years of experience as a toxicologist in industry (biopharma/pharma and other) conducting GLP/non-GLP nonclinical toxicology assessments for human health safety in support of global regulatory filings. She is author of 18 peer-reviewed journal articles, 2 patents and over 30 external presentations/conference chair positions.

Jonathan Stauber, PhD, ImaBiotech Dr. Stauber developed Precision Drug efficacy Laboratories in order to improve translational research and clinical services, optimize dose selection and anticipate lack of efficacy in animal models and patients. He oversees the development of innovative technologies of Mass Spectrometry Imaging and histology to quantify drug exposure and impact

into biomarkers simultaneously at the site of action in human and animal models. ImaBiotech conducted 400 drug development and clinical studies in two facilities in Boston and France.

Prior to establishing ImaBiotech, Dr. Stauber has contributed to the development of molecular imaging technologies by mass spectrometry as a routine technique with quantification and quality management FOM institute in Amsterdam. Dr. Stauber has received a PhD in biochemistry and analytical chemistry from the University of Lille in France and University of California in San Diego; as well as receiving a Masters degree in Business Administration from the University of Lille in France. He has more than 30 publications in international journals and awarded several patents based on the development of Quantitative Mass Spectrometry Imaging for drug discovery and development applications.

Richard Stebbings, PhD, AstraZeneca Dr. Stebbings is Director of Oncology Safety Science & Discovery at AstraZeneca supporting Immuno-Oncology therapeutics, Cell Therapies and Antibody Drug Conjugates (ICA), leading a team focused on early safety assessment and the development of new safety platforms. Richard is also the Nonclinical Lead for COVID-19 Vaccine AstraZeneca and has an extensive academic background in preclinical HIV vaccine efficacy studies in NHPs. His previous roles included Project Toxicologist at AstraZeneca, Visiting Professor at the University of Liverpool and Head of the Immunotoxicology Lab at the UK's National Institute for Biological Standards & Control. However, he is best known for his seminal work on understanding the cytokine release syndrome caused by the therapeutic mAb TGN1412 at a phase I clinical trial in the UK. This work is cited by FDA guidelines and the in-vitro assay he developed to predicted "cytokine storm" are now widely applied to therapeutic mAbs.

Jessica Sutherland, PhD, DABT, Alnylam Pharmaceuticals Dr. Sutherland is Senior Director of Toxicology at Alnylam Pharmaceuticals, Inc. in Cambridge, MA (USA) where she has conducted nonclinical safety evaluations of several candidate RNAi therapeutics including ONPATTRO™ which is the first approved RNAi therapeutic. Formerly, she was an Associate Director of Toxicology at Charles River Laboratories in Shrewsbury, MA. Dr. Sutherland is a Diplomat of the American Board of Toxicology (ABT) and is a member of the American College of Toxicology (ACT) and the Society of Toxicology (SOT). Dr. Sutherland received her BS in biology from Northern Michigan University, an MS in biology from the University of North Dakota and a PhD in Environmental Toxicology from the University of Wisconsin-Madison. She received a National Research Service Award from the National Institute of Environmental Health Sciences (NIEHS) and conducted her postdoctoral research in the department of Environmental Medicine at New York University.

John L. Vahle, DVM, PhD, DACVP, Eli Lilly Dr. Vahle received his veterinary medical degree from the University of Missouri in 1988 and his doctorate in veterinary pathology from Iowa State University in 1996. He became a Diplomate of the American College of Veterinary Pathologists in 1995 and joined Lilly as a senior pathologist in 1996. John has used his experience in pathology, toxicology, and regulatory affairs in a variety of roles including toxicology project leader, project pathologist, and regulatory toxicology advisor. In addition to nonclinical safety assessment, John has focused his efforts in the area of carcinogenicity assessments of biotherapeutics and bone toxicology and pathology. He currently serves as a Senior Research Fellow at Lilly. Dr. Vahle is active in various professional societies and consortia and is a past Councilor of the STP Executive Committee, past Chair of the BioSafe Organization, and a member of the steering committee for the international toxicologic pathology nomenclature initiative, INHAND. Based on his experience in carcinogenicity testing, John serves as a member of the ICH Expert Working Group assessing carcinogenicity testing paradigms for pharmaceuticals. Dr. Vahle's publications have focused on toxicologic pathology of the endocrine and musculoskeletal systems as well as approaches to carcinogenicity assessment.

Andy H. Vo, PhD, AbbVie Dr. Vo received a bachelor's degree in Cell and Molecular Biology from the University of Michigan and a doctorate in Developmental Biology at the University of Chicago. During his doctoral training, he investigated genetic landscapes relating to muscle and heart by integrating large genomic datasets and various computational methods. He is currently leading the computational toxicology team at AbbVie where he is focused on the development of artificial intelligence and machine learning methods to enhance drug safety evaluation through in silico methods. He has presented his research findings at multiple scientific conferences and has published over 20 peer reviewed manuscripts.

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 as predictive and mechanistic tools to help make safer 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.

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 and a debilitating addiction to home improvement projects.

Larry Whiteley, DVM, PhD, DACVP, Pfizer Dr. Whiteley has 30 years or experience in supporting the nonclinical safety assessment and clinical development of a broad range of therapeutic modalities. For the past 12 years he has had a focus on supporting modalities that modulate gene expression including viral based gene therapy. Larry's current role in Pfizer's nonclinical drug safety organization is the Global Pathology and Investigative Toxicology Scientific and Strategic Advisor, as well as Pathology Therapeutic Area Lead for Rare Disease. As an asset team toxicologist he has guided several gene therapy programs from preclinical to Phase III clinical development.

POSTER ABSTRACTS

A Combined in vitro Approach for the Dual Detection of Functional and Structural Cardiotoxicity

Stephanie Ryder, Samantha Bevan, Benjamin Park, Andrea Lavado, Paul Walker
Cyprotex Discovery Ltd, BioHub at Alderley Park, Cheshire, U.K. SK10 4TG

Cardiotoxicity is a major cause of drug attrition during pre-clinical and clinical drug development. Drugs can exhibit functional changes defined as an acute alteration in the mechanical function of the myocardium or structural in nature as defined by morphological damage to cardiomyocytes and/or loss of viability. In recent years in vitro strategies have been developed to allow the high throughput assessment of functional cardiomyocyte changes through kinetic monitoring of calcium transients, while structural morphology can be monitored in a high throughput manner using high content imaging (HCI). Here we describe a combined method to allow the morphological analysis of cardiomyocytes alongside calcium transient assessment in order to better define functional and structural cardiotoxicity events. Human induced pluripotent cardiomyocytes (hiPS-CMs) are seeded in 384 well plates for a minimum of 10 days before incubation with EarlyTox Cardiotoxicity (Molecular Devices) fluorescent dye. Following a 2 hour incubation, compound is applied at 8 concentrations in triplicate utilising a compound set comprising structural and functional cardiotoxins alongside dual toxicity compounds and non-cardiotoxins (total of 14 compounds). Fast kinetic fluorescent reading is then performed on a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek). High content imaging of calcium homeostasis (EarlyTox) and mitochondrial function (TMRE) is then performed using an ArrayScan XTI HCI reader (ThermoScientific). Finally, cells are lysed for the quantification of intracellular ATP using CellTiter-Glo (Promega). This combined assay approach allows the detection of acute functional cardiotoxicity; epinephrine and isoproterenol increase calcium transient peak frequency (MEC; 0.006 μM & 0.005 μM, respectively) while verapamil decreases peak frequency and amplitude (MEC; 0.03 μM & 0.02 μM, respectively). Early signs of morphological changes can also be detected; sunitinib is a dual toxicity compound (functional and structural) which exhibits a decrease in peak calcium transient amplitude (MEC; 88 μM) alongside cell morphology changes (MEC; 144 μM). This study shows a combined cellular assessment strategy can improve the in vitro to in vivo translation and risk assessment of the potential for novel compounds to elicit functional and structural cardiotoxic events.

Transcriptomic Profiling of in vitro 2D and 3D Models to Predict Drug Induced Liver Injury (DILI)

Paul Walker, Ruediger Fritsch, Alicia Rosell-Hidalgo, You Feng, Rene Rex, Maiara Severo Witte, Timur Samatov, Andrea Lavado, Stephanie Ryder, Ryan Barton, Christopher Strock

Drug-induced liver injury (DILI) remains a major concern for drug development programs, due to the risk of late stage clinical trial failures and post-marketing withdrawal. A variety of in vitro approaches have been used in an effort to improve prediction of DILI earlier in discovery, such as organotypic three-dimensional (3D) microtissue combined with High-Content Analysis (HCA). Mechanistic understanding of DILI can be gained by combining the most predictive and physiologically relevant in vitro models with analysis through high-throughput RNA sequencing to deliver more comprehensive toxicity profiles. Transcriptomics has been shown to play an important role in determining differentially expressed genes (DEGs), mechanisms of action and induced cell stress pathways associated with drug exposure. A pipeline has been established to fully automate screening compound libraries, a 128 reference drug library with and without clinical DILI compounds have been profiled across an eight dose response range at multiple time points including 14 day incubations. The models evaluated are HepaRG cells and primary human hepatocytes (PHH) in both 2D and as 3D organoids. The transcription profiles obtained allowed grouping of DILI positive and negative compounds into functional clusters by PCA (principal component analysis) and t-SNE analysis. DEGs in a dose-dependent manner were observed for DILI compounds and was shown to be mechanism and DILI rank dependent. Machine Learning combined with transcriptomic profiling of PHH allowed the identification of DILI compounds with 81 % of sensitivity, 86% specificity and 82 % accuracy. Also specific gene signatures could be associated with individual mechanisms of action. In summary, predictive toxicogenomics combined with organotypic models can be used to profile novel chemical entities to determine DILI risk, providing insight in to the potential mode of action implicated in the drugs toxicity.

The Human Liver-Chip as a Model for Alcoholic Steatosis

Sushma Jadalannagari[1], Jake Chaff[1], Michael Carleton[2], Heather Hsu[2], James Velez[1], Sannidhi Joshipura[1], David Conegliano[1], S. Jordan Kerns[1], Lorna Ewart[1]
[1] Emulate Inc., 27 Drydock Avenue, Boston, MA 02210, USA
[2] IniPharm,500 108th Ave NE, Bellevue, WA 98104, USA

PURPOSE:
Non-alcoholic and alcoholic fatty liver disease (NAFLD and AFLD) are a growing public health concern1, especially in the United States where it affects one quarter of the adult population[2]. The accumulation of excess fat in the liver causes progressive cell damage and inflammation which in severe forms of steatohepatitis can be toxic and lead to end-stage liver disease. Due to the lack of human relevant preclinical disease models to test lead candidate drugs, there are no clinically approved therapies targeting these diseases. To overcome this, we are developing a model for studying alcoholic steatosis using the Human Liver-Chip to induce cytotoxic levels of lipid accumulation by treating with increasing concentrations of ethanol, an important mediator of disease pathogenesis in AFLD patients.

METHODS:
Human Quad-Culture Liver Chips (n=3 for each condition) were coated with a mixture of rat tail collagen type I and bovine fibronectin. Primary human hepatocytes from two donors (HU8305 and CYC) were seeded at a density of 3.5 million cells/mL in the upper parenchymal channel and later overlaid with Matrigel and incubated at 37°C with 5% CO2. In the lower vascular channel on the opposite side of the porous membrane, human liver sinusoidal endothelial cells (LSECs) (3 million cells/mL), human liver Kupffer (0.5 million cells/mL) and stellate cells (0.1 million cells/mL) were seeded. Two days later, the Chips were connected to the Zoë® Culture Module (Human Emulation System®), and both the Chip channels were perfused at a constant flow of 30 µL/h. On Day 7 post seeding, the Liver-Chips were treated with ethanol at 0.16% or 0.5% (v/v). The Chips were maintained for 11 days with imaging and effluent collection on days 1, 3, 7, 10 and 11. On Day 11 post-treatment, the experiment was terminated, and Chips were fixed for immunofluorescent imaging with AdipoRed™ for lipid droplet accumulation and DAPI in the top channel and α-Smooth muscle actin (SMA) for activated stellate cells and DAPI in the bottom channel.

RESULTS:
A severe time- and concentration-dependent toxic response was observed in both hepatocyte donors, post ethanol treatment. Qualitatively, the ethanol treated groups demonstrated a concentration-dependent increase in lipid droplet accumulation and activated stellate cells, in the HU8305 hepatocytes indicative of cell damage. Similarly, the ethanol treated groups demonstrated an increase in lipid accumulation in the CYC hepatocytes, with similar stellate cell activation between the control and ethanol treated groups in this donor. Additionally, the 0.5% ethanol treated group showed a significant decrease in albumin release and an increase in ALT and triglyceride export in the CYC donor suggesting cytotoxicity from Day 7 post-treatment.

CONCLUSION:
The Human Quad-Culture Liver – Chip model demonstrated a time- and concentration-dependent increase in intracellular hepatic lipid accumulation (steatosis) following ethanol treatment indicative of toxicity. Activation of hepatic stellate cells, albumin secretion, ALT release and triglyceride export were also affected by ethanol treatment along with variability between the donors. Thus, based on the promising preliminary results from this study, further investigation is needed to enable evaluation of therapeutic agent efficacy in this Liver-Chip model of alcohol induced steatosis.

Steatosis as Risk Factor for Drug-induced Liver Injury (DILI)

Radina Kostadinova, Wolfgang Moritz, Bruno Filippi, Agnieszka Pajak, Katarzyna Sanchez, Armin Wolf
InSphero AG, Wagistrasse 27, 8952 Schlieren

BACKGROUND:
The sensitizing effect of steatosis and NASH as general risk factors for human DILI is receiving a lot of attention. Reasons are the high incidence of patients with metabolic diseases in our population and the need for a more differentiated patient specific risk assessment of new drug candidates. Patients with steatosis might be more sensitive and more fragile as healthy subjects. Until now the relationship between DILI and underlying steatotic diseases has not been systematically investigated. The steatosis DILI risk factor hypothesis appears very plausible but still needs clinical endorsement, as well supportive data from in vitro experiments.

METHODS:
Human 3D liver microtissue, containing scaffold free co-culture of primary hepatocytes, Kupffer cells and liver endothelial cells when exposed to free fatty acids in media, high levels of sugars and insulin displayed substantial accumulation of lipids within 7 days of treatment as detected by Nile red staining and triglyceride levels. 3D human liver microtissues have been validated by 108 clinically tested FDA-annotated DILI drugs by the determination of cellular ATP levels showing high specificity and sensitivity (Archives of Toxicology, vol. 91, 2849-2863, 2017).

RESULTS:
It was the purpose of the present study to evaluate the cholestatic drug Chlorpromazine and the non-cholestatic drug Acetaminophen under steatosis and non-steatosis conditions in human 3D liver microtissues. Under steatosis conditions the cytotoxicity of the cholestatic drug Chlorpromazine was significantly enhanced compared to non-steatosis conditions. The cytotoxicity of the non-cholestatic Acetaminophen under same conditions was not enhanced.

CONCLUSION:
These preliminary results suggest that drug-induced cholestasis together with underlying steatosis can enhance DILI and might be a risk factor for the expression of DILI in patients. The increased cytotoxicity of cholestatic drugs might come from the intracellular accumulation of cytotoxic bile acids. To better understand this relationship, further investigations of cholestatic and non-cholestatic drugs under steatotic and non-steatotic conditions are necessary.

ABOUT OUR SPONSORS

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

EMULATE INC. is a privately held company that creates living products for understanding how diseases, medicines, chemicals, and foods affect human health. Our Human Emulation System™ — which is comprised of Organ-Chips, instrumentation, and apps — sets a new standard for recreating true-to-life human biology and is being used to advance product innovation, design, and safety across a range of applications including drug development, agriculture, cosmetics, food, and chemical-based consumer products.

STEMCELL TECHNOLOGIES Contract Assay Services (CAS) is a contract research organization established within STEMCELL Technologies that performs assay services based on in vitro and in vivo primary cell-based assays. CAS specializes in the in vitro hematopoietic colony-forming unit (CFU) assay (also known as the colony-forming cell (CFC) assay) for assessing hematotoxicity. New assays include 96-well HemaTox™ assays to assess lineage-specific effects of test articles on hematopoietic progenitor cells, and T-cell focused immune assays. CAS also performs mesenchymal, immunological and custom cell-based assays. Stop by our booth to learn more about how we can help you with your drug discovery and development needs.

Thank you to all of our Organizers, Speakers, Sponsors and Delegates! Without your dedication, support and participation APT 2021 would not be possible. We greatly value your comments regarding APT 2021 as well as thoughts or suggestions for improving future conferences. Please take the time to fill out our online survey when we send it to you next week.

Sincerely,

The Boston Society