APPLIED PHARMACEUTICAL TOXICOLOGY

2019

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ORGANIZERS' WELCOME

Welcome to the 2019 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.

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Chair-Elect: Zoe Zhong, Genentech

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Eunice Musvasva, Boehringer Ingelheim
Vito Sasseville, Novartis
Nardos Tassew, Genentech

APT 2019 CONFERENCE AGENDA

TUESDAY, MAY 14

12:00 - 1:00 Registration

1:00 - 1:10 **Conference Opening and Plenary Lecture Introduction**
Christine Karbowski, Amgen

1:10 - 1:55 **Plenary Talk: Big Data Meets Drug Discovery**
Astrid Ruefli-Brasse, 23andMe

DISCOVERY TOXICOLOGY WORKSHOP

SESSION I: Machine Learning/AI/Computational Approaches in Toxicology

Chairs: Yoav Timsit, Blueprint Medicines and Christine Karbowski, Amgen

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

2:00 - 2:30 **Modeling of Big and Complex Data in Pharmaceutical Target and Chemical Safety Assessment**
Matt Martin, Pfizer

2:30 - 3:00 **Practical use of Computational Approaches to Impact Pipeline Programs with Potential Toxicology Concerns**
Falgun Shah, Merck

3:00 - 3:30 **So You Think You've Got Hematotoxicity...**
Mary Huber, STEMCELL Technologies

3:30 - 3:55 Break

3:55 - 4:25 **Using Data Science and AI to Improve the Safety of New Drug Candidates**
Nigel Greene, AstraZeneca

4:25 - 4:55 **Utility of Digital Pathology, Image Analysis, and Machine Learning in Toxicologic Pathology**
Famke Aeffner, Amgen

4:55 - 6:25 **POSTER SESSION AND RECEPTION**

WEDNESDAY, MAY 15

7:00 - 8:00 Registration & Breakfast

DISCOVERY TOXICOLOGY WORKSHOP

SESSION II: Non-Clinical to Clinical Translation

Chairs: Padma Narayanan, Ionis Pharmaceuticals and Jon Maher, Genentech

8:00 - 8:05 **Session Introduction**

8:05 - 8:35 **Non-clinical to Clinical Translation of Toxicity Associated with an Antibody-Drug Conjugate**
Lise Loberg, AbbVie

8:35 - 9:05 **Investigation of the Mechanism of a Small Molecule-Associated Coagulopathy**
Satoko Kakiuchi-Kiyota, Genentech

9:05 - 9:35 **Clinical Translation of Non-clinical Target Organ Toxicity: The When and Why Nots**
Ken Frazier, GSK

9:35 - 9:55 **Break**

SESSION III: Organ on a Chip/3D Cultures

Chairs: Michael Santostefano, Merck and Zoe Zhong, Genentech

9:55 - 10:00 **Session Introduction**

10:00 - 10:30 **Application of iPS-Derived Neural Spheroids In Drug Discovery**
Matthew Wagoner, Takeda

10:30 - 11:00 **Utility of Complex in vitro Liver Systems in Assessing the Safety of Drugs**
Jose Lebron, Merck

11:00 - 11:30 **An Industry Perspective on use of MPS in Drug Safety Assessment**
Terry Van Vleet, AbbVie

11:30 - 11:50 **Panel Discussion**

11:50 - 12:50 **Lunch**

DEVELOPMENT WORKSHOP

12:50 - 1:00 **Intro to Development Workshop and Plenary Lecture**
Florence Lorget, Sangamo Therapeutics

1:00 - 1:40 **Plenary Talk:** **Preclinical Development of Gene Therapy Products: CBER Considerations**
Mercedes Serabian, FDA

SESSION IV: Cell and Gene Therapy/New Modalities/Regulatory Interactions

Chairs: Vito Sasseville, Novartis and Eunice Musvasva, Boehringer Ingelheim

1:40 - 1:45 **Session Introduction**

1:45 - 2:15 **Use of Animal Models of Disease to Optimize Translation of Cell and Gene-Based Therapies**
Joy Cavagnaro, AccessBio

2:15 - 2:45 **Biodistribution and Immunogenicity Assessments to Support Gene Therapies: Challenges and Opportunities**
Mark Milton, Novartis

2:45 - 3:15 **Cell and Gene Therapy Product Development: A Funder's Perspective**
Abla Creasey, CIRM

3:15 - 3:35 **Break**

3:35 - 4:05 **Nonclinical Studies Supporting Development of ZFN-Based Genomic Medicines for MPS II**
Kathy Meyer, Sangamo Therapeutics

4:05 - 4:35 **Regulatory Considerations for Nonclinical Safety Assessment of GalNAc-conjugated siRNAs**
Joe Chichocki, Alnylam

4:35 - 4:55 **Panel Discussion**

4:55 - 6:25 **POSTER SESSION AND RECEPTION** Biomere reachbio RESEARCH LABS

THURSDAY, MAY 16

7:00 - 8:00 Registration & Breakfast

DEVELOPMENT TOXICOLOGY WORKSHOP

SESSION V: Predictive Toxicology

Chairs: Paul Cornwell, Eli Lilly and Nardos Tassew, Genentech

8:00 - 8:05 **Session Introduction**

8:05 - 8:35 **Immunogenicity Prediction - Strategies and Challenges**
Valerie Quarmby, Genentech

8:35 - 9:05 **Predictive Value of Nonclinical Studies To Support FIH**
Tom Monticello, Amgen

9:05 - 9:35 **Privacy-preserving Knowledge Transfer from Corporate Data to Federative Models**
Thierry Hanser, Lhasa Limited

9:35 - 9:55 Break

SESSION VI: Big Data and New Technologies

Chairs: Rebecca Erickson, Denali Therapeutics and Lauren Mihalcik, Amgen

9:55 - 10:00 **Session Introduction**

10:00 - 10:30 **Toxicological Transfer Learning in AI: Combining Classification Datasets to Make Better Predictions**
Thomas Hartung, Johns Hopkins Blomberg School of Public Health

10:30 - 11:00 **Accurate Prediction of Animal Toxicity Endpoints: All Things Considered in the Age of Big Data**
Ivan Rusyn, Texas A&M

11:00 - 11:30 **Using Population-Scale Genetic Databases to Inform Target and Drug Safety**
Luke Ward, Alnylam

11:30 - 12:00 **3D Printing of Pharmaceuticals and Accelerating Drug Development**
Jae Yoo and Don Wetherhold, Aprecia Pharmaceuticals

12:00 - 12:20 **Panel Discussion**

12:20 - 12:25 **Closing Remarks**

ABSTRACTS

PLENARY

Big Data Meets Drug Discovery
Astrid Ruefli-Brasse, 23andMe

23andMe, the leading personal genetics company, exists to help people access, understand and benefit from the human genome. Through it's research program 23andMe has an engaged cohort of millions of consented participants who have contributed a wealth of genotypic and phenotypic information, with the goal of improving human health through advances in genomics. 23andMe's research platform is currently the world's largest consented, re-contactable database for genetic research. To date, we have collected over two billion phenotypic data points. These data will lead to a better understanding of the biological mechanisms of disease, and accelerate the discovery of novel treatments through human genetics.

DISCOVERY TOXICOLOGY WORKSHOP

SESSION I

Practical Use of Computational Approaches to Impact Pipeline Programs with Potential Toxicology Concerns
Falgun Shah, Merck

In recent years, number of publications has highlighted an emerging role of predictive toxicology approaches in reducing compound attrition due to safety aiming towards a fewer animal tests. This presentation will focus on how we at Merck are using prioritized computational toxicology tool set to guide both reactive problem solving, and proactive early avoidance of toxicity risk to deliver better molecules faster. We will describe how we leverage in silico toxicology models on high safety risk targets in combination with ancillary pharmacology and in vitro experiments to flag potential toxicology liabilities. We will also illustrate a use case on how mining of preclinical safety/post-market data can help validate/overcome a perceived safety risk and enable progression of a medicinal chemistry project further in a research pipeline.

So You Think You've Got Hematotoxicity...
Mary Huber, STEMCELL Technologies

Hematotoxicity (neutropenia, anemia or thrombocytopenia) is common in anti-proliferative drugs such as cancer and anti-viral therapeutics. This undesired side effect can be mitigated during the drug development pathway, but in some cases, hematotoxicity can manifest unexpectedly in clinical trials. Various in vitro assays exist to probe the effects of therapeutics on the proliferation and differentiation capacity of the hematopoietic system. The power of these assays lies in the use of physiologically relevant primary cell types, including hematopoietic stem cells and progenitors from bone marrow, cord blood, and peripheral blood. The unique advantages of each of these hematopoietic assays and their appropriate applications in the drug development pathway will be discussed.

Using Data Science and AI to Improve the Safety of New Drug Candidates
Nigel Greene, AstraZeneca

Drug discovery and development is a multi-parameter optimization problem that requires a fine balance between efficacy, absorption, distribution, metabolism, excretion and safety. Although the number of clinical safety failures has been reduced through the introduction of in silico, in vitro and early in vivo screening, there are still some significant improvements that could be made. There are strong economic drivers to reduce the costs of discovering new medicines and data science and artificial intelligence is a potential method to both improve the safety profile of new drugs as well as reduce the costs and time to bring these to the clinic. This talk will highlight some of the current investments in computational methods and highlight some of the key gaps in realizing these benefits.

Utility of Digital Pathology, Image Analysis, and Machine Learning in Toxicologic Pathology
Famke Aeffner, Amgen

Digital pathology comprising whole slide imaging (WSI) has been transformative in clinical diagnostics and research, however, it is still in rather earlier adoption stages in some

aspects of toxicologic pathology. Nevertheless, the "future of digital pathology" is here, and remote slide evaluation, effective computational digital tissue image analysis, and implementation of machine learning approaches in the context of toxicologic pathology in drug development are becoming increasingly common. Utilization of these powerful platforms has also created avenues to use these tools in novel innovative ways. This presentation gives a brief overview of the present status of digital pathology and tissue image analysis, as well as highlight examples of machine-learning approaches in pathology by pharma companies.

SESSION II

Investigation of the Mechanism of a Small Molecule-Associated Coagulopathy

Satoko Kakiuchi-Kiyota, Genentech

Although prothrombin time (PT) and activated partial thromboplastin time (aPTT) are routinely measured to assess blood coagulation in preclinical general toxicity studies, there are few reports of small molecules with off-target activity that results in clinically significant prolongation in these screening tests. However, after 7-day repeat dosing of compound X in the Cynomolgus monkey, dose- and time-dependent increases in PT and aPTT times were identified, which correlated with clinical signs of a coagulopathy; and macroscopic/microscopic evidence of hemorrhage in multiple organs were noted at all doses. These findings were not recapitulated in rats at comparable exposures. To determine the mechanism of toxicity, more in depth coagulation studies were performed using aliquots of banked study citrated plasma. Coagulation factor assays revealed marked decreases in Vitamin (Vit) K-dependent factors (FII, FIX, FX, Protein C) without significant decreases in non-Vit K-dependent factors (FV, FXI), suggesting selective perturbation of Vit K metabolism. When high-dose group plasma was mixed with control group plasma (1:1), PT times corrected to normal, indicating that hemorrhage and prolonged clotting times were due to factor deficiencies and not factor inhibition. This observation correlated with results from follow-up in vitro spiking studies whereby compound X was added to normal monkey or human plasma prior to PT testing. No compound-induced in vitro prolongation of clotting time occurred, demonstrating that compound X does not directly interact with clotting factors. Our results to date suggest that compound X may impair the activity of recycling enzymes associated with hepatic Vit K metabolism. One metabolite with some structural similarity to anti-coagulants (e.g. warfarin), which inhibit Vit K epoxide reductase, a key enzyme of the Vit K cycle, was formed in higher amounts in cyno compared to in other species. Taken together, this metabolite may account for coagulation time prolongation and the observed species differences.

Clinical Translation of Nonclinical Toxicities: The When and Why Nots

Ken Frazier, GSK

It has been noted that a positive concordance rate of 71% can be achieved when data from both rodent and nonrodent toxicity studies are utilized to predict adverse drug reactions in human clinical trials. However, the predictivity of rodent studies alone is significantly less, and certain organ toxicities (hematologic, ocular or injection site) are much more predictive (with correlations reaching 70%) than others, with some organ system findings showing poor predictive correlates of less than 30% (such as musculoskeletal, respiratory, neurologic). Dogs are much more predictive than rodents, and nonhuman primates are much better at demonstrating efficacy endpoints than of toxicologic endpoints related to adverse drug reactions when assessing biologic therapies. Importantly, lack of toxicities in animals does not necessarily imply that no drug reactions are to be expected in humans. Differences in species susceptibility, organ distribution of drug, clearance and metabolism all can have a major effect on drug concentrations, and this may affect susceptibility of organ toxicity in patients in clinical trials. This seminar will discuss various reasons for the lack of clinical translation of nonclinical toxicities, in what organs correlations are good predictors of adverse reactions, and provide examples of and pathophysiologic explanations, to demonstrate when there is a disconnect between nonclinical and clinical toxicologic findings.

SESSION III

Application of iPS-Derived Neural Spheroids In Drug Discovery

Matthew Wagoner, Takeda

Drug-induced central nervous system (CNS) toxicity is a leading cause of safety-related attrition for therapeutics in clinical trials, resulting in unacceptable adverse events for patients and late stage clinical failures for project teams trying to address unmet medical needs. A key driver of the late stage attrition is the low concordance between in vitro & in vivo preclinical models of neurotoxicity and human adverse drug reactions observed in the clinic. Here we evaluated an iPSC-derived microbrain model using 84 structurally diverse pharmaceuticals with a robust clinical & preclinical dataset and varying levels of seizurogenic and neurodegenerative liability. Drug-induced changes in neuronal viability & function were assessed using seven endpoints based on Ca oscillation and cellular ATP levels. Across the 84 training and test set compounds, we find that this human microBrain model can separate those clinical molecules that contain labels for seizures / neurodegeneration with a specificity as high as 93.33% and sensitivity of 53.49%. Importantly, this high throughput model has very low false positive rate in the prediction of seizures, convulsions and neurodegeneration. This assay has the potential to be used as a predictive assay to detect neurotoxicity hazard identification in early drug discovery.

Utility of Complex in vitro Liver Systems in Assessing the Safety of Drugs

Jose Lebron, Merck

The majority of attrition during preclinical drug development is due to toxicities observed in liver, heart, kidney, and central nervous system. Despite multiple efforts and recent advances, preclinical de-risking efforts not always predict clinical outcomes. In fact, drug-induced liver injury (DILI) remains a major safety concern in pharmaceutical development, accounting for a significant cost burden to the drug development process. Thus, deployment of physiologically relevant in vitro liver models earlier in the drug discovery process, where only milligram quantities of drugs are needed, may enable assessments of a compound's liver safety profiles reducing cost and resources. We have focused our efforts on characterizing and qualifying Ascendance's Hepatopac® micropatterned hepatocyte-fibroblast complex in vitro co-culture model, which provides stable, functional hepatocyte cultures for weeks. We will summarize these efforts and discuss how we have used the Hepatopac® model, in conjunction with molecular and metabolomics approaches, to inform on the potential DILI risk of drug candidates. Lastly, we will lay out the challenges for future model development, and the next steps to refine such in vitro tools to optimize their potential.

An Industry Perspective on use of MPS in Drug Safety Assessment

Terry Van Vleet, AbbVie

This presentation provides a Pharma Industry perspective on potential advantages and challenges for incorporation of microphysiological systems (MPS) into drug safety assessment. Examples of niche contexts of use and opportunities to improve current safety screening as well as challenges related to cost/value and throughput needs are discussed. The importance and level of characterization for some likely initial contexts of use are also presented. Models for hazard identification, understanding of mechanism, and translation potential may have substantially different requirements.

PLENARY

Preclinical Development of Gene Therapy Products: CBER Considerations

Mercedes Serabian, FDA

The conduct of a clinical trial for an investigational gene therapy (GT) product is guided by the Code of Federal Regulations (CFR) Title 21, Part 312 to ensure the safety and rights of subjects in all phases of clinical investigation. According to 21 CFR 312.23(a)(8), sufficient information derived from preclinical pharmacology and toxicology studies is needed to support the decision that a clinical trial in human subjects is reasonably safe and scientifically feasible to conduct. Although the general principles for safety assessment of traditional biologics also apply to GT products, the preclinical testing platform to evaluate the safety and effectiveness of each investigational GT product can vary based on the biological properties of the GT product, the proposed clinical trial design, and other factors. The regulatory review of GT products employs a weight-of-evidence, science-based, tiered approach to determine the potential benefits and the risks of a particular GT product in the planned clinical context-of-use. This presentation will provide an overview of the regulatory considerations

for preclinical development programs assessing the safety and activity of GT products, to include recent guidances released by FDA/CBER for this diverse product class.

DEVELOPMENT WORKSHOP

SESSION IV

Use of Animal Models of Disease to Optimize Translation of Cell and Gene-Based Therapies

Joy Cavagnaro, AccessBio

The utility of animal models of disease for assessing the safety of novel therapeutic modalities has become an increasingly important topic of discussion as research and development efforts focus on improving the predictive value of animal studies to support accelerated clinical development (Cavagnaro and Silva Lima 2015). The advantages of using animal models of disease include potential increased prediction of exposure and toxicity relevant to the clinical indication, the possibility of direct estimation of therapeutic index, the identification of potential biomarkers of efficacy and safety, the potential for increased sensitivity, all with the goal of supporting more efficient clinical trials.

Animal models of disease are commonly used in the development of cell and gene-based therapies to assess both efficacy and safety. Models include naturally-occurring, chemically- or surgically-induced, and genetically-engineered disease models. Justification of models in a development program are based upon considerations of the similarities and differences between the pathophysiology of the disease/injury animal model and the pathophysiology of the disease/injury of humans and the timing of administration relative to the onset of disease. Several examples will be discussed.

Biodistribution and Immunogenicity Assessments to Support Gene Therapies: Challenges and Opportunities

Mark Milton, Novartis

In the past few years there has been a resurgence of interest in the development of gene therapies, although we have been developing gene therapies for approximately 30 years. Much has changed (e.g. the viral vectors used as the delivery mechanism for the gene) but many of the techniques and approaches used to support the development of gene therapies have remained the same. Two key elements of the characterization of a gene therapy are the description of the biodistribution and immunogenicity of the gene therapy. This presentation will provide an overview of why we perform these assessments and how they are currently performed. It will also provide some insights as to how such assessments could be performed in the future, if they should be conducted at all.

Regulatory Considerations for Nonclinical Safety Assessment of GalNAc-conjugated siRNAs

Joe Chichocki, Alnylam

With the recent regulatory approval of ONPATTRO®, RNA interference (RNAi) has officially emerged as a novel therapeutic modality. Specific regulatory guidance does not currently exist for oligonucleotide therapeutics, including those harnessing the power of RNAi, such as small interfering RNA (siRNA). RNAi therapeutics employ the use of synthetic, non-naturally-occurring nucleotides, and are thereby bound by regulatory guidance surrounding "small molecules" [e.g. ICH M3(R2)], despite being about 16 kDa in size. The lack of specific regulatory guidance for RNAi therapeutics has created a challenge for appropriate safety assessment and nonclinical development strategy. The goal of this talk is to shed light on some of the nonclinical challenges that have been faced in development of RNAi therapeutics by providing case studies and discussing regulatory inquiries.

SESSION V

Immunogenicity Prediction - Strategies and Challenges

Valerie Quarmby, Genentech

Every biotherapeutic has the potential to elicit unwanted immune responses, and these may compromise safety and efficacy. To minimize immunogenicity risk, biotherapeutics have traditionally been designed to maximize "self" human sequence content. However, extensive engineering of candidate biotherapeutics is now possible and may be necessary to enhance best-in-class potential.

Several types of in silico, in vitro and in vivo approaches can

be used during lead selection and optimization to assess the likelihood that a biotherapeutic may be immunogenic. This talk will review immunogenicity risk assessment systems in the context of biotherapeutic development.

Predictive Value of Nonclinical Studies To Support FIH

Tom Monticello, Amgen

The International Consortium for Innovation and Quality in Pharmaceutical Development (IQ), is a not-for-profit organization of biopharmaceutical companies with a mission of advancing science and technology. The mission of the DruSafe Leadership Group within the IQ, is to advance nonclinical safety sciences and impact the global regulatory environment. To that end, DruSafe created and analyzed an industry-wide nonclinical to clinical translational database to determine how safety assessment in animals translate to clinical risk. The database of 182 molecules contained animal toxicology data coupled with clinical observations from phase I human studies. Concordance statistics were performed by organ system and test species. Our results indicate that while animal studies can demonstrate great value in the positive predictive values for certain species and organ categories, the negative predictive value was the more impactful measure. Concordance of safety pharmacology animal studies and their predictive value for human safety will also be discussed. As the contribution of animal testing in drug development is continually debated and challenged, the IQ database results may also help provide context for emerging alternate models in support of the 3Rs.

Privacy-preserving Knowledge Transfer from Corporate Data to Federative Models

Thierry Hanser, Lhasa Limited

Latest innovation in artificial intelligence (AI) has provided a large spectrum of methodologies applicable to drug discovery and drug development. The critical common need across these applications is access to good quality data to allow machine learning algorithms to extract relevant knowledge and produce useful and predictive models. One of the main challenges in AI is therefore to compile such valuable datasets. Collecting high quality data is challenging, especially in the pharmaceutical universe due to the confidential nature of the chemical structures defining a drug. Although we have access to limited public data, the most important knowledge is found in corporate data. Unfortunately, this knowledge cannot be easily shared without disclosing private information. As a consequence, valuable knowledge remains locked in private silos for privacy reasons despite the willingness of industry to share non-competitive knowledge. To overcome this issue, Lhasa Limited has developed a methodology to facilitate the transfer of knowledge from corporate data into federative models whilst preserving the privacy of the original information. The method is based on the Teacher-Student approach [1] adapted to the domain of predictive toxicology. In this presentation, we will show how this methodology can be successfully applied to transfer knowledge from confidential hERG data from pharmaceutical companies into a useful and accurate model without disclosing any chemical structures. This approach opens the way to a new generation of enhanced predictive models that leverage public and corporate data in concert.

[1] Semi-supervised Knowledge Transfer for Deep Learning from Private Training Data. Nicolas Papernot, Martín Abadi, Úlfar Erlingsson, Ian Goodfellow, Kunal Talwar. International Conference on Learning Representations (ICLR) 2017.

SESSION VI

Accurate Prediction of Animal Toxicity Endpoints: All Things Considered in the Age of Big Data

Ivan Rusyn, Texas A&M

For over 40 years, prediction of toxicity from chemical structures has been an active area of research at the intersection of toxicology, chemistry, molecular modeling and regulatory science. Over the decades, many tools have been built that enable use of chemical structure to quantitatively or qualitatively "read-across" information on various adverse health effects between data rich and data poor compounds. Predictive models, such as quantitative structure-activity relationship (QSAR), facilitate replacement and reduction of animal testing in toxicology and risk assessment; however, many subject-matter experts acknowledge that these methods are not always reliable and must be assessed on their individual merit for the compound and context in question. In the past decade, the availability of data for predictive modeling burst with new information from massive in vitro screening programs and better cataloging of the chemical safety data submitted by registrants to the regulatory authorities around the world. Some have reasoned that this heralded the era of "big data" in predictive modeling for safety of drugs and chemicals and that new tests in animals may become

obsolete. This presentation will describe considerations on whether "big data" negate current principles for predictive modeling. Also, we will describe recent efforts to combine chemical features with data from bioactivity screening and development of publicly-available models that calculate a "threshold value" rather than a "class label" for safety endpoints.

Acknowledgements: The work presented was conducted in collaboration with the laboratories of Alexander Tropsha (UNC-Chapel Hill) and Weihsueh Chiu (Texas A&M) and funded by grants and cooperative agreements from NIH and EPA.

Using Population-Scale Genetic Databases to Inform Target and Drug Safety

Luke Ward, Alnylam

Human genetic data is used by the biopharmaceutical industry in two major ways: to discover new targets, by correlating variation in genes with diseases of therapeutic interest, and to study idiosyncrasies in drug response, by correlating patient genotypes with positive or adverse drug responses. Increasingly, however, human genetics is being used to understand drug safety through phenome-wide association study (PheWAS) and mendelian randomization (MR) methods, which uses "natural experiments" at population scale to characterize target biology. In a retrospective analysis we have validated this approach, demonstrating that variation in drug targets can be used to anticipate target-mediated side effects (Nguyen et al., Nat. Comm., 2019). I will show several recent examples from the literature, where massive genetic databases such as the UK Biobank have been used to investigate target safety and suggest best practices for interpreting these data (Ward et al., Drug Safety, 2018). Finally, I will discuss off-target effects, and describe recent work showing that secondary pharmacology screens can be improved by prioritizing assays on the basis of human genetic evidence (Deaton et al., Tox. Sci., 2018).

3D Printing of Pharmaceuticals and Accelerating Drug Development

Jae Yoo and Don Wetherhold, Aprecia Pharmaceuticals

Innovative approaches to speed the drug development process can result in significant savings of time and money and lead to shorter time for a pharmaceutical product to reach the market. Clinical trials (both animal and human) are an important phase of drug development, but can be both costly and time-consuming, making them ideal for innovative approaches. Three-dimensional (3D) printing of pharmaceuticals provides an opportunity to speed the drug development process, particularly during clinical trials due to its agile manufacturing process, allowing for rapid prototyping and potentially on-demand production. Binder-jet 3D printing (also known as powder-liquid) has been used successfully to produce an FDA-approved pharmaceutical product (Spritam®), demonstrating the commercial feasibility of this process. In addition to commercial production, this binder-jet process lends itself to small batch production and can be used to rapidly prototype several different formulations within a short period of time (minutes to hours) more efficiently than traditional tableting processes. Furthermore, clinical trial materials can be produced on-demand, reducing the need for stability studies and inventory. This rapid-prototyping/production provides an opportunity to accelerate drug development throughout the animal and human clinical trial phase of development.

SPEAKER BIOGRAPHIES

Famke Aeffner, DVM, PhD, DACVP, Amgen Dr. Aeffner received her DVM from the University of Veterinary Medicine, Hannover, Germany, and completed a 5-year combined PhD and residency program in veterinary anatomic pathology from The Ohio State University. She is a Diplomate of the American College of Veterinary Pathologists (anatomic). Dr. Aeffner currently is a Principal Pathologist at Amgen Inc. in South San Francisco, California.

Prior to joining Amgen, she was the Associate Director of Pathology at Flagship Biosciences, a digital image analysis service provider. In her previous role, she served as lead pathologist supporting ongoing and retrospective clinical trials.

Dr. Aeffner's special expertise is in the field of digital image analysis of human and animal tissue sections, especially to investigate oncology, immuno-oncology, medical devices and animal models of human disease. She has extensive experience in biomarker assay development, validation, and interpretation of chromogenic and fluorescent immunohistochemistry.

Dr. Aeffner founded the Digital Image Analysis Special Interest Group of the Society of Toxicologic Pathology and is currently service as it's first chair. She is also an active member of the Education Committee of the Digital Pathology Association and has authored and co-authored several of the organization's white papers.

Most recently, she was recognized for her work in the field of digital image analysis with the 2017 Distinguished Early Career Award from the Society of Toxicologic Pathology.

Joy Cavagnaro, PhD, DABT, Alnylam Pharmaceuticals Dr. Cavagnaro is the President of Access BIO where she consults on development strategies for novel drug, biologics and device combinations. Her career spans academia, the CRO and biotechnology industries and government. During her tenure at CBER/FDA she was appointed to the SBRS and served as FDA's safety topic lead and rapporteur for "ICH S6." She was the first to advocate the "case-by-case" science based approach to preclinical safety evaluation. Dr. Cavagnaro is Past Chair of RAPS and the National Capital Area Chapter of SOT. In 2011 she received SOT's Biotechnology Specialty Section first Career Achievement Award and in 2019 the Arnold J. Lehman Award. She is Founder, Past Chair and current ex officio member of the leadership committee of BioSafe, an expert preclinical science committee within BIO. She is a Past Chair of the Clinical and Regulatory Affairs Committee and Translational Science & Product Development Committee of the ASGCT. She was a member of the Scientific Advisory Committee on Alternative Toxicological Methods and is currently an advisor and member of the Grants Working Group of the California Institute of Regenerative Medicine. Dr. Cavagnaro is currently a Chair of Advarra, an independent IRB. She serves on multiple SAB's and consults and lectures internationally on translation and risk assessment of novel therapies. She has co-authored numerous white papers and chapters related to various aspects of preclinical safety assessment. The book she edited "Preclinical Safety Evaluation of Biopharmaceuticals A Science-Based Approach to Facilitating Clinical Trials" published by John Wiley & Sons, NJ, 2008 is commonly referred to as the "BioBible".

Joseph Cichocki, PhD, Alnylam Pharmaceuticals Dr. Joseph Cichocki is a board-certified Senior Toxicologist in the Department of Early Development at Alnylam Pharmaceuticals. In his current role, he provides scientific support to multiple Program Teams as the Nonclinical Safety Representative. In addition to his regulatory toxicology duties, he also works closely with the investigational toxicology group to support the development of lead candidates.

Dr. Cichocki has authored or co-authored twenty peer-reviewed manuscripts and book chapters and serves as an ad hoc reviewer for multiple toxicology journals. He is currently the Senior Councilor of the Northeast Chapter of the Society of Toxicology and is a member of the Society of Toxicology and the American College of Toxicology.

Dr. Cichocki received his Ph.D. in Pharmacology/Toxicology from the University of Connecticut in 2014. He performed postdoctoral work at Texas A&M University and was funded, in part, by a National Research Service Award (F32) through the National Institute of Environmental Health Sciences.

Shah Falgun, PhD, Merck Dr. Falgun is currently an Associate principal scientist and computational toxicology domain lead within the discovery chemistry group at Merck, West Point. His interest lies in applying machine learning and data mining approaches to identify potential ADME and safety liabilities in chemotypes. His current role involves influencing medicinal chemistry project teams to utilize in silico ADMET approaches during post-HTS work-up to enable them to bring high-quality chemical series forward. Prior to Merck, Falgun worked in compound safety prediction group at Pfizer for 4.5 years where he deployed in silico and informatics approaches to identify new mechanisms of organ toxicities, in particularly drug-induced liver injury and cardiotoxicity. He has over 25 publications in peer-reviewed journals and multiple invited presentations. Falgun is trained as a pharmacist and holds a Ph.D. in medicinal/computational chemistry.

Ken Frazier, PhD, GlaxoSmithKline Dr. Ken Frazier is a director of pathology and senior fellow at GlaxoSmithKline where he has been for over 17 years. His primary duties at GSK are both as a senior toxicologic consultant and toxicologic study pathologist, and he is a member of the Nephrology Safety Panel. His current scientific interests involve mechanisms of drug-induced toxic responses in the urinary system. He holds a BS in biology from Wichita State University, a DVM from Kansas State College of Veterinary Medicine and a PhD in molecular biology from the University of Miami. He completed a residency in comparative pathology at University of Miami's Jackson Memorial Hospital and is board certified in both Pathology and Toxicology. He is a fellow of the International Academy of Toxicologic Pathology. Dr. Frazier has held staff appointments at the University of Miami School of Medicine and at the University of Georgia School of Veterinary Medicine. His focus while in academia was on development of rodent models of kidney failure and mechanisms of both toxic and infectious renal disease. He has authored over 80 scientific journal publications and book chapters, chaired many committees and meetings for the STP, ACVP, ACT and other organizations, and has been on the editorial board for multiple scientific journals. In 2015, he spent 6 months on a PULSE assignment in Ghana working in an African diagnostic laboratory for the Tropical Laboratory Initiative of the Millennium Villages Project.

Nigel Greene, PhD, AstraZeneca Nigel Greene leads the Data Science and Artificial Intelligence department in Drug Safety & Metabolism at AstraZeneca and is interested in the application of artificial intelligence methods to understand of mechanisms of drug-induced toxicity and their translation to a clinical patient population. Previously Dr. Greene was a head of the Predictive Compound ADME and Safety group at AstraZeneca. Dr Greene also spent 14 years at Pfizer Inc. where he started in Drug Safety R&D and later transitioned to the Compound Safety Prediction group in Medicinal Chemistry. In his early career, Dr. Greene worked for Lhasa Ltd. where he pioneered computational toxicology, and for Tripos Inc.

Nigel's other activities outside of AstraZeneca have included being the Chair of the Board of Trustees for Lhasa Ltd. and he has served on multiple National Research Council committees sponsored by the US Environmental Protection Agency, US Food and Drug Administration, and the National Institutes of Health.

Dr. Greene received his B.Sc. and PhD from the University of Leeds in the UK.

Thierry Hanser, PhD, Lhasa Limited Dr. Hanser is leading the molecular informatics group at Lhasa Limited. Thierry obtained a PhD in cheminformatics at the university of Strasbourg (1992) and joined Prof Elias James Corey's group at Harvard university (1995) for a postdoctoral position followed by a second postdoctoral period with Prof. Peter Johnson at the university of Leeds (1996). In 2004, Thierry started his own company Ixelis developing innovative solutions for chemical information management. Thierry joined Lhasa Limited in 2006 where he initially led the design and development of Lhasa's Cheminformatics platform; his current research activity includes bridging cheminformatics and AI/Machine Learning in order to design new knowledge discovery and modelling methodologies in predictive pharmacology/toxicology.

Thomas Hartung, MD, PhD, Johns Hopkins Bloomberg School of Public Health Dr. Hartung is the Doerenkamp-Zbinden-Chair for Evidence-based Toxicology with a joint appointment for Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health, Baltimore. He holds a joint appointment as Professor for Pharmacology and Toxicology at University of Konstanz, Germany; he also is Director of Centers for Alternatives to Animal Testing (CAAT, http://caat.jhsph.edu) of both universities. He is the former Head of the European Commission's Center for the Validation of Alternative Methods (ECVAM), Ispra, Italy, and has authored more than 550 scientific publications.

Satoko Kakiuchi-Kiyota, PhD, Genentech Dr. Kakiuchi-Kiyota is currently a Scientist/Toxicologist in Safety Assessment at Genentech, where she provides nonclinical toxicology representation to small and large molecule programs across multiple therapeutic areas. Her primary responsibilities include lead optimization and characterization of early-stage molecules, and she represents preclinical drug safety on project teams until candidate nomination for progression into IND-enabling toxicology studies. In parallel, she also is involved in cross-functional collaborations, including a key role in evaluating in silico safety prediction models. Satoko earned her PhD in Toxicology from University of Nebraska Medical Center and completed her postdoctoral training at Pfizer before beginning her career in Compound Safety Prediction (Medicinal Chemistry) at Pfizer. Before moving to United States, she served as a Toxicologist, Study Director, and Study Monitor at Teijin Pharma, Japan supporting small molecule discovery programs and contributed to INDs and IBs.

Jose A. Lebron, PhD, Investigative Laboratory Sciences Dr. Lebron is an Executive Director who leads the Investigational Laboratory Sciences Group in the Safety Assessment and Laboratory Animal Resources (SALAR) Department at Merck in West Point, PA. His group is responsible for investigative and mechanistic toxicology problem solving for both discovery and development programs; cellular toxicity and immunotoxicity model development; applied metabolomics and proteomics; toxicology tissue and translational biomarkers; and genetic toxicology assessments.

Jose received his B.S. in Chemistry with a minor in Biology from the University of Puerto Rico in 1993. After graduation, he worked for one year as a chemist for Roche Pharmaceuticals as a chemist. He then attended the California Institute of Technology in Pasadena, where he graduated in 1999 with a PhD in Biochemistry, Molecular Biology, and Immunology. Shortly thereafter, he joined the BioAnalytical department at Merck Research Laboratories, where he led a group of increasing size working on the development of analytical assays for the characterization of adenovirus-based HIV vaccines for first-in-human clinical trials. In 2003, Jose transferred to the Safety Assessment department where he served for four years as the nonclinical compound leader of several vaccines in early- and late-stage development. During that time, he also managed a team dedicated to supporting cell- and QPCR-based analyses for peptides and biologics, and biodistribution studies of plasmids, viral-vectors, and siRNAs in support of regulatory filings. He then spent six years leading all nonclinical safety in vitro and in vivo efforts supporting the development of siRNA therapeutics. In 2013, Jose joined the Investigative Laboratory Sciences group as a Director, leading the Molecular and Investigative Toxicology group responsible for the development of in vitro-based predictive toxicology assays for mitochondrial toxicity, liver toxicity, and embryo-fetal development toxicity. In 2016, Jose was promoted to Executive Director and became the head of the Investigative Laboratory Sciences group. During his ~20 years at Merck, Jose has been part of > 20 development teams, and has successfully filed > 15 investigational new drug (IND) applications and 1 Biologics License Application (BLA). Jose has extensive experience in drug development of small molecules, biologics, peptides, and oligonucleotide-based therapeutics.

Lise I. Loberg, PhD, DABT, PMP, AbbVie Dr. Loberg has over 15 years' experience as a toxicologist and project manager in the biopharmaceutical industry. Dr. Loberg supports preclinical safety evaluation of compounds in development, from lead selection to late-stage clinical trials and marketed drugs. She has experience with large molecule biotechnologies including antibody-drug conjugates and small molecule drugs ranging across several therapeutic areas including neuroscience, oncology, immune-oncology and renal disease. Dr. Loberg is a Diplomate of the American Board of Toxicology and has earned the Project Management Professional (PMP) certification. She served as Treasurer (2011-2013) and Councilor (2010-2011) for the Midwest Regional Chapter of SOT and has been on the planning committee for Applied Pharmaceutical Toxicology

meetings in 2012-2018. Dr. Loberg is an employee at AbbVie, Inc., previously Abbott Laboratories, where she has worked in Preclinical Safety at three research & development sites (Ludwigshafen, Germany; Redwood City, California; and Lake County, Illinois) and three years in Drug Development Project Management. Prior to AbbVie/Abbott, Dr. Loberg supervised a Molecular Toxicology laboratory at IIT Research Institute (1996-1999). Dr. Loberg earned her Ph.D. in Toxicology at the University of Cincinnati (1996) and her B.S. in Psychology/Neuroscience at John Carroll University.

Matt Martin, PhD, Pfizer Dr. Martin is Director and Computational Toxicology Domain Lead within Pfizer's Drug Safety Research & Development organization focusing on safety applications of predictive modeling, data infrastructure, bioinformatics and NGS data analysis. Prior to joining Pfizer, Dr. Martin was a Research Biologist (and proud Tar Heel) at the US EPA's National Center for Computational Toxicology leading ToxCast and ToxRefDB data analysis efforts with over 60 peer-reviewed publications.

Kathleen E. Meyer, MPH, PhD, DABT, Sangamo Therapeutics Dr. Kathleen Meyer is Vice President of Nonclinical Development at Sangamo Therapeutics and leads the nonclinical development of Sangamo's genomic medicines including gene editing, gene therapy, cell therapy and gene regulation therapeutic candidates. Dr. Meyer provides leadership on nonclinical safety evaluation strategy and oversees the bioanalytical sciences development of assays supporting nonclinical and clinical studies since 2014. She has over 18 years of industry experience in nonclinical safety evaluation, bioanalytical development, pharmacokinetics and supported development of ZFN-based gene editing, small molecule, monoclonal antibody, enzyme replacement, botulinum toxin, gene and cell therapies. Prior to joining Sangamo, Dr. Meyer served as Principal Scientist, Pharmacology and Toxicology at BioMarin Pharmaceutical Inc. where she guided small molecule and biologic drug candidates through the nonclinical development process supporting clinical trials and marketing authorization. From 2009 to 2012, she served as Senior Director, Nonclinical Safety Evaluation at XOMA LLC and, prior to that, held positions as a Scientist and Principal Scientist at Elan Pharmaceuticals from 1997-2003. Before joining industry, she worked as a post-doctoral fellow evaluating non-viral methods of gene delivery at the University of California, San Francisco. Dr. Meyer received an A.B. in Physiology, a Master's degree in Public Health specializing in Toxicology and Epidemiology, and her Ph.D. in Environmental Health Science/Toxicology from the University of California, Berkeley. Dr. Meyer is a Diplomat of the American Board of Toxicology.

Mark N. Milton, PhD, Novartis Dr. Milton received a B.Sc. in Biochemistry and Soil Science from UCNW, Bangor, a M.Sc. in Toxicology and Ph.D. in Biochemical Toxicology from the University of Surrey, England. Mark has over 25 years' experience in the nonclinical and clinical PK/PD of NCEs and Biologics. Prior to joining Novartis in 2009, Mark worked at GD Searle, Millennium Pharmaceuticals, and Tempo Pharmaceuticals. At Novartis Mark provides nonclinical and clinical PKPD support to the development of biologics (antibodies, therapeutic proteins, gene therapies and cell-based therapies) and is currently Global Head, Ophthalmology Therapeutic Area in the PK Sciences Department.

Mark is the past-chair of the BioSafe PKPD EWG, a member of the BioSafe LC, IQ Board of Directors, a member of the AAPS pre-existing antibody and Immunogenicity Risk Assessment Working Groups and was the BIO observer to the ICHS3A Q&A WG. He has published over 30 peer-reviewed publications and book chapters and presented extensively on the development of both NCEs and Biologics. His current interests include the PK/PD and immunogenicity of biotherapeutics, the contribution of PK and IG to the development of gene and cell-based therapies, ocular immunology, the selection of the clinical starting dose based upon the MABEL calculation, and alternative designs for FIH Clinical Trials of monoclonal antibodies in Healthy Volunteers.

Thomas Monticello, DVM, PhD, DACVP, Amgen Dr. Monticello received his DVM from the Michigan State University College of Veterinary Medicine. He completed a residency in veterinary pathology at the North Carolina State University School of Veterinary Medicine followed by a post-doctoral fellowship in toxicology and toxicologic pathology at the Chemical Industry Institute of Toxicology in Research Triangle Park, NC. Tom concurrently completed a PhD in comparative pathology from Duke

University. He began his career in the pharmaceutical industry at Bristol-Myers Squibb in the department of Experimental Pathology supporting drug discovery and regulatory safety assessment. Tom then moved to Aventis Pharmaceuticals (US) as Head of Pathology and later Head of Nonclinical Projects and then deputy Head of Drug Safety for sanofi-aventis. Later, Tom moved to Merck and Co. where he was the Worldwide Head of Toxicological Sciences, responsible for nonclinical testing for general and reproductive toxicology and safety pharmacology for sites in the US, France and Japan. He relocated to sunny California and is currently at Amgen as Executive Director in Comparative Biology and Safety Sciences. Dr. Monticello is a member of the Society of Toxicology and American College of Toxicology, a diplomate of the American College of Veterinary Pathologists, past president of the Society of Toxicologic Pathology and currently serves as the Chair of the IQ DruSafe Leadership Group.

Valerie Quarmby, PhD, FAAPS, Genentech Dr. Valerie Quarmby is a Staff Scientist in Development Sciences at Genentech.

Dr. Quarmby received her B.Sc. and Ph.D. from the University of London (UK). She was an NIH Fogarty International Postdoctoral Fellow at the National Institute of Environmental Health Sciences and did postdoctoral work in the Department of Pediatric Endocrinology at UNC-Chapel Hill. Dr. Quarmby worked in the field of clinical diagnostics prior to joining Genentech.

Dr. Quarmby has contributed to IND, BLA and related filings for many approved medicines at Genentech. She is past Chair of the American Association of Pharmaceutical Sciences (AAPS) "Therapeutic Product Immunogenicity Focus Group" and was a member of the 2010-2015 United States Pharmacopeia "Immunogenicity Testing Expert Panel". Dr. Quarmby has presented and published extensively in the areas of bioanalysis and biopharmaceutical development. In 2014, in recognition of her many contributions to the pharmaceutical industry, Dr. Quarmby was awarded AAPS Fellow Status.

Astrid Ruefli-Brasse, PhD, 23andMe Dr. Ruefli-Brasse is the Director of Drug Discovery at 23andme Therapeutics and is responsible for leading the in vitro and in vivo biology, small molecule, biomarker discovery, protein science and antibody engineering groups. Before coming to 23andMe Astrid worked at Roche working as the Senior Group Leader for Molecular Targeted Therapies in the Pharma Research and Early Development Group. Her team focused on drug discovery for cancer immunotherapy and targeted molecular therapies at the company. Before her time at Roche, Astrid spent several years as a principal scientist at Amgen, in the department of hematology and oncology. Astrid received her Ph.D. in Pathology from the University of Melbourne and was a post-doctoral research fellow at Genentech. Astrid brings over 15 years of industry experience with broad expertise in drug discovery research and development.

Ivan Rusyn, PhD, Texas A&M University Dr. Rusyn is Professor in the Department of Veterinary Integrative Biosciences in the College of Veterinary Medicine & Biomedical Sciences, Chair of the Interdisciplinary Faculty of Toxicology, Director of an NIEHS T32 training program in "Regulatory Science in Environmental Health and Toxicology," and Director of the Superfund Research Center at Texas A&M University in College Station. Prior to joining Texas A&M University in 2014, he was Professor of Environmental Sciences and Engineering at the University of North Carolina in Chapel Hill. His laboratory has an active research portfolio with a focus on the mechanisms of chemical toxicity, genetic determinants of susceptibility to toxicant-induced disease and the use of new approach methods in decision-making. His studies on health effects of chemical agents resulted in over 225 peer-reviewed publications which were cited over 16,000 times (h-index=65). He has served on many US National Academies committees, World Health Organization/International Agency for Research on Cancer monograph working groups (as an overall chair, or a chair of "Mechanistic and Other Relevant Evidence" sub-group) and on the Expert Taskforce for the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). His other notable service commitments include serving on the Board of the Scientific Councilors of the United States National Institute of Environmental Health Sciences, the advisory board for Texas Department of Public Health, and membership on the Research Committee of the Health Effects Institute. Dr. Rusyn received a doctor of medicine degree from Ukrainian State Medical University in Kyiv and a Ph.D. in toxicology from the University of North Carolina at Chapel Hill. He conducted postdoctoral research at the Massachusetts Institute of Technology and Heinrich-Heine University in Dusseldorf. Dr. Rusyn's laboratory has been funded by grants and

cooperative research agreements from the National Institutes of Health and US Environmental Protection Agency, institutional funding from Texas A&M University, the industry, and other sources.

Mercedes Serabian, M.S., DABT, FDA Mercedes Serabian holds a M.S. degree in Toxicology and is a Diplomat of the American Board of Toxicology (DABT). She currently serves as Chief of the Pharmacology/Toxicology Branch 1 in the Division of Clinical Evaluation and Pharmacology/Toxicology (DCEPT) in the Office of Tissues and Advanced Therapies (OTAT) in the Center for Biologics Evaluation and Research (CBER) at the USFDA. She is responsible for overseeing the pharmacology/toxicology review, regulation, and policy development for cellular therapy products, gene therapy products, hemostatic products, selected plasma derivatives, and/or tissue-engineered products submitted to FDA/CBER. Ms. Serabian championed the "Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products" (November 2013), which was the first comprehensive outline of FDA recommendations on preclinical data to support clinical studies of cellular and gene therapy products. She has participated in several expert working groups under the International Conference on Harmonisation (ICH) and has presented to outside parties on preclinical regulatory considerations for cellular and gene therapy products.

Terry Van Vleet, PhD, Abbvie Dr. Van Vleet received a Bachelor's degree in Zoology from Weber State University and a doctorate in Toxicology studying mechanisms of carcinogenesis and metabolic activation of carcinogens, at Utah State University. His postdoctoral training was at the Medical University of South Carolina, studying mechanisms of renal toxicity with an emphasis in mechanisms of mitochondrial dysfunction. Dr. Van Vleet is a Diplomate of the American Board of Toxicology. He worked at Bristol Myers Squibb in Mt Vernon, Indiana, for 11 years in positions of increasing importance culminating as the Head of the Molecular and In Vitro Toxicology Group. Currently he works at Abbvie in Investigative Toxicology and Pathology (Preclinical Safety) where he is responsible for the Molecular and Computational Toxicology groups.

Matt Wagoner, PhD, Takeda Pharmaceuticals Dr. Wagoner leads the Global Investigatory Toxicology network for Takeda Pharmaceuticals, where their team applies microphysiological systems (MPS) 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 MPS tools in support of projects.

In the academic arena, Matt co-teaches 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 three kids and the canine equivalent of a frappuchino called a 'Woodle'.

Luke Ward, PhD, Alnylam Pharmaceuticals Dr. Ward is a Principal Scientist at Alnylam Pharmaceuticals where he works on human genetic analysis and application to target and patient discovery. Previously, he worked at Amgen and deCODE genetics, where he applied human genetics and functional genomics to safety issues in preclinical and clinical development. Before that he was a bioinformatics scientist at a custom software company, 5AM Solutions. He did postdoctoral research in computational genomics at MIT, completed his Ph.D. in Biology at Columbia, and his B.S. in Chemistry at the University of Virginia.

Donald Wetherhold, Aprecia Donald Wetherhold has served Aprecia as a Senior Vice President and an Advisor to the Chairman since January 2018. He was previously the company's Chief Executive Officer from November 2013 to December 2017. From October 2012 until September 2013 he served as Senior Vice President, Long-Term Care at Omnicare, Inc. Prior Omnicare, Mr. Wetherhold was the Corporate Commercialization Officer at Aprecia from November 2010 until September 2012. From January 2007 to September 2012, he served as Advisor to the Chairman at Prasco and Senior Vice President and General Manager of Hampton-Laine, Prasco's branded pharmaceutical business. Prior to joining Prasco and Hampton-

Laine, Mr. Wetherhold was the President of RxPedite LLC and prior to that role served as President of Sales and Marketing Services at Cardinal Health, Inc. Mr. Wetherhold holds a B.S. from West Chester University and a M. Ed. from Florida Atlantic University.

Jaedeok Yoo, PhD, Aprecia Dr. Jae Yoo is serving as Chief Technology Officer for Aprecia Pharmaceuticals, LLC since August 2018. His 3DP journey started at MIT in the early 1990's where he developed and used an inkjet printing based 3DP process to fabricate advanced ceramic materials with compositional gradient. He explored and demonstrated a wide range of pharmaceutical and biomedical applications of 3DP while working for Therics, Inc., a VC backed company that licensed the technology from MIT. In 2003, he co-founded Aprecia Pharmaceuticals and headed its research and engineering efforts to develop a high-speed, additive manufacturing process suitable for cGMP operation. His work set the foundation for first ever FDA approval of a 3D printed pharmaceutical product, SPRITAM®, manufactured and marketed by Aprecia. He was part of GlaxoSmithKline (2014-2018) and explored automation for R&D productivity gain and evaluated platform capabilities for Advanced Manufacturing Technology initiative. He is a co-inventor of many US and international patents on additive manufacturing of pharmaceutical products and medical devices. Prior to his graduate work at MIT, he studied Metallurgical Engineering and Materials Science at Carnegie Mellon University. He holds MBA from Wharton School at University of Pennsylvania.

ORGANIZER BIOGRAPHIES

Marc Bruder, DVM, DABT, Merck Dr. Bruder is a certified veterinary pathologist who graduated from the Veterinary Schools of Lyon (DVM) and Toulouse (toxicologic pathology) in France. He is also a DABT toxicologist.

Marc worked as a study pathologist for several large pharmaceutical companies initially on small molecules and after joining Novartis he focused on large molecules with an oncology indication (ADCs). Marc then took a position as Drug Development Director with Covance, interfacing biotechs with the larger Covance organization to provide a development strategy and preclinical expertise to young companies. Coming back to California in 2016, Marc joined Compugen, a company in the I/O field and after heading their nonclinical effort for about 18 months Marc joined Merck in South San Francisco as senior Discovery Program Leader to help transition projects from Discovery to non-clinical Development as fast and efficiently as possible.

Paul Cornwell, PhD, Eli Lilly Dr. Paul Cornwell is a Principal Research Scientist in Nonclinical Safety Assessment at Eli Lilly and Company. Prior to joining Lilly, Paul was a research scientist at the former Rosetta Inpharmatics LLC, a wholly owned subsidiary of Merck & Co. Inc. Paul received his bachelor's degree from Wabash College and a Ph.D. in pharmacology from Indiana University. After his graduate work, he completed a postdoctoral fellowship at the former CIIT Centers for Health Research in Research Triangle Park, NC. Paul is currently a member of the Society of Toxicology and the American College of Toxicology and is a Diplomate of the American Board of Toxicology. He has 13 years of experience in the development of small molecule pharmaceuticals and biopharmaceuticals.

Jodi Goodwin, Takeda Pharmaceuticals Jodi leads a global team of discovery toxicologists supporting the Oncology, Neuroscience, and GI portfolios at Takeda. Prior to Takeda, Jodi led a compound safety profiling team at Pfizer, whom developed a discovery toxicology strategy for each program based on chemotype-dependent safety risks coupled with in vitro safety screening and computational predictive models.

Jodi has 20 years' experience in drug discovery engaged in multidisciplinary programs involved in many facets of the industry including program leadership, target validation, mechanistic assay development, phenotypic screening and deconvolution, and early safety assessment and strategy.

Christine Karbowski, PhD, Amgen Dr. Christine Hegedus Karbowski is a Principal Scientist in Comparative Biology and Safety Sciences at Amgen, Inc. She is a member of the Society of Toxicology, an organizing committee member for APT since 2014, and a previous member of the American Association for Cancer Research and the Environmental Mutagen Society. Dr. Karbowski received her B.A. in Molecular and Cell Biology and her Ph.D. in Molecular Toxicology, both from U.C. Berkeley. Her research has focused on applying 'Omics technologies such as transcriptomics, proteomics, and metabolomics for hazard identification in preclinical toxicology studies. During her 10 years at Amgen, Dr. Karbowski has served as a subject matter expert utilizing gene expression and genetic data for understanding potential target based liabilities as well as provided guidance for development of internal databases to query and visualize such data. Currently Dr. Karbowski serves as a project team representative for programs across both large and small molecule modalities.

Bruce E. LeRoy, DVM, PhD, AbbVie Bruce is currently a Senior Principal Pathologist in Preclinical Safety at AbbVie. Bruce joined Abbott/AbbVie in 2010, and provides early and late-stage toxicology/pathology support to both small molecule and biologics programs in immunology, oncology, and infectious disease therapeutic areas. Bruce also contributes clinical pathology interpretation across the AbbVie pipeline, and oversees the zebrafish tox testing laboratory. Bruce is a Diplomate of the American College of Veterinary Pathology. He received his DVM from the University of Georgia, and completed a residency in veterinary clinical pathology and a PhD in Veterinary Biosciences from The Ohio State University. Prior to joining AbbVie,

Bruce was an Associate Professor of Pathology at the UGA College of Veterinary Medicine. He has been a member of the APT organizing committee since 2018.

Florence Lorget, PhD, Sangamo Therapeutics Florence Lorget is currently the Senior Director for nonclinical safety evaluation at Sangamo Therapeutics, a genomic medicine company. She oversees the pharmacology, toxicology and PK activities for a large portfolio of gene editing, gene regulation and gene therapy programs in various therapeutic areas including metabolic diseases and CNS.

From 2013 to 2018, Dr Lorget was a Senior Scientist in the Safety Assessment department at Genentech. There, she was the toxicology therapeutic area lead for ophthalmology. She also led the Ocular Platform Team, a cross-functional team focusing on the early development of long acting delivery strategies for ocular delivery.

Prior to joining Genentech in 2013, Dr. Lorget was a Senior Pharmacologist/Toxicologist at BioMarin where she was a key contributor to the nonclinical development of Vimizim®, an enzyme replacement therapy for Morquio syndrome, a rare lysosomal storage disorder, and of Vosoritide, a C-type natriuretic peptide for the treatment of Achondroplasia. Dr. Lorget started her industry career at Amgen working on the Avimer™ technology.

Dr. Lorget obtained a Master in Bioengineering from the University of Technology of Compiegne (France). She received a Pharm D and a PhD in Cellular and Molecular Biology from the University of Picardie- Jules Verne (France). Her postdoctoral work at the Nestle Research Center (Lausanne, Switzerland) and at UCSF focused on osteoclast biology and the role of TGF-b on mesenchymal stem cell biology, respectively.

Dr. Lorget has been a Diplomate of the American Board of Toxicology since 2014.

Stephanie S. Powlin, PhD, DABT, Takeda Pharmaceuticals Dr. Powlin is a board-certified toxicologist with over 20 years of experience in the pharmaceutical industry. She received her PhD from the University of Rochester in 1997 and conducted a postdoctoral fellowship with Jon Cook at E. I. DuPont de Nemours, Haskell Laboratory. She joined Bristol-Myers Squibb in 1998 where she held various roles as a study director/monitor, project representative, principal scientist, and group leader. Following BMS, Stephanie worked for 4 years at Bausch and Lomb on ophthalmic pharmaceuticals and medical devices, then joined Takeda Pharmaceuticals in 2014 as a Scientific Fellow. She has worked in several different therapeutic areas, but has focused primarily on oncology compounds, contributing to every stage of drug development (from pre-IND to NDA). She is also focused on reproductive and developmental toxicology and preclinical aspects of pediatric drug development. Stephanie is a member of the Society of Toxicology, American College of Toxicology and Teratology Society and is a Diplomate of the American Board of Toxicology.

Michael J. Santostefano, MS, PhD, DABT, Merck Dr. Santostefano received a B.S. in Biochemistry from the Univ. of Scranton, M.S. in Regulatory Science from the University of Southern California (USC), and a Ph.D. in Toxicology from Texas A&M University. After post-doctoral work at the Univ. of North Carolina, Michael joined GlaxoWellcome, Inc. as a senior toxicologist and held various roles as a study director/monitor, head of toxicology, project representative, and research investigator until his departure from GlaxoSmithKline in Oct., 2006. From 2006-2014, Michael worked as a principal scientist at Amgen and provided target liability advice, strategic planning, study design, and effective management of discovery and development toxicology programs/projects to support the development of biologics and small molecules. In 2014, he joined Merck in Boston and is currently the therapeutic area leader in preclinical safety assessment supporting the Business Development & Licensing organization. He is responsible for working with potential partners in conducting the due diligence non-clinical and regulatory reviews of in-licensing candidates (such as small molecules, mAbs, vaccines, and devices) and facilitating transfer of information for out-licensing candidates. He also serves as a compound leader for a marketed product in the immunology portfolio and various discovery projects in various therapeutic areas. In addition, he has provided oversight for regulatory

and business partner audits and submissions to international and national regulatory agencies while at Amgen, GSK, and Merck throughout his ~20 years in the pharmaceutical career. His academic and pharmaceutical career has generated over 35 peer-reviewed manuscripts in the field of mechanistic toxicology. Michael is a Diplomate of the American Board of Toxicology and a member of the Drug Information Association (DIA), American College of Toxicology (ACT), and the Society of Toxicology (SOT), including chapters in North Carolina, the Pacific Northwest, and the Northeast. He also serves on the editorial advisory board for Toxicology and Applied Pharmacology and has served as a symposium organizer/chairperson for many scientific organizations. He serves on the membership committee for ACT, is on council for the Northeast SOT and Applied Pharmaceutical and Toxicology, and was previously the vice president of the North Carolina Chapter of the SOT.

Yoav Timsit, PhD, Blueprint Medicines Yoav has just joined Blueprint Medicines as an Associate Director in the Quantitative Pharmacology and Drug Safety Group. He is a directing Investigative and Discovery Toxicology efforts and supporting regulatory submissions. Yoav most recent role was at AstraZeneca where he supported discovery and early development projects. While at AstraZeneca, he directed in vitro safety assays, secondary pharmacology, safety pharmacology, genetic toxicology, in silico modeling, and investigative in vivo toxicology studies to select the optimal lead drug candidates for entry into GLP studies and subsequently FIH trials.

Prior to joining Astra Zeneca in 2016, Yoav spent 9 years at Novartis (NIBR) where he was the Discovery and Investigative Safety representative for multiple project across various therapeutic areas spanning all stages of drug development. While at Novartis, he was awarded the 2011 NIBR Catalyst Award for his investigative in vivo toxicology work on Odomzo®, and supported nonclinical safety assessment for a range of therapeutics that included CAR-T, Fc and/or PEG-conjugated peptides, and inhibitors of M-CSF and Wnt pathways.

Yoav is a Board Certified Toxicologist with the American Board of Toxicology. He obtained his MSc and PhD in Pharmacology & Toxicology from the University of Toronto , followed by postdoctoral training as Visiting Fellow at NIEHS, NIH, Research Triangle Park NC.

Yu (Zoe) Zhong, PhD, Genentech Dr. Zhong is currently the Head of Small Molecule Discovery Toxicology, Safety Assessment, Genentech where she oversees lead optimization and safety assessment strategies for the small molecule discovery portfolio across multiple therapeutic areas. She has significant knowledge of safety strategies related to small molecule development, including target safety assessment, safety lead optimization, candidate selection, and mechanistic investigations. Zoe is a member of safety assessment toxicology leadership team with accountability for safety strategies, processes, and functional review of portfolio programs within Genentech. She has solid technical and disease area skillsets and knowledge in toxicology, safety pharmacology, oncology, immunology, infectious diseases, genitourinary, neuroscience and pain. Zoe has > 15 years' pharmaceutical R&D experience. She began her industry career at Roche in 2002 after completing the Wellcome Research Fellowship training at Kings College London. Zoe earned her PhD in Pharmacology from the School of pharmacy, University of London in 1997, and completed postdoctoral training at University College London. Zoe has contributed to numerous INDs and IBs, and authored/co-authored 22 peer-reviewed papers, review articles and patents in the field of pharmacology and toxicology. Zoe is a Diplomate of the American Board of Toxicology, and a member of the Society of Toxicology (SOT) and several specialty sections.

POSTER ABSTRACTS

Utilization of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes on an MEA Platform for Prediction of Liabilities with Chronic Drug Treatment

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The use of microelectrode array technology (MEA) has proven to be a very powerful tool for prediction of proarrhythmic liabilities in iPSC derived cardiomyocytes. This work has focused primarily on the prominent cardiac ion channels with an emphasis on hERG. To this end, the Comprehensive In Vitro Proarrhythmia Assay (CIPA) initiative was established and has been tasked with prediction of proarrhythmic compounds in an in vitro setting with acute short term dosing. Although most of the focus on the early work has been on arrhythmias driven primarily by hERG block, the real power of iPSC-derived cardiomyocytes on an MEA platform is predicting unexpected liabilities and long term chronic effects in vitro. These type of studies are currently performed in telemerized dogs at great expense. Here we show examples of compounds whose liabilities were identified with chronic dosing of compounds in an MEA assay. Due to the ability to measure and maintain cells over long periods of time, this platform is ideal for evaluating short and long term exposures early in drug development. Examples of hERG trafficking effects are demonstrated at 24 and 48 hours. The hepatitis C drug BMS-986094, which failed in clinical trials, is shown to progressively deteriorate cardiomyocyte health as compared to safe hepatitis C drugs such as sofosbuvir in 14-day studies. We also show examples of compounds that have other unexpected responses such as delayed onset arrhythmias and Na amplitude effects that are not observed acutely and cannot be predicted by ion channel screening. These results demonstrate that iPSC derived cardiomyocytes on an MEA platform are an effective tool for screening compounds to identify unexpected long term liabilities before expensive preclinical animal experiments are performed, thus improving the chances of moving forward with a safe compound.

Evaluating the Effects of the Muscarinic Receptor Agonist Pilocarpine on Neural Network Activity Using Three Cell Types on Different Days in vitro on a Microelectrode Array Platform

Jenifer A. Bradley and Christopher J. Strock

Pilocarpine is a muscarinic receptor agonist used for a rat model of epilepsy. It induces status epilepticus at a relatively high dose (380 mg/kg) within 10-30 minutes and after a prolonged duration of status epilepticus, brain injury and neuronal loss occur, producing an epileptic phenotype. We tested pilocarpine in an in vitro model for seizure liability using a microelectrode array platform in three different neuronal cell types; cryopreserved rat cortical neurons, cryopreserved rat hippocampal neurons and a co-culture of human iPSC-derived glutamatergic neurons with astrocytes. We determined that this drug, up to concentrations of 400 µM, did not produce seizurogenic-like responses in 14 DIV rat cortical neurons or 14 DIV rat hippocampal neurons. In the 14 DIV GlutaNeuron/Astrocyte co-culture, however, there was a robust response at 31.6 µM, with decreasing intensities at lower concentrations in a dose response manner. At 31.6 µM, pilocarpine caused up to 2-fold decreases in firing rates, 3-fold increases in percent isolated spikes (spikes occurring outside of bursts) and 5 to 10-fold increases in median/mean ISI and median ISI (indicators of burst structure deterioration) in the 14 DIV GlutaNeuron/Astrocyte co-culture. In subsequent experiments, we retested pilocarpine in the rat cortical neurons and hippocampal neurons at 21 DIV, which produced a much more robust response. The changes in the rat cortical neurons included 2-fold decreases in the number of spikes in bursts and 2-fold increases in the median ISI, both responses indicating a deterioration in burst organization. We also observed a breakdown in network synchrony. This pattern was consistent with the iPSC neuron response observed at 14 DIV. Alternatively, the 21 DIV rat hippocampal neurons responded with an increase in regularity characterized by changes in endpoints such as 3-fold increases in the number of spikes in bursts, 3-fold increases in burst duration and 2-fold decreases in the MAD burst spike number, which indicates an increase spike train regularity. When changes in muscarinic receptor expression over time were measured, expression changes in maturing cells were the cause of the significant differences. In conclusion, understanding the maturity and response patterns of different neuronal cell types is important for determining whether a model is correct for identifying liabilities associated with specific receptors.

Liver-Targeted AAV Gene Therapy Vectors Produced at Clinical Scale Result in High, Continuous Therapeutic Levels of α-GalA Enzyme Activity and Effective Substrate Reduction in a Mouse Model of Fabry Disease

Authors: Marshall W Huston[1], Makiko Yasuda[2], Silvere Pagant[2], Susan St. Martin[1], Carolyn Gasper[1], Thomas Wechsler[1], Kathleen Meyer[1] and Robert Desnick[2]
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Purpose:
Fabry disease (FD), an X-linked lysosomal storage disease, is caused by mutations in the GLA gene encoding the α-galactosidase A (α-GalA) enzyme. FD is characterized by progressive systemic accumulation of the enzyme's substrates, globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3), in blood and tissues leading to renal, cardiac and/or cerebrovascular disease and culminating in premature demise. The current standard of care is enzyme replacement therapy, which requires a lifetime of biweekly infusions and may not clear all substrate from tissues. A more effective and long-lasting treatment would benefit FD patients.

Methods:
An AAV-mediated, liver-targeted gene therapy approach was evaluated in a knock-out mouse model (GLAKO) of Fabry disease that lacks α-GalA activity and accumulates high levels of substrates in plasma and tissues. This strategy employs an episomal AAV (serotype 2/6) vector encoding human GLA cDNA (hGLA) driven by a liver-specific promoter and manufactured using a clinical scale production method. One-time administration of the AAV-hGLA vector leads to hepatic production and secretion of α-GalA into the bloodstream, where the enzyme can be taken up by tissues via mannose 6-phosphate receptor mediated endocytosis.

Results:
In a 3-month pharmacology and toxicology study, one-time intravenous administration of increasing amounts of AAV-hGLA vector to GLAKO mice (age 2-3 months) was well tolerated and resulted in supraphysiological expression of plasma α-GalA (over 300-fold of normal levels) that was maintained for the duration of the study. Dose-dependent increases in tissue α-GalA activity were achieved in liver, heart and kidney. At the end of the study, Gb3 and lyso-Gb3 content was quantified in plasma and tissues via mass spectrometry. GLAKO mice in the high dose group generally had undetectable levels of Gb3 in the plasma, liver, spleen and heart and below 10% of substrate remaining in kidney compared to untreated GLAKO mice. This initial AAV-hGLA vector was compared to an improved vector, designated ST-920. Both vectors were manufactured using a clinical scale production method and compared in a 28-day study in wild type C57BL/6 mice. ST-920 was well tolerated and produced up to 7-fold higher levels of plasma α-GalA activity than mice administered the same dose of the initial vector. In this study, a single administration of ST-920 was able to achieve plasma α-GalA activity over 1,500-fold of normal levels.

Conclusions:
The high levels of α-GalA activity seen in these studies, along with the concomitant marked reduction in the accumulated Gb3/lyso-Gb3 in key tissues of the GLAKO mouse model, provide preclinical proof-of-concept for AAV-mediated targeting of hepatocytes to express therapeutic levels of human α-GalA. The clinical scale manufacturing process developed for these studies will enable rapid production of clinical-grade ST-920 material for a planned Phase I/II trial.

High-throughput 3D Brain Model of iPSC-derived Neurons and Glia for Prediction of Neurotoxicity and Safety Pharmacology

Chiwan Chiang, Karlijn J. Wilschut, Christian Ramakers, Arnaud Nicolas, Thomas Olivier, Dwayne Dexter, Henriëtte L. Lanz, Sebastiaan J. Trietsch, Jos Joore & Paul Vulto

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Purpose:
Prediction of neurotoxicity remains challenging due to the lack of relevant human-based 3D in vitro models of the brain and rely heavily on ex vivo and in vivo animal testing. We show the application of a human in vitro 3D CNS models developed in the OrganoPlate® that can be used for risk assessment during drug discovery and development.

Methods:
The high-throughput OrganoPlate platform consists of 96 microfluidic individual chips supporting 3D co-cultures of human iPSCs-derived neurons and astrocytes embedded in Matrigel with an adjacent easy accessible channel for medium perfusing and compound exposures.

Results:
During long-term culturing, the 3D mature CNS cultures show the presence of both GABAergic and glutamatergic neurons with astrocytes observed by plate-based immunostainings. Calcium fluctuations were detected using a calcium sensitive dye reflecting spontaneous electrophysiological activity after one week of cell culturing. Modulation of the neuronal activity was achieved by exposure to GABA, as well as neurotoxicant Methylmercury. The seizurogenic predictivity was shown after 4-AP exposure resulting in increased bursting patterns.

Conclusions:
Together with the multiplexing of assays to determine mitochondrial membrane potentials and neuronal viability, and additional neurite integrity, this CNS platform allow us to study different classes of neurotoxicants. Using automated high content microscopy this functional 3D neuronal model can be applied to high-throughput screening of specific regions of the human brain to better predict neurotoxicity and safety pharmacology, while additionally contributing to diminish the use of animal models.

Development of High-throughput Liver-on-a-chip for Drug Toxicity Screening

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Purpose:
In vitro tissue models have been used for decades as a tool to understand the mechanism of the drug toxicity. However, the current 2D and most of the 3D models lack the physiological relevance and tissue complexity to that of the in vivo situation. Better predictive in vitro screening models early in the drug discovery pipeline are critically required to increase success of drug candidates, reduce development costs of new medicines, and reduce animal studies.

Methods:
Here, we developed a 3D liver-on-a-chip model comprised of HepaRG and primary human hepatocytes using the OrganoPlate® platform. This microtiter-based platform consists of 40 to 96 individual microfluidic chips supports the development of an human hepatocyte co-culture with hepatic non-parenchymal cell in a membrane-free and pump-free perfusion system.

Results:
Besides having long-term high viability, this liver-on-a-chip model also shows liver functionalities such as synthesis of albumin, detoxification of ammonia, formation of bile acid and metabolism of drugs by cytochrome P450. Next to that, this model is applicable to determine concentration-dependent cytotoxicity and mitochondrial dysfunction upon the exposure to acetaminophen drug. The hepatic cultures show polarization leading to the formation of rhythmically contracting bile canaliculi that are able to disrupted upon the exposure of the cholestatic drug, a Y-27632 ROCK inhibitor analogue, fasudil. Moreover, this model also shows the accumulation of lipids after exposed to the steatosis-inducing drug cyclosporine A.

Conclusion:
These findings indicate that this model is suitable for understanding compound-specific hepatic toxicity and applicable for disease-modelling studying drug efficacy.

3D NephroScreen: High Throughput Drug-induced Nephrotoxicity Screening on a Proximal Tubule-on-a-chip Model

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Purpose:
Renal toxicity remains a major issue in clinical trials, and stresses the need for more predictive models fit for implementation in early drug development1. Here, we describe a perfused, leak-tight renal proximal tubule cell (RPTEC) model cultured within a high throughput microfluidic platform (Mimetas' OrganoPlate®)2, along with recent results from a 12-compound nephrotoxicity screen performed within the "NephroTube" CRACK IT consortium in collaboration with sponsors and the NC3Rs.

Methods:
Human RPTEC (SA7K clone, Sigma) were grown against a collagen I ECM in a 3-channel OrganoPlate®, yielding access to both the apical and basal side. Drug-induced toxicity was assessed by exposing kidney tubules to 4 benchmark and 8 blinded compounds with known clinical effects supplied by the sponsors for 24 and 48h. The tightness of the barrier was evaluated by diffusion of a dextran dye from apical to basal compartment. Parallel to this, cell viability with a WST-8 assay and the presence of LDH in the supernatant were assessed. Finally, kidney tubules were lysed, and RNA was extracted for gene expression analysis of acute kidney injury markers.

Results:
Upon perfusion flow, RPTEC form leak-tight confluent tubular structures against the collagen I ECM in the OrganoPlate®. The NephroScreen revealed significant decreased barrier tightness and cell viability in 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL was observed in 9, 5 and 7 out of 12 compounds respectively whereas claudin-2 showed a decrease in 6 out the 12. Overall, more effects were observed after 48h in comparison to 24h exposure.

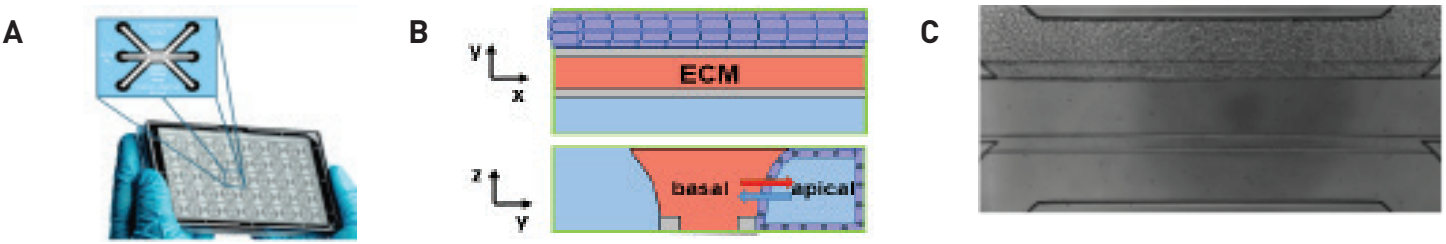

Figure 1. A) The 3-lane OrganoPlate® platform with 40 microfluidic cell culture chips embedded in a standard 384-well microtiter plate. **B)** Schematic overview of RPTC cells cultured against a collagen I ECM in a 3-lane OrganoPlate® yielding access to both apical and basolateral side. **C)** Phase-contrast image of RPTC cultured in the top channel of the OrganoPlate® at day 7.

Conclusion:
The kidney-on-a-chip model in the OrganoPlate® provides a promising in vitro renal toxicity tool to answer the desire to provide a better alternative to animal studies in terms of throughput, costs and predictivity and ultimately will be commercialised after further validation.

1. Wilmer, M. J. et al. Kidney-on-a-Chip Technology for Drug-Induced Nephrotoxicity Screening. Trends in Biotechnology 34, 156–170 (2016).
2. Trietsch, S. J., Israëls, G. D., Joore, J., Hankemeier, T. & Vulto, P. Microfluidic titer plate for stratified 3D cell culture. Lab Chip 13, 3548–3554 (2013).

Toward a More Universal HuLa Assay for Immunotoxicity Assessment

Neeraja Idamakanti, Yvonne Dragan, Matt Wagoner

Purpose:
The Human lymphocyte activation assay (HuLA) has demonstrated value as an in vitro model of immune suppression and activation – but requires ready access to recently immunized blood donors and their vaccine. The purpose of this study is to develop an off-the-shelf HuLA assay to monitor the effects of new pharmaceuticals on naturally-acquired immunity to infectious agents using frozen stocks of PBMCs to provide an early indicator of potential immunosuppression.

Methods:
Isolated human PBMCs from normal healthy donors were exposed to pharmaceuticals for 120 or 150 hours in the presence of peptide pools, each corresponding to a defined HLA class I or class II-restricted T cell epitope against infectious agents (CMV, EBV, influenza and tetanus toxoid) to monitor T cell health or TLR9 agonist, CpG ODN-2006, to monitor B cell health. Cell proliferation was measured by EdU incorporation assay using flow cytometry.

Results:
T cell stimulation by infectious agent peptides and B cell stimulation by TLR agonists resulted in proliferation as measured by 5-ethynyl-2´-deoxyuridine (EdU) incorporation. This proliferation was inhibited by clinically known immunosuppressive drugs (Tofacitinib, Prednisolone, Cyclosporin, Sirolimus, Ruxolitinib). The IC50 values obtained in this modified HuLA assay aligned well with the plasma concentrations at therapeutic doses of these drugs suggesting that these results may mirror the relevant mechanisms & concentrations that induce immunosuppression clinically.

Conclusion:
Results from our modified HuLA assay demonstrate that peptide-specific T cells and TLR agonist stimulated B cells proliferative responses are sensitive to immunosuppressants exposure and the concentrations are similar to Cmax levels. As peptides can be made easily and HLA-typed PBMCs from many different donors can be readily selected from commercial PBMC banks, this HuLA assay may be easily performed and readily adaptable to high throughput screening. Proliferative responses to peptide pools are robust since peptides cover many infectious agents, this helps easily differentiate immunosuppressant from non-immunosuppressant drugs. Therefore our HuLA assay method can be confidently incorporated into early stage drug development for immunotoxicity assessment.

Molecular Mechanisms of Cisplatin-Induced Toxicity to Acute Promyelocytic Leukemia Cells

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Purpose:
Cis-diamminedichloroplatinum (II) (cisplatin) is a widely used anti-tumor drug for the treatment of a broad range of human malignancies with successful therapeutic outcomes for head and neck, ovarian, and testicular cancers. It has been found to inhibit cell cycle progression and to induce oxidative stress and apoptosis in acute promyelocytic leukemia (APL) cells. However, its molecular mechanisms of cytotoxic action are poorly understood. We hypothesized that cisplatin induces cytotoxicity through DNA adduct formation, oxidative stress, transcriptional factors (p53 and AP-1), cell cycle regulation, stress signaling and apoptosis in APL cells.

Methods:
We used the APL cell line as a model, and applied a variety of molecular tools (cytotoxicity and oxidative stress assays, western blot analysis, flow cytometry, and confocal microscopy) to elucidate cytototoxic mode of action of cisplatin.

Results:
We found that cisplatin inhibited cell proliferation by a cytotoxicity, characterized by DNA-adduct formation, oxidative stress, cell cycle arrest, stress signaling and apoptosis in APL cells. Cisplatin also activated p53 and phosphorylated activator protein (AP-1) component, c-Jun at serine (63, 73) residue simultaneously leading to cell cycle arrest through stimulation of p21 and down regulation of cyclins and cyclin dependent kinases (cdks) in APL cell lines. It strongly activated the intrinsic pathway of apoptosis through alteration of the mitochondrial membrane potential, release of cytochrome C, and up regulation of caspase 3 activity by modulating p38MAPK pathway in APL cells.

Conclusion:
Overall the findings from this study provide novel targets of cisplatin mode of action that may be very useful in designing of new APL drugs.

Key words - Cisplatin, APL cell line, cell cycle modulation, AP-1, p53 and p38 MAPK signaling.

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Development of a Predictive in vitro Primary Proximal Tubule Platform for De-risking Nephrotoxicity Testing

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Purpose:
Nephrotoxicity is among the top five reasons for clinical attrition of compounds. Current in vitro models for detecting and de-risking nephrotoxicity are often inadequate as they lack apical and basolateral transporters responsible for compound uptake and disposition. Additionally, animal models suffer from poor predictivity for their human counterparts. Thus, better and more predictive models are needed which can be used for safety assessment of nephrotoxic compounds.

Methods:
In this study, we evaluated the utility of freshly isolated human primary proximal tubule cells (aProximateTM) seeded on Transwell 96-well plates for de-risking nephrotoxicity using kidneys from multiple different donors. Freshly isolated proximal tubule cells retained many of the key transport and metabolism functions that are both critical for toxicity and lost during cryopreservation. To assess the utility of aProximate cells, a variety of 36 mechanistically distinct pharmaceuticals were screened using the recently FDA qualified translational safety biomarkers; KIM-1, NGAL, and Clusterin to detect toxicity in vitro in addition to non-specific end points such as ATP depletion, LDH leakage, and TEER.

Results:
NGAL showed the highest predictivity with a sensitivity of 85%, specificity of 75% and area under the ROC curve of 0.86. Importantly, the model was able to correctly rank-order compounds from the same chemical class according to their clinical risk of causing drug-induced kidney injury. Using the cut-offs generated by the 30-compound dataset, an additional 10 Takeda internal compounds were screened, and the assay could distinguish nephrotoxic compounds from benign thus validating the predictivity of the current platform.

Conclusion:
Human proximal tubule cell monolayers retain a remarkable degree of differentiation and express a range of functional transporters and clinically relevant biomarkers of nephrotoxicity that are sensitive to nephrotoxin challenge over time. Human PTC monolayers show excellent potential as an in vitro predictive screening platform.

Predicting Hematotoxicity in Drug Development with HemaTox™ Assay

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In vitro colony-forming unit (CFU) assays allow for the assessment of hematopoietic progenitor cell growth and may be used to assess hematotoxicity in vitro. CFU assays have been validated for their ability to predict in vivo hematotoxicity, such as maximum tolerated dose for some hematopoietic progenitor cell lineages. However, while CFU assays are the gold standard for hematotoxicity evaluation, these semi-solid medium-based assays are low throughput and require expertise in colony identification. STEMCELL Technologies developed HemaToxTM assays to assess the toxicity of drugs on the growth and lineage-specific differentiation of human CD34+ hematopoietic stem and progenitor cells into one of three specific progenitor cell lineages (erythroid, myeloid, or megakaryocyte). These liquid medium-based assays, which show similar drug toxicity trends to those identified in CFU assays, can be performed in a 96-well format. Furthermore, HemaToxTM assays allow for flexible treatment regimens and improve the ability to evaluate effects of anti-proliferative drugs in vitro.

Characterizing a 384-well Throughput 14-color Flow Cytometry-based Model of Multi-lineage Hematopoiesis for Simultaneous Identification of Anemia, Thrombocytopenia, and Neutropenia Liabilities Throughout Drug Development

Authors: Nicholas Corr, Aaron Fullerton

Purpose:
To evaluate the sensitivity, specificity, and accuracy of a novel flow cytometry-based multi-lineage hematopoietic toxicity assay to simultaneously predict neutropenia, anemia, and/or thrombocytopenia liabilities by testing and reporting findings of a diverse clinical compound set.

Methods:
384-well throughput liquid-format simultaneous multi-lineage differentiation of primary bone marrow-derived CD34+ cells into erythroid, megakaryocyte, monocyte, neutrophil, and lymphoid blood lineages in the presence of a titrated compound of interest. After 6 days in culture, cells are harvested, stained with a 14-color flow cytometry panel and analyzed to evaluate compound impact simultaneously across 10 clinically relevant endpoints.

Results:
We report results of a >50 compound test set and observe simultaneous accurate prediction of anemia (80%), thrombocytopenia (92%), and/or neutropenia (95%), with additional evaluations ongoing.

Conclusions:
These results demonstrate utility of this assay to quickly and thoroughly characterize direct hematopoietic liabilities in early discovery and lead optimization stages of drug development as well as provide a means to identify drug mechanisms of action to help inform on dose scheduling and combinability of therapies all in a highly cost-effective manner.

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NOVA The NOVA team has been involved in ion channel research since the 1980's. They have a long history of providing data for regulatory guidelines. Nova has conducted the hERG screening for the original ILSI/HESI safety study which became the groundwork for the current S7B guidelines, and members of our team have been contracted by the FDA to provide ion channel screening in support of the new CiPA safety assessment guidelines. NOVA has worked with 100's of companies along with multiple government agencies to help de-risk compounds.

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