ABSTRACTS FROM THE ISSX/DMDG 2023 MEETING

June 11-14, 2023
University of Hertfordshire, UK

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Contents

Sunday, June 11, 2023

Monday, June 12, 2023

Tuesday, June 13, 2023

Wednesday, June 14, 2022

SC1.1 - INTRODUCTION AND APPLICATIONS OF PBPK MODELING

Neil Parrott
F. Hoffman La Roche, Switzerland

Over the last 15 years, PBPK modeling has changed from an academic curiosity to a key component of small molecule pharmaceutical development with regulatory impact. What exactly is meant by PBPK? When is it useful? Who can use it? This lecture will cover the basic principles of PBPK modelling and give example of impacts in drug discovery and development projects.

SC1.2 - HUMAN DOSE PREDICTIONS USING PBPK MODELING IN HV

Claire Jackson
GSK, United Kingdom

Human pharmacokinetic (PK) predictions are routinely used in the pharmaceutical industry and are vital in drug discovery for selecting quality candidate compounds and in drug development for predicting the likely PK, first within healthy volunteers (HV) before extending to the target patient population. Physiologically Based Pharmacokinetic (PBPK) modelling is increasingly being used in this arena to make more informed predictions and guide programs through discovery and development phases. This short course will cover some of the practical steps and considerations in this process, combining *in vitro*, *in vivo* and *in silico* knowledge with industry PBPK platforms and provide examples and case studies. You will learn why we begin with HV predictions, what data inputs are required, how to critically analyse and interrogate this data using PBPK, how to translate the results between species and combine this knowledge with pharmacodynamic and potency data to predict likely efficacious human doses.

SC1.3 - CONSIDERATIONS OF HUMAN DOSE PREDICTIONS IN SPECIFIC AND PATIENT POPULATIONS USING PBPK

Gareth Lewis
GSK, United Kingdom

Physiologically Based Pharmacokinetic (PBPK) modelling is routinely used in the pharmaceutical industry. It is now not only an expectation by Regulatory Agencies upon submission, but also an increasing expectation to apply PBPK models to the target patient population and/or specific populations and not just consider healthy volunteers (HV). PBPK is being used in this arena to make model-informed drug development and decision-making predictions (MID3) and guide programs through discovery-development phases, clinical trial designs and to consider the impact on the human efficacious dose predictions. This short course will cover some of the practical steps and considerations in this process, combining *in vitro*, *in vivo* and *in silico* knowledge with industry PBPK platforms and provide examples and case studies. You will learn why specific and/or patient populations may differ with HV predictions, what data inputs are required, how to critically analyse and interrogate this data using PBPK, how to translate the results between specific and patient populations, drug-drug interactions, or disease-drug interactions.

SC1.4 - PBPK OF MONOCLONAL ANTIBODIES AND NOVEL MODALITIES

Armin Sepp
Certara, United Kingdom

The recent decade has seen introduction of old and new modalities into drug discovery pipelines to tackle ever more challenging therapeutic targets. On one hand, in addition to typical monoclonal antibodies, novel biologics often come with substantial modifications that enhance not only target binding affinity but also provide properties beyond what exists in nature, like bispecificity, enhanced or controlled Fc-related functionality like extended half-life, complement and immune system activation, controlled attachment of covalently bound small molecule payload molecules in novel conjugation formats etc. In addition, new drugs are appearing that combine both small and large molecule characteristics, e.g. peptides and oligonucleotides that are chemically synthesized and heavily modified, yet share many PKPD properties with biologics. All this poses new challenges and invites ever more complex models to accommodate the tissue distribution, absorption and elimination properties of these compounds, as well as their mechanisms of action and pharmacological effects downstream from target binding. The sheer complexity of these models has now reached a threshold were new approaches are required to model assembly: principally in the form of automated model-building capability for full PBPK and beyond.

SC2.1 - WHY BRO5 COMPOUNDS HAVE POOR PK AND WHAT CAN BE DONE ABOUT IT?
Donglu Zhang
Genentech, United States

Why bRo5 Molecules Have 'Poor' PK Profiles and What Can We Do About It? New modality molecules e.g. MCPs, PROTACs, ASOs usually show limited oral absorption, high clearance, and low exposures, but have potential to modulate difficult-to-druggable targets. Their physicochemical properties include high molecular weight (MW), high polar surface area (PSA), high number of rotatable bonds that are beyond the Lipinski's rule-of-5 (bRo5) space. Their large MW and flexible structures have prevented effective optimization of *in vitro* and *in vivo* pharmacokinetics profiles without affecting their cell potency and selectivity. Fundamental questions one can ask 1) How can we deliver these molecules to the targets, 2) will these molecules show uptake in tissues and what are the tissue distribution patterns, 3) are they metabolically stable *in vivo*, 4) are they retained in tissues or tumors, 5) can we develop a practical dosing regimen in patients? This presentation will focus on delineate the key physicochemical and ADME properties like solubility/permeability that determine pharmacokinetic profiles. Novel delivery strategies of bRo5 molecules will be discussed. In addition, the discussion will also include how to be an effective DMPK rep on a new modality discovery team.

SC2.2 - ADME CHALLENGES OF TARGETED PROTEIN DEGRADERS
Caroline Rynn
F. Hoffman La Roche, Switzerland

Targeted protein degraders (TPD) constitute a novel therapeutic modality which induce selective degradation of target proteins involved in disease pathogenesis, by capitalising on the cells own protein destruction machinery - the ubiquitin-proteasome system. The properties of TPDs, specifically bifunctional molecules, largely violate accepted physicochemical limits (e.g. Lipinski's Rule of 5) for oral bioavailability, which may lead to ADME optimisation challenges. This course is intended to give a broad outline of the physicochemical properties of TPDs and their impact on the absorption, distribution, metabolism, and excretion (ADME) profiles as well as to provide an overview of the available tools to characterize and quantify these properties.

SC2.3 - DMPK AND MODELING AND DDI CONSIDERATIONS FOR OLIGONUCLEOTIDES
Farzaneh Salem
GSK, United Kingdom

Oligonucleotide based therapeutics are a new and growing class of pharmaceuticals in development. Due to their ability to provide target specificity this class of drugs are valuable and attract attention, especially in the field of cancer treatment, genetic disorders, infectious diseases, and metabolic disorders. However, the pharmacokinetics and potential for this class to act as perpetrators of drug-drug interactions (DDIs) has not been extensively researched or reported in the literature. The limited clinical interaction studies conducted with victim drugs that are cleared by major CYPs, UGTs, renal elimination, or nucleoside kinases, none has shown a clinically significant DDI by therapeutic oligonucleotide. Although oligonucleotides are very different from small molecules in physicochemical and pharmacokinetic characteristics, the regulatory DDI guidance for oligonucleotide therapeutics considered to be the same as small molecule drugs. It is encouraged to evaluate their potential for DDI. To satisfy these recommendations, *in vitro* studies are typically carried out to evaluate whether a new chemical entity is a substrate of CYPs or transporters, or if it can inhibit or induce major them. The *in vitro* results are evaluated by basic models using intrinsic clearance. If the basic models flagged a risk, DDI potential should be investigated by mechanistic models or conducting a clinical DDI study with a sensitive substrate. The alternative mechanisms for causing DDI through modulation of cytokines or heme synthesis is less likely but still plausible. Overall, the totality of evidence confirms that inhibition or induction of drug-metabolizing enzymes and transporters mediated by oligos is not likely.

K1 - PHARMACOGENOMICS: FROM DISCOVERY TO IMPLEMENTATION
Munir Pirmohamed
University of Liverpool, United Kingdom

Pharmacogenetics/genomics has been around for a long time, and it is only now that we are beginning to think about large scale implementation. Discovery can be looked at from two perspectives: first, the identification of an association between phenotypes (drug efficacy and safety) and genotype. The use of genome-wide approaches has certainly helped in this area, particularly for the discovery of pharmacodynamic gene variants, but more work is needed. There is also a need to increase the diversity of people studied as this will reduce health inequalities and increase the number of drug-

gene associations. Second, the use of genomic data can also be used for drug discovery, with studies showing that such an approach increases the chances of a drug getting to market at least 2-fold. It is important that any discovery is also linked to translation into clinical practice. This has generally been slow and fragmented. Worldwide, however, there is now greater emphasis on implementation, with many different approaches and areas being investigated including pre-emptive genotyping. Implementation is being explored in many different parts of the world, but most frequently in the US. To date, no healthcare system in the world has implemented pharmacogenomics for the whole population. The recent PREPARE study showed that using a 12-gene pharmacogenetic panel was able to reduce adverse drug reactions by 30%, a clinically impactful result. Barriers to implementation are no longer related to the availability of genotyping technologies or to the lack of evidence, but are due to difficulties in integrating the whole process into healthcare systems. The presentation will consider the whole spectrum from discovery to implementation into clinical practice.

PL1 - IT TAKES A COMMUNITY: CREATING TOGETHER IN SCIENCE AND PRODUCT DEVELOPMENT

Suzanne Iverson Hemberg
Toxicology Knowledge Team Sweden AB, Sweden

This keynote address marks the announcement of the appointment of the fifth DMDG fellow. DMDG fellows are members who have made outstanding contributions, long term commitment and high-level service to the DMDG and related sciences. This year, the DMDG is delighted to announce the appointment of Suzanne Iverson Hemberg. An accomplished scientist in the fields of biotransformation, biomarkers of toxicity, drug distribution and the bioanalytical techniques needed to study these, Suzanne has also held leadership roles throughout her career. Championing the DMDG for many years, Suzanne presented in and chaired sessions at several open meetings, served on the committee, chaired the scientific organizing committees for two open meetings, co-edited a book in honour of the DMDG and not least playing a key role in forging and maintaining links between the DMDG and the Swedish Pharmaceutical Society (SPS).

S1.1- GENERATION OF BLOOD-BRAIN BARRIER TRANSPORTER SCALING FACTORS TO SUPPORT *IN VITRO-IN VIVO* EXTRAPOLATION (IVIVE)

Zubida Al-Majdoub
University of Manchester, United Kingdom

Understanding the quantitative implications of P-gp and BCRP efflux is a key hurdle in the design of effective, centrally acting drugs. Access of drugs to the central nervous system (CNS) is limited by the blood–brain barrier (BBB), and this in turn affects drug efficacy/toxicity.1 To date, most drug discovery optimization paradigms have relied heavily on *in vitro* transporter assays and preclinical species pharmacokinetic evaluation, which provide a qualitative assessment of human brain penetration.2 Transporters abundances at the BBB (brain microvessels; pre-clinical species) and *in vitro* systems can inform development of *in vitro-in vivo* extrapolation (IVIVE) integrated in physiologically-based pharmacokinetic (PBPK) models, enabling translation to human. Here, we have measured the expression of efflux transporters (P-gp and Bcrp) in rat brain microvessels and in transfected grMDCK, MDCKII or LLC cells) and wildtype (gMDCK, MDCKII or LLC-PK1 cells). The aim of this work was to generate scaling factors to develop a physiologically-based pharmacokinetic (PBPK) model of the brain in rats and to support *in vitro-in vivo* extrapolation (IVIVE) of *in vitro* P-gp transporter kinetics. To evaluate the model's ability to predict local brain concentration of drugs. Eventual goal: Translation of the model to human based on proteomics-informed IVIVE of *in vitro* P-gp and BCRP transporter kinetics.

References:

1. Taggi et al. Pharmaceutics. 2022; 14(7):1376
2. Di et al. Drug Metab Dispos. 2013; 41(12): 2018-2023

S1.2 - APPLICATION OF A RAT LIVER DRUG BIOACTIVATION TRANSCRIPTIONAL RESPONSE ASSAY THAT INFORMS ON POTENTIAL FOR DRUG-INDUCED LIVER INJURY

James Monroe
Merck, United States

A drug safety testing platform that relies on gene expression biomarkers has been developed that informs on the potential for clinical drug-induced liver injury (DILI) (Kang et al., 2020; Monroe et al., 2020). Clinical DILI is poorly detected in nonclinical studies and the method described identifies a source of hepatotoxicity risk for compounds in the early drug discovery process prior to drug candidate selection. The DILI gene expression biomarker panel, so-called liver response assay (LRA), provides a measure of the potential for reactive-metabolite mediated liver injury in clinical settings. We describe the development and qualification of the LRA and its application to both early *in vitro* (rat or human) screens or *in vivo* rat studies. Case studies will highlight how this approach is used in conjunction with other gene expression, ADME

and *in silico* tools. Refinement of the assay is ongoing for better informed risk assessment with consideration of clinical dose projections.

S1.3 - USE OF 'OMICS TO UNDERSTAND SPECIES DIFFERENCES IN SENSITIVITY TO DRUG INDUCED LIVER INJURY

Ian Copple
University of Liverpool, United Kingdom

To minimize unexpected toxicities in early phase clinical studies of new drugs, it is vital to understand fundamental similarities and differences between preclinical test species and humans. This presentation will describe a study conducted as part of the EU Innovative Medicines Initiative TransQST consortium, which combined physiologically-based pharmacokinetic (PBPK) modelling with 'omics approaches to enable comparison of tissue adaptive responses under conditions of equivalent chemical insult. To overcome the well-documented species differences in the metabolic bioactivation and detoxification of the model hepatotoxin acetaminophen, PBPK modelling was used to identify doses yielding similar hepatic burdens of the reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) in mice and rats. Following administration of acetaminophen at these doses, mice exhibited a greater degree of liver injury than rats, despite the confirmation of equivalent hepatic NAPQI burden. Transcriptomic and proteomic analyses highlighted the stronger activation of stress response pathways (including the Nrf2 oxidative stress response and autophagy) in the livers of rats. Components of these pathways were also found to be expressed at a higher basal level in the livers of rats compared with both mice and humans. Our findings exemplify a systems approach to understanding differential species sensitivity to hepatotoxicity, and have important implications for species selection and human translation in the safety testing of new drug candidates.

S2.1 - THE ROLE OF Kp,uu IN CNS DRUG DESIGN - CURRENT STATE AND HORIZON BEYOND

Scott Summerfield
Pharmaron, United Kingdom

The concept of brain Kp,uu has made a significant impact on CNS Drug Discovery helping to focus attention onto the importance of unbound drug concentrations in the brain rather than total concentrations. Kp,uu measures the unbound concentration gradient across the blood-brain barrier (BBB) thus enabling an understanding of the relative importance of passive diffusion, efflux and uptake. Like any disruptive concept Kp,uu has helped address some longstanding questions while also offering up new ones as we dig deeper into linking effect to the drug concentration bathing the target. New questions of note include translational aspects of Kp,uu to gain a better understanding of the likely brain penetration in humans, harmonisation and standardisation of lab workflows, and reducing the measurement errors to tighten the predictive value (e.g., is a Kp,uu of 0.3 – 0.5 an indicator of drug efflux or within the measurement variability for passive diffusion). Current research is investigating these and other aspects that will continue to enhance the place of Kp,uu in the CNS Drug Discover's Tool Box.

S2.2 - CAN CARBOXYLIC ACID DRUGS BE CNS PENETRANT

Hugh Walton, Jessie Stow, Nicola Wilsher
Astex Therapeutics, United Kingdom

Drug Metabolism and Pharmacokinetics (DMPK) plays a key role within drug discovery, aiding the progression of drug candidates from hits-to-leads through to the clinic. In early drug discovery, *in vitro* techniques are applied to predict the *in vivo* characteristics of compounds. Then, *in vivo* profiling of compound efficacy and safety assists the development of potential clinical candidates.
Therapies that target the Central Nervous System (CNS) have an additional obstacle to overcome, namely the Blood-Brain Barrier (BBB). Therefore, the BBB has become a particular focus for CNS targeted projects and *in vitro* compound screens have been designed to improve prediction of drug BBB penetration. Characterisation of lipophilicity and permeability has become fundamental in assessing a compounds potential to enter the CNS, since a comprehensive model of the BBB is yet to be developed.
Particular chemical classes of compounds, i.e. acidic compounds, are considered more challenging to achieve efficacious concentrations in the brain. Here, we have evaluated some acidic drugs which target the CNS, and characterised their *in vitro* properties to predict *in vivo* BBB penetration. For a drug to be determined as potentially brain penetrant *in vitro*, it would need to be lipophilic, permeable across an MDCK monolayer, and not liable to efflux transporters.
However, we show that *in vivo* analysis of compounds which did not have the desired *in vitro* profile, demonstrated unexpected BBB penetration. These data indicate the potential limitations of using *in vitro* assays to definitively predict the BBB penetration properties of certain chemotypes. This study shows that a comprehensive evaluation of particular

chemotypes *in vitro* and correlation with *in vivo* data is required to avoid the incorrect elimination of potential CNS candidates during the lead optimisation process.

S2.3 - CHALLENGES AND LEARNINGS IN THE DEVELOPMENT OF BRAIN PENETRATING LARGE MOLECULE THERAPEUTICS

Niels Janssen
F. Hoffman La Roche, Switzerland

Proteins such as mAbs offer access to fundamentally different modes of action from small molecule drugs, making them intriguing modalities for the development of novel therapeutics. Despite the recent success of monoclonal antibodies in Alzheimer's disease, limited access to the central nervous system (CNS) continues to be an intrinsic limitation of conventional mAbs. We developed “Brain Shuttle”, a versatile platform to enhance brain delivery of therapeutic proteins and other cargos across the blood brain barrier via Receptor mediated transcytosis. We share challenges and solutions through the lens of a DMPK scientist from the 10 year journey of bringing the first Brain shuttle therapeutics to patients.

PS1.1 - OPTIMISING PROTACS FOR ORAL DRUG DELIVERY: A DRUG METABOLISM, PK AND PKPD PERSPECTIVE

Dermot McGinnity
AstraZeneca, United Kingdom

Proteolysis-targeting chimeras (PROTACs) are an exciting new therapeutic modality with the potential to open target space not accessible to conventional small molecules via a degradation-based mechanism. However, their bifunctional nature can result in physicochemical properties that breach commonly accepted limits for small-molecule oral drugs. In this lecture I offer a drug metabolism and pharmacokinetics (DMPK) perspective on the optimisation of oral PROTACs across a portfolio established within Oncology R&D at AstraZeneca, highlighting some of the challenges presented to established screening cascades. I will demonstrate that acceptable oral PK properties for this modality are achievable despite the physicochemical property challenges they present. Furthermore the experience of implementing translational pharmacokinetic-pharmacodynamic (PKPD) strategies for PROTACs is less mature compared to conventional small molecule antagonists. The relationship between circulating compound levels and target degradation is complex due to the interplay of the target protein-of-interest, an ubiquitin E3 ligase and the PROTAC to form a ternary complex. I will present key insights and modelling approaches that may improve our predictive ability, hence enabling acceleration of PROTAC drug discovery programs and increased probability of clinical success.

PS1.2 - PHYSCHEM AND DESIGN ASPECTS RELATED TO DRUG DISCOVERY OF PROTACS: A FOCUS ON ADME PROPERTIES

Giuseppe Ermondi
University of Turin, Italy

PROTACs (Proteolysis-Targeting Chimeras) are heterobifunctional molecules capable to induce protein degradation via the recruitment of the ubiquitin-proteasome system. PROTACs are made by three moieties: a protein of interest (POI) ligand (often called warhead), an E3 ubiquitin (E3) ligase-recruiting ligand and a linker connecting the two components. PROTACs can form a stable ternary complex involving the E3 ligase and the POI simultaneously. In this way, the POI and the E3 ligase are in close proximity, inducing polyubiquitination of the POI and thus degradation by the proteasome. Because PROTACs are made of three different building blocks, they result in large structures for which the simultaneous optimization of pharmacodynamics and ADME is quite arduous and time-consuming. Therefore to accelerate the discovery of new PROTAC drugs a well-thought design strategy is required mainly to overcome permeability and solubility issues. PROTACs belong to the so-called 'beyond the rule of 5' (bRo5) chemical space where they form a distinct cluster. In particular, PROTACs are generally flexible and rich in donor and acceptor hydrogen bond moieties, resulting in molecules with a large number of accessible conformations, generally prone to form intramolecular interactions. Capturing the relevant physicochemical properties of this class of compounds calls for calculated molecular descriptors and methodologies that should consider the three-dimensional structure of the molecules and that will be presented here. In addition, the irreplaceability of experimental descriptors will be also discussed by examining a set of chromatographic determinants developed specifically for the bRo5 chemical space.

PS1.3 - PK/PD-MODEL BASED GUIDANCE OF PROTAC OPTIMISATION
Andreas Reichel
Bayer, Germany

The talk will elaborate on how the optimization of the pharmacological properties of PROTACs can be guided by PK/PD-modeling. A comprehensive modeling framework has been developed to integrate experimental data, as routinely available in early project phases, with the aim: (1) to assess PROTACs based on accurate degradation metrics, (2) to guide compound optimization of the most critical parameters, and (3) to link target protein degradation to downstream pharmacodynamic effects. The presented framework contains a number of first-time features, such as a mechanistic model to fit the hook effect in the PROTAC concentration-degradation profile, the quantification of target occupancy and its role in the PROTAC mechanism of action, and the ability to deconvolute the combined effects of target degradation and target inhibition on the overall pharmacodynamic response of PROTACs. The applicability of the modeling framework is shown using project examples illustrating how it can be used for a better experimental study design as well as a better understanding of the data, ultimately leading to a more successful PROTAC discovery.

PS1.4 - TRANSLATIONAL PK/PD MODELING OF PROTACS: A CASE STUDY OF STAT3 DEGRADERS
Andreas Harsch
Kymera, United States

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

PS2.1 - PK ENHANCING CO-THERAPIES, CASE STUDY OF A DESIGNED CO-THERAPY (INQOVI) VERSUS REPURPOSED DRUGS
Aram Oganesian
Astex Pharmaceutials, United States

Decitabine is a hypomethylating agent (HMA) and was approved by FDA for treatment of intermediate/high-risk MDS for IV infusion and by EMA for treatment of AML patients not fit for intensive induction chemotherapy. Oral administration of decitabine, or other HMAs as single agent, has proven challenging as high first-pass due to metabolic degradation by cytidine deaminase (CDA) results in low bioavailability. Cedazuridine, a synthetic analog of tetrahydrouridine, is a potent inhibitor of CDA and oral combination with decitabine enhances the oral bioavailability of decitabine. Astex developed INQOVI, oral decitabine+cedazuridine, and achieved approval of the label with the same indication as IV decitabine, using clinical pharmacology data as primary endpoint in the registrational phase-3, targeting pharmacokinetic (AUC) equivalence against reference IV decitabine to demonstrate biological comparability. Additional supportive pharmacodynamic data (LINE-1 demethylation) were also used. Clinical efficacy and safety were secondary endpoints in phase-3. Details of development of oral decitabine/cedazuridine fixed-dose combination including nonclinical background; regulatory considerations; and clinical pharmacology endpoints comparing oral vs IV will be presented.

PS2.2 - APPLICATION OF MICE HUMANISED FOR PATHWAYS OF DRUG DISPOSITION TO RESOLVE SPECIES DIFFERENCES IN DRUG DISCOVERY AND DEVELOPMENT
Roland Wolf, Kenneth Macleod, and Kevin Read
University of Dundee, United Kingdom

When testing new drug candidates or new chemical entities (NCEs) in human subjects, the time, cost and regulatory hurdles can increase exponentially. Therefore, significant emphasis has been placed on developing preclinical methodologies that provide more predictive information about human drug metabolism, pharmacodynamic responses and drug safety. The major pathways involved in human responses to drugs and environmental chemicals have been elucidated predominantly through studies in rodents. They are also of vital importance in furthering our understanding of human disease by serving as disease models, often engineered to reflect a human disease, for drug efficacy testing and, more recently, as a model system for the personalization of the treatment of diseases such as cancer (1,2). However, the fundamental differences between rodents and humans in pathways of drug metabolism and disposition, both qualitative

and quantitative, can, at least in part, explain why preclinical studies frequently do not extrapolate to the clinic.
In order to circumvent the limitations of animal models to study drug efficacy and drug disposition we have created a mouse model that closely reflects human pathways of drug disposition, we have substituted 33 murine P450s from the major gene families involved in drug disposition, together with Car and Pxr, for human CAR, PXR, CYP1A1, CYP1A2, CYP2C9, CYP2D6, CYP3A4, and CYP3A7 (the 8HUM model). The applications of this model in drug development , which could include the replacement of conventional mouse models in drug discovery and the more informed design of clinical trials will be discussed in this presentation.

PS2.3 - ORGAN-ON-CHIPS FOR IMPROVED PREDICTION OF HUMAN ADME PARAMETERS
Yassen Abbas
CN-Bio Innovations, United Kingdom

Accurately predicting ADME parameters in humans during pre-clinical development is crucial as it forms the basis for setting safe and efficacious doses in the clinic. Due to their simplicity, most current in-vitro ADME assays are limited by poor physiological relevance whereas animal models circumvent this but are in turn limited by a host of interspecies differences. Organ-on-a-chip (OOC) technologies have been developed to bridge the gap between traditional in-vitro assays and in-vivo tissue functionality in humans. Here, we show the predictive potential of OOC compared to standard approaches with a focus on a multi-organ model for the determination of human oral bioavailability.

PL2 - NO WAY BACK: MOVING FORWARD WITH PBPK MODELING (WHY? HOW?)
Aleksandra Galetin
University of Manchester, United Kingdom

Physiologically-based pharmacokinetic (PBPK) modelling is a powerful translational tool which has now established its acceptance in regulatory submissions with a variety of applications. PBPK-informed predictions support decisions on the necessity, timing and design of clinical trials, exploration of untested/“what-if” scenarios or to complement unavailable/ limited clinical data in certain patient populations (e.g., pediatrics). The plenary presentation will illustrate current status and emerging areas of PBPK modelling, namely expansion of PBPK modelling to disease and special population to inform the inclusion of patients in late-phase clinical trials.
Integration of PBPK modelling with imaging, biomarker and exosome-based approaches to support evaluation of transporter-mediated drug-drug interactions, assessment of disease-related changes in transporter function and associated inter-individual variability will be illustrated.

S3.1 - SWIFTPK: DEVELOPMENT AND IMPLEMENTATION OF A HIGH THROUGHPUT PBPK FRAMEWORK TO INFORM EARLY DRUG DISCOVERY
Andrés Olivares-Morales
F. Hoffman La Roche, Switzerland

Physiologically-based pharmacokinetic (PBPK) modeling has been extensively used over the past two decades as a tool to inform and accelerate drug development and to generate mechanistic insights regarding the interplay between physiological and compound properties driving pharmacokinetics (PK) and pharmacodynamics (PD). PBPK reduces unnecessary experimentation (be it in animals or humans) and allows the assessment of previously untested scenarios and has gained both academic and regulatory acceptance. Health agencies accept PBPK modeling in lieu of human clinical trials, particularly in the field of drug-drug interactions, special populations and absorption modeling. Despite its widespread acceptance in drug development, PBPK use as part of the medicinal chemistry and DMPK toolbox in early drug discovery (from compound design towards clinical lead selection) is limited, mainly due to a lack of awareness and the complexity of implementation. Over the last years at Roche we embarked on a journey to incorporate PBPK modeling as part of the standard toolbox in early teams. We have validated the approach and facilitate its use by creating a fully automated pipeline that connects a simulation interface to our internal data sources, allowing simulations of PK, PK/PD and human doses at very early stages that allow compound prioritization and selection. This talk discusses the journey, implementation and impact that this novel pipeline and approach has generated so far in some of our early small molecule project teams, reducing cycle times and providing insights that were previously unavailable.

S3.2 - IMPLEMENTING MACHINE LEARNING AND EARLY PBPK MODELS IN EARLY DRUG DISCOVERY

Yanran Wang
Genentech, United States

In the early phase of small molecule discovery research, *in silico* and *in vitro* ADME models are widely used as screening tools. At this stage, molecular properties (e.g. MW, charge, lipophilicity), *in vitro* ADME endpoints (e.g. PPB, permeability), potency against the intended targets are all taken into consideration in subjective scoring methods. The high-dimensionality of the problem makes it complex and difficult to constrain the number of pre-clinical *in vivo* experiments needed to identify promising candidates. PBPK models are mechanistic frameworks using physiological parameters and differential equations to define the PK of drugs. We validated the feasibility of introducing PBPK to early space and gained learnings in applying this technology in ongoing projects.

S3.3 - VIRTUAL DRUG DISCOVERY AND DEVELOPMENT COMBINING MACHINE LEARNING AND QUANTITATIVE SYSTEMS PHARMACOLOGY APPROACHES

Helle van den Maagdenberg[1], J.G.C. van Hasselt[1], P.H. van der Graaf[1,2], and G.J.P. van Westen[1]
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Many promising machine learning techniques have been developed and successfully applied to optimize target affinity of molecules for the discovery of novel drug candidates. However, clinical efficacy of drug candidates is dependent on more than just target affinity. Quantitative systems pharmacology (QSP) models can describe the relationship between pharmacokinetics, receptor activation and biomarkers for efficacy and toxicity. The therapeutic area of immuno-oncology is one area where the mechanistic link between target binding and effect is complex and poorly characterized. In this context, adenosine A2aR blockade has been proposed as a source of novel immune checkpoint inhibitors[1]. In this case study, we demonstrate how integration of QSP models in the virtual drug discovery workflow can be used to inform discovery of novel adenosine A2a receptor (A2aR) inhibitors.
Novel ligands were generated for the adenosine A2aR using DrugEx [2], which is a de novo drug generation model using recurrent-neural network-based reinforcement learning. The scoring in this framework was performed by several quantitative structure-property relationship models (QSPR) models for target binding and pharmacokinetic properties. QSPRpred, a novel QSPR modelling tool, was used to build models for adenosine receptor affinity, clearance, volume of distribution and unbound drug fraction. In addition to their use as the molecular scorers in the de novo drug design, these four models form the input for a QSP model. This model by Voronova et al. [3] uses ordinary differential equations to capture tumour size dynamics in mice for the A2aR inhibitor AZD4635. Here, the pharmacokinetic profile of AZD4635 was replaced by the predicted pharmacokinetics of the generated compounds. Through simulations, this model was then used to compare tumour inhibition efficacy of the predicted inhibitors.
In this presentation, the performance and limitations of the different QSPR models will be discussed. The characteristics of the generated A2aR inhibitors including QSP simulation results will be outlined and compared to known A2aR inhibitors. Finally, challenges of integrating pharmacokinetics and QSP models in the de novo drug discovery pipeline will be explored. In addition, future prospects and more potential use cases will be highlighted.

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2. Liu, X. et al. Journal of Cheminformatics (2021) 13 (1) 85
3. Voronova, V. et al., Frontiers in Immunology. (2021), 12 (1)

S4.1 - THE TRANSBIOLINE SAFETY BIOMARKER PIPELINE AND FOCUS ON MIRNAS

Sophia Samodelov
University of Zurich, Switzerland

The Translational Biomarker Pipeline (TransBioLine) focuses on the development and regulatory qualification of safety biomarkers for implementation in drug development in the context of use of phase I clinical trials. This international consortium consists of academic and pharma industry researchers, clinicians, regulatory, and sample/data management specialists in the ambitious endeavors of qualifying novel urine and blood-derived biomarkers with the FDA and EMA aimed at the more sensitive and specific detection of drug-induced injury of the liver, kidney, vasculature, pancreas, and nervous system. These efforts focus on aiding and streamlining decision-making during drug development, offering tools in conjunction with standard biomarkers of drug-induced injury, towards ultimately safer and more robust drug development processes and patient safety. A key avenue of TransBioLine's work is the focus on miRNAs in a pan-consortium way: untargeted NGS sequencing of roughly 300 normal healthy volunteer samples, along with samples collected from patients with vascular, nervous system, liver, and pancreatic injury of various etiologies, has facilitated the large-scale collection of robust miRNA data to study organ damage and establish reference ranges in healthy individuals.

Lead candidate selection of potential miRNA biomarkers for each of the organ injury working groups has been largely completed, with confirmatory studies using quantitative RT-PCR for select candidates being foreseen within the project. In addition, the feasibility of cross-organ work package analyses of NGS data are being explored. TransBioLine work on miRNA research in the frame of biomarker development has implemented streamlined sample collection and preparation, standardized quantitative NGS analysis, and robust statistical analysis approaches dependent on the sample matrix and obtained data across patient cohorts.

S4.2 - MIRNA BIOMARKER DEVELOPMENT: PITFALLS AND BEST PRACTICES FOR DISCOVERY AND VALIDATION

Matthias Hackl
TaMiRNA, Austria

MicroRNAs feature several characteristics that render them promising and useful biomarkers to study the adverse effects of xenobiotics on cells and tissues, including cross-species conservation, high and stable presence in biofluids, tissue specificity, and a highly regulated transcription that shapes cellular homeostasis.
Adapted next-generation sequencing (NGS) workflows termed small RNA-sequencing allow the quantification of thousands of microRNAs in parallel in biological samples and enable unbiased selection of novel biomarker candidates. For the TransBioLine project, we implemented a small RNA-seq workflow with sufficient sensitivity to detect very low abundant microRNAs in human plasma samples. We followed a fit-for-purpose validation protocol to characterize the analytical variability and sequencing bias of the NGS workflow. Further, we developed a bias-free spike-in control set (miND®) to enable quality control and normalization of microRNA NGS data.
We applied the miND(R) small RNA-seq assay for genome-wide analysis of microRNAs in >400 plasma samples from healthy volunteers to characterize the magnitude and sources of biological variation in normal populations. To understand pre-analytical variability, we investigated sample matrix effects that can derive from variations in plasma preparation protocols, and we performed a prospective 36-month stability study with plasma from normal and chronic liver disease patients. Our data suggest that this discovery assay is fit for the purpose of discovering novel miRNA biomarker signatures, which will be shown in the example of neurotoxicity microRNA biomarkers in CSF and serum samples.

S4.3 - MIRNAS AS CLINICAL BIOMARKERS OF DRUG-INDUCED LIVER AND PANCREATIC INJURY

Warren Glaab
Merck & Co., Inc., United States

MicroRNAs (miRNAs) are currently being evaluated as biomarkers of drug-induced tissue injury. Characteristics of these miRNAs include high abundance in tissues, tissues specificity for individual miRNAs, and the relative stability in plasma following release from injured tissues. Liver and pancreas miRNAs have been identified and sensitivity and specificity to drug-induced injury demonstrated nonclinically, further providing supporting evidence for their translation to clinical settings to monitor safety liabilities in early clinical trials. This presentation will highlight previous efforts demonstrating their performance in nonclinical studies, correlation with microscopic histopathology, and then focus on the translation and early performance in human samples with liver and pancreas injury. Leveraging tissue-specific miRNAs in this manner provides one directed approach to translate key miRNA candidates and supplements the TransBioLine approach to generate profiles and identification of additional miRNAs as a signature for drug-induced injury in these tissues.

S4.4 - STATISTICAL AND BIOINFORMATIC STRATEGIES FOR EVALUATING MIRNAS AS NEW BIOMARKERS

Anthony Evans
University of Liverpool, United Kingdom

The Translational Safety Biomarker Pipeline (TransBioLine) IMI2 consortium aims to discover, verify, and qualify novel drug safety biomarkers for four organ systems (liver, pancreas, vascular and central nervous systems). MicroRNAs (miRNAs) are small non-coding RNA molecules whose abundance and stability in liquid biopsies offers promise as candidate biomarkers. Strategies will be presented to identify circulating miRNAs as potential safety biomarkers to be put forward for regulatory qualification. Particular focus will be given to those predicting drug-induced liver injury and pancreatitis severity, in plasma from the well-characterised TransBioLine cohorts.

PS3.1 - DMPK/ADME CONSIDERATIONS IN ADC DEVELOPMENT

Kevin Beaumont
AstraZeneca, United Kingdom

Antibody Drug Conjugates (ADCs) are an established modality in oncology. The ADC modality generally consists of a targeting antibody linked to several small molecule cytotoxic molecules (payload). The ADC gains access to the tumor cell via binding to an internalizing protein. Subsequent processing in the cell leads to release of the cytotoxic which causes cell killing.
The ADC therapeutic principle is to target tumors selectively using an antibody against a tumor expressed protein with minimal expression in healthy tissues. The aim is to deliver locally high concentrations of the cytotoxic to the tumor whilst sparing healthy tissue.
ADC access to the tumor to deliver the payload is an underlying driver of successful therapy. High expression of the internalizing target protein can represent a barrier to penetration deep into the tumor, with cells closer to the tumor vasculature removing the ADC prior to further penetration. In addition, solid tumors tend to show heterogeneous expression of the target internalizing protein. It is important that the released payload can diffuse out of the targeted cell into adjacent cells that do not express the targeted protein to cause cell kill (the bystander effect).
The combination of large and small molecules creates challenges for the ADME/DMPK scientist with consideration of aspects of antibody pharmacokinetics/distribution in addition to small molecule distribution/elimination. This presentation will discuss the challenges posed by ADCs and propose approaches whereby incorporation of ADME/DMPK techniques can facility the discovery and development of future ADCs.

PS3.2 - MULTI-MODAL IMAGING TO ACCELERATE DRUG DISCOVERY AND DEVELOPMENT R&D

Georges de Violente
Servier, France

Multimodal Molecular Imaging can be described as a combination of signals from more than one imaging technique to maximize the value from a study. Spatially resolved transcriptomics, on tissue proteomics & mass spectrometry imaging among others can be applied to a single sample or adjacent tissue slices to better understand pathophysiology, toxicology and drug/metabolite-to-target effect. Multimodal imaging is often utilized in *in vivo* clinical evaluation for disease diagnostic, prognostic and treatment follow up using the same instrumentation or in parallel studies. These *in vivo* imaging techniques are also utilized for preclinical applications such as studies related to pathophysiology and animal model validation and are key for the target validation purposes as well as drug discovery and candidate selection. Molecular imaging approaches such as Mass spectrometry imaging, spatial proteomics and transcriptomics and their combination can be applied to the quantification of the distribution of drugs, and drug metabolites, as well as changes in protein expression, endogenous metabolites such as lipids. Emerging technologies can provide resolution up to even sub-cellular level depending on the purpose and techniques used. Spatial biology combined with digital pathology & artificial intelligence make it possible to relate drug and metabolite exposure to specific cells to obtain the optimum benefit for the drug discovery and development activities. Many of the current imaging instruments can help making the distinction between drug and related metabolites to help provide mechanistic insights to their activity and/or toxicity. Some of the techniques require the structural modification of the drugs or metabolites such as isotope labelling, or addition of zirconium cages for isotopic imaging techniques (eg PET). Applying multiple modalities in imaging comes with many challenges as well, from sample preparation for ex vivo imaging to the implementation of new instrumentations to cover the vast amount of molecular information as well as new strategies and tools for data registration, data stratification and analysis. This presentation will highlight some of the benefits of multimodal imaging and current challenges regarding the application of multimodal imaging in drug discovery and development with emphasis on the potential of these combined approaches to accelerate R&D processes.

PS3.3 - TAKING AN HBV OLIGO INTO PH3 - REFLECTIONS FROM THE ADME TEAM

Steve Hood
GSK, United Kingdom

In 2019, the WHO estimating that Chronic Hepatitis B (CHB) effects over 250M patients worldwide, with less than 10% diagnosed and only 1.7M of those undergoing treatment. In 2019 GSK reviewed the Phase 2a data from two Ionis Gapmers designed to target the HBV viral genome; both had the same core sequence but one was GalNAc targeted to hepatocytes. Against all expectations, the non-GalNAc version was more efficacious and was selected for further development. In 2022, GSK shared Ph2b data with the regulators and initiated a global Ph3 study – this is the story of the journey and the ADME learnings on the way.

PL3 - ADME TACTICS IN THE DISCOVERY/DEVELOPMENT OF ORAL COVID-19 PROTEASE INHIBITORS
Amit Kalgutkar
Pfizer, United States

The SARS-CoV-2 3C-like protease (3CLpro) inhibitor PF-07321332 (Nirmatrelvir), in combination with ritonavir (PaxlovidTM) was recently granted emergency use authorization for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients, who are at high risk for progression to severe COVID-19, including hospitalization or death. The presentation will dwell into the discovery ADME strategies that were adopted for bespoke SARS-CoV-2 3CLpro inhibitor designs that seeked to improve the poor oral absorption traits of the previously identified SARS-CoV-1 3CLpro clinical candidate PF-00835231. While a reduction in hydrogen bond donor count, which was thought to be the principal cause for the poor oral absorption of PF-00835231, ultimately proved to be the winning medicinal chemistry strategy, alternate approaches such as prodrug analogs of PF-00835231 were also evaluated. The presentation will also discuss details of Nirmatrelvir disposition, particularly highlighting a predominant role for CYP3A4 in its metabolism, which proved useful in designing clinical studies involving co-administration of nirmatrelvir with the CYP3A4 inhibitor ritonavir. Finally, a de novo approach that examined human mass balance and disposition of Paxlovid using 19F-NMR spectroscopy (instead of the traditional 14C human ADME) will be outlined. The 19F NMR approach in studying mass balance/disposition has been accepted by regulatory agencies worldwide and enabled the timely approval of the emergency use authorization.

PS4.1 - DIGITAL TWINS AND ORGAN-ON-CHIPS. THE KEY TO HUMANIZING DRUG DISCOVERY?
Christian Maass
esqLABS GmbH, Germany

Organ-on-chips (OoCs) have emerged as a promising innovation in the realm of drug development, holding great potential to embody human physiology *in vitro* with greater precision than animal models. Yet, to unlock their full potential, the comparative advantage of OoCs over other *in vitro* models and animal testing in predicting and translating to human *in vivo* outcomes must be established.
To meet this challenge, we developed a cutting-edge workflow that marries OoC data with computational modeling, offering a powerful tool to accelerate drug discovery. The synergy between these two technologies provides a deeper mechanistic understanding of the biological principles underpinning drug mechanisms of action. This knowledge can then be applied to optimize experimental designs, speeding up the drug discovery process and paving the way for more effective treatments, reliably, sustainable, and at reduced cost.
Predicting the safety and efficacy of novel drugs before they are tested on humans, reducing the risk of adverse outcomes and accelerating the time to market is the outcome of our powerful approach that leverages the power of digital twins of organ-on-chips and patients.
The potential impact of this visionary approach is vast. By enhancing the accuracy and efficiency of drug development, it could reduce the reliance on animal models and lead to better health outcomes for humans. This lecture will showcase inspiring literature-based examples and future prospects to highlight the need, impact, and added value of this innovative workflow. With OoCs and computational modeling, we can revolutionize drug development and transform the future of medicine.

PS4.2 - GI ORGANOID MODELS FOR SAFETY
Carrie Duckworth
University of Liverpool, United Kingdom

Abstract not available.

PS4.3 - MATHEMATICAL MODELING OF DRUG EXPOSURE IN GI ORGANOID MODELS
Carmen Pin
AstraZeneca, United Kingdom

In vitro advanced cell systems can provide high-resolution measurements of multiscale nature to advance our understanding of basic biology and pharmacology. Moreover, these measurements can enable modelling strategies to generate quantitative clinical predictions at early stages of the drug development pipeline.
The clinical interpretation of microphysiological systems derived datasets is often not straightforward because recapitulating clinical drug concentration profiles and/or understanding the physiological meaning of in-vitro endpoints can be challenging. Translational modelling strategies can enable the integration of the drug effect, quantified in in-vitro settings, into modelling frameworks suitable to describe the dynamic interaction of multiple organic processes in patients responding to relevant drug concentration profiles. To develop such models, it is essential to understand dynamic

changes of the drug concentration as well as of relevant biological efficacy or safety endpoints in the in-vitro system. We will show several examples of the application of in-vitro advanced cell systems and mathematical models to assess investigational drugs and support clinical trial design.

PS5.1 - THE STORY OF RISDIPLAM: FROM CONCEPT TO A FIRST-IN-CLASS ORAL SMALL-MOLECULE MRNA SPLICING MODIFIER

Agnes Poirer
F. Hoffman La Roche, Switzerland

Spinal muscular atrophy (SMA) is a severe, progressive neuromuscular disease that affects individuals with a broad age range and spectrum of disease severity. SMA is caused by insufficient levels of the survival of motor neuron (SMN) protein due to homozygous deletion of – or loss-of function mutations within – the SMN1 gene. Pre-mRNA of a paralogous gene, SMN2, undergoes alternative splicing that excludes exon 7 from the majority of the mRNA to produce only low levels of functional SMN protein, which are insufficient to compensate for the loss of SMN1.
Risdiplam (EVRYSDI®) is the first approved oral small-molecule mRNA splicing modifier for the treatment of SMA, representing a turning point in drug discovery of small molecules. A novel screening process was used in its development, resulting in a flat molecule with a conjugated ring system and no attached halogens, and a high specificity for SMN2 pre-mRNA.
As an oral medication, risdiplam is centrally and peripherally distributed, with tissue distribution in peripheral organs. Thus, in animal models, comparable increases in SMN protein were observed in the blood, brain, muscle and spinal cord. Blood samples were therefore used as a surrogate for functional SMN protein in the central nervous system in clinical trials. A 2-fold SMN protein increase in animal models at the no-observed-adverse-effect level in toxicology studies gave a clear dose rationale, which enabled a fast move into pivotal clinical trials.
The inclusion of two unique, seamless Phase 2/3 studies in the clinical trial programme has enabled risdiplam to be raced from the first in-human healthy volunteers study to approval in less than four years. Meanwhile, risdiplam has been approved for the treatment of SMA in over 80 countries, based on confirmed efficacy results from a clinical development programme encompassing infants, children, teenagers, and adults with SMA. This research & development journey with risdiplam reveals potential for the research and development of other diseases for which RNA splice modulation plays a key role.
From lead optimisation to registration, the ADME & PK/PD development of Risdiplam has been a challenging and truly exciting scientific story with a focus on the pediatric indication, and along the way, more plasma protein binding measures than you have ever imagined.
Risdiplam is a low turnover compound whose metabolism is mediated through a non-cytochrome P450 (CYP) enzymatic pathway. Some of the main ADME challenges to be discussed will include: plasma protein binding, predicting *in vivo* hepatic clearance, determining *in vitro* metabolites with regard to metabolites in safety testing (MIST) guidelines, elucidating enzymes responsible for clearance, and estimating potential drug-drug interactions. A combination of *in vitro* and *in vivo* results was successfully extrapolated and used to develop a robust PBPK model of risdiplam. These results were verified through early clinical trial studies, further strengthening the understanding of the ADME properties of risdiplam in humans, making it a success story.
Authors: Stephen Fowler, Andreas Brink, Yumi Cleary, Andreas Guenther, Katja Heinig, Christophe Husser, Heidemarie Kletzl, Nicole Kratochwil, Lutz Mueller, Mark Savage, Cordula Stillhart, Dietrich Tuerck, Mohammed Ullah, Massimiliano Donzelli, Kenichi Umehara, Agnès Poirier

PS5.2 - TRANSLATIONAL PKPD: BATTLES WON BUT THE FIGHT GOES ON

James Yates
GSK, United Kingdom

Translational PKPD modelling has positively impacted the productivity of drug discovery and development as evidenced in Pfizers 3 pillars and AstraZeneca's 5Rs publications. There are many concepts, translational assumptions, and approaches that we would now take as accepted. So, is it business as usual, or are there battles we are yet to fight? In this talk I will review the tools in the PKPD modeller's arsenal as well as the gaps in our armour. In particular the talk will highlight the current challenges in our understanding that, if overcome, will gain us significant ground in the quest for translational perfection.

PS3.2 - COMPLEX PHARMACOKINETICS OF LYS006 POSE CHALLENGES IN TRANSLATION TO HUMAN BUT ENABLE WAIVING A HADME STUDY

Patrick Schweigler
Novartis, Switzerland

LYS006 is a potent leukotriene A4 hydrolase inhibitor for long-term treatment of various neutrophil-driven inflammatory conditions. Results of a first-in-human study with LYS006 combined with *in vitro* characterization work revealed unique PK properties.
In particular, LYS006 displayed non-linear pharmacokinetics caused by saturable binding to the target, which is highly expressed in blood cells. Consequently, a long terminal T1/2 was observed without relevant accumulation, reflecting a strong impact of target binding on drug distribution at lower concentrations.
In human, LYS006 was predominantly excreted unchanged into urine, triggering investigations of the involved renal transporter. Identified metabolites in plasma and urine comprised only a small fraction of the administered dose. The recovered amounts of parent LYS006 and metabolites in urine revealed a near-complete mass balance at steady-state in the FIH study, potentially allowing to waive a human radiolabeled ADME study.
The presentation will highlight the PK characteristics of LYS006 and cover challenges in translating preclinical data to human.

Reference:

1. B.Poller, H.M. Weiss et al Drug Metab Dispos. 2022 Dec;50(12):1472-1482 Human Pharmacokinetics of LYS006, an Oral Leukotriene A4 Hydrolase Inhibitor Displaying Target-Mediated Drug Disposition.

PRE-DOCTORAL/GRADUATE POSTER FINALISTS (A1 – A4)

A1 - AUGMENTATION OF THE CYP INDUCTION TEST METHOD IN THE HUMAN HEPATIC HEPARG™ CELL LINE AND APPLICATION TO METABOLISM DISRUPTING COMPOUNDS
Elodie Person, INRAE Toxalim, Toulouse, France

A2 - ABSTRACT WITHDRAWN

A3 - 3D SPHEROID PRIMARY HUMAN HEPATOCYTES FOR OCT1 (SLC22A1) TRANSPORTER KINETICS AND LONG-TERM MODULATION STUDIES
Evgeniya Mickols, *Uppsala University, Uppsala, Sweden*

A4 - PYRIDOXIC ACID PBPK MODEL DEVELOPMENT TO EVALUATE THE EFFECT OF PROBENECID INHIBITION AND CHRONIC KIDNEY DISEASE ON OAT1/3 ACTIVITY
Shawn Pei Feng, University of Manchester, Manchester, United Kingdom

POSTDOCTORAL POSTER FINALISTS (A5 – A10)

A5 - THE CHANGING LANDSCAPE FOR HAME: PRACTICAL EXPERIENCES FROM A DATA ANALYSIS OF 500 STUDIES
John Kendrick, Labcorp, Harrogate, United Kingdom

A6 - METABOLISM AND TRANSPORT IN A PDMS-BASED MICROPHYSIOLOGICAL SYSTEM: ADDRESSING THE CHALLENGES OF COMPOUND-LOSS
Patrick Carius, Boehringer Ingelheim Pharma GmbH und Co. KG, Biberach an der Riß, Germany

A7 - A LIQUID BIOPSY APPROACH FOR THE CHARACTERIZATION OF PHARMACOKINETICS AND PHARMACODYNAMICS PROTEIN TARGETS IN EXOSOMES FROM PSEUDOMYXOMA PERITONEI PATIENTS
Areti-Maria Vasilogianni, *University of Manchester, Manchester, United Kingdom*

A8 - DEVELOPING PBPK FRAMEWORK FOR ALDEHYDE OXIDASE: FOCUS ON CAPMATINIB
Nihan Izat, University of Manchester, Manchester, United Kingdom

A9 - ESTABLISHMENT OF AN *IN VITRO* MODEL TO INVESTIGATE THE INTERPLAY OF THE HUMAN TRANSPORTER OATP2B1 AND THE RAT METABOLIZING ENZYME CYP3A1
Anima Schäfer, University of Basel, Basel, Switzerland

A10 - THE USEFULNESS OF COMBINING CLINICAL THERAPEUTIC DRUG MONITORING DATA WITH BOTTOM-UP SYSTEM DATA TO UNDERSTAND THE EFFECT OF RENAL IMPAIRMENT ON THE NON-RENAL CLEARANCE OF DRUGS: TACROLIMUS AS A DRUG EXAMPLE
Eman El-Khateeb, Certara UK Limited (Simcyp Division), Sheffield, United Kingdom

BIOAVAILABILITY (P1 and P2)

P1 - A PRIMARY JEJUNUM AND PRIMARY HEPATOCYTE MULTI-ORGAN MPS: A PROMISING TOOL FOR MORE PREDICTIVE STUDIES OF HUMAN DRUG ADME AND ORAL BIOAVAILABILITY
Yassen Abbas, CN-BIO Innovations, Cambridge, Cambridgeshire, United Kingdom

P2 - *IN VITRO* MEMBRANE AFFINITY MEASUREMENTS MAY INCREASE THE PREDICTABILITY OF CALCULATED PERMEABILITY PROPERTIES OF MACROCYCLES
Hinnerk Boriss, Sovicell, Leipzig, Saxony, Germany

BRAIN PENETRANT MOLECULES (Kp,uu,BRAIN) (P3)

P3 - IMPROVING SUCCESS IN THE DISCOVERY OF A NOVEL BRAIN-PENETRANT CHEMOTHERAPEUTIC AGENT
Martina Nibbio, IRBM spa, Pomezia, Roma, Italy

CLEARANCE PREDICTION (P4)

P4 - IMPROVING *IN VITRO* TO *IN VIVO* CORRELATION (IVIVC) FOR HIGHLY PLASMA PROTEIN BOUND MOLECULES
Markus Trunzer, Novartis Pharma AG, Basel, Switzerland

CNS PENETRATION (P5)

P5 - PREDICTING HUMAN CNS PENETRATION: UNDERSTANDING SPECIES DIFFERENCES IN EFFLUX TRANSPORTERS
Elnaz Gozalpour, Pharmaron, Hoddesdon, United Kingdom

CYTOCHROME P450 (P6 and P7)

P6 - SULFENIC ACID AS REACTIVE INTERMEDIATE DURING THIENOPYRIDINES ACTIVATION: COMPARISON OF SULFENIC ACID TRAPPING METHODS. CHEMICAL METHOD OF FORMATION
Patrick Dansette, Université de Paris Cité, Paris, France

P7 - *IN SILICO* OFF-TARGET PREDICTIONS IN DRUG SAFETY ASSESSMENT: CLOSING THE LOOP FOR PREDICTED CYP17 INHIBITION OF A DRUG CANDIDATE
Audrey Fischer, Novartis Institutes for BioMedical Research, Basel, Switzerland

DIFFERENCES IN METABOLISM (SPECIES, GENDER, AGE, DISEASES) (P8 and P9)

P8 - PROTEOMICS PROFILE AND QUANTITATION OF PHARMACOKINETICS AND PHARMACODYNAMICS TARGETS IN SMALL INTESTINE AND COLON CANCER
Areti-Maria Vasilogianni, The University of Manchester, Manchester, United Kingdom

P9 - USE OF TRANSCRIPTOMICS TO UNDERSTAND HOW DISTURBED CHOLESTEROL SYNTHESIS DUE TO KNOCKING OUT CONSECUTIVE GENES ALTERS DRUG METABOLISM PATHWAYS N HUMAN HEPATOMA CELLS
Damjana Rozman, University of Ljubljana, Ljubljana, Slovenia

DILI RISK ASSESSMENT (P10)

P10 - ASSESSMENT OF DRUG-INDUCED LIVER INJURY (DILI) RISK OF TWO NOVEL PHARMACEUTICALS BASED ON *IN VITRO* COVALENT BINDING TO HUMAN LIVER PROTEIN MEASUREMENTS AND REACTIVE METABOLITE TRAPPING
Clair Stroud, Labcorp Early Development Laboratories Ltd, Huntingdon, Outside North America, United Kingdom

DISPOSITION (P11)

P11 - EVALUATION OF PERFLUOROOCTANESULFONIC ACID (PFOS) DISTRIBUTION IN AN ALBUMIN DEFICIENT (ALB-/-) MOUSE MODEL
Emily Kaye, University of Rhode Island, Kingston, Massachusetts, United States

DRUG DISCOVERY AND DEVELOPMENT (P12-P17)

P12 - OPTIMIZING HUMAN CNS AVAILABILITY IN THE CONTEXT OF RODENT VS NON-RODENT SPECIES DIFFERENCES IN IN-VIVO BRAIN KPUU
Stephanie Harlfinger, AstraZeneca, Cambridge, United Kingdom

P13 - ULTRA-HIGH THROUGHPUT MASS SPECTROMETRY BASED ANALYSIS BY DIRECT INJECTION (ECHO™ MS) FOR PLASMA PROTEIN BINDING ASSAY
Xiaotong Li, WuXi AppTec, Shanghai, China

P14 - ESTABLISHMENT OF THE FLUX DIALYSIS METHOD FOR HIGHLY BOUND COMPOUNDS IN HUMAN PLASMA AND RAT BRAIN HOMOGENATE
Xiaotong Li, WuXi AppTec, Shanghai, China

P15 - IMPACT OF THE PHYSICAL-CHEMICAL PROPERTIES OF POLY(LACTIC)-POLY(ETHYLENE GLYCOL) POLYMERIC NANOPARTICLES ON BIODISTRIBUTION AND PHARMACOKINETICS
Aaron Smith, AstraZeneca, Cambridge, Essex, United Kingdom

P16 - DE-RISKING *IN VIVO* PK ATTRIBUTES OF THERAPEUTIC ANTIBODY LEAD PANELS USING HIGH THROUGHPUT *IN VITRO* APPROACHES AS PART OF AN EARLY DRUG DISCOVERY AND HUMAN DOSE PREDICTION STRATEGY
Daniel Rycroft, GSK, Stevenage, United Kingdom

P17 - ACCELERATION OF INFECTIOUS DISEASE DRUG DISCOVERY AND DEVELOPMENT USING A HUMANIZED MODEL OF DRUG METABOLISM
Kenneth MacLeod, University of Dundee, Dundee, United Kingdom

DRUG INTERACTION (P18)

P18 – ABSTRACT WITHDRAWN

ENDOCRINE DISRUPTION (P19)

P19 - APPLICATION OF A GLP PROTOCOL FOR THE MEASUREMENT OF 17B-ESTRADIOL AND TESTOSTERONE BY LC-MS/MS IN THE H295R STEROIDOGENESIS ASSAY, TEST NO 456
Connor McKeever, Concept Life Sciences Limited, Dundee, United Kingdom

ENZYME INDUCTION (P20)

P20 - MODULATION OF HEPATIC XENOBIOTIC METABOLIZING ENZYMES FOLLOWING CHRONIC LOW-DOSE EXPOSURE TO PFOA IN MICE UNDER CHRONIC STRESS
Nicolas Cabaton, INRAE, Toulouse, France

ENZYME INHIBITION/INACTIVATION (P21-P22)

P21 - COMPETITIVE INHIBITION OF OROXYLIN A ON THE CARBOXYLESTERASE MEDIATED HYDROLYSIS OF IRINOTECAN
Zuo Zhong, The Chinese University of Hong Kong, Shatin, Hong Kong

P22 - APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING FOR PREDICTION OF DRUG-DRUG INTERACTIONS USING THE STRONG CYP2D6 INHIBITOR TERBINAFINE.
Harry Moore, Certara UK, Sheffield, United Kingdom

HEPATOCYTES (P23)

P23 - ANALYTICAL APPROACHES IN METABOLITE SAFETY TESTING (MIST) AND THEIR CONTRIBUTION TO HUMAN METABOLISM EVALUATION
Dimitri Colato, Evotec, Verona, Italy

IN SILICO (P24-P26)

P24 - FROM MS SIGNAL ANALYSIS TO AI BASED METHOD: SOLUTIONS FOR METABOLISM DATA PROCESSING AUTOMATION
Ismael Zamora, Lead Molecular Design, S.L., Sant Cugat del Vallés, Barcelona, Spain

P25 - OPTIMISATION OF ADME PROPERTIES OF SARS-COV-2 MPRO INHIBITORS USING MACHINE LEARNING
Lucy Ellis, Exscientia, Oxford, United Kingdom

P26 - BIOTRANSFORMATIONS FOR THE PREDICTION OF RING-FORMING AND RING-OPENING REACTIONS
Ausrine Marinskaite, Lhasa Limited, Leeds, United Kingdom

***IN VITRO IN VIVO* EXTRAPOLATION (IVIVE)** (P27-PP29)

P27 - MICRORNA PROFILING OF LIVER CELLS IN A PRECLINICAL DRUG-INDUCED TISSUE INJURY MODEL USING METHOTREXATE
Shiva Seyed Forootan, University of Liverpool, Liverpool, United Kingdom

P28 - PLANNING AND INTERPRETATION OF PKS IN VARIOUS SPECIES WITH THE AID OF SIMDOC2, AN INHOUSE-DEVELOPED WEB APPLICATION FOR INTEGRATED PK AND DOSE PREDICTIONS
Zhizhou Fang, Merck Healthcare KGaA, Darmstadt, Germany

P29 - HIGHER UNBOUND INTRINSIC CLEARANCES IN HUMAN LIVER MICROSOMES RELATIVE TO HEPATOCYTES EXPLAINED BY DIVERGENT CYTOCHROME P450 ACTIVITIES FOR 5-AZAQUINAZOLINE IRAK-4 INHIBITORS
Tashinga Bapiro, AstraZeneca, Cambridge, Cambridgeshire, United Kingdom

***IN VITRO* TECHNIQUES** (P30-P39)

P30 - USE OF LONG-TERM CO-CULTURED HUMAN HEPATOCYTES FOR ESTIMATION OF FM,CYP3A BY CHEMICAL INHIBITION ASSAY
Nicholas Njuguna, F. Hoffmann-La Roche, Basel, Switzerland

P31 - METABOLISM CONSIDERATIONS IN NEXT GENERATION RISK ASSESSMENT: CHALLENGES OF FIT-FOR-PURPOSE EXPERIMENTAL DESIGN USING COUMARIN AS A CASE STUDY
Sandrine Spriggs, Unilever, Bedford, United Kingdom

P32 - PLASMA PROTEIN BINDING OF NINE ANTISENSE OLIGONUCLEOTIDES BY ULTRAFILTRATION
Jie Wang, WuXi AppTec (Shanghai) Co ltd, Shanghai, China

P33 - VALIDATION OF A FULLY AUTOMATED 384-WELL ASSAY FOR SCREENING OF HUMAN HEPATOCYTE CLEARANCE IN DRUG DISCOVERY
Ursula Mueller-Vieira, Boehringer-Ingelheim, Biberach an der Riß, Germany

P34 - OPTIMIZATION OF METHOD FOR MEASURING AGP-MEDIATED PROTEIN BINDING IN DOG PLASMA
Janis Kuka, Pharmaron UK, Hoddesdon, England, United Kingdom

P35 - NEXT LEVEL DRUG RESEARCH IN AN EX VIVO TISSUE GUT-ON-A-CHIP MODEL: ADVANCED APPLICATIONS OF

THE INTESTINAL EXPLANT BARRIER CHIP
Joanne Donkers, TNO, Leiden, Zuid-Holland, Netherlands

P36 - ACCURATE DETERMINATION OF PLASMA PROTEIN BINDING OF HIGHLY BOUND COMPOUNDS WITH THE USE OF 18-HOURS RAPID EQUILIBRIUM DIALYSIS METHOD
Dawid Gogola, Jagiellonian University, Kraków, Poland

P37 - INTESTINAL TISSUE ORGANOIDS TO STUDY DRUG TRANSPORT AND METABOLISM FROM INFANTS TO ADULTS
Eva Streekstra, Radboudumc & TNO, Leiden, Netherlands

P38 - DEVELOPMENT OF AN *IN VITRO* TEST SYSTEM FOR CYP AND UGT INHIBITION STUDIES USING CRYOPRESERVED HUMAN HEPATOCYTES
Zsolt Fekete, Charles River Laboratories Hungary Ltd., Szeged, Hungary

P39 - HIGH-THROUGHPUT GLP-COMPLIANT *IN VITRO* ASSAY TO DETERMINE RELATIVE TOXICITY OF LIPOSOMAL FORMULATIONS OF AMPHOTERICIN B
Ritika Mukherjee, Eurofins Advinus Biopharma Services India Pvt. Ltd., Bangalore, Karnataka, India

METABOLISM (P40-47)

P40 - MECHANISM OF ACTIVATION OF CLOPIDOGREL INTO ITS ACTIVE THIOL METABOLITE: P450-CATALYZED OPENING OF THE 2-OXO-CLOPIDOGREL THIOLACTONE : S-OXIDATION OR BAEYER-VILLIGER OXIDATION?
Patrick Dansette, Université de Paris Cité, Paris, France

P41 - DISULFIDE RE-ARRANGEMENT LEADING TO CHAIN SPLITTING: A DEGRADATION MECHANISM IMPACTING THE SPECIES-SPECIFIC POTENCY OF INSULIN DETEMIR?
Christian Cramer, Novo Nordisk A/S, Maaloev, Copenhagen, Denmark

P42 - DAR DISTRIBUTION DETERMINATION FOR ANTIBODY-DRUG-CONJUGATES (ADCS) BY LC-HRMS IN *IN VITRO* AND *IN VIVO*
Weiqun Cao, WuXi AppTec, Shanghai, Shanghai, China

P43 - FROM QUANTUM MECHANICS TO METABOLIC PATHWAYS
Peter Hunt, Optibrium, Cambridge, United Kingdom

P44 - IMPACT OF A FLUORINE ON THE CNS PENETRATION AND METABOLIC FATE OF AZD9574
Andy Pike, AstraZeneca, Cambridge, United Kingdom

P45 - 14C-RADIOLABELLED HUMAN AME: A REVIEW OF CLINICAL MASS BALANCE RATES AND ROUTES OF ELIMINATION
Jessica Richardson, Pharmaron UK Ltd., Rushden, United Kingdom

P46 - SOFTWARE-BASED METABOLITE SCREENING: SEPARATING THE WHEAT FROM THE CHAFF
Martha Green, Charles River Laboratories, Tranent, East Lothian, United Kingdom

P47 - CYTOCHROME P450 REACTION PHENOTYPING OF LOW CLEARANCE COMPOUNDS USING SELECTIVE CHEMICAL INHIBITORS IN HµREL® CO-CULTURE PLATES
Joanne Wilcock, Cyprotex, Macclesfield, United Kingdom

NON-CYP ENZYMES (P48)

P48 - QUANTIFICATION OF DRUG-METABOLIZING ENZYMES IN THE HUMAN KIDNEY CYTOSOL
Annika Tillmann, University of Manchester, Manchester, United Kingdom

PHARMACOKINETIC AND PHARMACODYNAMICS (P49-P53)

P49 - INTESTINAL PGP-INDUCTION BY HYPERFORIN IN RATS - A COMPARISON OF TWO ST. JOHN'S WORT FORMULATIONS
Valerio Taggi, University of Basel, Basel, Switzerland

P50 - AN INTEGRATED MODELLING APPROACH FOR TARGETED DEGRADATION: INSIGHTS ON OPTIMISATION, DATA REQUIREMENTS AND PKPD PREDICTIONS FROM SEMI- OR FULLY-MECHANISTIC MODELS AND EXACT STEADY STATE SOLUTIONS
Pablo Morentin Gutierrez, AstraZeneca, Cambridge, United Kingdom

P51 - APPLICATION OF MASS SPECTROMETRY IMAGING IN CHIMERIC MICE WITH HUMANIZED LIVER FOR SPECIES DIFFERENCES ANALYSIS OF HEPATOTOXICITY BY A PROTOPORPHYRINOGEN OXIDASE INHIBITOR, EPYRIFENACIL
Kohei Matsunaga, Sumitomo Chemical Co.,Ltd., Konohana-ku, Osaka, Japan

P52 - QUANTITATIVE DOSE-RESPONSE MODELING USING METABOLOMICS TO DISCLOSE HEPATOTOXIC EFFECTS AND METABOLISM OF ENNIATIN B IN HEPARG CELLS
Estelle Dubreil, ANSES Laboratoire de Fougères, Javene, France

P53 - THE USE OF INTERACTIVE R SHINY APPLICATIONS IN THE ASSESSMENT OF NEW TARGETS
Adrian Devitt-Lee, UCB, Slough, United Kingdom

PHARMACOKINETIC MODELING (54-57)

P54 - FULL TMDD PK MODEL OF MAB BINDING TO PROTEIN WITH A SOLUBLE AND A MEMBRANE-BOUND VARIANT IN CYNOMOLGUS MONKEY
Mie Broberg, LEO Pharma, Ballerup, Denmark

P55 - COMPARISON OF IMMUNODEFICIENT AND IMMUNOCOMPETENT MOUSE STRAINS FOR PHARMACOKINETIC ASSESSMENT AND SELECTION OF MOLECULES FOR PHARMACOLOGICAL STUDIES IN DRUG DISCOVERY
Georges Da Violante, Servier, Gif Sur Yvette, France

P56 - PREDICTION OF ABSORPTION OF PROTEIN DEGRADERS
Stefan Eirefelt, LEO Pharma A/S, Ballerup, Denmark

P57 - OPTIMIZED NORMOTHERMIC MACHINE PERFUSION OF LIVER AND KIDNEY USED TO PREDICT HUMAN PHARMACOKINETICS
Evita Van de Steeg, TNO, Leiden, Netherlands

PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) (P58-P64)

P58 - PREDICTION OF PH ALONG THE NEPHRON TUBULE USING THE MECHANISTIC KIDNEY MODEL (MECH KIM): MEMANTINE AS CASE EXAMPLE
Liam Curry, Certara UK, Sheffield, South Yorkshire, United Kingdom

P59 - DAPAGLIFLOZIN PBPK COMPOUND FILE TO PREDICT CHANGES IN ITS EXPOSURE WITH DRUG-DRUG INTERACTIONS, VARIOUS ETHNICITIES, AND SPECIAL DISEASE POPULATIONS.
Eman El-Khateeb, Certara, Sheffield, United Kingdom

P60 - PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING OF ADALIMUMAB TO PREDICT PHARMACOKINETICS IN PEDIATRICS
Pradeep Sharma, AstraZeneca, Cambridge, United Kingdom

P61 - PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING OF UGT1A4 PROTOTYPICAL DRUG LAMOTRIGINE IN PREGNANCY POPULATION
Pradeep Sharma, AstraZeneca, Cambridge, United Kingdom

P62 - PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL DEVELOPMENT AND VALIDATION OF MITIPERSTAT AND APPLICATION TO PREDICT DRUG-DRUG INTERACTION (DDI) WITH CYP3A4 INDUCER
Pradeep Sharma, AstraZeneca, Cambridge, United Kingdom

P63 - PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING OF SENSITIVE CYP2C8 SUBSTRATE ROSIGLITAZONE FOR DRUG-DRUG INTERACTION PREDICTIONS
Nilesh Gaud, Jagiellonian University Medical College, Krakow, Poland

TRANSPORTERS (P65-P70)

P64 - THE QUEST TO DEFINE CANCER-SPECIFIC SYSTEMS PARAMETERS FOR PERSONALIZED DOSING
Areti-Maria Vasilogianni, The University of Manchester, Manchester, United Kingdom

P65 - CHLOROQUINE AND HYDROXYCHLOROQUINE, AS PROTEASOMAL INHIBITORS, UPREGULATE THE EXPRESSION AND ACTIVITY OF ORGANIC ANION TRANSPORTER 3
Guofeng You, Rutgers, The State University of New Jersey, Piscataway, New Jersey, United States

P66 - ABUNDANCE OF RENAL TRANSPORTERS IN HEALTHY CAUCASIANS: A META-ANALYSIS
Matthew Harwood, Certara UK Ltd, Sheffield, South Yorkshire, United Kingdom

P67 - THE INHIBITOR PREINCUBATION EFFECT IS UNIVERSAL TO SLC TRANSPORTER ASSAYS AND IS ONLY PARTIALLY ELIMINATED IN THE PRESENCE OF EXTRACELLULAR PROTEIN
Zsuzsanna Gáborik, Charles River Laboratories, Budapest, Hungary

P68 - INVESTIGATING CROSS-SPECIES DIFFERENCES OF MDR1-MEDIATED DRUG TRANSPORT
Zsuzsanna Gáborik, Charles River Laboratories, Budapest, Hungary

P69 - ORGANIC ANION TRANSPORTER 2 OVER EXPRESSION STABLE CELL LINE FOR OAT2 SUBSTRATE AND INHIBITOR EVALUATION
Jing Lai, Pharmaron Beijing Co. Ltd., Beijing, China

P70 - EVALUATING THE ACTIVE CONTRIBUTION TO CREATININE RENAL CLEARANCE IN RENALLY IMPAIRED PATIENTS USING PBPK MODELING
Olha Shuklinova, Certara UK Limited, Simcyp Division, Sheffield, United Kingdom

A1 - AUGMENTATION OF THE CYP INDUCTION TEST METHOD IN THE HUMAN HEPATIC HEPARG™ CELL LINE AND APPLICATION TO METABOLISM DISRUPTING COMPOUNDS

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Many human xenobiotic metabolizing enzymes (XME) are inducible by a number of drugs and environmental chemicals. The induction of Cytochrome P450 (CYP) enzyme activities can have an impact on the fate as well as the biological effect(s) of xenobiotics. In addition, since many CYPs are also involved in endogenous metabolic pathways of anabolism and catabolism, the modulation of their activity can also impact endogenous physiological functions, energy metabolism and signalling pathways (including hormones metabolism). Within the GOLIATH project (EU Horizon 2020, grant n°825489), investigations are conducted to examine the potential of model Metabolism Disrupting Compounds (MDCs) to exert their effects through the modulation of functional XME activities, including both phase I and phase II activities. The former are notably assessed with the aim to examine the potential of model MDCs to induce key CYP activities: CYP1A2, CYP3A4 and CYP2B6. The CYP induction test method, first developed with pharmaceutical proficiency chemicals [TM2009-14 (EU)], was optimized and applied to six model MDCs at six concentrations, including both persistent and non-persistent chemicals: bisphenol A (BPA), perfluorooctanoic acid (PFOA), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (p,p'-DDE), triclosan (TCS), tributyltin (TBT) and triphenyl phosphate (TPP). The test method was implemented in the human hepatic cell line HPR116 (HepaRG™) and three different cell batches were used. Solubility and cytotoxicity assays were performed. Single chemicals were applied in a 48h exposure regime, followed by the addition of a cocktail of specific probe substrates. Subsequent LC/MS-MS analyses were performed for the quantification of metabolites. The test method revealed that five out of six of these MDCs are able to induce at least one specific CYP activity. TCS was determined not to induce any of the tested activities. BPA and p-p'-DDE were both found to induce CYP3A4, as well as CYP2B6 only for BPA and CYP1A2 only for p-p'-DDE. As regards PFOA, TBT and TPP the three CYP activities assessed were found to be induced. Results obtained for the set of model MDCs provide very interesting information about the possible involvement of CYP induction mechanisms that are triggered by some of these compounds and may contribute to their Mode of Action.

A2 - ABSTRACT WITHDRAWN

A3 - 3D SPHEROID PRIMARY HUMAN HEPATOCYTES FOR OCT1 (SLC22A1) TRANSPORTER KINETICS AND LONG-TERM MODULATION STUDIES

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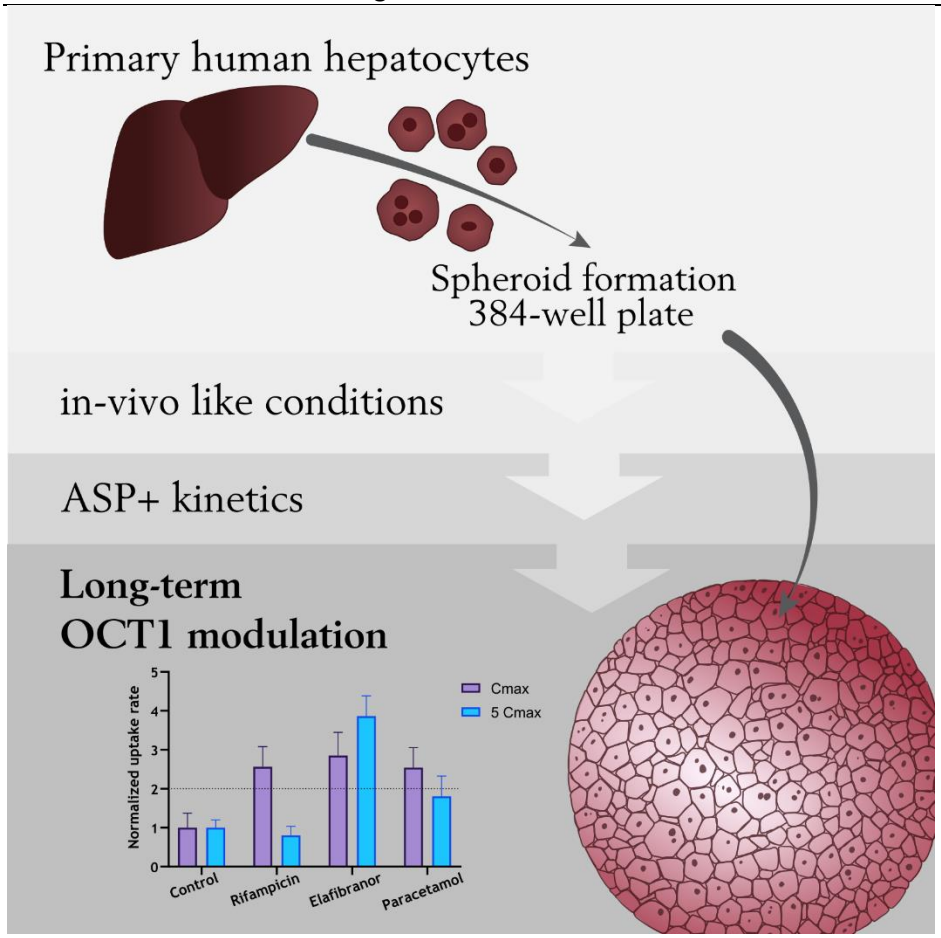
Isolated primary human hepatocytes (PHH) are considered the gold standard *in vitro* model for the human liver. 3D spheroids of primary human hepatocytes (3D PHH) keep a differentiated phenotype with retained metabolic function and proteome fingerprint for weeks in culture and are widely used to predict hepatic drug-drug interactions and long-term xenobiotics effects. Nonetheless, the transporter function has not yet been investigated in PHH spheroids. Here we show the applicability of 3D spheroids for the organic cation transporter 1 (OCT1/SLC22A1) transporter kinetics and long-term modulation studies. The 3D PHH were cultured for two weeks and the OCT1 transport activity was assessed using the fluorescent model substrate ASP+ and known OCT1 inhibitors. Moreover, the ASP+ uptake assay was together with proteomics used for evaluating the potential modulation of OCT1 expression after prolonged exposure to selected xenobiotics. With this approach, we could show that spheroids indeed have the expression and activity of ADME-related transporters. An affinity of ASP+ for OCT1 transporter (Km) of 17.4 (± 1.17) µM and the maximal turnover rate (Vmax) of 347 (±9.5) RFU/min was determined. Six well-known OCT1 inhibitors reduced the uptake of ASP+ in the 3D PHH spheroids, resulting in 40 to 60% of the maximal uptake rate of ASP+. The long-term exposure to elafibranor, a dual PPARα/δ agonist, induced OCT1 protein expression and ASP+ uptake by 2.7 fold. Our results show for the first time that 3D PHH spheroids express fully active OCT1 and that transporter kinetics can be studied in individual spheroids using fluorescent probes. We anticipate our study to be a starting point for developing short and long-term ADME-relevant hepatic transporter studies in a 3D physiologically relevant format.



A4 - PYRIDOXIC ACID PBPK MODEL DEVELOPMENT TO EVALUATE THE EFFECT OF PROBENECID INHIBITION AND CHRONIC KIDNEY DISEASE ON OAT1/3 ACTIVITY

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Monitoring endogenous biomarkers is increasingly used to evaluate transporter-mediated drug-drug interactions (DDIs) in early clinical trials and may potentially be applied to elucidate changes to transporter activity in disease. 4-pyridoxic acid (PDA) has been identified as the most sensitive plasma endogenous biomarker of renal organic anion transporters (OAT1/3) in humans [1, 2]. Increase in PDA baseline concentrations has been observed after administration of probenecid, a strong clinical inhibitor of OAT1/3, as well as in chronic kidney disease (CKD) populations. The aim of this study was to develop and verify a physiologically-based pharmacokinetic (PBPK) model of PDA and predict the CKD-related changes in PDA concentrations by accounting for disease effect on OAT1/3 activity. Initially, the PDA PBPK model was developed in healthy population; the model was informed by previous population pharmacokinetic modelling [3]. A mechanistic kidney model was used by incorporating a top-down estimate of PDA OAT1/3-mediated intrinsic clearance and experimentally measured passive diffusion clearance. Probenecid PBPK model was adapted from the SimCYP database, optimised, and re-verified to capture dose-dependent pharmacokinetics of this inhibitor across 0.5 to 2.0 g single/multiple dose range (n = 9 studies). The PDA PBPK model successfully predicted PDA maximal plasma concentration (Cmax), area under the curve (AUC) and renal clearance (CLr) in healthy subjects at baseline and after single/multiple probenecid doses. Predicted PDA CLr, Cmax and AUC ratios following probenecid administration were within 1.5-fold of the observed data, building confidence in biomarker informed PBPK modelling of OAT1/3 DDI. For simulations in CKD patients, the severe CKD population in the SimCYP database was modified. Previously estimated additional 50% decline in OAT1/3 activity beyond the proportional decrease in GFR [4] was implemented, in addition to a 60% increase in PDA fraction unbound in plasma in severe CKD. As a result, the PDA model was able to recover the increase in PDA plasma concentration in severe CKD within two-fold of the observed data. The PDA PBPK model developed in the current study supports future robust evaluation of OAT1/3 DDI in drug development and can guide the decision on necessity for a dedicated DDI study. The PDA model predicted CKD-related changes in its baseline levels,

increasing further our confidence in predicting the exposure of a drug eliminated via OAT1/3 renal secretion in this disease.

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A5 - THE CHANGING LANDSCAPE FOR HAME: PRACTICAL EXPERIENCES FROM A DATA ANALYSIS OF 500 STUDIES

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Coincidental with the intensified regulatory and industry focus on the design and conduct of hAME studies in the past 12 months, we have recently completed our 500th cohort involving radiolabelled test item administration to humans. We therefore wanted to build upon a recent industry white paper in this journal (Young et al, 2022) and share some of our own experiences as a Contract Research Organisation (CRO) based upon collaborations with numerous pharma companies and their differing approaches to design and timing, to add further context to the discussion regarding hAME studies and the pivotal role that DMPK plays. In this article, we explore how both changing relationships/structures within the industry and shifting regulatory guidelines are impacting strategies, and compare EU and US pre-study approval requirements, before evaluating the trends from over 500 studies conducted at our global facilities. The aims and endpoints of hAME studies, along with the necessary planning and preparation, are reviewed before considering how some of the challenges specific to such studies may be overcome by aligning preclinical research activities with the clinical program. To support this discussion, we present over 30 years of historical data (indications, dose routes, radioactive dose level, panel size, gender, study duration), which also demonstrate some of the trends we have noted. Finally, we evaluate how improved technical capabilities and strategies are influencing the design and conduct of hAME studies, before speculating on some of the driving factors (artificial intelligence, modelling, use of other instrumentation/isotopes, biologics) which may shape the direction they take in the future.

A6 - METABOLISM AND TRANSPORT IN A PDMS-BASED MICROPHYSIOLOGICAL SYSTEM: ADDRESSING THE CHALLENGES OF COMPOUND-LOSS

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Microphysiological systems (MPS) comprise human-relevant cellular models representing single or multiple tissues embedded in a biomimetic *in vitro* platform that introduce physiologically important stimuli such as dynamic stretch or fluid flow (1). With regards to ADME applications, the features offered by MPS may improve predictions of the pharmacokinetics (PK) and PK-pharmacodynamic (PK/PD) relations of drug molecules (2). However, many MPS are made of polydimethylsiloxane (PDMS) which strongly ab- and adsorbs a wide range of small molecules (3) and can lead to inaccurate determinations of metabolism and/or clearance parameters in such systems. Considering concepts from the ADME field led to the following HYPOTHESIS: Within PDMS-based MPS, metabolism and/or clearance parameters can be accurately predicted if the ab- and adsorption is corrected for compound-specific, chip-intrinsic clearance. METHODS USED: Experiments were conducted utilizing the Emulate® gut-on-chip MPS, comprised of Caco-2 cells (epithelial layer) and HUVEC cells (endothelial layer). Following a 1-h incubation at a flow rate of 200 µl/h, compound-loss to PDMS was initially determined for 16 substances (10 µM) with a range of different physical/chemical properties. Testosterone was identified as a tool compound with moderate PDMS-absorption, which was additionally determined over a total of 135 minutes in 15-minute intervals in chips with and without cells. This procedure allowed a differentiation between chip-intrinsic clearance due to compound-loss and cellular metabolic clearance. Testosterone metabolites formed in the gut-on-chip were identified and quantified using LC-MS/MS.

RESULTS: After passage through the gut-on-chip without cells, testosterone-loss amounted to ~30%, calculated from the difference between inlet and outlet concentrations after 1 h. A steady state concentration was, however, observed from 75-135 minutes. Identifying a timeframe where testosterone-absorption to PDMS was constant in chips without cells (chip-intrinsic clearance at steady state) was key to calculate metabolic clearance in experiments with cells. Additionally, the metabolic clearance of testosterone (109±10 µl/h) also corresponded well to an experimentally determined clearance derived from the quantified metabolites 4-androstenedione and testosterone-glucuronide (107±16 µl/h). Lastly, the P-gp substrate Apafant and a proprietary compound with no efflux and low permeability, were utilized as controls in bidirectional transport experiments to determine drug transport and barrier properties. The learnings from the testosterone study further led to the efficient inhibition of P-gp using Elacridar as an inhibitor including a 75-minute pre-incubation, which was not possible using standard protocols.CONCLUSION: This study showed for the first time that metabolic clearance of testosterone can be determined in a PDMS-based MPS when accounting for substantial compound-loss in the system. Moreover, a practicable protocol to experimentally assess compound-loss in similar systems is proposed.

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A7 - A LIQUID BIOPSY APPROACH FOR THE CHARACTERIZATION OF PHARMACOKINETICS AND PHARMACODYNAMICS PROTEIN TARGETS IN EXOSOMES FROM PSEUDOMYXOMA PERITONEI PATIENTS

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Pseudomyxoma peritonei (PMP) is an orphan disease affecting 1 person per million. This rare cancer type usually originates from the appendix and metastasizes to the peritoneum. Aggressive cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy is used for treatment. However, this approach is associated with morbidity and mortality. Additionally, only 25% of patients are eligible for this treatment. Therefore, there is a high need to discover new therapeutic targets that would treat as well as diagnose PMP. A liquid biopsy from plasma is a minimally invasive approach that enables identification and quantification of circulating biomarkers in plasma with diagnostic and therapeutic value. This study investigated, for first time, the proteome in exosomes from plasma from PMP patients and healthy controls to suggest important pharmacokinetics (PK) and pharmacodynamics (PD) diagnostic and therapeutic protein markers for PMP. Extraction of exosomes from plasma from 19 PMP patients and 6 healthy subjects was performed. Filter-aided sample preparation (FASP) protein digestion protocol was used for the digestion of the exosomes. Global liquid chromatography–mass spectrometry proteomics was used to identify and quantify all the proteins in exosomes. The Total Protein Approach (TPA) was used to quantify all the identified proteins. Additionally, 4 QconCATs (concatenated peptides) for the quantification of specific protein targets were used; MetCAT for the quantification of cytochrome P450 enzymes (CYPs) and glucuronosyltransferases (UGTs), TransCAT for the quantification of ATP-binding cassette transporters (ABCs) and solute carrier transporters (SLCs), NuncCAT for the quantification of non-CYP, non-UGT drug-metabolising enzymes (DMEs), and KinCAT for the quantification of receptor tyrosine kinases (RTKs). The abundance of all the identifiable proteins was measured, and the inter-individual and technical variability was assessed. The TPA revealed 79% overlap of the proteins found in exosomes from PMP and healthy subjects. The principal component analysis showed that the proteins in PMP and healthy subjects form 2 distinct clusters. 83 proteins were exclusively identifiable and quantifiable in exosomes from PMP patients. These have prognostic value (favourable and unfavourable) for different cancer types, and 12 of them were liver-enhanced. Additionally, 51 of these proteins were found to be potential druggable targets with 19 of them being enzymes. The targeted approach (QconCATs) showed that most of PK targets and RTKs were below the limit of quantitation. Among the targets that were quantifiable in at least 3 samples were CYP1A1 (lower in PMP), ADH1B (exclusively found in PMP), ALDH1A1 (similar expression), AOXA (higher in PMP), CES1, CES2 (exclusively found in PMP), EPHX1, MGST1, NAT1 (exclusively found in healthy), UGT1A1 (exclusively found in PMP), UGT2B7/2B4, P-gp, MRP3, and VGFR3 (exclusively found in PMP). Overall, this study elucidates, for first time, the proteome in the exosomes of PMP patients and studies the perturbation of the abundance of PK and PD markers in PMP. It also suggests proteins that would serve as diagnostic markers and therapeutic targets for PMP.

A8 - DEVELOPING PBPK FRAMEWORK FOR ALDEHYDE OXIDASE: FOCUS ON CAPMATINIB

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The present study aimed to develop a physiologically-based pharmacokinetic (PBPK) model for capmatinib, a dual aldehyde oxidase (AO)/CYP450 substrate (1), using Simcyp Simulator v21 (Certara UK Ltd., Sheffield, UK). A stepwise approach was used to verify the absorption and distribution components of the initial capmatinib PBPK model. The *in vitro-in vivo* extrapolation (IVIVE) of unbound intrinsic clearance (CLint,u) from *in vitro* systems (human hepatocytes and cytosol) using either solely physiological scaling factors, or with additional empirical scaling factors (ESFs) (2), were evaluated to predict metabolic elimination. Subsequently, human mass balance data were used to inform fraction metabolized by AO (fmAO) and CYP3A4 (fmCYP3A4). Oral pharmacokinetic data and CYP3A4 drug-drug interaction (DDI) studies were used to refine CLint,u and verify the PBPK model. IVIVE of microsomal and cytosolic data predicted *in vivo* oral maximum plasma concentration (Cmax) and area under the curve to infinite time (AUCinf) of capmatinib were within 2-fold of observed values with predicted fmAO of 5%. In contrast, IVIVE of CLint,hepatocytes (with/without AO inhibitor hydralazine) resulted in under-prediction of observed CLoral by 5-fold, with predicted fmAO of 10%. After applying ESF for CL predictions in human hepatocytes, CLoral was over-predicted by 2-fold. Since predicted total CL and/or fmAO did not agree with observed data, the model was refined by back calculating CLint,u from *in vivo* oral PK study data, assuming fmAO and fmCYP3A4 of 40% and 60% respectively, based on human mass balance study. The simulated oral pharmacokinetic profile of capmatinib was consistent with observed data; predicted Cmax and AUCinf were within 2-fold of observed values. Independent DDI studies with rifampicin and itraconazole verified the fmCYP3A4, and in both cases predicted AUCinf ratio was within 1.5-fold of the observed data. In conclusion, capmatinib case study highlights the importance of totality of mass balance and *in vitro* reaction phenotyping data together with available clinical CYP DDI studies to evaluate parallel metabolic pathways and development of robust PBPK models for AO substrates.

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A9 - ESTABLISHMENT OF AN *IN VITRO* MODEL TO INVESTIGATE THE INTERPLAY OF THE HUMAN TRANSPORTER OATP2B1 AND THE RAT METABOLIZING ENZYME CYP3A1

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The organic anion transporting polypeptide (OATP) 2B1 (gene name SLCO2B1) is an uptake transporter that facilitates cellular accumulation of its substrates. In order to assess the transporter's *in vivo* relevance, we have generated Slco2b1-/- knock-out and SLCO2B1+/+ knock-in rats applying the CRISPR/Cas9-technology. Pharmacological phenotyping with the OATP2B1 substrate-drug atorvastatin suggested increased activity of rCYP3A1, the rodent orthologue of the human CYP3A4, in humanized animals. Considering our previous findings showing species specificity in substrate recognition especially for sulfated steroids of endogenous origin, we hypothesize that the human transporter facilitates cellular accumulation of an endogenous molecule that modulates transcription of rCYP3A1 in rat liver, whereby linking the function of OATP2B1 to activity of this major drug-metabolizing enzyme. In this study we sought to establish an *in vitro* model to further investigate this hypothesis. However, at first, we validated our observation of increased activity of rCYP3A1 in the liver of humanized SLCO2B1+/+ rats compared to Slco2b1-/- animals by Western blot analysis. Subsequently, we tested and optimized an *in vitro* system. The latter is based on the hepatic H4IIE cells of rat origin, which under normal culture conditions exhibited moderate or negligible expression of rCYP3A1 or rOATP2B1, respectively. For heterologous expression in the hepatic cell line we used adenoviral gene transfer. Adenoviral dose-dependent expression of OATP2B1 in H4IIE cells was validated by Western blot analysis and real-time PCR. Transport experiments with estrone-3-sulfate supported adenoviral dose-dependent functionality of the transporter. Ad-eGFP served as control in our experiments. In this study we optimized a heterologous expression system to further investigate the mechanism linking OATP2B1 and rCYP3A1. We expect that understanding the molecular mechanism of this interplay will provide new insights into modulation of drug metabolizing enzyme.

A10 - THE USEFULNESS OF COMBINING CLINICAL THERAPEUTIC DRUG MONITORING DATA WITH BOTTOM-UP SYSTEM DATA TO UNDERSTAND THE EFFECT OF RENAL IMPAIRMENT ON THE NON-RENAL CLEARANCE OF DRUGS: TACROLIMUS AS A DRUG EXAMPLE

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The impact of chronic renal disease (CRD) on the liver's ability to metabolize CYP3A substrates is not well established. This work aims to show the benefit of supporting therapeutic drug monitoring (TDM) clinical, demographic, biochemical data for patients suffering from CRD with system pharmacology and physiologically-based data to understand the alteration in the hepatic unbound CYP3A intrinsic clearance (CL,u,h,int) of tacrolimus. Tacrolimus minimum concentrations at steady state in blood (Cmin,ss) were collected from the records of 40 patients who performed renal transplantation surgeries at different occasions. Additionally, patient's demography and relevant blood tests were also collected with each TDM reading. Blood binding, distribution volume and drug affinities to tissues have been calculated for every patient to allow the estimation of CL,u,h,int. Kidney function related parameters such as eGFR and the stage of CRD were introduced as covariates in the model. The results revealed a significant ($p=0.0005$) positive relationship ($r= 0.21$) between eGFR and tacrolimus CL,u,h,int. The unbound clearance was dropping slowly moving from normal kidney function to end-stage renal disease to reach a maximum drop by 37%. No difference was noticed statistically in the CL,u,h,int for patients who failed transplantation compared to those with stable transplanted kidney function. In conclusion, the estimation of the degree of drop in CL,u,h,int with renal disease can be important clinically in order to adjust the dose of hepatically CYP3A eliminated drugs in CRD patients especially those with narrow therapeutic window such as tacrolimus. The strategy of bottom-up individualization of pharmacokinetic parameters using previously defined system components can assist in the determination of unknown parameters with higher certainty instead of depending only on clinical datasets.

P1 - A PRIMARY JEJUNUM AND PRIMARY HEPATOCYTE MULTI-ORGAN MPS: A PROMISING TOOL FOR MORE PREDICTIVE STUDIES OF HUMAN DRUG ADME AND ORAL BIOAVAILABILITY

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ADME studies are a key part of drug discovery, as the evaluation of pharmacological properties determines the efficacy and safety of a given compound. Efforts to improve the *in vitro* to *in vivo* translation of drug efficacy and safety data has led to the emergence of more complex microphysiological systems (MPS) that consist of multiple organs that are fluidically linked [1]. Here, we introduce a multi-organ MPS, that consists of six wells, each with two compartments; a Transwell®-based intestinal epithelial monolayer (RepliGut® Planar) and a liver microtissue. Liquid flow can be independently controlled in each compartment and in the interconnecting channel from the liver to intestinal compartment. In this *in vitro* model, we used cells that are of primary human origin, because immortalized liver and intestinal cell lines have absent or low levels of metabolic enzyme expression, and thus fail to predict first pass human metabolism. The RepliGut® Planar model is established by seeding human jejunum stem/progenitor cells onto a biomimetic scaffold coated Transwell® insert. After reaching confluence and being subjected to differentiation cues, the cell layer forms a polarized barrier and is capable of mucus secretion. For the liver, primary human hepatocytes (PHH) are seeded on a 3D collagen-coated scaffold and form microtissues. We developed a chemically defined media that supports hepatic metabolic functionality and intestinal barrier integrity in the multi-organ MPS. Expression of metabolic enzyme and transporter genes that are known to be highly expressed by human jejunum was confirmed in the primary intestinal cells by RT-qPCR. To demonstrate improved predictive capacity, we investigated two drugs where current models fail to predict human ADME behaviour. Temocapril, which is a prodrug and is designed to be resistant to intestinal hydrolysis [2] and midazolam, which is known to undergo intestinal clearance [3]. The parent drugs and metabolites were quantified by LC/MS from media samples taken in the liver and the intestinal apical compartments over a 48-hour period. For temocapril, we correctly observed resistance to intestinal hydrolysis by the primary jejunum model with subsequent clearance by PHHs. In contrast, Caco-2 cell carboxylesterase enzyme expression is abnormal and thus vastly overpredicts intestinal metabolism. With midazolam, we saw greater clearance when PHHs were co-cultured with a primary jejunum barrier, and this resulted in an improvement in the oral bioavailability prediction. In conclusion, we present a multi-organ MPS with both intestinal and liver cells from a primary human source. We demonstrate maintenance of cell functionality in co-culture and show its predictive potential for drug ADME and bioavailability studies.

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P2 - *IN VITRO* MEMBRANE AFFINITY MEASUREMENTS MAY INCREASE THE PREDICTABILITY OF CALCULATED PERMEABILITY PROPERTIES OF MACROCYCLES

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Macrocyclic peptides (MCPs) are gaining interest as potential therapeutics due to their notable target selectivity, binding affinity, and ability to disrupt protein-protein interactions. Permeability across plasma membranes is a major hurdle for MCPs and can limit access to intracellular targets and negatively impact oral absorption. Understanding how complex 3-dimensional conformations of MCPs interact with phospholipids and plasma membranes on a fundamental level is needed to design permeable MCPs. Methods: We optimized and automated the TRANSIL PC Intracellular Binding kit (TMP-0160-2029, Sovicell) on a Hamilton VANTAGE to assess the membrane affinity (Log MA) of MCPs. Four MCPs per cassette (0.5 µM/MCP) were added to wells containing phospholipids (PLs) covalently attached to silica beads. A constant concentration of MCP was added to wells with varying concentrations of PLs. Equilibrium between MCPs and PLs was reached by mixing the beads with the 96-channel head for 120 cycles (200 µL/mix, 250 µL/s). Supernatant was collected after centrifugation and analyzed on a Sciex ZenoTOF 7600. The phospholipid composition used in this study approximates that of MDCK cells; 50% phosphatidylcholine (PC), 17%, phosphatidylethanolamine (PE), 11% phosphatidylserine (PS), 2% phosphatidylinositol (PI), 20% other PLs. The Log MA was calculated using the algorithm supplied with the kit. Additionally, we ran the Schrodinger Prime Macrocycle Permeability calculation, which estimates the free energy for an MCP to move from water into a membrane (dG_insert). dG_insert has been shown to correlate with passive permeability for both small molecules and small macrocycles. Since bigger MCPs pose challenges due to the

large number of rotatable bonds and thus the size of the conformational space, dG_insert was calculated for MCPs with MWs < 1000. Results: As expected, Log MA is not predictive of apparent permeability obtained from our 96-well MDCK permeability assay (R2: < 0.1, n=219 MCPs). While dG_insert was highly correlated with EPSA (R2 = 0.82, n=74), it was not with MDCK Papp (R2: 0.36, n=85). The correlation between Log MA and dG_insert (R2: 0.36, n=113) was minimal. Out of 85 MCPs with MDCK Papp, Log MA and dG_insert values, 16 had MDCK Papps >= 0.5 10E-06 cm/s. A dG_insert value < 9 predicted 24 MCPs would have acceptable permeability (8 false positives). When dG_insert (< 9) and Log MA (2.5 – 4.5) were used together as cut-offs, 14 out of 16 MCPs were predicted to have acceptable permeability (87.5%). Therefore, the Log MA from the Transil membrane affinity assay may increase predictability when combined with calculated properties over either alone.

P3 - IMPROVING SUCCESS IN THE DISCOVERY OF A NOVEL BRAIN-PENETRANT CHEMOTHERAPEUTIC AGENT

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The ADME strategy adopted to establish a resource-efficient process to progress brain penetrant molecules involved the use of two primary BBB assays, namely MDCK line transfected with both main human efflux transporters (P-gp and BCRP), and an iPSC-derived BMECs (iBMECs) *in vitro* BBB model.
Compounds with optimal physicochemical properties (i.e., CNS-MPO, LogP, LogD, TPSA, MW, HBD and pKa) and low unbound human liver microsome clearance were screened in the *in vitro* BBB models at 0.1 µM, to mimic physiological concentrations, with the aim of identifying those which were not substrates for efflux transporters i.e., efflux ratio <2.
Abbreviations: Kpu,u: unbound brain-blood equilibrium distribution, BBB: blood brain barrier
Molecules of interest, which were not efflux substrates in the MDCK-MDR1-BCRP assay or that showed efflux ratios equal or lower than 1 in the iPSC-derived BMECs (iBMECs) *in vitro* BBB model, were progressed into an *in vivo* rat cassette Kp,uu,brain study to gain further understanding of brain penetrance. The rat cassette Kp,uu,brain assay involved co-dosing up to 5 compounds in 2 rats following IV administration at 0.2 mg/kg and sampling brain and plasma at 8 h (Kp = Cbrain/Cplasma). Kp,uu was then corrected for brain and plasma fraction unbound. Using rat IV cassette data molecules were rank ordered in terms of Kp,uu,brain and early human dose prediction.
While making such early human dose predictions, unbound potency (IC50,u) was used as a surrogate for the desired degree of target coverage at 24 h (Ctrough-based target coverage hypothesis) prior to the elucidation of the PK/PD relationship. The dose required to cover IC50,u (nM) has been derived using the rat cassette unbound volume of distribution (Vssu, L/kg), the plasma clearance (CLp, mL/min/kg), and assuming a bioavailability of 50%.
Only molecules with promising Kp,uu,brain and early human dose prediction were progressed into an *in vivo* mouse PK study involving the dosing of 12 mice PO at 10/30/100 mg/kg and sampling brain and plasma across a time course from 0 to 24 h (Kp,uu,brain = AUC,u brain/AUC,u plasma).
This approach based on *in vitro/in vivo* BBB studies and early human dose calculations during the compound optimization stage improves the visibility of the assumptions made for medicinal chemists, and the simplicity of such a model allows teams to devise an effective optimization strategy and focus on improving parameters that are most beneficial for lowering projected human dose.

P4 - IMPROVING *IN VITRO* TO *IN VIVO* CORRELATION (IVIVC) FOR HIGHLY PLASMA PROTEIN BOUND MOLECULES

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It is a common practice in drug discovery and development to predict the (human) *in vivo* clearance based on *in vitro* incubations with liver microsomes or hepatocytes. The well-stirred model (WSM) is an established approach for the translation to *in vivo* using the measured *in vitro* intrinsic clearance values (CLint) corrected for binding to the incubation matrix (fumic or fuhep) and plasma proteins (fup). However, an internal analysis showed this approach fails for acids which are highly bound to plasma proteins. In the classical well-stirred model, correcting for plasma protein binding and incubation binding results in an underprediction. We challenged the implicit assumptions that are made with the standard approach and tested an alternative assay design to account for binding.
Incubations using rat liver microsomes (RLM) were performed in the presence of varying concentrations of rat plasma. A plasma concentration of 5% was found to be sufficient to reach the maximal effect of CLint,u. In total, 68 proprietary compounds were selected including 21 highly bound compounds with PPB>99% and poor IVIVC (only 3 compounds within 3-fold, an average fold error (AFE) of 0.08 and an absolute average fold error (AAFE) of 13.0). Incubations with 5% plasma improved the IVIVC with 13 compounds within 3-fold and an AFE of 0.32 and an AAFE of 3.1. In contrast, only minor impact of plasma was observed for 47 compounds with PPB ≤99% with a similar AFE of 0.53 and slightly improved AAFE of 2.4 compared to 2.5.
Our data suggests that incubations in RLM in the presence of 5% plasma resulted in an improved IVIVC for highly plasma protein bound compounds with minimal impact on compounds that were not highly bound. Based on these promising results the applicability of this approach for predicting human clearance will be explored.

P5 - PREDICTING HUMAN CNS PENETRATION: UNDERSTANDING SPECIES DIFFERENCES IN EFFLUX TRANSPORTERS

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The blood-brain barrier (BBB) is a neurovascular unit consisting of microvascular endothelial cells, astrocytes, pericytes, and microglia. Therapeutic drugs targeting the central nervous system (CNS) need to overcome the BBB which protects the CNS against endogenous compounds and xenobiotics by its physical and biochemical barrier function driven by tight junctions and efflux transporters such as P-glycoprotein (P-gp, MDR1) and breast cancer resistance protein (BCRP, ABCG2). MDR1 and BCRP expression levels vary across pre-clinical species and humans leading to potential differences in the disposition of some drugs. During the drug discovery phase, MDR1- or BCRP-expressing cell lines and brain kpuu values in rodents are used as tools to predict human CNS penetration. Therefore, it is essential to understand the translational characteristics of the different methods.
In this study, we extracted data from the literature on a panel of 44 drugs with known human brain kpuu values. Of this panel pre-clinical data in rats (n=44) and cyno primates (n=16) were also available in literature with the Kpuu values measured by different techniques including CSF and non-CSF (post-mortem, PET imaging, CNS infusion) sampling methods. We measured the efflux ratios (ER) of the drugs using NIH MDCK cells expressing MDR1 and in-house BCRP-expressing MDCK cells and subsequently explored the correlation with brain kpuu values across different species. Relative activity factor (RAF) and relative expression factor (REF) [1-3] methods were applied to compare the correlation between efflux ratios and kpuu values within and across species.
The comparison of protein sequences across species showed that cyno and human MDR1 (96.4%) had greater similarity than rat and human (87.34%). Additionally, rat brain kpuu values showed a superior correlation with MDR1 efflux ratio than with human brain kpuu values. Moreover, the panel of drugs included several BCRP substrates, while the BBB of humans and cyno primates expresses both MDR1 and BCRP, rodents BBB have limited expression of BCRP. Therefore, the BCRP-mediated efflux ratios were utilized to further improve the correlation between human brain kpuu values and efflux by BBB transporters [4]. Furthermore, the impact of CSF and non-CSF methods, used to determine kpuu values, on this correlation was investigated across different species. In conclusion, the relevance of different species to predict CNS penetration in humans will be discussed, including next steps to enhance the correlation between human brain kpuu values by incorporating efflux by BBB transporters.

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P6 - SULFENIC ACID AS REACTIVE INTERMEDIATE DURING THIENOPYRIDINES ACTIVATION: COMPARISON OF SULFENIC ACID TRAPPING METHODS. CHEMICAL METHOD OF FORMATION.

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Thienopyridine antiaggregants are activated into a thiol metabolite that inhibits the ADP platelet receptor P2Y12. This activation proceeds through an 2-oxo-thienopyridine (a thiolactone) further oxidized by cytochrome P45O, into an unstable thiolactone sulfoxide that hydrolyses to a sulfenic acid further reduced by reductase or excess reductant (GSH, thiols, DTT, Ascorbate, Phosphines (TCEP) or Arseniate) (1,2).
In presence of a sulfenic trapping agent like dimedone one can isolate an adduct. In presence of reducing agents, formation of thiol competes with the trapping reaction.
The aim of this study is to compare newly introduced sulfenic acid trapping agents (3) and to show the formation of the sulfenic acid by chemical oxidants.
Method: incubations of 2-oxo-prasugrel (100 µM) in presence of rat liver microsomes (1mg/mL) NADPH (1 mM) were done accordingly to reference 2. In absence of NADPH, oxene donors were tested (tBuOOH, CuOOH) in absence or presence of microsomes. For chemical oxidation 2-oxo-thienopyridines were reacted with diverse oxidants in presence of dimedone. The trapping agent were nucleophiles: thiols, cyanide, thiocyanate, dimedone, cyclopentane1,3-dione, and analogs or strained alkene or alkyne (ethyl-maleimide, norbornenes and BCN).
For nucleophiles, adducts corresponded to addition of the sulfenic acid on the nucleophile with water elimination.
For strained alkenes, adducts corresponded to addition of the sulfide oxide on the double bond giving a sulfoxide product. In addition an adduct that could derive by cycloaddition of the olefine to a conjugated thione (dehydrated sulfenic acid) was found.
In absence of NADPH, and in presence of dimedone and oxene donors, 2-oxo-ticlopidine was converted to the dimedone adduct only in presence of microsomes.

Chemical Formation: 1) 2-oxo-ticlopidine in presence of dimedone and periodate, gave the adduct. The reaction was concentration and time dependent. 2) In CH2Cl2 in presence of dimedone the 2-oxo-ticlopidine was converted to the dimedone adduct by TFA and mCPBA. 3) Reaction first in CH2Cl2, BF3 etherate with mCPPA then extraction with NaHCO3 containing dimedone also afforded the same adduct.
The results of the above study support the pathway: oxidation of the thiolactone into a reactive thiolactone S-oxide that is opened by water to an acid-sulfenic acid then trapped by trapping agents. In presence of thiols, mixed disulfide are formed then reduced further to the active thiol. DTT, ascorbate, TCEP and arseniate make directly the active thiol. Dimedone and analogs, cyanide and thiocyanate give stable adducts. Finally, NEM or norbornene carboxylic and BCN acid give sulfoxide adducts and another dehydrated compound.

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P7 - *IN SILICO* OFF-TARGET PREDICTIONS IN DRUG SAFETY ASSESSMENT: CLOSING THE LOOP FOR PREDICTED CYP17 INHIBITION OF A DRUG CANDIDATE

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Early drug development in the pharmaceutical industry is constantly evolving to increase the likelihood of success and reduce the time to bring a drug candidate to the market. Artificial intelligence (AI) and *in silico* prediction tools leveraging internal and external “big data” play an emerging role in early safety assessment.
Here, we report the *in silico* identification and *in vitro* validation of a drug off-target that could explain some of the histopathological findings observed for the drug candidate NVP001 in a non-rodent toxicity study.
Using a Naïve Bayes multi-category model trained with chemical fingerprints as descriptors and biological targets as categories, CYP17A1 & CYP11B1/2 were identified as potential off-targets for NVP001. This compound notably displayed high chemical fingerprint similarities with an internal dual CYP17/CYP11B1/2 inhibitor (NVP002). Both compounds induced hypertrophy in the adrenal glands in non-rodent toxicity studies. Inhibition of those cytochrome P450 could impair adrenal corticosteroid synthesis and may explain the observed histopathology findings in that organ.
To investigate this hypothesis, we have established an enzymatic LC-MS *in vitro* inhibition assay using recombinant CYP17A1 enzyme and the positive controls abiraterone (CYP 17 inhibitor, approved in prostate cancer treatment) and NVP002 to benchmark potencies. In addition, we developed a similar inhibition assay with rat adrenal homogenates to assess potential effects on the Cyp11b1/2 aldosterone biosynthesis pathway. Our results confirmed off-target inhibition of recombinant CYP17A1 *in vitro* by NVP001 with an IC50 in the mid to high micromolar range, whereas, as expected, the two positive controls showed much higher potencies at low nanomolar concentrations. In contrast, no clear inhibition of aldosterone synthesis was observed with NVP001 in rat adrenal homogenates.
In conclusion, *in silico* off-target predictions based on chemical structural similarity flagged an internal drug candidate for potential effects on adrenal steroidogenesis, which was confirmed by a tailored *in vitro* inhibition assay, providing a mechanistic explanation for the observed cortical hypertrophy in the zona fasciculata of the adrenals in a 2-wk non-rodent toxicity study.

P8 - PROTEOMICS PROFILE AND QUANTITATION OF PHARMACOKINETICS AND PHARMACODYNAMICS TARGETS IN SMALL INTESTINE AND COLON CANCER

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Small intestine cancers account for less than 0.6% of all cancers, but they are rising in the last decades. Despite the important role of the small intestine in the absorption of orally administered drugs, the abundance of important pharmacokinetics (PK) proteins (cytochrome P450 enzymes - CYPs and glucuronosyltransferases - UGTs, ATP-binding cassette transporters - ABCs and solute carrier transporters - SLCs) and their changes in small intestine tumours have not been studied. Similarly, the perturbations of pharmacodynamics (PD) proteins, such as receptor tyrosine kinases (RTKs) have not been investigated. On the other hand, colorectal cancer is a leading cause of death, and is the third most common cancer type. Despite its high incidence, the abundance of PK and PD proteins and their alterations in colorectal cancer have not been investigated. This study aimed to investigate, for first time, the protein expression of PK and PD protein targets in small intestine and colon cancer tissues and study their alterations compared with non-tumour

(histologically normal) and healthy tissues. Tissue homogenisation and extraction of epithelial cells was performed. Pooled homogenates from 5 healthy small intestine tissues, 7 non-tumour peri-carcinomateous small intestine tissues, and 8 tumour small intestine tissues were prepared along with 5 healthy colon tissues, 2 non-tumour peri-carcinomateous colon tissues and 3 tumour colon tissues. Filter-aided sample preparation (FASP) protein digestion protocol was used for the digestion of the homogenate samples. Global liquid chromatography–mass spectrometry proteomics was used to identify and quantify proteins. CYPs and UGTs were significantly decreased in small intestine cancer tissues compared with healthy controls, by ~ 11- and 17-fold for CYPs and UGTs, respectively. UGTs were significantly decreased in colon cancer tissues compared with healthy controls (more than 40-fold). CYP2J2 was 3-fold lower, but interestingly, CYP3A4 was more than 2-fold higher in colon cancer tissues compared with healthy controls. Other DMEs (non-CYP, non-UGT enzymes) were significantly lower in both cancer types compared with healthy controls, with the exception of CES1 that was significantly higher in small intestine cancer tissues compared with healthy controls. Generally, ABC transporters were lower in small intestine cancer (up to 10-fold), including P-gp and BCRP. However, P-gp and BCRP were similar between colon cancer and healthy controls. Among SLC transporters, MCT1 was significantly higher in small intestine cancer compared with healthy controls, while OSTA was significantly lower in colon cancer compared with healthy controls. Among RTKs, INSR and ERBB2 were significantly decreased in small intestine and colon cancer patients compared with healthy volunteers, while EGFR decreased in colon cancer and was not identifiable in small intestine. Additionally, potentially druggable targets were identified and quantified in small intestine and colon cancer with most of them being enzymes. Overall, this study highlights impaired drug metabolism and drug transport and altered abundance of cancer markers in small intestine and colon cancer. These data could be used in the future to inform PK/PD models in cancer populations to accurately predict PK and drug response. Finally, potential druggable targets are highlighted.

P9 - USE OF TRANSCRIPTOMICS TO UNDERSTAND HOW DISTURBED CHOLESTEROL SYNTHESIS DUE TO KNOCKING OUT CONSECUTIVE GENES ALTERS DRUG METABOLISM PATHWAYS N HUMAN HEPATOMA CELLS

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The cross-talk between cholesterol homeostasis and drug metabolism is complex and bidirectional. On one hand xenobiotics modulate cholesterol metabolism while cholesterol levels affect the expression and activity of drug-metabolizing enzymes. Herein we investigated how the disturbed cholesterol synthesis due to knockouts (KO) in consecutive genes of the sterol part of cholesterol synthesis affects gene regulatory networks. Hepatoma cells HepG2 were chosen as a model. Knocking out CYP51A1, DHCR24 and SC5D by CRISPR-Cas9 resulted in production KO cells with depleted activities of selected enzymes from cholesterol synthesis. This resulted in accumulation of different sets of sterols measured by LC-MS. By transcriptome analysis followed by gene and transcription factor enrichment analysis we identified genes and pathways altered by individual sterols. Sterol intermediates of cholesterol synthesis are supposed to be dedicated to cholesterol. However, we show that they influence multiple downstream gene regulatory pathways, indicating interactions with drug metabolism. The overlap of deregulated genes in the three KO cell lines was only 9% in terms of steroid metabolism and proliferation control, with majority of pathways changed in just one KO. The CYP51 KO with highly elevated 24,25-dihydrolanosterol showed significantly higher proportion of cells in the G2+M phase, in line with enriched cancer and cell cycle pathways, and elevated LEF1 by activation of WNT/NFKB/SMAD signalling. In contrast, SC5D and DHCR24 KOs (with elevated lathosterol or desmosterol) slowed cell proliferation and promoted apoptosis with downregulated E2F, mitosis, cell cycle transition, and enriched HNF1A tumor suppressor. Transcription factor with the lowest p-value upon ChEA enrichment analysis was FOXA2 (also HNF3B), which regulates bile acid metabolism and is associated with ER stress. Nuclear receptors enriched in all KOs were LXR (Liver X Receptor), PPARA (Peroxisome Proliferator Activated Receptor Alpha) and RXR (Retinoid X Receptor), which are known to act as heterodimers in regulating lipid homeostasis and also drug metabolism (Figure 1). The DHCR24 KO the LXR enrichment was the highest (lowest p value). The CYP51 and DHCR24 KOs also share enriched CJUN and NR1I2 (also PXR-pregnane X receptor). These findings demonstrate that sterols from cholesterol synthesis control distinct gene regulatory pathways and with drug metabolism associated nuclear receptors. From the cytochrome P450 part CYP3A5, CYP19A1 and CYP24A were most upregulated in CYP51A1 KO and while DHCR24 KO caused upregulation of a larger number of UDP-glucuronosyltransferase 2 genes. Genes associated with synthesis of fatty acids and the PPAR signalling were most upregulated in SC5D KO. In conclusion, we show that different sterols, while structurally similar, activate different signalling pathways and drug metabolism associated nuclear receptors. Better understanding of the cross-talk of cholesterol synthesis and drug metabolism is essential for predicting and minimizing drug-related adverse effects and for developing new therapeutic strategies, including therapies for cholesterol-related disorders.

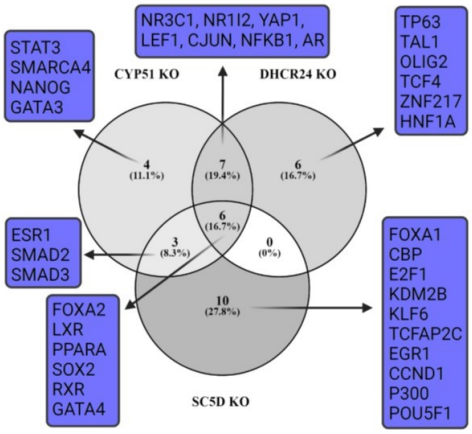

Figure 1. Transcription factor enrichment analysis using CheEA analysis. The Venn diagram of enriched TFs in each KO cell line is presented, showing the number and fraction (%) of TFs in each group.

P10 - ASSESSMENT OF DRUG-INDUCED LIVER INJURY (DILI) RISK OF TWO NOVEL PHARMACEUTICALS BASED ON *IN VITRO* COVALENT BINDING TO HUMAN LIVER PROTEIN MEASUREMENTS AND REACTIVE METABOLITE TRAPPING

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Idiosyncratic drug-induced liver injury (DILI) is a major cause of safety-related drug withdrawal post-market for small molecule medications and a major cause of attrition during drug development. Prediction of DILI remains difficult, and the underlying mechanisms are not fully understood. However, many drugs causing DILI are considered to form reactive metabolites and covalently bind to cellular macromolecules in the liver. In these studies, the potential covalent binding of two 14C-labelled novel pharmaceuticals (denoted Compound X and Compound Y) to human liver proteins and the formation of potential 'trapped' electrophilic metabolites of 14C-labelled Compound X [GSH-adducts] was investigated using human liver microsomes as the *in vitro* test model. Covalent binding was of drug related material was measured by liquid scintillation counting, and trapped reactive metabolites were detected using high-resolution, accurate mass, mass spectrometry. The results were compared with three positive controls and one negative control. The results showed the covalent binding of Compound X and Compound Y and metabolites thereof to be low relative to the controls, and no GSH-adducts (representing reactive metabolites) were detected. Therefore the risk of DILI due to the formation of reactive metabolites and binding to macromolecules is considered to be negligible, and adds confidence that this potential liability is de-risked for these compounds.

P11 - EVALUATION OF PERFLUOROOCTANESULFONIC ACID (PFOS) DISTRIBUTION IN AN ALBUMIN DEFICIENT (ALB-/-) MOUSE MODEL

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Perfluorooctanesulfonic Acid (PFOS) is a perfluoroalkyl substance (PFAS) ubiquitously found in the environment, with exposure occurring through the consumption of drinking water, food (via contact materials), consumer product use/exposure, and industrial use. PFOS is detected in the serum of ~95% of the United States adult population and has a half-life of 4.1-8.67 years. Multiple human and animal studies have found adverse health outcomes associated with PFOS exposure, such as, liver injury (increased ALT), decreased immunity (decreased serum antibody response to vaccination), dyslipidemia (increased serum cholesterol), and obesity. In comparisons of PFOS accumulation in whole blood and plasma, it was revealed that PFOS accumulates in the non-cellular blood fraction. Albumin is a protein synthesized by the liver that aids in moving endogenous substances and xenobiotics throughout the blood. Because albumin is abundant in plasma and binds PFOS with high affinity *in vitro*, it was hypothesized that albumin is a major carrier for PFOS, and likely other PFAS, in the plasma. An analbuminaemic mouse model(Alb-/-) was chosen for this study to test the hypothesis that albumin is a critical mechanism for PFOS retention in plasma, a determinant of PFOS tissue distribution, and inducer of adverse liver outcomes (increased plasma ALT and AST, liver triglycerides, and liver pathology). We hypothesized that Alb-/- mice would have lower plasma PFOS concentrations and increased tissue PFOS concentrations. Adult male C57BL/6J wild-type (WT) mice and albumin null (Alb-/-) mice were administered either vehicle (0.5% Tween 20, VEH, n=8 for 10 mg/kg, n=12 for 0.5 mg/kg) or PFOS in VEH (n=8 for 10 mg/kg, n=12 for 0.5 mg/kg) p.o. daily for 7 days. Plasma and tissues were processed using RoQ QuEChERS for subsequent LC-MS/MS analysis. PFOS treatment significantly

increased liver weight 2-fold in both Alb+/+ (2.17 g) and Alb-/- (2.22 g), however, a lack of albumin didn't cause a significant change in liver weight. Plasma PFOS concentrations were significantly lower in Alb-/-(29.69 µg/mL) as compared to Alb+/+(85.72 µg/mL). Seeing as PFOS is 99.999% bound to albumin *in vitro*, in the absence of albumin *in vivo*, there is more free drug circulating available to readily enter into tissues. Liver PFOS concentrations were significantly higher in Alb-/- (349.74 ng/mL) as compared to Alb+/+ (277.92 ng/mL). These findings align with the plasma PFOS concentration data and support our hypothesis that a lack of albumin will increase liver PFOS concentrations, likely utilizing protein and phospholipid binding to stay in the liver. Additionally, qPCR revealed that a lack of albumin significantly upregulated genes related to lipid metabolism/accumulation in PFOS treated mice, Ehhadh (33-fold), Cd36 (21-fold), Acot2 (3-fold), and Fabp1 (2-fold). In conclusion, data herein suggests that albumin is a critical factor for PFOS plasma concentrations and tissue distribution *in vivo*. Albumin however was not a critical factor for PFOS induced liver enlargement at concentrations >250 ng/mg, which may be due to exceeding a minimum threshold for inducing liver enlargement. Along with this data, a repeat of this study at a dose of 0.5 mg/kg BW will be presented to investigate exposure relevant effects.

P12 - OPTIMIZING HUMAN CNS AVAILABILITY IN THE CONTEXT OF RODENT VS NON-RODENT SPECIES DIFFERENCES IN IN-VIVO BRAIN KPUU

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The central nervous system (CNS) as a target tissue for drugs has always been challenging for drug discovery teams. The blood-brain-barrier (BBB) is a tight endothelium expressing high levels of efflux transporters limiting access to the CNS for xenobiotics. The molecular property space of molecules amenable to permeate across the BBB is well known and in-vitro systems to reduce active efflux potential via human BBB transporters have been established. Promising molecules enter pre-clinical in-vivo studies to confirm CNS availability. In this case study, we present an example of a candidate drug (AZ'7717) with low efflux potential in a human P-gp and BCRP double transfected MDCKII cell line (efflux ratio (ER): 1) but displaying higher P-gp in-vitro efflux ratios for rat (ER: 8) and monkey (ER: 3). In-vivo AZ'7717 was characterized by a profound difference in CNS availability between rodent and non-rodent species. Rat brain Kpuu were determined based on several surrogate matrices (total brain homogenate, cerebrospinal fluid, brain micro-dialysate) and were similarly low, ~0.1 and reproduced in mouse. A monkey brain PET (positron emission tomography) study using a 11C-labelled tracer revealed high CNS availability (brain Kpuu 0.7), and dog brain Kpuu was estimated at 0.4. The quantitative results from the monkey brain PET study position AZ'7717 well within the range of approved CNS drugs. In view of the multiparameter optimization requirements in drug discovery, CNS-campaigns may not arrive at molecules fully devoid of active efflux potential. This example demonstrates how a CNS strategy rooted in optimizing human in-vitro efflux and passive permeation properties is complemented by cross-checking in-vivo animal brain availability, which may diverge between pre-clinical species.

P13 - ULTRA-HIGH THROUGHPUT MASS SPECTROMETRY BASED ANALYSIS BY DIRECT INJECTION (ECHO™ MS) FOR PLASMA PROTEIN BINDING ASSAY

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Plasma protein binding (PPB) assays are routinely performed during drug discovery. In our lab, a fully automated PPB assay was developed using equilibrium dialysis device and Hamilton Microlab STAR workstation. Therefore, an ultra-high throughput mass spectrometry (MS) analysis platform needs to be developed to keep up with automated PPB assay. A simple and reliable ADE-OPI-MS/MS (Echo™ MS) platform was established by optimizing MS detection conditions and sample preparation. PPB samples in 384-well plates were directly injected with nanoliter size sample droplet ejection into a mass spectrometer connected to an electrospray ionization (ESI) source, without the addition of internal standard and protein precipitation. The optimized carrier solvent was 2mM NH4F in MeOH at a flow rate of 400 µL/min. The following MS parameters were used for analysis: ion source temperature at 300 °C, spray voltage at 5000 V, nebulizer gas (GS1) at 90 psi and heater gas (GS2) at 45 psi, respectively. To ensure adequate peak points and accurate peak integration over the signal duration, the analyte dwell time was 90 msec and the pause time (additional delay time) was 2000 msec to achieve high quantitation precision and data accuracy. Thirty-nine compounds in human, dog, rat monkey, and mouse plasma samples with matrix-matched buffers in PPB assays were analyzed by Echo MS and conventional LC-MS/MS. The results from comparing unbound% generated by Echo MS (5 nL injection volume) versus LC-MS/MS (2-5 µL injection volume) for all compounds showed good correlation with R2 =0.9896. Throughput for a 384-well plates with the Echo MS used in this study at 19.2 min (3 sec/sample) was approximately 20 times faster than LC-MS/MS analysis at 384 min (1 min/sample). Moreover, successful direct analysis of samples without extraction steps significantly simplifies sample preparation.

P14 - ESTABLISHMENT OF THE FLUX DIALYSIS METHOD FOR HIGHLY BOUND COMPOUNDS IN HUMAN PLASMA AND RAT BRAIN HOMOGENATE

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The fraction unbound (fu) data in plasma and tissue are important to predict drug interaction, develop PK/PD relationships, and estimate therapeutic index. For compounds with high molecular weight, high lipophilicity, and severe non-specific binding, instability in matrix and other complex properties, they are usually highly protein bound with fu values being less than 0.01. Therefore, it is difficult to be determined accurately using standard assays such as equilibrium dialysis, ultrafiltration, and ultrafiltration. In this experiment, the flux dialysis was applied to determine fu in human plasma and rat brain homogenate for commercial compounds with highly bound, instability and other complex properties. Drug-spiked biological matrix and blank matrix were added on the donor and receiver side of the membrane of the 96-well equilibrium dialysis device, respectively. Samples on both sides of the membrane were taken after incubation at 37°C for different time points up to 120 hours, and the drug concentrations were analyzed by LC-MS/MS. The drug concentration ratios between the receiver and the donor side were fitted with incubation time. After that, the initial slope of the concentration ratio-time curve was obtained by nonlinear regression, and the fu value was further calculated. The human plasma fu of 18 compounds and rat brain homogenates fu of 9 compounds measured in this experiment correlated well with those reported in the literature (R2>0.98). In conclusion, we established a flux dialysis method and verified the accuracy and reliability of this method by determining the fu of commercial compounds in human plasma and rat brain homogenate. With improved detection range and accuracy of determined fu, as well as no requirement of reaching equilibrium, this method can be especially applied for highly bound and plasma unstable compound.

P15 - IMPACT OF THE PHYSICAL-CHEMICAL PROPERTIES OF POLY(LACTIC)-POLY(ETHYLENE GLYCOL) POLYMERIC NANOPARTICLES ON BIODISTRIBUTION AND PHARMACOKINETICS

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The use of nanotechnology as a small molecule drug delivery tool in the pre-clinical space has seen increased prevalence in the literature over recent years. This has not however translated into an increase in successful clinical candidates and raises questions over the predictability and translatability of the preclinical studies. Nanoparticle (NP) formulations are inherently polydisperse, making their structural characterization complex. It is essential, however, to gain an understanding of the physico-chemical properties that drive performance *in vivo*. To elucidate these properties, drug-containing poly(lactic acid) (PLA)–poly(ethylene glycol) (PEG) block polymeric NP formulations (or PNPs) were sub-divided into discrete size fractions and analyzed using a combination of advanced techniques. Together, these techniques revealed a uniquely detailed picture of PNP size, surface structure, internal molecular architecture and the preferred site(s) of incorporation of the hydrophobic drug, properties which cannot be accessed via conventional characterization methodologies. PNP size distribution was established as important in determining drug loading, with the presence of the smallest PNPs containing significantly less drug than their larger sized counterparts, reducing overall drug loading, while PNP molecular architecture was critical in understanding the nature of *in vitro* drug release. The effect of PNP size and structure on drug pharmacokinetics and biodistribution was determined by administrating selected PNP size fractions to mice, with the smaller sized NP fractions increasing the total drug-plasma concentration area under the curve and reducing drug concentrations in liver and spleen, due to greater avoidance of the reticuloendothelial system. In contrast, administration of unfractionated PNPs, containing a large population of NPs with extremely low drug load, did not significantly impact the drug's pharmacokinetic behaviour - a significant result for nanomedicine development where a uniform formulation is usually an important driver. We also demonstrate how it is not practicable to validate the bioanalytical methodology for drug released *in vivo* due to the formulation properties, a process which is applicable for all drug-releasing nanomedicines. In conclusion, this work details a strategy for determining the effect of formulation variability on *in vivo* performance, thereby accelerating the translation of PNPs and other NPs, from the laboratory to the clinic.

P16 – DE-RISKING *IN VIVO* PK ATTRIBUTES OF THERAPEUTIC ANTIBODY LEAD PANELS USING HIGH THROUGHPUT *IN VITRO* APPROACHES AS PART OF AN EARLY DRUG DISCOVERY AND HUMAN DOSE PREDICTION STRATEGY

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It is well established that small sequence differences between therapeutic monoclonal antibodies can affect the potential manufacturability of drug candidates. However, it is only relatively recently that the pharmaceutical industry has started to observe that similar intrinsic biophysical properties, such as charge or non-specific binding interactions, can impact the pharmacokinetics of candidate therapeutic mAbs in preclinical species and human (1-4). Our aim was therefore to

develop, embed and industrialise a high throughput comprehensive biophysical *in vitro* screen, with the capability of testing the *in vivo* suitability of lead panels of candidate antibodies from our antibody discovery projects. The screen was designed to include various orthogonal assays so that all major non-target-mediated clearance risks for antibodies could be assessed, including FcRn inefficiency, non-specific binding and molecule *in vivo* instability. The screen was validated with a panel of clinically relevant antibodies on a common IgG1 backbone, with the aim of comparing the observed assay outputs to reported human clearance. This benchmarking work allowed us to build confidence in the predictability of our new *in vitro* capability while also defining suitability thresholds for subsequent *de novo* antibody campaigns. With the *in vitro* screen operationalised and embedded into our antibody discovery process, we subsequently took the opportunity to consider our human dose prediction strategy for biopharmaceuticals and refine our recommendations on the use of NHP PK/PD studies. We developed a new risk-based strategy that considers the antibody platform used (e.g. typical mAb, Fc modifications etc) and what we may or may not know about the target and its potential role on exposure at the site of action and anticipated dose. Taken together this provides an appraisal on the relative value of performing an NHP PK/PD study as part of our human dose prediction package (assuming appropriate species cross-reactivity). The i*n vitro* translational screen we now perform is therefore a critical piece of our new dose prediction strategy, allowing us to assume IgG1-like pharmacokinetics by first intent in some cases and providing opportunities to progress to GLP toxicology studies without any in vivo PK/PD data at all. Our combined new *in vitro* screen and dose prediction strategy is therefore providing key 3Rs benefits by eliminating potentially unvaluable NHP studies, appropriately prioritising their use in an environment where NHP access remains challenging. It is also allowing us to accelerate some projects when the relative risk profile allows, by removing potentially lengthy studies from the critical path of our project plans.

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3. Datta-Mannan et al 2015 (mAbs, 7:3, 483-493): "Balancing charge in the complementarity-determining regions of humanized mAbs without affecting pI reduces non-specific binding and improves the pharmacokinetics” [DOI: 10.1080/19420862.2015.1016696]
4. Kelly et al 2016 (mAbs, 8:7, 1269-1275): “Target-independent variable region mediated effects on antibody clearance can be FcRn independent” [DOI: 10.1080/19420862.2016.1208330]

P17 - ACCELERATION OF INFECTIOUS DISEASE DRUG DISCOVERY AND DEVELOPMENT USING A HUMANIZED MODEL OF DRUG METABOLISM

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A key step in drug discovery and development workflows common to many disease areas is preclinical demonstration of efficacy in a mouse disease model. However, translation of results from these studies to the clinic can be impeded by species difference in drug metabolism. Furthermore, medicinal chemistry resource must be given to ensuring compounds survive metabolic pathways specific to the mouse. Here, for a validation set of 28 approved anti-infective medicines, we demonstrate that a transgenic mouse line extensively genetically humanized for cytochrome P450s, “8HUM”, can circumvent these problems. *In vitro*, using hepatic microsomes and primary hepatocytes isolated from 8HUM, species differences in rates of intrinsic clearance were removed for the vast majority of compounds. *In vivo*, alignment of drug pharmacokinetic (PK) profile with that reported for human was greatly improved in 8HUM, primarily due to slower elimination rates than in WT mice. Furthermore, the susceptibility to and magnitude of drug-drug interaction (DDI) of combinations were also more predictive of the human situation. Translational relevance of metabolite profiles in 8HUM was greatly improved over WT mice, both in terms of metabolite identities and in the relative abundance of metabolites across profiles. To determine whether 8HUM could be used in place of WT mice in models of infection applied routinely for efficacy assessment of New Chemical Entities (NCEs), we carried out pilot infection studies. The course of infection of 8HUM with Trypanosoma cruzi, Leishmania donovani and Mycobacterium tuberculosis matched that of the WT strains. In an exemplification of our new cascade, lead compounds from a chemical series under active development – compounds with WT mouse-specific metabolic liabilities – were found to exhibit (i) metabolic stability in incubations with 8HUM hepatic microsomes, (ii) favorable PK properties in 8HUM *in vivo* and (iii) efficacy in the 8HUM T. cruzi infection model. Separately, in an 8HUM model of acute M. tuberculosis infection, we found that the PK interaction of bedaquiline (approved for the treatment tuberculosis) with efavirenz (approved for the treatment of the common co-morbidity, HIV), the resultant impact on bedaquiline efficacy, and the ratio of bedaquiline to its major human (active) metabolite, N-desmethybedaquiline, in peripheral blood were well-aligned with clinical observations. Collectively, our results demonstrate that the elimination of species differences in drug metabolism through the application of 8HUM – in place of WT mice – during preclinical drug development has the potential to significantly increase the proportion of compounds that can be considered for progression during lead optimization and the translational relevance of the data generated.

Note, all animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

P18 – Abstract Withdrawn

P19 - APPLICATION OF A GLP PROTOCOL FOR THE MEASUREMENT OF 17B-ESTRADIOL AND TESTOSTERONE BY LC-MS/MS IN THE H295R STEROIDOGENESIS ASSAY, TEST NO 456

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Regulators have concerns about environmental chemicals, such as agrochemicals and their metabolites, and how to recognize their potential ability to disrupt the endocrine system1. These concerns led to the publication of a series of OECD *in vitro* test guidelines for the assessment of potential endocrine disrupting chemicals (EDCs). We have developed an extraction procedure and tandem LC-MS method1 to support one of these tests, TG4562, which investigates compound effects on the synthesis of the steroid hormones Testosterone (T) and 17β-estradiol (E2). The test uses the human H295R adreno-carcinoma cell line, as it encodes for all the key enzymes in the steroidogenesis pathway. Our robust GLP bioanalytical method is highly sensitive and can detect 10-5000 pg/mL of Testosterone and 17β-Estradiol from the same sample of spent cell media, in which the H295R cells were incubated with the investigated compound. The cells are incubated across 7 concentrations of the test item, alongside a QC plate containing the strong inducer, Forskolin, and the strong inhibitor, Prochloraz, to confirm the H295R cell performance. Testosterone and 17β-estradiol in the spent cell media are extracted using supported liquid extraction (SLE) and analysed by tandem mass spectrometry (LC-MS/MS), allowing for the avoidance of test item related interference issues as might be seen with immunoassay-based detection. Analysis of the measured concentrations of Testosterone and 17β-Estradiol in the spent cell media allows for the determination of the lowest observed effective concentration (LOEC) of the test item, and the maximum fold change at each concentration.

References:

1. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC)No 1107/2009. DOI https://doi.org/10.2903/j.efsa.2018.5311
2. Test No. 456: H295R Steroidogenesis Assay, https://www.oecd.org/env/test-no456-h295r-steroidogenesis-assay-9789264122642-en.htm

P20 - MODULATION OF HEPATIC XENOBIOTIC METABOLIZING ENZYMES FOLLOWING CHRONIC LOW-DOSE EXPOSURE TO PFOA IN MICE UNDER CHRONIC STRESS

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Perfluoroalkylated substances (PFAS) are used in a wide range of industrial applications including coatings for cookware, food contact papers, waterproofed clothing, and fire-fighting fluids. PFOA (perfluorooctanoic acid) has raised major public health concerns over the last few years and still does. Although its industrial usage is decreasing, consumers are still exposed to this extremely persistent and bioaccumulative substance. Dietary intake is reported as the main source of human exposure to PFOA, with seafood and freshwater fishes being the highest contributors. Epidemiological surveys and results from *in vivo* studies in rodents suggest that PFAS can induce hepatotoxicity and exert metabolic effects, including effects on lipid metabolism. However, the mechanisms underlying these effects remain largely unexplored.
In this study, we assessed the effects of low doses of perfluorooctanoic acid (PFOA) on mice hepatic xenobiotic metabolizing enzyme (XME) activities under chromic stress. XMEs are involved in the biotransformation of xenobiotics, playing an essential role in the protection of organisms, and are also involved in a number of endogenous anabolic and catabolic processes. This study aimed at investigating the effects of chronic low doses of PFOA on mice hepatic phase I and II functional XME activities under chronic stress, better characterizing the mechanisms underlying PFOA effects, and determining specific enzyme isoform modulation following incubations with hepatic subcellular fractions and probe substrates combined with different analytical strategies.
Adult C57BL/6 male mice were exposed via drinking water to 1.5 ng/kg body weight/day (n=10 per group) for 90 days (chronic exposure) alone or in combination with corticosterone (35 mg/L the last 10 d, to mimic stress). At the end of the 90 d-period, mice were then sacrificed, and livers were collected and perfused. Subcellular fractions (microsomal and cytosolic fractions) were prepared. Following protein content assessment, *in vitro* incubations with specific probe substrates and cofactors were carried out to measure key phase I and II activities. Specific CYP450 isoform activities were assessed in microsomal fractions, including CYP2A4, CYP2C37, CYP3A11 and CYP4A10. Phase II enzyme activities such as glutathione S-transferases (GST) were also investigated using microsomal and cytosolic fractions.
Our study has demonstrated a significant impact of *in vivo* exposure to PFOA on hepatic phase I and phase II XME activities. Stress conditions can affect enzyme activities such as CYP4A10.

P21 - COMPETITIVE INHIBITION OF OROXYLIN A ON THE CARBOXYLESTERASE MEDIATED HYDROLYSIS OF IRINOTECAN

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Our previous studies systematically screened the potential inhibitory effect of the major flavones from Radix Scuttelarine on the Carboxylesterase (CES) mediated activation of seven clinically used prodrugs. It was identified that Oroxylin A (OXA) had the most significant inhibition potential toward the CES mediated hydrolysis of irinotecan, leading to decreased CES enzyme activities as well as the formation of SN-38 in plasma and tissues. The current mechanistic study is designed aiming to investigate the enzyme inhibition kinetics involved in the CES mediated hydrolysis of irinotecan by OXA in plasma, intestine and liver, the three major CES containing organs in rats.

Briefly, the *in-vitro* incubation studies were carried out in 200 μL mixtures containing irinotecan (2.5, 5, 10 and 20 μM), rat plasma/intestine S9/liver S9 (at protein concentration of 1mg/mL) and PBS (100 mM, pH7.4) with 15 minutes preincubation in absence and presence of OXA (50 and 200 μM) at 37°C. After a 30 min incubation at 37°C, all the reactions were terminated by adding 600 μL of ice-cold acetonitrile followed by determination of the formation of SN-38, the hydrolyzed metabolite of irinotecan, by our developed LC/MS/MS method [1]. Each experiment was repeated in triplicate. The enzyme kinetic parameters including Km and Vmax values were determined by fitting the Michaelis-Menten plot and the enzyme inhibition mode were identified by creating Lineweaver–Burk plot using Prism 8.0 software (GraphPad Software Inc., La Jolla, CA, USA).

Figure 1 demonstrated the profiles of inhibition of SN-38 formation in different biomatrices with all the enzyme kinetic parameters shown in Table 1. The Lineweaver–Burk plot resulted in a family of straight lines with the same y-axis intercept, suggesting the competitive inhibition of OXA on irinotecan metabolism in rat plasma, intestine, and liver S9 incubation system. Based on the Ki, OXA indicated the strongest inhibition) on the formation of SN-38 from irinotecan in plasma with the smallest Ki value of 3.107 μM than that from liver S9 (Ki of 56.31 μM) and intestine S9 (Ki of 38.23 μM), suggesting a significant decrease in the formation of its active metabolite SN-38 in the plasma. Our present study for the first time demonstrated the competition inhibitory effect of OXA on the CES meditated hydrolysis of irinotecan in rat plasma, intestine, and liver. Clinical impact of co-administration of OXA or its containing herbs on the efficacy of irinotecan treatment warrants further attention.

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Reference:

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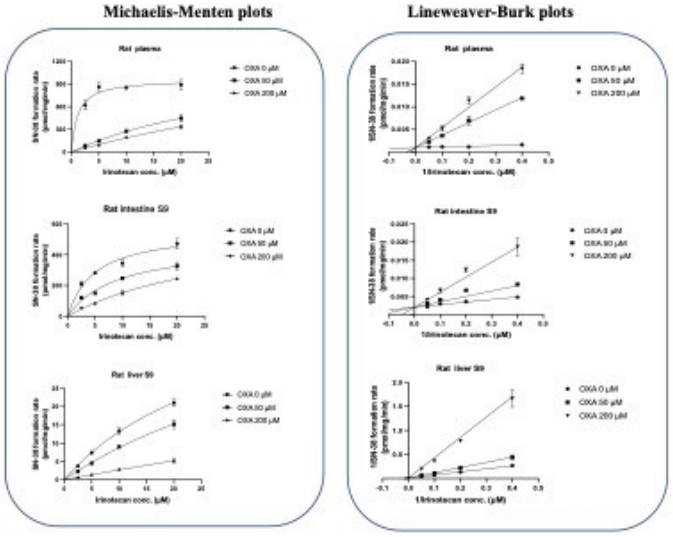

Figure 1. **Michaelis-Menten plots (Left) and Lineweaver-Burk plots (Right) for the formation of SN38 in rat plasma, intestine S9 and liver S9 after incubation of irinotecan with and without OXA.**

Table 1. Enzyme kinetics parameters of SN-38 formation in different biomatrices after incubation of irinotecan with and without OXA.

Biomatrices	Kinetic parameters	OXA (Mean±SE, n=3)		
		0 μM	50 μM	200 μM
Plasma	V_{max} (pmol/mg/min)	964.3 ± 36.39	1299 ± 275.3	1881 ± 708.5
	K_m (μM)	1.15 ± 0.15	37.62 ± 8.17	91.82 ± 41.9
	Ki (μM)		3.107	
Intestine S9	V_{max} (pmol/mg/min)	565.9 ± 44.97	483.2 ± 88.65	652.2 ± 100.1
	K_m (μM)	5.02 ± 0.98	9.66 ± 3.32	32.02 ± 8.43
	Ki (μM)		38.23	
Liver S9	V_{max} (pmol/mg/min)	54.35 ± 3.85	60.95 ± 14.17	34.22 ± 3.98
	K_m (μM)	51.47 ± 1.28	59.27 ± 12.51	119.9 ± 9.4
	Ki (μM)		56.51	

P22 - APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING FOR PREDICTION OF DRUG-DRUG INTERACTIONS USING THE STRONG CYP2D6 INHIBITOR TERBINAFINE

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Terbinafine increases the exposure of drugs that are metabolised by cytochrome P450 2D6 (CYP2D6) following co-administration. Thus, terbinafine is categorised by the FDA as a strong inhibitor and can be used for assessment of drug-drug interactions (DDIs) involving CYP2D6. However, a simple competitive inhibition of the parent compound alone is not able to explain the observed DDIs. In addition, there is no evidence of a mechanism-based inhibition. Several researchers suggested that metabolites may have equal or greater inhibition potential against CYP2D6 than the parent compound (Akiyoshi 2015; Mikami 2017). The aim of the current study was to generate a PBPK model for terbinafine and its carboxylic metabolite, carboxylbutylterbutaline (CBT), a metabolite that is slowly eliminated from the plasma, and to evaluate, if the additional competitive inhibition of a metabolite can explain observed DDIs. Prior *in vitro* information on the metabolism of terbinafine were combined with physicochemical data in a PBPK model implemented in the Simcyp Population-based Simulator (V22). The absorption was described using a mechanistic permeability (MechPeff) model within the Advanced Dissolution, Absorption, and Metabolism (ADAM) model, while the distribution was simulated using a full PBPK model. The metabolite, CBT, was also simulated using a full PBPK model; however, a simple clearance was included to describe the elimination of the metabolite. Concentration-time profiles of terbinafine and when available for CBT following single (SD) and multiple (MD) oral doses were simulated over a range of doses (125 to 750 mg) to assess the quality of the model to recover terbinafine and CBT exposure compared with observed data. Simulations of terbinafine were performed using the study design described in the individual clinical studies. Then a DDI between desipramine and terbinafine was simulated, where measured competitive inhibition parameters for terbinafine were also included for the CBT metabolite. *In vitro-in vivo* extrapolation (IVIVE) of enzyme kinetic data for terbinafine was able to recover the observed clearance and concentration-time profiles of terbinafine and its carboxylic metabolite in healthy volunteer populations for all evaluated SD and MD studies. The predicted increases in AUC(0-inf) and Cmax of desipramine following administration of terbinafine were 3.63 and 1.7-fold, which were reasonably consistent with observed values of 4.94 and 1.92-fold. Further applications of the validated model allowed other trial designs to be investigated, such as DDIs for other ethnicities and other CYP2D6 substrates. IVIVE-linked PBPK modelling in conjunction with a mechanistic absorption model and reliable *in vitro* metabolism and competitive inhibition data, can be used to assess the impact of complex DDIs with the CYP2D6 inhibitor terbinafine, after accounting for the additional inhibition of relevant terbinafine metabolites.

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P23 - ANALYTICAL APPROACHES IN METABOLITE SAFETY TESTING (MIST) AND THEIR CONTRIBUTION TO HUMAN METABOLISM EVALUATION

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Nowadays, there is an increased focus on human drug metabolites due to their potential contribution to pharmacological activity, safety, and drug-drug interactions. The Regulatory Agency recommends evaluate any differences in drug metabolism between animals, used in non-clinical safety assessment, and humans. Consequently, Metabolite Safety Testing (MIST) has become a hot topic in the drug development process. The request for an early evaluation of relevant human circulating metabolites has led to implementing appropriate approaches required for human metabolites identification, semi-quantitation and, when necessary, preliminary toxicological evaluation. The main challenge is how to balance this recommendation with the need to appropriately invest resources according to the drug development plan. The adoption of “alternative approaches” such as 1H- or19F-NMR and LC-UV-MS allows for preliminary metabolite semi-quantification, without the need of radio-label compound, metabolite synthetic standards and validated assays. NMR has been demonstrated to be useful in quantitating biological metabolites, provided a suitable dose level had been administered or sufficient sample is available. Alternatively, metabolites can be semi-quantified using the UV response if the parent drug chromophore offers sufficient selectivity and is not altered by metabolism. Additionally, a “radio-metabolites” approach could be a valuable tool when the other approaches are not applicable. By means of radiolabelled metabolite(s), generated in-vitro or in-vivo, which should reflect the same metabolite(s) observed in human, this approach exploits the hyphenation of MS and radio-detection to determine the metabolite MS response factor. Data generated in several MIST studies has helped design an appropriate strategy to assess safety of relevant human metabolites: preliminary data on coverage of human circulating metabolites in preclinical species were accepted by the Regulatory

Agency to support the start of the clinical Phase II of the project. Moreover, data obtained from MIST investigation resulted to be consistent with those subsequently obtained in human AME studies, corroborating the value of the MIST approach.

P24 - FROM MS SIGNAL ANALYSIS TO AI BASED METHOD: SOLUTIONS FOR METABOLISM DATA PROCESSING AUTOMATION

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The Mass Spectrometer (MS) is usually the final detector for many Drug Metabolism and Pharmacokinetic measured end points and it is used in in-vitro ADME calculation like clearance, metabolite identification or in-vivo Pharmacokinetic quantification. We are showing in this presentation how signal analysis algorithms are applied for LC-MS data processing for molecules of any modality (small molecule, PROTAC, Macrocyclic Peptide, peptides, Oligonucleotides and Antibodies). The algorithm modifications needed to transition from small to macro molecule is also described an applied to one case per compound modality covering: atom-based analysis versus monomer-based analysis, peak selection based on monoisotopic m/z versus Most Abundant Mass or Average Mass or the incorporation of Charge deconvolution to enhance sensitivity and avoid complexity. In addition to the signal analysis, we are also showing in this presentation the development of Machine Learning models that learns from user peak selection criteria (peak quality, multiple sample kinetic, MS and MSMS spectra quality) and the intended usage of the data processing (soft Spot analysis, Metabolite identification) to automatically select/hide Chromatographic peaks. The Machine learning model is therefore introduced into a self-learning mechanism (AI) where the data for user peak selection is incorporated into the model building and predictions are refined with new data. This feature will be shown with 2 different datasets one dedicated for multiple time point analysis (Soft Spot ID) and another one for GSH trapping experiment. Finally, an application of Machine Learning models in the field of MS based analysis of DMPK data will also be introduced for the selection of the m/z transition in a typical MRM experiment by the automatic selection of m/z to be used in a QQQ instrument try to avoid the experimental condition optimization, using the predicted transitions to compute the compound of interest Area that is used in DMPK end points computation like Clearance and Papp determination.

P25 - OPTIMISATION OF ADME PROPERTIES OF SARS-COV-2 MPRO INHIBITORS USING MACHINE LEARNING

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The main protease (Mpro) of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) plays an important role in viral replication and has been the focus of recent global drug discovery efforts. A significant challenge for designing MPro inhibitors has been generating compounds with low human clearance to negate the need for co-dosing with the CYP inhibitor, ritonavir in the clinic. Here we describe the optimisation of ADME properties of Mpro inhibitors, including human clearance, using our AI algorithms and machine learning models. Using multi-parametric optimisation, we generated compounds with good potency and favorable ADME properties. Within 5 design cycles, we identified our first highly promising compound, EXS7063 (Table 1). This initial compound was potent in FRET assays, showed good permeability in the MDCK-WT cell line and had moderate solubility. However, rat bioavailability at 3 mg/kg was 23% despite a calculated fraction absorbed (Fa) of 82%. The rat bioavailability was limited by the moderate clearance of EXS7063. Subsequent design cycles focused on tackling these ADME issues. Each cycle provided additional ADME data that was fed back into the machine learning models to further refine them, and potentially enable acceleration of the drug discovery process. Compounds with improved properties were generated, exemplified by EXS9031 (Table 1). Rat bioavailability of EXS9031 at 3 mg/kg was 78%, a significant improvement on EXS7063 (F = 23%). This was mainly driven by a reduction in intrinsic clearance and an increase in the calculated Fa to 100%. The increase in Fa was due to increased solubility from 43 µg/ml (EXS7063) to 101 µg/ml (EXS9031), as the permeability in the MDCK-WT cell line was maintained. FRET potency also remained consistent. In human hepatocytes, we were able to significantly reduce the intrinsic clearance from 57 (EXS7063) to 8 (EXS9031) µl/min/million cells. The work described here shows how we can harness the power of machine learning and AI to develop optimized compounds at speed to tackle COVID-19 and future pandemics.

Table 1: Potency, physiochemical and ADME properties of two M^{Pro} inhibitors.

Property	EXS7063	EXS9031
Cycle	5	14
SARS-Cov2 FRET potency IC_{50} (nM)	14	10
logD	3.6	2.8
Semi-thermodynamic solubility pH 7.4, PBS (µg/ml)	43	101
Human / Rat Clint hepatocytes (µl/min/million cells)	57 / 105	8 / 31
MDCK-WT AB/BA/ER (Papp x 10^{-6} cm/sec)	14.7 / 17.3 / 1.2	13.3 / 22.7 / 1.7
Rat F (%) at 3 mg/kg	23	78
Calculated rat Fa (%) at 3 mg/kg	82	100

P26 - BIOTRANSFORMATIONS FOR THE PREDICTION OF RING-FORMING AND RING-OPENING REACTIONS

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The *in-silico* prediction of the metabolites of a chemical has a number of important applications including providing early insight into the potential for metabolism-related toxicity. Meteor Nexus is an expert system designed to predict the likely metabolic fate of a xenobiotic based on its chemical structure. It contains a dictionary of biotransformations to predict the structures of potential metabolites and a scoring model to determine those which are most likely. The prediction of experimentally known ring-forming and ring-opening metabolic reactions by Meteor has been assessed. Recent metabolism data consisting of metabolic reactions and associated experimental details were collected from the primary literature and added to an existing database. These data included *in vivo* and *in vitro* studies in humans, other mammals and occasionally microflora. Substrates for ring-forming and ring-opening reactions were processed in Meteor and the predicted metabolites were compared with those observed experimentally. Meteor predicted reported ring-opening reactions for both aliphatic and aromatic heterocycles by oxidative, reductive, hydrolytic and conjugation pathways. Oxidative ring-opening of aliphatic heterocycles was the most common. Meteor also predicted reported ring-forming epoxidation reactions. However, ring-forming reactions of other types in the experimental data were rare. The analysis highlighted new biotransformation which can be implemented in Meteor to further improve coverage such as the hydrolytic ring-opening of oxetane rings and the ring-forming conjugation of nitriles. Periodic review of biotransformation dictionaries used in metabolite prediction against recent experimental data ensures continued relevance of *in silico* metabolism solutions to new areas of chemical space.

P27 - MICRORNA PROFILING OF LIVER CELLS IN A PRECLINICAL DRUG-INDUCED TISSUE INJURY MODEL USING METHOTREXATE

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Introduction: Drug induced liver injury (DILI) is an important problem in the clinic and in drug development. One important reason for this is the lack of sensitive and specific biomarkers. Methotrexate (MTX) is a widely used drug, at a high dose, in the treatment of different type of cancer and at low dose for a variety of dermatological and rheumatic disease. MTX causes DILI in 0-30% of all patients (acute & chronic). Circulating microRNAs (miRs) represent a potential new set of markers for drug discovery and development. Our aim is to develop a panel of novel safety biomarkers that will reliably indicate and locate DILI, specifically for MTX, initially drawing on the potential of miRs to do this.
Methods: Hepatocytes, cholangiocytes, liver sinusoidal endothelial cells (LSECs), and plasma were collected from eight male Wistar rats. miRNA was extracted using QIAGEN miRNA extraction kit and Illumina small RNA-seq was performed by TAmiRNA (TAmiRNA Gmbh, Vienna, Austria) (Fig 1). A rat MTX pre-clinical model was then developed to assess miRNA biomarker candidates under conditions of MTX hepatic perturbation, using both acute and chronic treatment. Acute MTX was administered 20 mg/kg single dose i.p. or saline control (n=5), whilst chronic was administered single p.o. dose daily at 0.125 mg/kg and 0.25 mg/kg or saline for 28 days (n=5).
Results: Following RNA-seq, we observed clusters of miRNAs selective for each cell/tissue and plasma (Fig 1). *In silico* analysis yielded a cell-selective panel of miRs enriched in each cell type (Fig 2). Methotrexate caused minimal changes in alanine aminotransferase/aspartate aminotransferase. Histopathology revealed sinusoidal dilation and hepatic atrophy at 20 mg/kg acute administration, whilst 0.25 mg/kg daily chronic administration caused mild micro-vesicular steatosis. In

liver tissue, chronic treatment caused elevations in VEGFR2, VEGFR3, NFκB, TNFa and ABCB1a -markers of endothelial cell and inflammation pathways- at the gene level and in CD31- marker of the endothelial pathway- and iNOS- marker of immune system- at the protein level. Acute administration of MTX showed increased in oxidative stress response markers and decreased VEGFR2/VEGFR3 gene expression and a slight increase in the protein abundance of CD31 and NQO1 (a marker of oxidative stress). The plasma of acute MTX administration showed increases in hepatocyte-selective miR-122 and LSEC-selective miR-335. Chronic administration led to dose-dependent plasma increases in miR-126 and decreases of miR-335 and miR-362-3p.
Conclusions: These data identified miRs that are organ- and cell-selective/-enriched in the rat. After administration of MTX, changes in circulating miRs have potential to act as sensitive markers of MTX-related liver insult in the absence of significant aminotransferase increases. These data in an extensively characterized model of MTX-DILI now require testing and validation in MTX clinical cohorts.
Acknowledgement of IMI support: The TransBioLine project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 821283. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA".

Fig1, Unsupervised clustering of all sequenced samples, 443 miRNAs shown

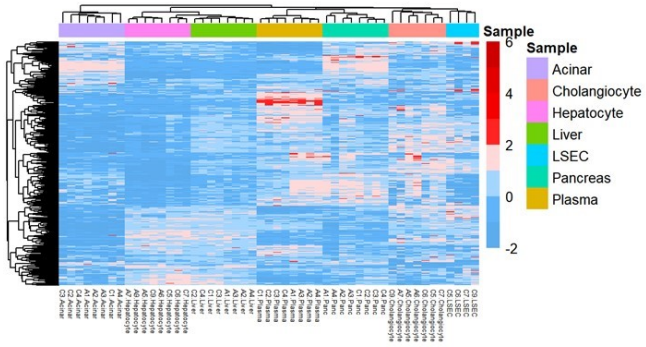
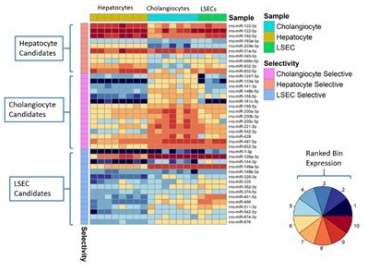


Figure 2 – Panel of cell-selective miR biomarker candidates of hepatocyte, cholangiocyte and LSEC injury



P28 - PLANNING AND INTERPRETATION OF PKS IN VARIOUS SPECIES WITH THE AID OF SIMDOC2, AN INHOUSE-DEVELOPED WEB APPLICATION FOR INTEGRATED PK AND DOSE PREDICTIONS

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Predictions of efficacious dose and PK are more valuable for evaluating the potential success of drug candidate compounds than individual endpoints such as potency or clearance (CL). In order to make these endpoints accessible to project teams, Merck Healthcare R&D has developed a web-based application that automatically extracts PK and PD data from the company data base and performs automated human and animal PK and dose predictions. It is based on standard pharmacokinetic principles to extrapolate basic PK parameters, and mainly employs extensively validated methods (mostly PBPK models).

- Human volume of distribution (VDss) is predicted using Oie-Tozer if data from non-rodents is available. In absence of such data, a QSAR model based on Rat is employed (1).
- Human CL is predicted through extrapolation from *in vitro* systems (IVIVE) when an acceptable *in vivo-in vitro* correlation (IVIVC) was shown in pre-clinical species. If a poor IVIVC was observed, allometric methods are employed (4). All *in vitro* systems (microsomes, hepatocytes) and species (human, mouse, dog, monkey) were calibrated to adjust for systematic underestimations, ensuring accurate assessment of IVIVC/IVIVE. The IVIVC is displayed graphically using an 80% confidence interval based on reference compounds (2,3).
- The prospective accuracy of human VDss and CL is displayed using Monte-Carlo simulations based on the accuracy of the reference set for each system and method used (5)
- Graphical illustrations of target engagement in animals and humans at user-selected dose levels and dosing regimens

SimDoC2 has become an essential tool for all discovery project teams to select compounds and plan PK studies based on its predictions, using internally calibrated *in vitro* data. The SimDoC tool has been in everyday use in prospectively predicting and retrospectively interpreting *in vivo* data for hundreds of compounds in various species over the last 10

years, including multiple clinically developed candidates. In the poster, we will show examples how SimDoC2 has helped identify key optimization parameters by identifying elimination pathways. Identification of compounds with transporter-mediated clearance in mouse will be shown demonstrating the link between fecal levels, permeability and lack of IVIVC for a set of ~400 compounds. Additionally, we will illustrate how SimDoC2 can be used to easily assess the impact of different PKPD scenarios through examples along the value chain.
The poster will demonstrate the benefits of a validated PBPK system which is user-friendly for DMPK reps in project teams, and is directly connected to the Merck database, thereby eliminating potential for errors and inconsistencies in data retrieval and treatment. The individual accuracy of each system has been reported elsewhere (1-3). In this poster we will show a prospective accuracy based on Monte-Carlo simulations with ~10 development compounds for individual end-points (CL and VDss). We will demonstrate that integrated parameters such as dose and t1/2 are consistent with these predictions, and that compounds are inside the predicted confidence interval. Pros and cons of using point estimates for dose and t1/2 as opposed to the current Monte Carlo simulation-based approach (5) will also be discussed.

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P29 - HIGHER UNBOUND INTRINSIC CLEARANCES IN HUMAN LIVER MICROSOMES RELATIVE TO HEPATOCYTES EXPLAINED BY DIVERGENT CYTOCHROME P450 ACTIVITIES FOR 5-AZAQUINAZOLINE IRAK-4 INHIBITORS

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Metabolic stability, expressed as intrinsic clearance, is determined *in vitro* using human liver microsomes (HLM) and cryopreserved human hepatocytes (HH) and is key in identifying suitable compounds for development in drug discovery programs. The scaled unbound intrinsic clearance (CLint,u) corrected for microsomal protein, cellularity per gram of liver and non-specific incubational binding can differ between the two systems. Higher HH CLint,u values are expected when non-microsomal enzymes are significant contributors to metabolism of a compound. However, some compounds exhibit higher CLint,u values in HLM versus HH that may be difficult to explain and pose a challenge as to which system is more predictive of *in vivo* clearance. In this study, a series of interleukin-1 receptor-associated kinase 4 (IRAK-4) inhibitors with a 5-azaquinazoline core, including a subset showing a significant disconnect (HLM: HH CLint,u ratio 2-26), were used to build a better understanding of the disconnect. Metabolic rates and metabolites were investigated in various liver matrices to explore the hypothesis that the disconnect in CLint,u was due to significantly lower cytochrome P450 (CYP) activity in HH relative to HLM. The compounds had metabolic liabilities to microsomal CYP/flavin monooxygenase (FMO) and/or liver cytosol (HLC) aldehyde oxidase (AO) resulting in two groups with either CYP/FMO or AO-driven metabolism based on CLint,u values in HLM and HLC. There was a lack of concordance between CLint,u determined in HLM and HH contrasted with an excellent correlation of AO dependent CLint,u determined in HLC and HH (y=0.71x+0.34, r2 = 0.95, P < 0.0001). AO and FMO-mediated metabolites formed in subcellular fractions were detected in intact HH while those formed by CYPs were absent or present at low levels in intact HH. Data from lysed HH, as with other subcellular fractions fortified with NADPH, exhibited significantly higher CYP activities relative to intact HH (CLint,u of 71 vs 5 mL/min/kg in lysed and intact HH, respectively, for a compound with the largest disconnect) which supports the conclusion that intrinsic CYP activities are lower in intact HH relative to HLM and further studies are required to understand the underlying cause for the divergent activities.

P30 - USE OF LONG-TERM CO-CULTURED HUMAN HEPATOCYTES FOR ESTIMATION OF FM,CYP3A BY CHEMICAL INHIBITION ASSAY

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Introduction/Background: Determination of the enzymes principally responsible for the metabolism of drug candidates is crucial in predicting potential unwanted drug interactions early in the development process. Estimation of the relative contribution of individual hepatic CYP450 enzymes to overall metabolism (fm,CYP) significantly guides downstream victim drug DDI risk assessment but is especially challenging for compounds that exhibit low metabolic turnover. Establishing the role different isoforms play in the metabolism of such substrates is difficult using currently available incubation systems such as hepatocyte suspensions or liver microsomes whose inherent instability limits the duration *in vitro* assays can be reliably performed. This drawback may be circumvented using a long-term hepatocyte co-culture system that allows *in vitro* incubations to be conducted for longer than previously possible without loss of enzyme activity.
Methodology: Optimization and validation of 96 h incubation conditions for selective chemical inhibitors (ketoconazole, itraconazole, ritonavir and Posaconazole) of CYP3A4 was performed using midazolam as the probe substrate in HepatoPac® kits. Intrinsic clearance values of the inhibitors were first determined to ensure their ability to sustain inhibition of the target isoform in 96 h experiments. The optimized setup was subsequently used to conduct fm, CYP3A4 estimation assays on 13 clinically used drugs at concentrations below Km. Concentrations of the test parent drug remaining in the incubations were determined using LC-MS followed by calculation of the *in vitro* metabolic intrinsic clearance (CLint). The ratios of the difference of CLint in incubations with/without inhibitor representing fm, CYP3A4 were compared against reference values.
Results and Discussion: Itraconazole was found to be the most suitable chemical inhibitor of CYP3A4-mediated midazolam metabolism in 96 h HepatoPac® incubations by exhibiting low clearance, thereby retaining consistent unbound concentration *in vitro* compared to ketoconazole, ritonavir or Posaconazole. Additionally, negligible cross-reactivity for other CYP isoform metabolism was demonstrated. Using the optimized chemical inhibition assay format, the *in vitro* fm, CYP3A4 for 10 of the 13 different drugs with varying clearance values exhibited good correlation with clinical reference data, lying within the 0.5-2.0-fold range. This estimation strongly predicted drug-drug interaction risk for such drugs in patients co-administered with strong CYP3A4 inhibitors.
Conclusion and Future Perspectives: Long-term hepatocyte co-culture incubation systems are a powerful tool for the *in vitro* determination and estimation of fm, CYP3A4 for metabolically intransigent compounds. Using optimized chemical inhibitors of other CYP450 enzymes, this platform may be extended to improve the estimation of contributions of different isoforms to metabolism in early drug discovery.

P31 - METABOLISM CONSIDERATIONS IN NEXT GENERATION RISK ASSESSMENT: CHALLENGES OF FIT-FOR-PURPOSE EXPERIMENTAL DESIGN USING COUMARIN AS A CASE STUDY

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Next Generation Risk Assessment (NGRA) integrates estimations of internal exposure (e.g. plasma Cmax) with measures of bioactivity detected across a range of *in vitro* bioassays, to inform the safety of a new chemical for a defined consumer use scenario. Within this framework, there is a need to consider metabolism-driven toxicity. Thus, when *in silico* tools predict the potential formation of reactive metabolites, based on the chemical structure of the parent compound alone, a tiered approach is taken to confirm metabolite formation and define the best model for bioactivity detection. To demonstrate the application of this tiered approach for assessing the safety of parent and metabolites in the context of a cosmetic risk assessment, we used coumarin as a case study chemical present at 0.1% in a hypothetical face cream. One of the first steps in the risk assessment was to use *in silico* tools, which predicted rapid metabolism of coumarin and formation of reactive metabolites (e.g. epoxides) with alerts for DNA and protein binding, which are molecular initiating events for genotoxicity and skin sensitization respectively. Reactive metabolites can also be responsible for other systemic effects such as drug-induced liver injury. Next steps involved *in vitro* liver and skin metabolism studies to further elucidate the metabolic pathway of coumarin, particularly to understand if the reactive metabolites were formed at concentrations relevant to consumer exposure. A mixture of Human liver S9 and primary hepatocyte incubations, with or without inhibition of CYP2A6, showed that reactive metabolites (coumarin-3,4-epoxide and o-hydroxyphenylacetaldehyde) were only formed if the pathway for metabolism of coumarin to 7-hydroxycoumarin was saturated (1mM Coumarin). This corroborated the results obtained in other *in vitro* assays, where no significant difference in points of departure obtained from a range of assays (cellular stress pathways and transcriptomics) between 2D HepaRG (24h) or 3D HepaRG (7 days) was observed, when the dosing regimen was below 1mM. From the skin exposure perspective, there was little evidence of metabolism of coumarin in *ex vivo* cultured skin (20µL of 500mM Coumarin applied topically, 24h) through minimal formation of 7-hydroxycoumarin (<0.01% of the dose applied), providing supporting evidence that safety data obtained from parent alone would be sufficient for the risk assessment. Finally, the safety decision that 0.1% coumarin in a

hypothetical face cream would be safe to use, obtained using new approach methodologies, was comparable to the traditional decision which would have been made using toxicity data from published animal studies.

P32 - PLASMA PROTEIN BINDING OF NINE ANTISENSE OLIGONUCLEOTIDES BY ULTRAFILTRATION

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Plasma protein binding (PPB) data are known to be important for understanding the pharmacokinetic (PK) and pharmacodynamic (PD) behaviors of antisense oligonucleotides (ASOs). However, ASOs present unique challenges in fraction unbound determination through traditional techniques considering their properties such as relatively high molecular weight, linear structure, and nonspecific binding. We described an accurate and reliable ultrafiltration (UF) method combined with LC-MS/MS analysis for determining the PPB of ASOs in relevant preclinical species using 9 commercially available ASO compounds, including phosphonothioates and phosphonodiamidite morpholino oligomers. The Amicon Ultra 50 kDa molecular-weight cutoff centrifugal filters were used in our method and 0.1% Tween-20 in buffer was used for the filter membrane pretreatment, without the need of pretreatment using structure-different oligonucleotides to block nonspecific binding sites. Centrifugation conditions are optimized to ensure sufficient separation of free ASOs as well as low protein leakage in the ultrafiltrate. Post-centrifugation samples are matrix-matched in the filter tubes before transferring to minimize nonspecific binding. After that, samples were extracted by liquid-liquid extraction or protein precipitation. Then the extracts were analyzed by LC-MS/MS. Results showed high equilibrium rate and low nonspecific binding in the tubes when ASOs were spiked in buffer. Protein leakage rate, which was evaluated by comparing protein concentration in the ultrafiltrate and plasma, ranged from 0.27 to 0.88% in mouse, rat, monkey and human plasma. The fraction unbound in plasma are consistent with the previously reported data. The ultracentrifugation (UC) performance of separating free ASO from plasma was also evaluated in this study. The fraction unbound in buffer as well as plasma are much lower in UC method compared to UF, suggesting potential sedimentation of ASO molecules during ultracentrifugation. In conclusion, the established ultrafiltration coupled with LC-MS/MS method for ASO PPB determination is simple, accurate and reliable, suitable for widely use in drug discovery programs.

P33 - VALIDATION OF A FULLY AUTOMATED 384-WELL ASSAY FOR SCREENING OF HUMAN HEPATOCYTE CLEARANCE IN DRUG DISCOVERY

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Human hepatic clearance is an important determinant of every relevant pharmacokinetic parameter in drug design, including half-life, oral bioavailability, and the estimation of the human efficacious dose. Intrinsic clearance assays using cryopreserved human hepatocytes to predict clearance are well established and are arguably the most optimal *in vitro* system because they contain the full range of phase 1 and phase 2 metabolic enzymes, transporters and cofactors required for drug metabolism. However, optimization of hepatic clearance still remains a challenging objective in small molecule drug discovery projects.
During the last decades, assay miniaturization, automation, and robotics have led to significantly increased throughput and optimization of timely data delivery to identify drug candidates with suitable DMPK properties. However, miniaturization and automation of hepatocyte clearance assays is generally challenging since cryopreserved hepatocytes are fragile after thawing and cellular integrity can be significantly impaired by vigorous and repeated liquid handling steps, leading to reduced cell viability and loss of functional activities. To date, mainly semi-automated hepatocyte clearance assays have been described excluding automated cell dispensing [1, 2].
In order to optimize our current hepatocyte clearance assay, a fully automated, high-throughput 384-well-based assay was established on the basis of a Tecan Fluent automated workstation with specific custom modifications. The system is operated with custom-written scripts to enable a fully automated workflow including cell dispensing, incubation, sampling, and sample preparation. The Tecan platform is combined with a Certus Flex liquid dispenser that is equipped with a customized Bioshaker unit to facilitate automated dispensing of thawed cells, incubation medium, and serum protein into 384-well plates, and an automated tailor-made valve-cleaning station that requires no manual interaction.
Overall, the automated cell dispensing step did not compromise cell viability and the validation of the entire system using 85 Boehringer-Ingelheim compounds demonstrated an inter-assay variability of <20% for 82.0% of all tested compounds (N=3), and a mean intra-assay variability of 12.9% (n=3) in the absence of plate or edge effects. In comparison to the historically used lower-throughput system, a correlation with R^2 of 0.8 was achieved for 160 compounds and up to 93.1% of compounds were ranked into similar binning categories. The ability to predict human *in vivo* clearance was investigated for 80 marketed drug and demonstrated *in vitro* clearances that were within two-fold of the *in vivo* clearance for the majority of the drugs depending on the serum protein content in the incubation. Overall, the completely automated hepatocyte clearance assay in 384-well format allows a significant increase in throughput, 80% reduction in hands-on time, and 75% savings with regard to human hepatocytes.

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P34 - OPTIMIZATION OF METHOD FOR MEASURING AGP-MEDIATED PROTEIN BINDING IN DOG PLASMA

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Inter-individual differences in fraction unbound mediated by Alpha-1-Acid-Glycoprotein (AGP) levels are characteristic for dog plasma protein binding (PPB), particularly for neutral and basic compounds. There is a relationship between dog plasma AGP levels and PPB (1 - Pike A, Jones B, Markandu R, O'Neill D. Impact of Interindividual Differences in Plasma Fraction Unbound on the Pharmacokinetics of a Novel Syk Kinase Inhibitor in Beagle Dogs. Drug Metab Dispos. 2021 Sep;49(9):736-742.) and here we have investigated this relationship across several batches of plasma from male beagle dogs. Non-labelled-propranolol and 3H-propranolol were selected as an AGP substrate and AGP levels in several batches of dog plasma were determined using Dog AGP ELISA Kit ab205082 from Abcam. Dialysis assembly HTD 96B and 12-14kDa MWCO membranes (HTDialysis) were used to determine PPB by equilibrium dialysis against phosphate buffered saline at pH 7.4 determined at 5% CO2 and 37°C for 18 hours. AGP levels in dog plasma varied 2.5-fold from 77 to 194 mg/L and correlated (Pearson r = -0.8614, p=0.027) the unbound fraction of propranolol in plasma (ranging 10.7 to 21.8 %) showing that differences in AGP levels between dogs may give rise to variability in pharmacokinetics in dogs for some compounds, due to differences in free fraction.
Most PPB assays are based on measuring test compound concentration in dialysate versus concentration in spiked-dialysed plasma. However, Banker et al. (2 - Michael J Banker, Tracey H Clark, Plasma/serum protein binding determinations, Curr Drug Metab. 2008 Nov;9(9):854-9) showed that comparable results can be obtained when measuring compound concentration in plasma dialysate and dialysate from compound in buffer. In a higher throughput setting, this simplified methodology may prove advantageous as no matrix matching and no deproteinization are required prior to bioanalysis. Some potential disadvantages are differences in non-specific binding between control equilibrium buffer alone and plasma and insolubility of some compounds. We hypothesized that this technique could be a viable alternative to determine protein binding of AGP substrates, across a range of dog plasma samples. No significant difference was observed between total measured propranolol levels (recovery) in dialyzed dog plasma and buffer when compared to dialyzed buffer only. Moreover, no significant difference was observed between PPB determined using unlabelled propranolol compared to that determined using 3H-propranolol (75-84% binding) determined by scintillation counting. Altogether, these findings indicate that PPB determination by measuring compound concentration in plasma dialysate compared to dialyzed buffer alone is viable and potentially more cost-efficient alternative when compared to the use of standard method for compounds with moderate overall PPB. Further work is warranted, and additional control compounds need to be tested to establish useful binding range when use of equilibrium buffer control would be beneficial.

P35 - NEXT LEVEL DRUG RESEARCH IN AN EX VIVO TISSUE GUT-ON-A-CHIP MODEL: ADVANCED APPLICATIONS OF THE INTESTINAL EXPLANT BARRIER CHIP

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Gut-on-a-chip systems are increasingly gaining more attention since they offer more translational value compared to conventional *in vitro* models. The Intestinal Explant Barrier Chip (IEBC) integrates small *ex vivo* intestinal tissue explants between two microchannels. By maintaining the *in vivo* distribution and properties of the different intestinal cell types in their correct structure and architecture, intestinal processes can be studied in an advanced physiological model. Previously, we have described the applicability of the IEBC to study intestinal permeability of small molecule drugs [1]. Here, we report on the added value of using the IEBC to study advanced research questions in the field of drug absorption, metabolism, and host-microbe (immune) responses. Human jejunum, ileum or colon tissue explants were fixed in the IEBC and apically exposed to drugs dissolved in standard cell culture medium or in fecal water containing microbial excretion products (iscreen technology) for 24 hours. Additionally, the combination of human tissue explants with iscreen spent medium was used to map host-microbe interactions. The addition of an aerobic-anaerobic interface to the IEBC took host-microbe interaction studies one step further as the apical anaerobic environment, similar to the gut lumen, allowed inclusion of living gut microbiome. Phase 1 and phase 2 metabolism over a period of 24h was demonstrated in human jejunum tissue using coumarin and 7-OH coumarin. Coumarin was predominantly hydroxylated to 7-OH coumarin (via CYP2A6) at the apical side of the intestinal tissue and demonstrated an apparent permeability (Papp) rate of 2 x 10-6 cm/s. Next, 7-OH coumarin was metabolized further into 7-OH coumarin glucuronide and 7-OH coumarin sulfate via phase 2 conjugating enzymes and appeared basolateral at high rates with Papp values of 41.1 and 24.2 x 10-6

cm/s, respectively. Human colon tissue (n=3 donors) was exposed to short chain fatty acids (SCFA) or iscreen spent medium collected under favorable conditions for microbial SCFA production. Average results showed improved tissue barrier integrity measured by a 1.7-3.5-fold reduction in FITC-dextran 4000 (FD4) leakage and 1.4-2.1 lower transcellular and paracellular transport of antipyrine and atenolol, respectively. Furthermore, the release of pro-inflammatory cytokine TNF-α was significantly reduced under these circumstances. Tissue explants cultured in the aerobic-anaerobic interface showed proper tissue functionality (transcellular/paracellular transport >2), intact tissue integrity (FD4 leakage <0.5%/h) and significant differences in microbial beta diversity compared to aerobic tissue conditions, with a higher abundance of e.g., Bifidobacterium and lactobacillus species. Tissue functionality and integrity remained intact upon co-culture with Bifidobacterium animalis, for which growth and tissue-attachment were shown by targeted qPCR analysis. In conclusion, we successfully demonstrate the use of the IEBC for advanced drug absorption and metabolism questions as well as presenting a novel way to understand host-microbe effects on tissue functionality and local inflammation in the human intestine.

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P36 - ACCURATE DETERMINATION OF PLASMA PROTEIN BINDING OF HIGHLY BOUND COMPOUNDS WITH THE USE OF 18-HOURS RAPID EQUILIBRIUM DIALYSIS METHOD

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Plasma protein binding (PPB) is one of the most fundamental pharmacokinetic parameter used to establish PK/PD relationships, predict drug-drug interactions, and evaluate drug candidates toxicity. Rapid equilibrium dialysis is a gold standard method for determination of plasma protein binding. In the manufacturer's recommendations, time of 4-6 hours of dialysis is sufficient to reach equilibrium and measure the free fraction. However, this time seems to be insufficient for highly bound compounds. The main goal of this study was to develop methods capable of accurately determining the binding of compounds with high protein binding (greater than 95%) to human plasma proteins. 24 reference compounds reported to have high binding (from fu = 0.011 for doxazosin to fu = 0.000003 for venetoclax) were tested. The plasma protein binding in human plasma was assessed using RED method with determination of compounds in both compartments using LC/MS/MS technique. The time needed for reaching the equilibrium was studied by evaluation of dialysis time in the chosen conditions (drug added to plasma, study equilibration with only buffer). Reference compounds with highest plasma protein binding characteristics were studied at concentration range (3-300 uM) to measure unbound fraction and check the possibility of plasma proteins saturation. Additionally, for all the reference compounds, kinetic solubility assay was performed for 18 hours, in order to confirm whether whole amount of compound is in dissolved state in PPB incubations. Results show that dialysis time of 18 hours was adequate to achieve equilibration for compounds with high protein binding and allow to determine fraction unbound with good accuracy. Obtained results for reference compounds (from fu = 0.031 for doxazosin to fu = 0.000003 for venetoclax) are in good correlation with previously published data. In conclusion, the developed method is useful in the precise determination of plasma protein binding of highly bound compounds.

P37 - INTESTINAL TISSUE ORGANOIDS TO STUDY DRUG TRANSPORT AND METABOLISM FROM INFANTS TO ADULTS

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For the development of drugs, a decent understanding of intestinal absorption, including possible regional differences and age-related effects, is paramount to reach appropriate bioavailability and related drug efficacy. Most drugs are prescribed orally, however, translational preclinical models to investigate oral drug exposure in various age groups are lacking. This study aimed to explore the use of tissue-derived intestinal organoids from adults and children to assess regional drug metabolism and transport differences, as well as the possible impact of age. Organoid lines from fresh adult (jejunum n=3, ileum n=3) and pediatric tissues (ileum n=5, age range: 5-52 weeks) were established, cultured and expanded as 3D self-organizing organoids in Matrigel. For studying oral drug absorption, the intestinal organoids were made single-cell and grown as an epithelial monolayer on a permeable membrane. After differentiation, carrier-mediated drug transport by MDR1 and BCRP was determined in bidirectional transport assays and presented as apparent permeability (Papp), the substrates talinolol and rosuvastatin were used, respectively. Transformation of midazolam to 1OH-midazolam was used to assess CYP3A4 metabolic conversion rate. Gene expression of ABCB1/MDR, ABCG2/BCRP and CYP3A4 in fresh intestinal tissue was determined and compared to expression in 3D grown intestinal organoids.Drug transport studies showed uptake, active efflux transport of talinolol and rosuvastatin and metabolism of midazolam in the intestinal organoid

model. The adult epithelial barriers showed region-specific trends in efflux transport, with higher efflux transport (basolateral to apical direction) of talinolol and rosuvastatin by MDR1 and BCRP in ileum (Papp MDR1, b-a: 7.5 ± 0.7; Papp BCRP, b-a: 7.0 ± 0.5 x10-6 cm/s) compared to the jejunum region (Papp MDR1, b-a: 5.3±1.4; Papp BCRP, b-a: 3.0±0.5 x10-6 cm/s). The intestinal organoid monolayers of children and adults both displayed CYP3A4 activity as measured by formation of 1-OH-midazolam, which was undetectable in Caco-2 monolayers. The rate of midazolam metabolism by CYP3A4 showed higher values in adult jejunum (7.8 ± 3.0 pmol/mg protein/min) compared to adult ileum organoids (3.7 ± 2.6 pmol/mg protein/min). No age-related differences in efflux transport and metabolism were observed (pediatric ileum: Papp MDR1, b-a: 6.2±1.4; Papp BCRP, b-a: 6.8 ± 1.4 x10-6 cm/s; metabolic rate: 9.4 ± 7.7 pmol/mg protein/min). Gene expression in fresh tissue compared to intestinal organoids derived from this tissue showed similar patterns for ABCB1/MDR1, ABCG2/BCRP) and CYP3A4. Our results show the potential of the application of tissue-derived intestinal organoids to study regional differences in drug transport in adults and children. In adult intestinal organoid monolayers, drug metabolism and transporter functionality appeared to be region-specific and the relative gene expression of ABCB1/MDR1, ABCG2/BCRP and CYP3A4 was found to be similar in organoids compared to fresh tissue. Future research will focus on including more samples from different donors covering a wider age-range, which is needed to confirm and further study age-related effects.

P38 - DEVELOPMENT OF AN *IN VITRO* TEST SYSTEM FOR CYP AND UGT INHIBITION STUDIES USING CRYOPRESERVED HUMAN HEPATOCYTES

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The inhibition of cytochrome P450 (CYP) and/or UDP-glucuronosyltransferase (UGT) enzymes are central mechanisms that might result in clinically significant drug-drug interactions (DDIs) thus early and accurate prediction of inhibition potential of drug candidates is crucial for drug development. Various *in vitro* systems are used for enzyme inhibition studies among which microsomes are the most widely used due to being cost-effective, simple to use and providing reliable data for DDI prediction for small molecule drugs1. More and more biological molecules (peptides, proteins, nucleotides etc.) have been developed recently in a wide range of therapeutic areas and soon become best-selling drugs. Available data of biologics have shown no significant pharmacokinetic DDIs with co-administered medicines. Testing some of these molecules, like phosphorothioate oligonucleotides for CYP and UGT inhibition using microsomes resulted in false positive findings which were proven to be a test system-dependent effect not occurring when incubations were repeated using hepatocytes2. These data suggest that hepatocytes provide a more clinically relevant inhibitory profile for use in *in vitro* to *in vivo* extrapolation of DDIs for biological drugs. The aim of this work was to develop and validate CYP and UGT enzyme inhibition assays on cryopreserved human hepatocytes. Experiments were performed in 96 well plates in triplicate using pooled (n = 50) cryopreserved hepatocytes. Incubations were conducted at 37°C with 95% humidity and 5% CO2/95% air. The enzyme-specific metabolite formation of the substrates was quantified by LC/MS/MS. First, time courses were established for the selected probe substrates for most relevant enzymes CYP1A2, (phenacetin), CYP2B6 (bupropion), CYP2C8 (amodiaquine), CYP2C9 (diclofenac), CYP2C19 (mephenytoin), CYP2D6 (dextromethorphan), (CYP3A4) midazolam and UGTs, (4-methyl-umbelliferon (4-MU)) to find the optimal incubation time, followed by determination of Km and Vmax for each probe substrate. Finally, the system was tested with reversible and time-dependent selective inhibitors (alpha-naphthoflavone and furafylline for CYP1A2, voriconazole and deprenyl for CYP2B6, quercetin and gemfibrozil glucuronide for CYP2C8, sulphaphenazole and tienilic acid for CYP2C9, nootkatone and S-fluoxetine for CYP2C19, quinidine and paroxetine for CYP2D6, and ketoconazole and troleandomycin for CYP3A4) using substrate concentrations near or below Km. Concentration-dependent inhibitory effect was observed in case of all inhibitors which was comparable to available literature data. In conclusion, we developed and validated CYP enzyme inhibition assays in cryopreserved human hepatocytes which can be used to evaluate the reverse and time dependent CYP inhibition potential of drug candidates.

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P39 - HIGH-THROUGHPUT GLP-COMPLIANT *IN VITRO* ASSAY TO DETERMINE RELATIVE TOXICITY OF LIPOSOMAL FORMULATIONS OF AMPHOTERICIN B

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Introduction: Amphotericin B remains the standard of care for life-threatening fungal infections. However, its use is limited due to its severe acute and chronic toxicity. Amphotericin B forms pores in sterol-containing membranes, resulting in leakage of monovalent ions such as potassium and other cell constituents. This is the primary mechanism of action for its anti-fungal activity as well as toxicity.
Amphotericin B liposomal formulations have been developed to improve this safety profile. These formulations achieve slow release of Amphotericin B and reduce the toxicity while retaining potency. During the COVID-19 pandemic, Amphotericin B liposomal formulation, was the single line of treatment for patients suffering from Mucormycosis. Due to high demand, generic companies are investing in making similar products.
One of the challenges is to develop a high-throughput *in vitro* assay to determine the relative potential toxicity by measuring the potassium released from red blood cells with high sensitivity. The current reported assay uses the clinical electrolyte analyzer for potassium measurement.1 Here, we have developed a high throughput assay performed in 96-well plates using rat and human red blood cells (RBCs) and replaced the traditional methods of potassium analysis with more sensitive Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In addition, the *in vitro* potassium release assay and bioanalytical method in rat and human RBCs were validated as per FDA guidelines. This GLP assay can be employed to prove bioequivalence of generic products to the innovator product.
Methods: Whole rat blood was centrifuged at 1100 x g for 5 minutes at 10 °C. The plasma and buffy coat layer were removed. The RBCs were washed with normal saline until a clear suspension was obtained. The cells were separated, counted, and diluted to the required number. To 540 µL of red blood cell suspension, different concentrations of test formulation were added. After incubation for 4 h, the plates were centrifuged, and supernatant collected. The supernatant was subjected to ICP-MS analysis to determine potassium concentration. The concentration of formulation that causes 50% release of potassium was considered K50 and was used as a measure of relative toxicity.
Results: The potassium released from RBCs showed a gradual increase with increase in concentrations of Amphotericin B formulations at 4 h. The %CV of the replicates was <20%. The bioanalytical method for potassium determination using ICP-MS was validated as per USFDA guidance document and the method met the acceptance criteria. The K50 value for the reference Amphotericin B formulation was 9.7 ± 3.3 µg/mL using rat RBCs at 4 h. This *in vitro* assay may substitute the need for *in vivo* studies to prove similarity of test formulations to the innovator product.

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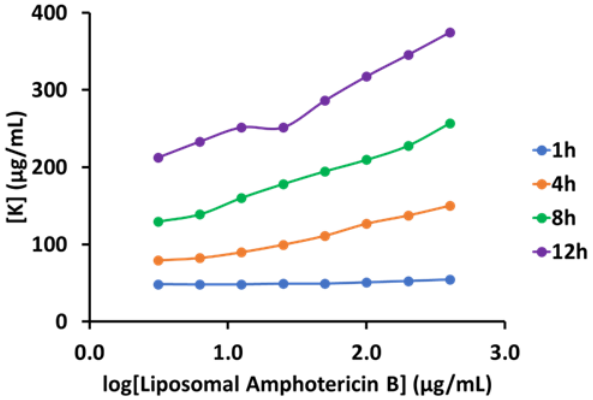

P40 - MECHANISM OF ACTIVATION OF CLOPIDOGREL INTO ITS ACTIVE THIOL METABOLITE: P450-CATALYZED OPENING OF THE 2-OXO-CLOPIDOGREL THIOLACTONE : S-OXIDATION OR BAEYER-VILLIGER OXIDATION?

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Clopidogrel and ticlopidine are activated to the thiol active metabolite in two oxidative steps and a reduction: First, P450 catalysed oxidation into thiolactones : 2-oxo-clopidogrel and 2-oxo-ticlopidine. Second, P450-catalyzed opening into a sulfenic acid (1). We have proposed that the thiolactone is oxidized to a thiolactone S-oxide (Pathway A) that hydrolyzes to a sulfenic acid, reacting with glutathione to give a mixed disulfide, further reduced (enzymatically or chemically) to the active thiol. Alternatively in presence of dimedone the sulfenic acid is trapped as a stable dimedone adduct (1, 2) or in

presence of thiols into mixed disulfides that are reduced by excess thiol into the active thiol and its isomers (1). In presence of ascorbic acid, TCEP or DTT, the sulfenic acid is reduced into the active thiol (2).
A recent paper by Zhu (3) challenges this proposal and propose an alternative Baeyer-Villiger oxidation of the thiolactone (Pathway B) followed by hydrolysis to afford the same sulfenic acid intermediate.
The aim of our study is to use as end product the sulfenic acid dimedone adduct to analyze incorporation of 18O from 18O water or 18O dioxygen, and compare it to the incorporation of 18O in other co-metabolites in incubations of clopidogrel (and 2-oxo-clopidogrel and 2-oxo-ticlopidine) with rat liver microsomes, to try to decide between these two hypothical pathways. The 18O incorporation should be different for each pathway as shown on the joined scheme. Thus starting from clopidogrel the carbonyle of the adduct comes from dioxygen. The hydroxyle of the carboxyle group comes from water in case of Pathway A, from dioxygen in case of Pathway B.
We will show that the results of these experiments point much more to the thiolactone-S-oxide pathway A and that the Baeyer–Villiger pathway B, if present, represents at most 10% of the reaction. The published results obtained for prasugrel and 2-oxo-prasugrel agree with this statement (4).

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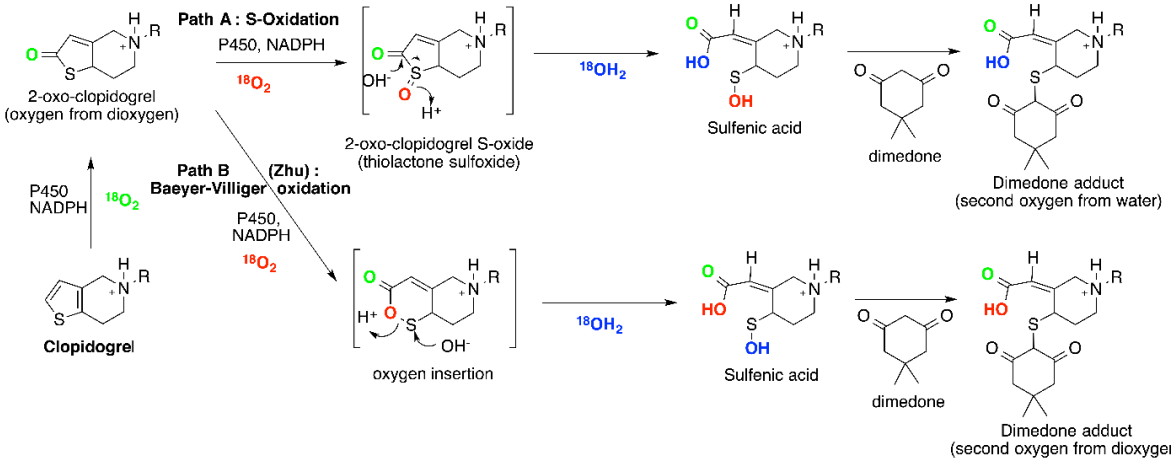

P41 - DISULFIDE RE-ARRANGEMENT LEADING TO CHAIN SPLITTING: A DEGRADATION MECHANISM IMPACTING THE SPECIES-SPECIFIC POTENCY OF INSULIN DETEMIR?

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Long-acting insulin analogues have been developed based on a strategy of lowering the insulin receptor affinity together with extending the elimination half-life by e.g., adding an albumin-binding moiety. To achieve a longer half-life, it is key to avoid or reduce non-insulin receptor mediated clearance, such as renal clearance and proteolytic degradation. Insulin detemir (desB30, B29N(eps)-myristoyl-Lys insulin) has reduced *in vivo* potency in rats and humans (approximate 10% and 25% potencies relative to human insulin, respectively, whereas it is 100% in dogs and pigs), but the underlying mechanism for the reduced potency and species difference has so far not been identified, although non-receptor mediated degradation has been proposed. Here, we report a novel insulin clearance mechanism, in which splitting of the insulin molecule into A- and B-chains occurs by a thiol-disulfide exchange reaction. With a redox assay, we demonstrate that the redox stability of the disulfide bonds in insulin detemir are less stable than in human insulin and that the rate of insulin detemir chain splitting in plasma from different species correlates with the observed *in vivo* potency. We demonstrate that a few selected amino acid substitutions in the insulin backbone can have a significant stabilizing effect towards this chain splitting mechanism. Chain splitting of both insulin detemir and human insulin was also demonstrated *in vivo* in rats and therefore we hypothesize that chain splitting is a mechanism relevant for the *in vivo* elimination and *in vivo* potency of insulin molecules in general, a mechanism which can explain the species dependent reduced *in vivo* potency of insulin detemir.

P42 - DAR DISTRIBUTION DETERMINATION FOR ANTIBODY-DRUG-CONJUGATES (ADCS) BY LC-HRMS IN *IN VITRO* AND *IN VIVO*

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The drug-to-antibody ratio (DAR) is an important characteristic of antibody-drug conjugates (ADCs). It is essential to determine and identify the change the DAR distribution and DAR value in drug metabolism and pharmacokinetics (DMPK) investigation. In this study, commercial HER2-targeting ADC, Trastuzumab deruxtecan (T-Dxd, DS-8201a) was incubated in *in vitro* plasma for stability study and dosed to SD rat for *in vivo* pharmacokinetics study to evaluate the change of DAR value and DAR distribution, and the relation between *in vitro* stability and *in vivo* pharmacokinetics study. Immunoaffinity capture method with streptavidin magnetic beads and biotinylated-anti-human IgG was used to extract the ADCs in biological matrix such as plasma. Reverse Phase Liquid Chromatography (RPLC) coupled with tandem High Resolution Mass Spectrum (HRMS) are applicate for the analysis. The deconvoluted mass of the subunits (heavy chain and light chain) of T-Dxd are used for qualitative determination each DAR species and the MS intensity of each DAR species is used for semi-quantitation. During the *in vitro* incubation in rat plasma for 7 days, in addition to the initial DAR distribution of the L1 (DAR= 0 in light chain) and H3 (DAR = 3 in heavy chain), the L0 (DAR= 0 in light chain), H2, H1 and H0 (DAR = 2, 1, 0 in heavy chain) species were generated progressively while the hydrolysis of maleimide ring was detected on the remaining high-DAR species. The DAR value decrease of ADC was correspondingly detected in *in vivo* PK study along with the catabolic reduction of the total antibody. The method was successfully developed for the evaluation of ADC plasma stability, which enabled a clear and complete exhibition of the distribution of different DAR species, as well as the changes during the plasma incubation and *in vivo* biotransformation, referring the therapeutic efficacy and safety concerns.

P43 - FROM QUANTUM MECHANICS TO METABOLIC PATHWAYS

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Unexpected metabolism can lead to the failure of many late-stage drug candidates or even the withdrawal of approved drugs. Therefore, during early research, it is important to predict the sites of metabolism and metabolites of potential drug-like molecules.
Historically, predictive models have targeted metabolism by human Cytochrome P450 (CYP) isoforms due to their well-documented importance in phase I metabolism.[1] Here, we will present methods to predict isoform-specific metabolism for a broad range of enzymes involved in phase I and phase II metabolism, including aldehyde oxidases (AOs)[2], flavin-containing monooxygenases (FMOs)[2,3], sulfotransferases (SULTs)[4] and UDP-glucuronosyltransferases (UGTs)[2,3] alongside CYPs. These models are based on a consistent framework, combining mechanistic quantum-mechanical simulations with machine learning, and are rigorously validated with experimental data. The resulting models predict if a potential site of metabolism on a compound is likely to be metabolised by the specified enzyme.
We will demonstrate how these site-of-metabolism models can be combined with models that predict which enzymes and isoforms[5] are likely to metabolise a compound. Applying models iteratively to a parent compound and its metabolites enables the prediction of metabolic pathways and the resulting metabolites observed *in vivo*. We validate these pathway predictions by comparison with experimentally observed metabolite profiles.

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P44 - IMPACT OF A FLUORINE ON THE CNS PENETRATION AND METABOLIC FATE OF AZD9574

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AZD9574 and the related PET ligand AZ3391 are selective inhibitors and trappers of PARP1 designed to be CNS penetrant. The introduction of a fluorine to a quinoxalinone ring system was key in reducing P-gp mediated efflux and improving rat brain Kpuu in the series. Direct CNS target engagement was later confirmed in cynomolgus monkey using the PET ligand AZ3391 and was consistent with a Kpuu of ~1. However, the fluorine substitution also led to an unexpected observation that AZD9574 and AZ3391 were, in contrast to previous leads which had shown oxidative metabolism only, principally metabolized in hepatocytes to a primary glucuronide. This poster will describe the impact of

the fluorine substitution on the physicochemical, CNS distribution and metabolism properties of these molecules vs their des-fluoro analogues and the structural elucidation of the metabolite.

P45 - 14C-RADIOLABELLED HUMAN AME: A REVIEW OF CLINICAL MASS BALANCE RATES AND ROUTES OF ELIMINATION

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Investigation of absorption, metabolism, and excretion (AME) is an essential step in the drug development process. The use of radiolabelled material (usually 14C) is integral to understanding the AME characteristics of a drug. Clinical mass balance studies and the evaluation of the safety of human metabolites was originally outlined over a decade ago with the FDA Metabolites in Safety Testing ('MIST') guidance document[1], and more recently (2022) in the FDA draft guidance Considerations for Human Radiolabelled Mass Balance Studies[2]. Human mass balance studies now represent a notable regulatory expectation and milestone within drug development. Since January 2014 Pharmaron UK have conducted the mass balance analysis on over 80 studies performed at Quotient Sciences, covering a range of drugs across a wide spectrum of therapeutic areas. For most studies, the oral dose route was selected, however intravenous infusion and subcutaneous injection were also selected on occasion. Other than the dose route, the overall study design for each radiolabeled study was remarkably similar with a small number of subjects (typically 6-8 healthy male volunteers [sometimes females depending on the indication]) administered with a single dose of radiolabeled drug, followed by collection of all urine and faeces. The latest draft guidance from the FDA[2] indicates that at least six 'evaluable subjects' should be included. The mass balance recovery was determined at Pharmaron UK by measurement of the radioactivity in the urine and faeces samples using liquid scintillation counting (LSC). A review of the overall mass balance recovery, along with the rates and routes of elimination, show that, despite the similar study design, the results vary depending on the AME characteristics of each individual drug. In over 88% of the clinical studies performed, a mass balance of greater than 80% was reached, and in 83% of the studies, the subject release criteria were achieved. The individual mean cumulative total recovery of 14C radioactivity was 89% (SD 10%); the latest draft guidance from the FDA[2] suggests that ideally the total recovery of radioactivity should be at least 90%.

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P46 - SOFTWARE-BASED METABOLITE SCREENING: SEPARATING THE WHEAT FROM THE CHAFF

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Metabolite identification plays a critical role in human pharmaceutical, veterinary pharmaceutical, and agrochemical development. Metabolites can contribute to efficacy and toxicity, and it is essential to understand the implications on human safety. Regulatory authorities require metabolites >10% drug-related exposure or >0.01 mg/kg in a crop/animal derived commodity to be identified. Typically, radio-LC-high resolution MS is used to quantify and identify metabolites. This, however, has its limitations. Producing a radiolabeled test item can be challenging and expensive and, while high resolution mass spectrometry can provide a wealth of structural information, it is not always possible to determine a definitive structure. This requires an authentic reference standard or NMR, which again, can be time consuming and costly. It would be useful to have an understanding of metabolism before embarking on radiolabeled work. Metabolite structure elucidation using non-radiolabelled materials comes with its own challenges. How are potential metabolites identified amongst the myriad of endogenous components in complex biological matrices, how do we separate the wheat from the chaff? Software-screening tools can be used to interrogate high resolution mass spectra to identify potential metabolites. In this presentation the use of software screening tools including Compound Discoverer™ (Thermo Scientific™), MetabolitePilot™ and Molecule Profiler (Sciex™) to differentiate metabolites from endogenous material are explored. A range of ex-vivo and in-vitro samples including urine, faeces, plasma, hepatocytes, microsomes and S9 by LC-MS screening tools were analyzed by LC-MS and the resulting data screened using either Compound Discoverer™ (Thermo Scientific™), MetabolitePilot™ or Molecule Profiler (Sciex™). There are notable differences in how the workflows are set up, e.g. MetabolitePilot™ requires screening parameters to be set prior to screening and requires a control sample for comparison while in Compound Discoverer the initial screening parameters are less specific but more filtering is applied to the data after application of the workflow and successful screening is not reliant on having a control sample. An overview of useful workflows, parameters and result filtering which allow efficient metabolite profiling will be presented.

P47 - CYTOCHROME P450 REACTION PHENOTYPING OF LOW CLEARANCE COMPOUNDS USING SELECTIVE CHEMICAL INHIBITORS IN HμREL® CO-CULTURE PLATES

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Assays to investigate routes of metabolism for a new chemical entity (NCE) have traditionally used recombinant enzymes or microsomes/hepatocytes with selective chemical inhibitors. With low clearance NCE's becoming increasingly prevalent, traditional matrices are not always able to provide sufficiently long incubation times to accurately determine intrinsic clearance (CLint). Although there have been advances in methods to determine CLint for low clearance compounds, *in vitro* tools for phenotyping the enzymes responsible for their metabolism are limited. Utilising our HμREL® co-culture low clearance assay, we have investigated the suitability of using selective chemical inhibitors over an extended incubation period. This will enable phenotyping of Cytochrome P450 (CYP) enzymes responsible for the metabolism of low clearance NCE's; which is vital for accurate prediction of clinical drug-drug interactions (DDIs), in addition to assessing any metabolic contribution by polymorphic CYP isoforms. CYP3A4 remains the primary biotransformation pathway for recently approved drugs(1). This, alongside the potential for DDIs with common co-medications or foods/dietary supplements that are CYP3A4 inhibitors or inducers, mean it is an important enzyme to evaluate. As a result, the present study has focused on identifying CYP3A4/5 metabolism by testing the effectiveness of a range of CYP3A4/5 selective inhibitors. In addition, the suitability of atipamezole was assessed, which has been reported as a more potent pan-CYP inhibitor than commonly used 1-aminobenzotriazole(2). The compounds studied included a panel of primarily CYP3A4/5 substrates and non-specific CYP substrates. Cells were pre-incubated with inhibitor for 2 hours, media removed and replaced with serum-free media containing substrate and inhibitor. Samples were taken at 0, 2, 6, 24, 48 and 72 hours, with inhibitor replenished as required. Substrate depletion was monitored via LC-MS/MS and CLint calculated. Azamulin was identified as the most appropriate selective CYP3A4/5 inhibitor, effectively inhibiting metabolism of the CYP3A4/5 sensitive substrate triazolam by >90% based on substrate depletion. Azamulin selectivity for CYP3A4/5 was also confirmed using a panel of isoform specific marker reactions for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6. Atipamezole demonstrated its effectiveness as pan-CYP inhibitor by inhibiting marker reactions for all CYPs, alongside the panel of substrates assessed by substrate depletion. Overall, we have validated the effective use of chemical inhibitors in a low clearance assay to differentiate CYP and CYP3A4/5 specific metabolism. Our future studies will focus on identifying suitable control substrate/selective inhibitor pairings and optimising conditions for a robust assay to investigate phenotyping of all CYP isoforms recommended by the FDA.

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P48 - QUANTIFICATION OF DRUG-METABOLIZING ENZYMES IN THE HUMAN KIDNEY CYTOSOL

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The extrahepatic drug-metabolizing enzymes (DMEs) have been of interest for their role in local and systemic drug disposition. Nonetheless, quantitative information on these is sparse at its best and non-existent in many other cases. Recent publications on extrahepatic DMEs have focused on the membrane-bound Cytochrome P450s (CYPs) and UDP glucuronosyltransferases (UGTs) in the small intestine and the kidney [1] [2]. Hence, information on expression of non-CYP and non-UGT enzymes, such as esterases, oxidases, and sulfotransferases is scarce [3]. Therefore, the current study focuses on the quantitation of non-CYP and non-UGT enzymes in the human kidney cytosol.
The human kidneys (n=20) had been collected from patients undergoing nephrectomy due to cancer [1]. The tissue harvested from sections contralateral of the tumour and were judged as "histologically normal". The cytosolic fractions of the renal tissue were digested according to our previous protocol [1].
The samples were analyzed using both a targeted QconCAT-based AMRT approach as well as global analysis with bovine serum albumin serving as external standard.
Based on the AMRT analysis, 24 proteins were quantified. Of these 10 are primarily located in the microsomal fraction. Among the non-CYP enzymes, the most abundant proteins in the kidney cytosol are aldehyde dehydrogenase 1A1 (8.19 ± 5.61 pmol/mg), alcohol dehydrogenase 1B (6.31 ± 5.03 pmol/mg), CYP1A1 (3.93 ± 5.04 pmol/mg), epoxide hydrolase 2 (2.24 ± 1.61 pmol/mg), and thiopurine S-methyltransferase (1.64 ± 0.96 pmol/mg).
The study elucidates the expression of several cytosolic non-CYP enzymes in the human renal tissue for the first time.

Generating such data is an essential resource for systems biology models to facilitate quantitative translation of renal drug disposition.

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P49 - INTESTINAL PGP-INDUCTION BY HYPERFORIN IN RATS - A COMPARISON OF TWO ST. JOHN'S WORT FORMULATIONS

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P-glycoprotein (Pgp, Abcb1) is an efflux pump expressed in various pharmacokinetically relevant tissues. In the intestine, Pgp limits drug absorption, in the liver enhances hepatobiliary drug elimination and at the blood-brain barrier reduces drug penetration into the CNS. One of the transcriptional regulators of Pgp is the pregnane X receptor (PXR). This nuclear receptor is ligand-activated by various xenobiotics and increases Pgp expression and activity. St. John's wort (SJW), an herbal medicine used to treat mild depression, contains several bioactive constituents of which hyperforin is known to activate the human but not the rat PXR [1]. Nevertheless, SJW treatment with SJW showed to increase intestinal Pgp expression in rats [2]. Of the various SJW formulations, Hyperiplant® is characterized by a high hyperforin concentration, while Rebalance® is known for its low hyperforin content and reduced interaction potential in humans [3].
The aim of this project was to compare the effect of a 10 days oral treatment with Hyperiplant® and Rebalance® on Pgp expression in rats, to further investigate the impact of SJW formulations in this species. Seven weeks old Wistar rats were subjected to a 10 days oral treatment with 400 mg/kg of Hyperiplant® or Rebalance® suspended in H2O containing 0,5% methylcellulose and 0,1% Tween 80. As a control, Wistar rats were treated with the suspension mixture only. After organ harvesting, Pgp expression and abundance was determined by real-time PCR, immunohistochemical staining and Western blot analysis. Quantifying Abcb1 transcript levels in brain, liver and small intestine revealed no modulation neither by Hyperiplant® nor by Rebalance® treatment. Immunohistochemical staining of Pgp applied to small intestine sections revealed the expected location of the transporter. Interestingly, in the small intestine of Hyperiplant® treated rats Pgp intensity appeared higher compared to animals treated with the control or Rebalance®. This notion was further supported when comparing the amount of Pgp protein in the small intestine by Western blot analysis. Here we were able to replicate the intestinal induction of Pgp in rats treated with the high hyperforin content formulation Hyperiplant®, while no such effect was observed in animals treated with Rebalance®. Moreover, probing the apical or basolateral membrane fractions isolated from small intestines of Hyperiplant® treated animals showed the transporter mostly residing on the apical side. Our findings show that the increased Pgp expression is only observed in animals treated with a SJW formulation containing high amounts of hyperforin, thereby mimicking the modulation in humans. However, as neither Hyperiplant® nor Rebalance® treatment had an impact on the Pgp mRNA expression, we conclude that the transporter induction is not linked to the nuclear receptor PXR.

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P50 - AN INTEGRATED MODELLING APPROACH FOR TARGETED DEGRADATION: INSIGHTS ON OPTIMISATION, DATA REQUIREMENTS AND PKPD PREDICTIONS FROM SEMI- OR FULLY-MECHANISTIC MODELS AND EXACT STEADY STATE SOLUTIONS

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This work proposes a novel pragmatic modelling approach for targeted protein degradation which captures the essence of the mechanism while retaining model simplicity. We propose a general integrated modelling approach which leverages

benefits while mitigating limitations of different modelling techniques: (1) Exact mechanistic steady state solutions can (i) provide insight on each system parameter role regardless of its specific value, (ii) suggest which mechanistic knowledge can be confidently extracted from single time point data, (iii) which additional data needs to be collected, and (iv) ultimately, inform compound optimization, data generation and resource prioritization; (2) Global sensitivity analysis can help identify key drivers of the response. For monovalent degraders we assume that endogenous protein synthesis and degradation are zero- and first-order processes, respectively. When compound is added, binding kinetics leads to the formation of a binary complex, which induces degradation at a first order rate. For bivalent degraders (e.g., PROTACs), ternary complex formation can happen from a PROTAC-target or PROTAC-ligase binary complex, where the extent of the contribution of each pathway is dictated by binding affinities, PROTAC concentration, and target and E3 ligase levels. Upon degradation of the target protein, PROTAC and E3 ligase are recycled back into the system. Exact solutions of monovalent degraders mechanistic models show how on/off binding rates and degradation rates are related to potency and maximal effect, which can be used to suggest a compound optimization strategy. For bivalent degraders, even the structure of convoluted exact steady state solutions suggests that the total remaining target at steady state, which is easily accessible experimentally, is insufficient to reconstruct the state of the whole system at equilibrium and observations on different species such as binary/ternary complexes are necessary. Furthermore, global sensitivity analysis of fully mechanistic models suggests that both target and ligase baselines (actually, their ratio) are the major source of variability in the response of non-cooperative systems, which speaks to the importance of characterizing their distribution in the target patient population. As a result, we propose a pragmatic modelling approach which incorporates the insights generated with fully mechanistic models into simpler turnover models to improve their predictive ability and in turn enable acceleration of drug discovery programs and increased probability of success in the clinic.

P51 - APPLICATION OF MASS SPECTROMETRY IMAGING IN CHIMERIC MICE WITH HUMANIZED LIVER FOR SPECIES DIFFERENCES ANALYSIS OF HEPATOTOXICITY BY A PROTOPORPHYRINOGEN OXIDASE INHIBITOR, EPYRIFENACIL

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One of the most challenging problems for the safety assessment of pesticides is to estimate the kinetics and toxicity in humans because it is impossible to assess toxicity by dosing pesticides directly to humans. A chimeric mouse with humanized liver produced by transplanting human hepatocytes into mouse liver has been used as a human model for the evaluation of pharmacokinetic parameters and chemical-induced hepatotoxicity. We conceived of an idea to combine the two technologies: chimeric mice with humanized liver with low replacement index of human hepatocytes and mass spectrometry imaging (MSI) for species differences analysis of pesticide-induced hepatotoxicity between mice and humans. In the liver of chimeric mouse, human hepatocytes can be exposed to chemicals under the same conditions as host mouse hepatocytes. Then, MSI analysis enables us to separately evaluate the effect of chemicals in human and mouse hepatocytes. We applied this strategy to estimate the risk of human hepatotoxicity by one of the protoporphyrinogen oxidase (PPO) inhibiting herbicides, epyrifenacil (ethyl[(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl]phenoxy}pyridin-2-yl)oxy]acetate). Previous studies revealed that hepatotoxicity was caused by an ester-cleaved epyrifenacil, S-3100-CA, which is a major metabolite in mammals. *In vitro* studies showed significant species differences in the profiles of S-3100-CA; less active uptake rate in hepatocytes and less PPO inhibitory activity in humans than in rodents, which suggested less sensitive to epyrifenacil-induced hepatotoxicity in humans than in rodents1. However, there was no information about how the species differences in the kinetics and dynamics of S-3100-CA affect hepatotoxic potential in humans *in vivo*. The chimeric mice which have liver with approximately 60% replacement with human hepatocytes were fed diets containing 40 ppm epyrifenacil for 7 days. The liver sections were analyzed by MSI to compare the exposure of S-3100-CA and accumulation of the endogenous biomarker of the PPO inhibition, protoporphyrin IX. As a result, MSI analysis revealed that both analytes were colocalized much more in the mouse hepatocyte region than in the human hepatocyte region. It was suggested that epyrifenacil has significantly less effect *in vivo* on human livers than on mouse livers because of the species differences in the active hepatic uptake and PPO inhibition of S-3100-CA. In conclusion, this strategy is a useful technique to evaluate the species differences in the toxicological key events occurring in human and mouse livers under the same conditions.

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P52 - QUANTITATIVE DOSE-RESPONSE MODELING USING METABOLOMICS TO DISCLOSE HEPATOTOXIC EFFECTS AND METABOLISM OF ENNIATIN B IN HEPARG CELLS

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To investigate a toxic event, dose response data generated by metabolomics are often analysed using univariate and multivariate chemometric tools to identify the relevant dysregulated metabolites and their associated biological pathways. Usually, metabolomic dose response studies are done with only few concentrations that can't reflect all trends of responses. For example, besides their own kinetic, interaction of metabolites with molecular target(s) and secondary compensatory processes may generate non-linear responses. Therefore, modeling the quantitative responses of metabolites over a gradient of concentrations can enhance the mechanistic understanding of molecular responses [1]. The resulting models are also useful to assess sensitivity thresholds, e.g. Effective Concentrations (ECx), Benchmark Dose (BMD), which are key parameters for risk assessment [2]. This promising approach has been recently proposed to support ecotoxicological risk assessment of chemicals with the development of the DRomics open-source software [3-4]. It was also used to assess and compare toxicological effects of chemicals in liver HepaRG cells [5-6]. In this study, we applied the DRomics dose-response framework to study the fate of Enniatin B (ENNB) mycotoxin in HepaRG cells. Enniatins produced by fungi belong to emerging mycotoxins commonly found in cereals and raising concern for food and feed. However, due to the lack of toxicokinetic and toxicodynamic data to derive a Health-Based Guidance Value, these mycotoxins are not regulated at the European level, [7]. Therefore, human HepaRG cells were treated with a large range of 10 concentrations (0-4 µM) of ENNB for 48h. Untargeted metabolomics was applied to assess simultaneously kinetic (xenometabolome, ENNB and (bio)transformation products) and dynamic (endometabolome, metabolites interlinked in specific processes) responses and to identify the pathways involved.

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P53 - THE USE OF INTERACTIVE R SHINY APPLICATIONS IN THE ASSESSMENT OF NEW TARGETS

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Mathematical PKPD models often appear as a "black box" to the scientists whose experiments are being simulated. These scientists can benefit greatly from proposing and visualizing what-if scenarios, especially at an early stage when in-house assays have not been developed and uncertainty looms over the biology itself. But difficulty understanding mathematical equations or simulation software often limits their use to one or two experts in a project team. This poster highlights the ways R Shiny applications can be employed to aid in the exploration and presentation of modelling results, especially for newly proposed targets. In this situation, we sought an antibody targeting a cellular receptor in peripheral tissue which could additionally be shed from the membrane. The desired action involves binding to the receptor form, but the soluble form is known to have further biological functions. Modelling and simulation allow prediction both of the dose requirements for sufficient binding to the receptor, as well as the likelihood of side effects related to modulation of the soluble target. This identification of therapeutic and adverse effects in the same modelling framework permits realistic comparisons. Beyond just delineating the parameter sensitivity of each outcome, modelling the therapeutic window (or one aspect of it) clarifies some fundamental limitations on the potential optimization of a biologic treatment. These modelling results were developed into an R Shiny application. The first page is a simulation dashboard – with a single click the scientist can generate PKPD curves for the default parameters over a range of doses and times. More complicated plots are separated into tabs. Such an application can be hosted on a private or public server and gives the

scientists proposing the new targets the opportunity to interactively understand the effect of system parameters or drug parameters on the PD outcomes. In addition to the model itself, the user interfaces of Shiny apps are built by the modeler, allowing one to prevent scientists from creating inappropriate scenarios or unreasonable assumptions. This kind of early assessment of new targets leads to the reprioritization of experiments around a few key parameters which will most likely determine the project's feasibility, both in terms of finding an efficacious dose, and in terms of potential adverse events.

P54 - FULL TMDD PK MODEL OF MAB BINDING TO PROTEIN WITH A SOLUBLE AND A MEMBRANE-BOUND VARIANT IN CYNOMOLGUS MONKEY

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Objectives: Development of a PK population model of an antibody binding to a soluble and a membrane-bound target in cynomolgus monkeys. Estimation of the concentration of membrane bound target in the central compartment.
Background: To address our objectives, we used a human IgG1 monoclonal antibody (mAb) with no binding to effector Fcγ receptors. This mAb binds to a membrane bound protein as well as a soluble variant in both human and cynomolgus monkeys. The affinity to the cynomolgus protein was measured in SPR to 6 pM. The plasma concentration of soluble target was measured to be approximately 26 nM however it was not possible to measure the concentration of membrane bound target as it cannot be found on the cells in plasma. Like other TMDD models (Ref 1, 2), our final model assumed stable target turnover for both targets.
Methods: IV and SC dosing of vehicle (n=2) or 0.6-60 mg/kg mAb (n=1-2) to 9 cynomolgus monkeys. The study was reviewed with regards to animal welfare and was in full compliance with animal ethical principles as well as local and national legislations. 15 blood samples per animal were collected over 42 days. Plasma samples were analysed in 4 ligand binding assays for measuring concentration of total soluble mAb, free mAb, total soluble target and free soluble target via Meso Scale Discovery instruments and software. Different PK models including Qss models and full TMDD PK models (Ref 1, 2) were evaluated and fitted to the data in Phoenix WinNonlin® (version 8.3.4) from Certara L.P. CV% of inter-individual variation (IIV) was calculated by sqrt(e^Omega-1).
Results: The observed PK profiles of the mAb suggested TMDD with increased clearance at lower plasma concentrations. This was seen e.g. as non-linear profiles of both total and free mAb. No free soluble target was detectable when the mAb concentration was above approximately 40 nM. A full TMDD PK population model with 17 parameters was the best fit to the data. Inter-individual varia¬tion was observed on the baseline plasma concentration of the soluble target (CV% 63%). Based on the established model, the typical concentration of the membrane bound target (nmol/L) in the central compartment was estimated to be approximately 2 nM.
Conclusions: A full population TMDD model was established describing the PK of an mAb that binds to a soluble and a membrane bound target in cynomolgus monkeys. The median concentration of the membrane bound target was estimated to be approximately 16 times less than the soluble target concentration.

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P55 - COMPARISON OF IMMUNODEFICIENT AND IMMUNOCOMPETENT MOUSE STRAINS FOR PHARMACOKINETIC ASSESSMENT AND SELECTION OF MOLECULES FOR PHARMACOLOGICAL STUDIES IN DRUG DISCOVERY

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In vivo pharmacokinetics studies in rodents are important in selecting the right molecules for pharmacological studies. Barr et Al (1) compared the *in vitro* and *in vivo* Pharmacokinetics (PK) parameters of eight marketed drugs in 3 different strains of wild type mice (BALB/c, C57Bl/6 and CD-1). The authors showed similar *in vitro*/*in vivo* PK properties between strains and suggested that a unique strain of mice should be enough, as a first approach, to characterize new drugs in early discovery stage. Based on these encouraging results, we extended the analysis by comparing PK parameters obtained in wild type mice (C57Bl/6) and nude mice (SCID), an immunodeficient mice extensively used in oncology domain for pharmacodynamic studies. Seven small molecules with different physicochemical properties (4 acids, 1 Neutral and 2 bases) were characterized: *in vitro* ADME (ie. metabolism in liver microsomes and plasma protein binding) and *in vivo* PK in order to assess the IVVC (*In vitro/In vivo* correlation). Three mice per compound and per route of administration were dosed by IV bolus (1mg/kg in PEG/Ethanol/NaCl, 40/10/50 V/V/V) and PO route (3 mg/kg in 1% HEC/1% Tween). Blood samples (n=6) were collected over 24h in each animal and plasma concentrations were

determined by Liquid Chromatography tandem Mass Spectrometry. Individual PK parameters were determined by non-compartmental analysis. Overall, the *in vivo* PK parameters were similar between both strains with the same range of systemic clearance (from 16 to 92 ml/min/kg in C57Bl/6 and 17 to 71 ml/min/kg in SCID mice), and with the same trends in oral bioavailability. The most significant differences were observed in the estimated volume of distribution (Vss) where 3 compounds (out of 7) showed differences slightly higher than 2-fold between strains. These results strengthen the rationale of using only one strain of mice to assess full PK parameters as well as ensuring sufficient exposure for pharmacological studies. This approach has allowed us to save time, resources and material. It also limited risk to select the unsuitable compound for a pharmacological study or to compromise the next step, prediction of the human active dose.

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P56 - PREDICTION OF ABSORPTION OF PROTEIN DEGRADERS

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The prediction of absorption of target protein degraders (TPDs) after oral administration to humans is widely recognized as a challenge in the drug design of new bifunctional TPDs. We have investigated the predictability of absorption *in vivo* (mouse and rat) from *in vitro* data (e.g., Caco-2 permeability and efflux, experimentally measured polar surface area (EPSA) and solubility in fed and fasted state simulated intestinal fluid – FeSSIF and FaSSIF). We also discuss the impact of species differences (mouse vs. rat) regarding the absorption of TPDs.
85 different TPDs were profiled for permeability in Caco-2 cell line at pH 7.4 (AtB, BtA and Efflux were estimated). EPSA was measured using supercritical fluid chromatography (SFC). Solubilities were measured from DMSO solutions in FeSSIF and FaSSIF at pH 5.8 and pH 6.5, respectively. Fraction of dose absorbed (Fabs) (in mouse and rat) were estimated from oral bioavailability and predicted first pass metabolism (from a combination of IV-clearance and *in vitro* metabolic stability data in mouse and rat liver microsomes and hepatocytes). All TPDs were dosed perorally (PO) and intravenously (IV) as solutions.
The impact of gastrointestinal (GI) permeability and pH, on absorption have previously been discussed by Escribano et al (2012) and Hatton et al (2015) and we will compare our findings with their conclusions.
The permeability in Caco-2 were low (0-1.5 x 10-6 cm/s) and the efflux ratio varied from (<)1 to 100. EPSA was relatively high (110-150 Å2). Solubility was higher in FeSSIF compared to FaSSIF and varied between (<)2 and 90 µg/mL. Fabs in mouse ranged from no absorption up to full absorption and was in general clearly lower in rats.
The were no significant correlations between Caco2 permeability and *in vivo* absorption, in mice or rats. There was a clear trend for better absorption for compounds with low EPSA and when also taking efflux into account, the predictability was improved.
Absorption (Fabs) was clearly higher in mouse compared to rat and we hypothesize that this is due to a combination of lower permeability and higher pH in the GI tract of rat.
It is a significant challenge to predict absorption and so far, there is no bioavailability measured for TPDs in the clinical setting, that can be used to validate the predictive models. We believe that mouse Fabs is most predictive for Fabs in humans and suggests using this in combination with EPSA, efflux ratio, and solubility in FaSSIF/FeSSIF, in the dose to man predictions.

P57 - OPTIMIZED NORMOTHERMIC MACHINE PERFUSION OF LIVER AND KIDNEY USED TO PREDICT HUMAN PHARMACOKINETICS

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Introduction: Good prediction of the absorption, distribution, metabolism and excretion (ADME) profile of drugs is of high importance in the drug development process. Ex vivo liver normothermic machine perfusion (NMP) of organs are a promising tool, especially when combined with physiologically-based pharmacokinetic (PBPK) modeling to reliably predict human exposure levels. The aim of the current study was 1) to optimize organ functioning during NMP by improving physiological resemblance of the liver through the addition bile acids, and 2) demonstrate prediction of ADME data of model drugs (rosuvastatin, digoxin, metformin and furosemide) based on NMP of liver and kidney combined with *ex vivo* intestinal absorption studies and PBPK modeling.
Methods: NMP of human discarded (n=4) and porcine livers (n=5) was performed with standard taurocholate infusion (0.1g/h) for 360min. Porcine liver (n=2) perfusion was performed with infusion of an (un)conjugated bile acid blend

(gCDCA, gCA, CA, CDCA) for 720 min. Biopsies were taken ate several time points for RNAseq expression analysis and bile was collected to study bile composition.
In order to predict ADME of the model drugs, three *ex vivo* models were applied using porcine organs 1) the inTESTine system (n=3) to study apical to basolateral intestinal transport, determining the apparent permeability (Papp) in duodenum, jejunum, ileum and colon tissue. 2) Normothermic machine perfusion of *ex vivo* liver (n=3) was used to determine the hepatic uptake, clearance and biliary excretion and 3) normothermic machine perfusion of ex vivo kidney (n=3) was used to determine the renal clearance and urinary excretion.
Results: Perfusion with taurocholate resulted in a decreased expression of bile acid synthesis related genes, increased gene expression of cholesterol metabolism related genes and a decreased expression in bile acid-dependent uptake and efflux transporters. Upon infusion of (un)conjugated bile acid pool, stable bile production, flow and lower AST and ALT values were achieved until 720 min of perfusion. Gene expression showed a limited and delayed increase/decrease compared to the taurocholate protocol.
Perfused porcine livers showed to rapidly clear rosuvastatin from the perfusate with a hepatic extraction ratio of 0.82 and 0.31 for digoxin, whereas for metformin and furosemide this was very low (~0.2). Biliary excretion of digoxin was the highest (53%), followed by rosuvastatin (23%). Minimal biliary excretion was obtained for furosemide and metformin, which were >90% excreted into urine by the kidneys.
These data will be incorporated into a generic PBPK model to estimate plasma concentration (Area Under the Curve (AUC)), plasma peak concentration (Cmax), hepatic clearance and bioavailability, which will be compared to human clinical studies.
Conclusion: NMP can be optimized to sustain organ functionality during perfusion. Portal infusion of (un)conjugated bile acids led to better liver functioning and stabilized gene expression. The combination of *ex vivo* gut, liver and kidney models with a generic PBPK model is a unique and powerful combination to predict ADME profile of (new) drugs, including the possibility to calculate the fraction that undergoes enterohepatic circulation. Future research is aimed at studying drug-drug interactions and the effects of disease processes.

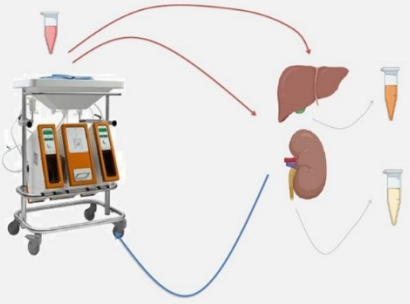

***Figure 1**. Graphical representation of combined ex vivo liver and kidney perfusion; generating perfusate, bile and urine fractions.*

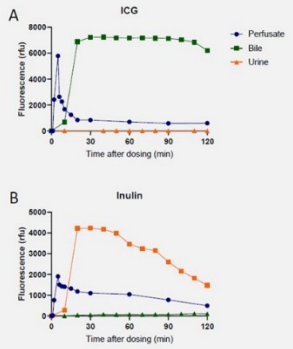

***Figure 2**. Hepatic and renal clearance of fluorescently labelled model compounds ICG (hepatic and biliary clearance) and inulin (renal and urinary clearance). (A) ICG is excreted into bile and not into urine (B) inulin excretion into urine and not into bile*

P58 - PREDICTION OF pH ALONG THE NEPHRON TUBULE USING THE MECHANISTIC KIDNEY MODEL (MECH KIM): MEMANTINE AS CASE EXAMPLE

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The kidneys play a key role in acid-base homeostasis, reabsorbing bicarbonate (HCO3-) from the blood and in turn secreting hydrogen ions into the urine, it is through this mechanism that the pH of the bloodstream is balanced and maintained. The mechanism of acid-base homeostasis results in a shift in pH starting from the blood, along the lumen of the kidney nephron, and finally the urine. In a PBPK model, accurate estimation of the pH in the urine and thus along the nephron is important when predicting the ionisation, passive permeability, and precipitation of compounds. In the present study the mechanistic kidney model (Mech KiM) in the Simcyp Simulator[1] was expanded to incorporate these changes in pH within the nephron lumen based on literature physiological data, rather than the existing assumption that the pH remains at 7.4. As part of this work, meta-analyses were carried out to establish the pH in both the tubular lumen of various nephron segments, and the urine. Segment-specific tubular lumen pH was collated from eight rat studies, where pH values relative to the distance along the nephron were analysed via linear regression. A decrease in pH was observed along the nephron from the plasma to the urine, enabling a non-monotonic equation to predict the pH at a given distance along the nephron (in mm) based on the plasma and urine pH to be derived. The distance of individual segments was assigned based on literature data[2]. An increased pH of 7.39 at the loop of Henle was observed, which has been speculated to allow for the dissociation of ammonium, allowing ammonia out of the tubule lumen and into the collecting duct fluid, where it is re-entrapped[3]. Analysis of 3 studies presenting data on human urine pH in healthy subjects allowed

for a mean human urine pH of 5.99 (n = 196, CV = 8%) to be determined, this was in line with a pH value of 5.92 (n = 160, CV = 6.12%) from rat data which confirmed the suitability of the rat studies in absence of human data to be used in deriving the regional pH. The along the nephron performance of the urine pH expansion to Mech KiM was verified using a memantine PBPK model, previously described by Burt et al.[4], where simulations were carried out in subjects (10 x 12 males, 22-31 years) with acidic and alkali (pH 5 and 8) urine pH while the segmental nephron pH was predicted based on these values. In both cases the observed amount of drug excreted over 24 hours and concentration-time profiles were recovered by the simulation, validating the equation used to predict the pH in the nephron segments.

P59 - DAPAGLIFLOZIN PBPK COMPOUND FILE TO PREDICT CHANGES IN ITS EXPOSURE WITH DRUG-DRUG INTERACTIONS, VARIOUS ETHNICITIES, AND SPECIAL DISEASE POPULATIONS.

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Dapagliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor indicated for use in patients with Type II Diabetes Mellitus. The drug is mainly metabolised by hepatic UGT1A9, UGT2B4/7, and CYP3A enzymes. We developed a PBPK model for dapagliflozin as a UGT1A9 probe substrate to predict the drug exposure in various scenarios such as drug-drug interactions and anticipating differences in various ethnic and special disease populations. Simcyp version 22 was used to develop the model. Physicochemical parameters such as molecular weight, octanol-water partition coefficient, blood-to-plasma ratio, and fraction unbound in plasma were collected from the literature. Reverse translational tool was used to calculate the intrinsic clearance for UGT1A9, UGT2B7, and CYP3A4 metabolic pathways. Drug distribution was modelled using Minimal PBPK model to capture the biphasic profile following intravenous administration. For the oral model, the ADAM model with Caco-2 permeability of 15.9×10-6 cm/sec was used. The developed model was able to recover drug exposure at different oral doses (2.5, 10, 20, 50, and 100 mg) within 2-fold. The model was also able to reproduce clinical studies involving various healthy and diseased populations. The healthy populations included North American populations studies (African, White, Asian, and Hispanic) and the Chinese population, while the disease populations involved studies of patients with hepatic and renal impairment. The model predicted all pharmacokinetic profiles of the above populations within 2-fold range. Lastly, the model accurately predicted the effect of the co-administration of rifampicin with dapagliflozin within 1.5-fold. However, as there is a lack of specific UGT1A9 inhibitors, we were unable to validate the model for drug interactions with UGT1A9. More *in vitro* and *in vivo* data for the UGT1A9 inhibitors are still required to validate UGT1A9 enzyme's contribution in metabolism of dapagliflozin. In conclusion, a dapagliflozin PBPK compound file was developed and validated in various populations.

P60 - PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING OF ADALIMUMAB TO PREDICT PHARMACOKINETICS IN PEDIATRICS

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Ethical regulations and limited pediatric participants may delay approval of monoclonal antibodies in pediatric by 6 years (1). Modelling and simulation is increasingly adopted for optimizing pediatric clinical studies, reducing patient burden. The classical modelling using body weight or body surface area-based allometric scaling may preclude rapidly changing physiology in paediatrics that affects mAb disposition, especially in younger infants (2). However, PBPK modelling accounts for ontogeny of key physiological processes in paediatrics (3). Currently, pediatric PBPK models of mAbs are limited, hence, this study aims to develop a pediatric PBPK model for mAb, Adalimumab. An adult full PBPK model integrated with target-mediated drug disposition was developed for Adalimumab using Simcyp software (Version 21, Certara UK Ltd) in healthy volunteers and rheumatoid arthritis population following intravenous or subcutaneous dosing. Drug and systems parameters were optimized via local sensitivity analysis (LSA). For model validation, demographic details (age, gender, health conditions) and clinical study design information (dosing regimen, route of administration, number of subjects) were extracted from clinical pharmacokinetic studies. SimCYP software was used to perform clinical trial simulations replicating the original study design. Observed pharmacokinetic data was overlaid over model-predicted pharmacokinetics data and model prediction accuracy was measured by geometric mean fold error (GMFE) and absolute average fold error (AAFE) between observed and predicted pharmacokinetic data. Upon confirming adult PBPK model possess reasonable prediction accuracy, pediatric PBPK model was developed for pediatric Crohn's disease population. Drug-specific input parameters were the same as adult PBPK model. However, system input parameters were based on ontogeny data of key physiological parameters extracted from pediatric clinical studies ranging from birth to 18 years of age. The developed adult PBPK model in healthy volunteers predicted drug concentration-time profiles reasonably well, with Cmax and AUC demonstrating GMFE and AAFE within satisfactory range (0.8-1.25). The adult PBPK model for rheumatoid arthritis population predicted Cmax and AUC within satisfactory (0.8-1.25) and acceptable range (0.5-0.8; 1.25-2-fold) respectively. The pediatric PBPK model for Crohn's disease population predicted drug concentration-time profiles reasonably well, with Cmax and AUC demonstrating GMFE and AAFE within satisfactory range (0.8-1.25). LSA reveals TNF-α target expression does not exhibit age-dependency in paediatrics. Overall, using adalimumab, this study demonstrates PBPK modelling strategy in capturing the complexities of age-dependent physiological changes in

paediatrics. Significantly, this is the first pediatric PBPK model developed for Adalimumab and offers a good starting point for further refinements. Lastly, the most recent ontogeny data on key physiological processes in pediatric were consolidated to facilitate exploration in future PBPK studies not limited to Adalimumab but other monoclonal antibodies.

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P61 - PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING OF UGT1A4 PROTOTYPICAL DRUG LAMOTRIGINE IN PREGNANCY POPULATION

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PBPK modelling of drugs metabolised by Phase I enzymes (CYPs) in pregnant population had been reported in past (1) while its use in Phase II (UGTs) is not known. Additionally, use of anti-epileptic drugs during pregnancy is major challenge as pharmacokinetics of these drugs is reported to be altered significantly in this population (2). There is sparse PK data of anti-epileptic drug lamotrigine (LTG) reported in clinical studies, which is primarily metabolized by UGT1A4 enzyme. The primary objective of this study was to apply PBPK modelling to predict PK of LTG during different trimesters in pregnant population. Previously reported minimal PBPK model of LTG (3) was adapted to full PBPK model and validated in non-pregnant population by 'middle out' approach by using Simcyp V21 (Certara Ltd, UK). Clinical trial simulations were conducted to predict the PK of LTG during different trimesters of pregnancy. Default pregnancy population available with Simcyp was adapted to reflect likely changes in expression of UGT1A4 enzymes based on literature reports ((4)). Model's prediction capability was assessed by comparing observed versus predicted PK parameters (Cmax, AUC, CL) and calculating the average fold error (AFE) and absolute average fold error (AAFE), respectively.
Full PBPK model well predicted plasma concentration profiles and PK parameters of LTG in non-pregnant population for after single-dose and multiple-dose oral administration. The AFE and AAFE values for predicted Cmax and AUC were found to be 1.05/0.99 and 1.13/1.24, respectively. The PBPK model predicted Cmax and AUC of 4.95 mg/L and 79.4 mg/L.h during third trimester and was close to observed values of 5.3mg/L and 43.4 mg/L.h, respectively. Additionally, percent changes in Cmax and AUC in 3rd trimester compared to baseline values in non-pregnant state were calculated and well reflected observed trends. Prediction error analysis revealed model's good ability to simulate LTG PK during pregnancy (AFE values of 0.97 for clearance, 1.06 for AUC and 1.04 for Cmax) respectively.
Our study demonstrates the successful development and validation of a PBPK model for LTG in pregnancy population. Future work with additional UGT1A4 substrate drugs using proposed changes in UGT1A4 activity may enable to validate pregnancy population model and its subsequent use for prospective prediction of PK.

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P62 - PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL DEVELOPMENT AND VALIDATION OF MITIPERSTAT AND APPLICATION TO PREDICT DRUG-DRUG INTERACTION (DDI) WITH CYP3A4 INDUCER

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Mitiperstat is an oral myeloperoxidase inhibitor being developed for the potential management of cardiovascular disease. *In vitro* studies and human mass balance study have shown that mitiperstat is metabolized by CYP3A4, although renal clearance is the major elimination pathway (1). The present study aimed to develop and validate a PBPK model of mitiperstat for the potential application of DDI prediction with CYP3A4 inducers.
Whole-body PBPK model, with first-order absorption, was developed for mitiperstat in Simcyp Simulator® (Simcyp version 21; Simcyp, Sheffield, UK). The PBPK model was developed based on physicochemical, *in vitro*, and clinical data of mitiperstat using 'middle-out' approach. The demographic details (age range, proportion of females) and duration of study for the virtual population were matched to the corresponding clinical studies (such as NCT03136991, NCT04232345, NCT05052710, NCT05236543, NCT04949438). The PBPK model was validated by comparing the model-predicted overall PK profiles and ratios of AUC and Cmax to observed clinical data. The model was then used to predict DDI between CYP3A4 inducers and mitiperstat (CYP3A4 substrate) using the simulation trial design (2 week inducer administration, followed by oral co-administration of the inducer and substrate) and doses (such as carbamazepine 300 mg b.i.d, phenytoin 300 mg QD) proposed by Chen et al (2).
PBPK model predictions of mitiperstat exposure parameters (geometric mean AUC and Cmax) were generally within 2-fold of the observed clinical data and it recovered the overall PK profile well. The predicted geometric mean ratio (mitiperstat with and without itraconazole) of Cmax, AUCt and corresponding 90% CI were 1.08 (1.08, 1.09) and 1.34 (1.32, 1.35) respectively. The observed geometric mean Cmax, AUCt (90 % CI) were 1.25 (1.02, 1.54) and 1.30 (1.26, 1.33) respectively. The model predicted Cmax, AUCt, AUCinf was about 1.18, 0.73, 0.97 fold of the observed data in severe renal impaired subjects. The validated model predicts DDI with alternate strong CYP3A4 inducers such as, carbamazepine, phenytoin will result in geometric mean ratio (with and without inducer, 90% CI) for Cmax of 0.85 (0.84, 0.85); 0.83 (0.82, 0.84) and AUCt 0.73 (0.72, 0.74); 0.67 (0.65,0.69) respectively.
A PBPK model for mitiperstat was developed, validated and used for DDI prediction as a victim of CYP3A4 induction to inform further clinical studies. The present model was developed to potentially support dosing recommendations in the product label of mitiperstat in DDI situations not evaluated in dedicated clinical DDI studies..

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P63 - PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING OF SENSITIVE CYP2C8 SUBSTRATE ROSIGLITAZONE FOR DRUG-DRUG INTERACTION PREDICTIONS

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Physiologically based pharmacokinetic (PBPK) models combine drug and physiological system related information together helping to build mechanistic models. PBPK modeling is a valuable tool to understand and quantitatively predict the pharmacokinetics of drugs and the threshold for drug-drug interactions (DDI's). During the development of novel drugs with potential DDI liability estimates using *in vitro* enzyme inhibition data, PBPK modeling results can significantly accelerate the process. Development of verified PBPK models of index perpetrator and victim drugs is helpful to predict the DDI potential of new drug candidates. In this work, we present the development and verification of a whole body PBPK model for the sensitive CYP2C8 substrate rosiglitazone using PKSim® software. Rosiglitazone PBPK model was developed and evaluated based on clinical data obtained from the scientific literature. To assess the prediction of DDI, the index CYP2C8 inhibitor Gemfibrozil along with glucuronide metabolites and the inducer rifampicin were considered. Developed PBPK models for perpetrator compounds were adopted without changes from the Open-Systems Pharmacology model repository. Model predicted PK parameters and DDI potential due to CYP2C8 modulation by selected perpetrators were in line with generally accepted 2-fold standards. As recommended in regulatory guidance documents, sensitivity analysis was also performed to evaluate key model parameters of rosiglitazone for their effect on model output. Developed rosiglitazone PBPK model will be useful in quantifying DDI potential of novel drug caused by modulation of CYP2C8 enzyme with implication of their design.

P64 - THE QUEST TO DEFINE CANCER-SPECIFIC SYSTEMS PARAMETERS FOR PERSONALIZED DOSING

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Clinical trials in oncology routinely recruit heterogeneous populations, without catering for all types of variability. The target cohort is often not representative, leading to large variability in pharmacokinetics (PK). To address enrolment challenges in clinical trials, physiologically-based pharmacokinetic (PBPK) models can be used as an alternative to clinical studies. PBPK models in combination with *in vitro–in vivo* extrapolation (*IVIVE*) techniques are used to predict absorption, distribution, metabolism, and excretion (ADME) of drugs, aiming to inform dosing guidance. These models require cancer-specific system data, which are scarce. This study reviews the system parameters (for example, serum albumin, alpha-1 acid glycoprotein, haematocrit, renal/hepatic function, inflammation, gastrointestinal complications) that affect PK in cancer patients and highlights important gaps in data, with liver cancer as a case example. Important parameters in pharmacology, such as microsomal protein content in liver tissue and changes in drug-metabolizing enzymes (DMEs) and transporters have not been fully investigated in several cancer types. Impaired expression of them can significantly affect patients' capacity for drug elimination. The importance of changes in DMEs, especially CYP3A, for the effectiveness of anticancer tyrosine kinase inhibitors is also reviewed here. Currently, there are 51 small molecule receptor and non-receptor tyrosine kinase inhibitors (TKIs) approved by the US FDA for the treatment of a wide range of cancer types. TKIs are widely used in cancer treatment and their metabolism vastly depends on CYP3A function. Finally, the use of PBPK modelling for precision dosing in oncology is highlighted in this study. Overall, it is concluded that model-informed precision dosing is useful for appropriate dosing in cancer sub-populations, which are excluded from oncology clinical trials. There is a lack of fully characterised systems parameters in cancer cohorts, which are required in PBPK models. It is believed that the increased use of liquid chromatography–mass spectrometry (LC-MS) proteomics will address gaps in obtaining measurements of key proteins in cancer patients. Additionally, emerging technologies, particularly liquid biopsy together with virtual twin models, are important advances for the application of personalized precision dosing in cancer. Generation of such data and application of cancer models in clinical practice should be encouraged.

P65 - CHLOROQUINE AND HYDROXYCHLOROQUINE, AS PROTEASOMAL INHIBITORS, UPREGULATE THE EXPRESSION AND ACTIVITY OF ORGANIC ANION TRANSPORTER 3

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Organic anion transporter 3 (OAT3), at the basolateral membrane of kidney proximal tubule cells, facilitates the elimination of numerous clinically important drugs. Earlier investigation from our laboratory revealed that ubiquitin conjugation to OAT3 leads to OAT3 internalization from the cell surface and subsequent degradation in proteasome. In the current study, we examined our hypothesis that chloroquine (CQ) and hydroxychloroquine (HCQ), two well-known anti-malarial drugs, may act as the proteasomal inhibitors and regulate OAT3 ubiquitination, expression, and function. We showed that in OAT3-expressing monkey kidney COS-7 cells and human embryonic kidney HEK293 cells treated with CQ and HCQ individually at 10 μM for 4 hours, the ubiquitinated OAT3 was enhanced by 50% (Values are means ± S.D. (n = 4). *P < 0.05), which correlated well with a decreased 20S proteasomal activity. Furthermore, in CQ- and HCQ-treated cells, OAT3 expression and OAT3-mediated transport of estrone sulfate, a prototypical substrate, were also increased by 47% (Values are means ± S.D. (n = 6). *P < 0.05). Kinetic analysis showed that the increase in OAT3 expression and transport activity by HCQ were accompanied by an increase in the maximum transport velocity (Vmax: 65.8 ± 1.3 pmol·mg−1·3 min−1 with HCQ-treated cells and 54.1 ±1.7 pmol·mg−1·3 min−1 with untreated cells) and a decrease in the degradation rate of the transporter by 38%. In conclusion, significant amounts of CQ and HCQ are eliminated in the kidney. Their plasma concentrations vary individually, ranging from 650-1300ng/mL (2.0-4.1 μM) for CQ and 1161-2436ng/mL (3.5-7.3 μM) for HCQ. Therefore, the concentrations (10 μM) used in the current study for both drugs are within the clinically therapeutic range. This study unveiled a novel role of CQ and HCQ in enhancing OAT3 expression and transport activity by preventing the degradation of ubiquitinated OAT3 in proteasome. This finding provides a new strategy in upregulating OAT3 function that can be used to accelerate the clearance of drugs, metabolites, or toxins and reverse the decreased expression under disease conditions. During comorbidity therapies, the use of CQ and HCQ may alter the function of OAT3, which will affect the therapeutic efficacy and toxicity of those drugs that are the substrates for this transporter.

P66 - ABUNDANCE OF RENAL TRANSPORTERS IN HEALTHY CAUCASIANS: A META-ANALYSIS

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Physiologically-based pharmacokinetic (PBPK) models use *in vitro-in vivo* extrapolation (IVIVE) scaling factors that account for differences in enzyme and transporter expression based on quantitative protein expression as a surrogate for activity. The Mechanistic Kidney Model (Mech KiM) in the human Simcyp Simulator has the capacity to assess the impact

of renal transporters impact on PK utilising relative expression/activity scaling factors[1]. This study derives quantitative abundance values for PK renal transporter proteins suitable for IVIVE-PBPK to enable transporter-mediated scaling of drug activity through the absolute abundances of renal transporters. A literature meta-analysis was conducted where original articles quantifying renal transporter absolute abundances were retrieved on PubMed through a keyword search (i.e., 'Human', 'Renal', 'Kidney' 'Transporter', 'Absolute' 'Relative', 'Protein', 'Expression', 'Abundance'). The complete database comprised 12 studies, including n=167 kidney samples and n=91 transporters probed for quantification. A refined database was constructed where exclusion criteria were applied based on: (a) samples <18 years of age, (b) ethnicity (non-White) where data was available, (c) sample overlap, (d) where the same samples were quantified in more than one study, (e) pooling of samples, (f) insufficient evidence of quantitative abundance values reported, and (g) underlying disease. The refined database included 105 kidney samples from 8 independent studies. Of the 20 transporters specifically named in Mech KiM, there were suitable abundance data for 16 proteins available in the refined database (weighted mean and CV). The abundance values directly reported in most studies were in the units of 'pmol transporter/mg crude membrane protein' or 'pmol transporter/mg microsomal membrane protein', or in one case tissue homogenate. To enable quantitative scaling of transporter activity in Mech KiM, the transporter abundances are required in 'pmol transporter/106 proximal tubule cells'. Hence, conversion factors were derived based on the available data in the literature for various fractionation methods or homogenate protein yield and applied accordingly to normalise and convert data to the required units, alongside the 'proximal tubule cells per gram of kidney' (PTCPGK; 99.4 x 106 cells/g kidney). The most abundant protein was SLC22A2 (URAT 1), with SLC47A1 (MATE1) and SLC22A2 (OCT2) also displaying moderately high expression. Sufficient data could not be sourced at present to assign abundance values to SLC10A2 (ASBT), SLC16A1 (MCT1), SLC19A2 (THTR1) and SLC19A3 (THTR2). Values are available in pooled kidney microsomes for ASBT and MCT1, but due to pooling these were not carried into the refined database. This comprehensive analysis of protein expression for a range of renal transporters based on absolute abundance quantification methods enables absolute scaling of transporter activity in Mech KiM. Efforts are on-going to utilize these data and scaling approaches to describe the renal elimination of compounds within the IVIVE-PBPK framework.

Reference:

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P67 - THE INHIBITOR PREINCUBATION EFFECT IS UNIVERSAL TO SLC TRANSPORTER ASSAYS AND IS ONLY PARTIALLY ELIMINATED IN THE PRESENCE OF EXTRACELLULAR PROTEIN

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Inhibition of transporters by experimental drugs is being assayed routinely *in vitro* to predict potentially hazardous drug interactions. However, lack of consensus over the exact methodology of transporter inhibition assays results in wide inter-laboratory variation of IC50/Ki data. In particular, although preincubation with the inhibitor was shown to potentiate the inhibitory effect in some SLC transporter assays, current guidelines only encourage sponsors to follow emerging literature but do not contain exact recommendations on inhibitor preincubation. Our aim was to clarify how generally preincubation should be considered in transporter inhibition assays, and whether the preincubation effect could be solely explained by strong protein binding of the respective inhibitors. We performed *in vitro* inhibition assays with and without preincubation on 29 structurally and mechanistically diverse SLC transporters (ASBT, ASCTs, CNTs, ENTs, LAT1, MATEs, MCTs, NTCP, OATs, OATPs, OCTs, RFVT3, SGLTs, URAT1) and 4 representative ABC transporters (BCRP, MDR1, BSEP, MRP2). We also tested the effect of adding extracellular protein in SLC assays. In SLC inhibition assays, a 30-minute preincubation in the absence of protein caused significant >2-fold decrease of IC50 for 15/23 inhibitors on 19/29 evolutionarily disparate transporters. The extent of the preincubation effect depended on the physicochemical properties of the inhibitor since it showed positive correlation with predicted plasma protein binding (ρ=0.668) and negative correlation with CLogS (ρ=-0.730) and the ratio tPSA/MW (ρ=-0.643). Preincubation in ABC vesicular transport assays had a >2-fold potentiating effect for only 2/20 inhibitors tested (valspodar and zosuquidar on MDR1), and it was practically inconsequential in BCRP and MDR1 monolayer assays performed with 3 and 4 standard inhibitors, respectively. In the SLC assays, the preincubation effect partly persisted upon addition of up to 5% bovine serum albumin to the assay buffer; hence, the lack of extracellular protein and consequent accumulation of highly protein-bound inhibitors within the cell could not fully explain the preincubation effect. We concluded that while preincubating in a protein-free buffer might lead to overestimation of the inhibitory potency, omitting preincubation altogether may miss clinically relevant inhibitors, and preincubation in the presence of protein may compromise the robustness of the assay and hamper its interpretation. Therefore, we propose that for robust and sensitive worst-case scenario models, a protein-free preincubation should be considered for all SLC inhibition assays. Broad implications of preincubation to ABC transporter inhibition assays seem unlikely, but this area needs further exploration.

P68 - INVESTIGATING CROSS-SPECIES DIFFERENCES OF MDR1-MEDIATED DRUG TRANSPORT

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MDR1, commonly referred to as P-gp, is an efflux transporter expressed in barrier tissues and serves major drug transport functions. It can mediate biliary and renal excretion, and occasionally direct gut secretion. In the gastrointestinal tract, placenta or brain it restricts the distribution of its substrates into the organs. Because of its essential role together with BCRP in preventing drug penetration through the blood-brain barrier (BBB), their function is an essential factor to consider in central nervous system (CNS) drug development. For any new chemical entities (NCE), *in vitro* and *in vivo* preclinical experiments are key to predict human transport processes. Although desirable, it is often difficult to extrapolate *in vivo* preclinical data to human ADME properties. Furthermore, there are numerous *in vitro* models available to predict human BBB penetration, but their predictive potential is also limited. To resolve discrepancies between animal and human data, it is important to create an assay platform where preclinical and human transport properties can be compared. Differences in expression levels and ligand specificities between tissues and species are already well documented for MDR1. Currently, inconsistencies in *in vitro* assay conditions applied by different laboratories cause further challenges in the prediction of substrate specificity and inhibitor profiles across species. To resolve some of these inconsistencies we generated canine ABCB1-knockout MDCKII cell lines stably expressing either the mouse, rat, monkey or human orthologs of MDR1 for transcellular transport assays.
Our first goal was to identify species differences in the substrate specificity and inhibitory effects of known MDR1 interactors. The 16 tested inhibitors comprise a representative sample in terms of ADME properties, and they inhibited MDR1-mediated digoxin and quinidine transport with different potency. The permeability of numerous CNS+ and CNS- drugs and known MDR1 substrates was also determined, and a correlation analysis of efflux results was carried out. It is important to highlight that using these cell lines MDR1 activity from each species can be studied independently, allowing for direct comparisons. Here we demonstrated that these *in vitro* models are applicable for mechanistic analysis of MDR1-mediated transport in early-phase drug discovery. The obtained interaction data could help to improve the prediction of brain penetration and inhibitory potency of NCEs across species.

P69 - ORGANIC ANION TRANSPORTER 2 OVER EXPRESSION STABLE CELL LINE FOR OAT2 SUBSTRATE AND INHIBITOR EVALUATION

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Organic anion transporter 2 (OAT2), a member of the family of organic anion transporters, is mainly expressed in liver and kidney, and mediates the transmembrane transport of endogenous substances such as creatinine and uric acid and a variety of exogenous drugs. It is the drug transporter with the second highest expression in liver. OAT2 has a wide range of substrates that mediate the transmembrane transport of a variety of endogenous and exogenous substances. In order to set-up assays for OAT2 substrate and inhibitor evaluation, an OAT2 over expressing HEK293 cell line was constructed. A total of 11 positive potential clones were screened by western blot, then followed by transport function test by using the following OAT2 substrates: estrone-3-sulfate Sodium Salt (E3S), creatinine, cGMP, penciclovir, nicotinic acid, acyclovir and non-substrates methotrexate. Ketoprofen and rifamycin SV were used as inhibitors. The amount of uptake of test compounds was determined by LC-MS/MS. Among the 11 existing clones, 4 clones showed uptake functionality, and finally one clone (HEK293-OAT2-5D5) with highest uptake rate and highest uptake ratio (OAT2/mock) was selected. The corresponding uptake ratio (without inhibitor) of E3S, creatinine, cGMP, penciclovir, nicotinic acid, acyclovir methotrexate were 99.50, 11122.86, 1035.58, 13719.68, 1743.71, 4757.38, 6.89 pmol/min/mg protein, respectively. The uptake ratio of HEK293-OAT2-5D5 to parental cell line was: 4.08, 12.34, 450.88, 155.5, 82.95, 57.82, 0.5-fold. In the inhibitor group, ketoprofen showed higher inhibition compared with rifamycin SV. These results indicate that the OAT2 over expressing cell line HEK293-OAT2-5D5 has high OAT2 activity suitable for OAT2 substrate and inhibition assays.

P70 – EVALUATING THE ACTIVE CONTRIBUTION TO CREATININE RENAL CLEARANCE IN RENALLY IMPAIRED PATIENTS USING PBPK MODELING

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Glomerular filtration rate (GFR) in physiologically-based population models is usually predicted based on serum creatinine (SCr) and covariates such as age, gender, and weight, assuming that only the passive component contributes to creatinine renal excretion. At the same time, 10-40% of creatinine has been reported to secrete actively (Chu et al 20161, Levey 19882, Breyer & Qi 20103). The purpose of our work was to evaluate the fraction of creatinine which is secreted actively in renally impaired patients using physiologically-based pharmacokinetic (PBPK) modeling.
The creatinine PBPK model was developed in Simcyp Simulator V22 using the mechanistic permeability-limited kidney model (MechKiM) with healthy volunteers as population. Besides the glomerular filtration, the model included active uptake via the organic cation transporter (OCT) 2 (34%) and the organic anion transporter (OAT) 2 (6%), and apical efflux

via the multidrug and toxin extrusion (MATE) 1 transporter. The significant contribution of the OCT2-transporter was verified by simulating the creatinine-drug interaction studies with OCT2 inhibitors such as cimetidine, trimethoprim, pyrimethamine, capmatinib, and fedratinib. The passive and active contribution to creatinine renal clearance was separated as follows. The creatinine synthesis rate was calculated based on creatinine baseline level and removal rate using the verified values in Simcyp population libraries (SCr and GFR). The baseline synthesis rate represented passive contribution only. Next, by using the previously developed creatinine compound file and baseline synthesis rate, we established the additional synthesis rate required to recover the observed values. The additional synthesis rate reflected the active contribution to creatinine renal excretion and was about 40% in healthy volunteers which agrees with literature data. The population specific transporter abundance and activity in renally impaired patients are not implemented in the default PBPK population models due to lack of reliable data, therefore this contribution needed to be determined indirectly. The additional synthesis rate in renally impaired patients was established in similar way to those in healthy volunteers. The calculated values were 72% and 69% of the baseline synthesis rate in mild and moderate renal impairment, respectively. It can be concluded that the active contribution to creatinine renal clearance in mild and moderately renal impaired patients decreases by around 30% compared to healthy subjects which agrees with the study of Han et al., 20224, where the OCT2 downregulation in kidney disease was shown.

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