# 1st Conference of the Society for the Study of Xenobiotics (SSX) India

## Affiliate of the International Society for the Study of Xenobiotics (ISSX)

# SSX-2016

**1st – 3rd September, 2016**
**JN Tata Auditorium, Indian Institute of Science (IISc)**
**Bangalore, India**

**Simcyp (Certara) Workshop, August 31st, 2016**
**Faculty of Pharmacy, M S Ramaiah University of Applied Sciences**
**Bangalore, India**

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# Program at a glance

| Day 1: 01st September 2016 | | |
|---|---|---|
| **Theme: Mechanisms of drug-induced toxicity** | | **Chair: Tom Baillie** |
| **Time** | **Title of Presentation** | ***Speakers, Organization*** |
| **0820-0830** | Opening Remarks | *Murali Subramanian President SSX* |
| **0830-0905** | A new look at target-based toxicity | *Bruce Car Bristol-Myers Squibb* |
| **0905-0940** | Metabolism-based Structure Activity Analysis for Identification of a Clinical Candidate Devoid of Off-Target Hepatotoxicity | *Raju Subramanian Gilead Sciences* |
| **0940-1000** | **Refreshment break; Poster Set-up** | |
| **1000-1050** | Reversible and Irreversible Covalent Drugs: Therapeutic Promise Versus Toxicological Risk | *Tom Baillie University of Washington* |
| **1050-1125** | Unraveling the Mysteries of Predicting Human Pharmacokinetics of Targeted Covalent Inhibitors | *Mike Zientek Takeda Pharmaceuticals* |
| **1125-1135** | Study of Metabolism Mediated Decrease in Hepatotoxicity of a Combination of Paracetamol and Diclofenac | *Oral Presentation by Young scientist: Shristy Tiwari, NIPER Mohali* |
| **1135-1150** | Panel Discussion; Car, Subramanian, Baillie, Zientek | |
| **1150-1205** | The Human Hepatic HepaRG™ Cells and New Readouts for Understanding and Predicting Cholestatic Side Effects of Drugs | *Vendor Talk; Chris Chesne, Biopredic* |
| **1205-1330** | **Lunch and Poster Viewing** | |
| **Theme: Drug disposition and drug interaction** | | **Chair: Peter Fan** |
| **1330-1405** | Predicting Human Clearance of Drugs Predominately Metabolized by Glucuronidation Using Monkey and Human Models | *Mike Sinz Bristol-Myers Squibb* |
| **1405-1440** | Role of IVIVE-Linked PBPK Modelling in DDI Predictions | *Masoud Jamei,Simcyp* |
| **1440-1500** | **Refreshment break; Poster Viewing** | |
| **1500-1550** | Rate-Determining and Rate-Limiting Steps in the Clearance of a Potent and Selective p21-Activated Kinase Inhibitor in Rat | *Peter Fan Genentech* |
| **1550-1625** | Interactions With Vitamin D metabolism: Can they be Translated to Optimize Vitamin D Actions? | *Subrata Deb Roosevelt University* |
| **1625-1700** | Importance of Determining Drug Distribution to Target | *Griff Humphreys Bristol-Myers Squibb* |
| **1700-1710** | Identification and Characterization of Silodosin Metabolites using Ultraperformance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry | *Oral Presentation by Young scientist: Johnsi Rani, NIPER Hyderabad* |
| **1710-1725** | Panel Discussion: Sinz, Jamei, Fan, Deb, Humphreys | |
| **1725-1740** | Small to Large Molecule Met ID - A Pragmatic Approach for Comprehensive Coverage using Novel Workflows | *Vendor Talk Anoop Kumar, Sciex* |

| Day 2: 02nd September 2016 | | |
|---|---|---|
| **Theme: Transporters** | | **Chair: Jash Unadkat& Berend Oosterhuis** |
| **Time** | **Title of Presentation** | ***Speakers, Organization*** |
| **0830-0905** | Why Basolateral Transporters Matter - Their Importance in Predicting Biliary Clearance and Hepatotoxicity | *Kenneth R. Brouwer, Qualyst Transporter Solutions* |
| **0905-0940** | Intrahepatic Drug Exposure: Experimental Determination and Focus on the Role of Transporters | *Pieter Annaert KU Leuven* |
| **0940-1000** | **Refreshment break & Poster Viewing** | |
| **1000-1050** | Transport vs. Metabolism: What Determines the Pharmacokinetics (PK) And Pharmacodynamics (PD) of Drugs? Insights from the Extended Clearance Model | *Jash Unadkat University of Washington* |
| **1050-1125** | Exposure or Safety: Where Do Transporter Evaluations Have the Greater Impact During Drug Development? | *Jasminder Sahi Sanofi* |
| **1125-1135** | Inhibitory Effect of Arjuna Bark Extracts on P-Gp Mediated Efflux | *Oral Presentation by Young scientist: Alice Varghese, NMIMS, Mumbai* |
| **1135-1150** | Panel Discussion: Brouwer, Annaert, Unadkat, Sahi, | |
| **1150-1205** | Novel Tools for Studying Transporter Mediated DDI, Tox and Clearance | *Vendor Talk; Berend Oosterhuis,Solvo* |
| **1205-1330** | **Lunch and Poster Viewing** | |
| **Theme: PK/PD modelling** | | **Chair: Eric Chan** |
| **1330-1405** | Preclinical PK/PD Modelling to Optimise Early Clinical Development Studies | *Sheila Peters Merck Darmstadt* |
| **1405-1440** | Added Value of Combining PBPK and PD Models | *Nikunj Kumar Patel Simcyp* |
| **1440-1500** | **Refreshment break; Poster Viewing** | |
| **1500-1550** | Drug-Drug Interactions Between Dronedarone and Rivaroxaban | *Eric Chan National University of Singapore* |
| **1550-1625** | Changing The Game of Biologics Discovery and Development Using PK-PD | *Punit Marathe Bristol-Myers Squibb* |
| **1625-1635** | PK-PD Modeling of Furosemide in Spontaneously Hypertensive and DOCA-Salt Induced Hypertensive Rats | *Oral Presentation by Young scientist: Mahendra Shukla, CDRI Lucknow* |
| **1635-1650** | Panel Discussion; Peters, Patel, Chan, Marathe | |
| **1700-1830** | **High Tea** | |

| Day 3: 03rd September 2016 | | |
|---|---|---|
| **Theme: Personalized Medicine** | | **Chair: Magnus Ingelman-Sundberg** |
| **Time** | **Title of Presentation** | ***Speakers, Organization*** |
| **0830-0905** | Pharmacometrics and Systems Pharmacology of Immune Checkpoint Inhibitor Nivolumab | *Sujit Nair*<br>*Amrita University* |
| **0905-0940** | Implementation of Genotype Guided Personalized Pharmacotherapy: Status in Korea | *Jae-Gook Shin*<br>*Inje University* |
| **0940-1000** | **Refreshment break** | |
| **1000-1050** | Novel Biomarkers for More Effective Individualized Drug Therapy Tomorrow | *Magnus Ingelman-Sundberg*<br>*Karolinska Institute* |
| **1050-1100** | Repurposing Bioenhanced Solid Dispersion of Tadalafil for Improved Efficacy in Pyelonephritis | *Oral Presentation by Young scientist: Sagar Bachhav, IICT Mumbai* |
| **1100-1115** | Panel Discussion: Nair, Shin, Ingelman-Sundberg | |
| **1115-1130** | | *Vendor Talk; Thermo* |
| **1145-1245** | **Lunch and Poster Viewing** | |
| **Theme: DMPK in drug discovery** | | **Chair: Larry Wienkers** |
| **1245-1315** | Novel Human Hepatocyte and Enterocyte Technologies for the Evaluation Of Human-Specific Drug Properties in Drug Development | *Albert Li*<br>*In Vitro ADMET* |
| **1315-1400** | Biochemical Aspects of Cytochrome P450 Mechanism-Based Inactivation | *Larry Wienkers*<br>*Amgen* |
| **1400-1415** | **Refreshment break** | |
| **1415-1445** | Analysis of the Reproducibility and Predictivity of Immortalized Hepatocyte-like Cells for CYP3A4 Induction and Hepatotoxicity | *Charles Crespi*<br>*Corning Life Sciences* |
| **1445-1515** | In Silico Tools in Understanding Ligand-CYP/UGT/P-Gp Complex Formation at The Atomistic Level | *Abhay Sangamwar*<br>*NIPER* |
| **1515-1530** | Panel Discussion: Li, Wienkers, Crespi, Sangamwar | |
| **1530-1535** | Raffle Award | |
| **1535-1545** | Vote of Thanks | *Jasminder Sahi* |

# Speaker Abstarcts

**Bruce D. Car (PhD)**
Vice President
Pharmaceutical Candidate Optimization (PCO)
& the Bristol-Myers Squibb Biocon Research
Center, India

Bruce Car received Veterinary Medicine (1983) and Masters in Pathology degrees (1985) at The University of Melbourne, Australia, and his Ph.D. degree in 1989 from Cornell University, NY, USA. He attained specialty certification in Anatomic and Clinical Pathology (1986, 90), and later with the American Board of Toxicology (1995), and was elected a Fellow, International College of Toxicologic Pathology (2002). Bruce undertook postdoctoral studies in immunology (1989-1994) at the Theodor Kocher Institute, University of Berne, and ETH/University of Zurich, Switzerland in immunology Bruce has now been with BMS and its legacy companies for 22 years, working across all therapeutics areas. In 2010, Bruce extended previous responsibilities to include Discovery Metabolism, Biotransformation, Pharmacokinetics, Analytical Sciences, Toxicology, and Pharmaceutics as Vice President, PCO. PCO's facilitates the creation of synthetic and biologic drug candidates with optimal developability profiles and shepherds those compounds through development. Bruce now also leads BMS' R&D campus in Bangalore, India (BBRC) and is collectively responsible for over 700 scientists. He is a frequently invited speaker in Translational Research, Drug Developability, Toxicology, and Drug Discovery fields.

**Abstract of the talk**

# Risk Assessment of Target-based Toxicity – scientific absolute or dogma with some wiggle room?

Historically, target-based toxicity identified Discovery Biology work, nonclinical toxicology studies, or clinical trials has led to the termination of approximately 20% of all targets, with the general assumption that full agonism or antagonist is the pharmacologic requirement for efficacy. Altered pharmacology with degrees of partial agonism or antagonism, modification of drug-disposition or peak-trough characteristics through novel delivery systems or other PK modifiers, or consideration of exaggerated preclinical species toxicology through physiologic susceptibility (X-IAP, p38 in dogs vs monkeys) may potentially "rescue" otherwise interesting targets. Poorly understood, pharmacologically promiscuous chemotypes with off-target activities may be mistakenly labeled as target-based, such as the "hepatotoxicity liability" of early p38 kinase inhibitors and Factor Xa inhibitors. This talk illustrates common pitfalls associated with the assessment of data sets relative to target-based toxicity, and provides safe paths for advancing some targets with potentially egregious target toxicity.

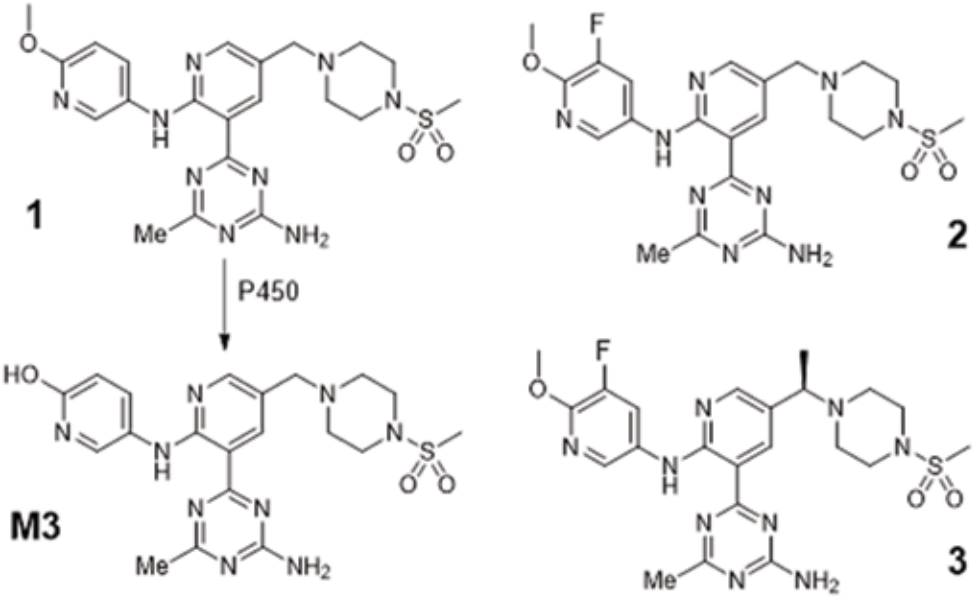
**Raju Subramanian (PhD)**
Director, Drug Metabolism and
Pharmacokinetics, Gilead Sciences,
Foster City, CA 94404, USA.

Raju Subramanian is currently a Director and head of the Pharmacokinetics group in the Department of Drug Metabolism and Pharmacokinetics (DMPK), Gilead Sciences. Raju has over 16 years of experience in DMPK supporting small molecule, peptide and large molecule modalities spanning the discovery and development continuum. He obtained his B. Sc. (Honors), Chemistry, St. Stephen's College, University of Delhi, India 1989; Ph. D., Physical Chemistry, SUNY at Stony Brook, NY 1994; and from 1994-1999 completed post-doctoral training at College of William and Mary (Physics) and University of Illinois at Urbana-Champaign, IL (Electrical and Computer Engineering). Following his post-doctoral training he joined Merck from 1999-2005 where he led a structure elucidation laboratory. He moved to Amgen and over this phase of his career (2005-June 2016), he led a biotransformation/ADME chemistry group and managed broader aspects of ADME science with molecules in discovery through regulatory filing and approval. Externally, Raju has +60 publications, participated in industry consortia and as a speaker, organizer and organizing committees for conferences.

**Abstract of the talk**

# Metabolism-based Structure Activity Analysis for Identification of a Clinical Candidate Devoid of Off-Target Hepatotoxicity

Pyridyltriazine containing compounds **1** and **2** are potent inhibitors of class Ia phosphatidylinositol 3-kinases, a pathway implicated in several human cancers. Rat hepatobiliary and hepatocellular toxicity was identified in exploratory repeat dose toxicity studies with these lead compounds - compound **1** in a 4-day study and compound **2** in a 14-day study. Metabolite(s) were hypothesized to be a contributor to the observed toxicity. Mechanistic studies with compound **1** determined a P450 demethylation metabolite **M3** caused the observed toxicity. Co-administration of the pan-P450 inhibitor 1-aminobenzotriazole (ABT) with **1** to rats significantly reduced the formation of **M3** and prevented liver toxicity, while direct administration of **M3** reproduced the toxicity. Metabolism based structure activity relationship was established in the pyridyltriazine series that reduced this demethylation pathway and alleviated hepatotoxicity and led to nomination of compound **3** as a clinical candidate. The M3 pathway was less significant for compound **3** and liver toxicity was not observed after 14-day treatment with compound **3**.



**Thomas A. Baillie (PhD)**
Professor & Dean Emeritus, Department of Medicinal Chemistry, School of Pharmacy, Box 357610, University of Washington Seattle, WA 98195-78610, USA

Professor Thomas A. Baillie is Dean Emeritus of the School of Pharmacy at the University of Washington in Seattle, WA, where he also served as Vice Provost for Strategic Initiatives. He was born in Scotland and educated at the University of Glasgow, where he earned B.Sc. (Hons) and Ph.D. degrees in Chemistry in 1970 and 1973, respectively. He also holds a M.Sc. degree in Biochemistry from the University of London (1978) and was awarded the degree of D.Sc. in Chemistry from the University of Glasgow in 1992.

Following postdoctoral research at the Karolinska Institute in Stockholm, Sweden (1973-75), Dr. Baillie held successive faculty positions at the University of London (1975-78), University of California San Francisco (1978-81), and University of Washington (1981-94). He then joined Merck Research Laboratories in West Point, PA, where he was Global Vice President of Drug Metabolism & Pharmacokinetics until 2008, at which point he returned to the University of Washington where he served as Dean of the School of Pharmacy until his retirement in 2016.

Dr. Baillie's research interests center on the application of mass spectrometry and allied techniques to mechanistic studies on the metabolism of foreign compounds, with particular emphasis on the generation of chemically-reactive, potentially toxic products of biotransformation. He has co-authored over 200 peer-reviewed publications, serves on the Advisory Boards of a number of journals and academic research centers, and acts as a consultant to several companies in the pharmaceutical and biotechnology industries. Currently, he is President Elect of the International Society for the Study of Xenobiotics (ISSX).

Dr. Baillie was awarded a Fogarty Senior International Fellowship from the NIH in 1988, was the recipient of the James R. Gillette Award from the American Society for Pharmacology & Experimental Therapeutics (2001), and received the Lifetime Achievement Award from the International Isotope Society (2009). In 2010, he was elected as a Fellow of the Royal Society of Chemistry and a Fellow of the Japanese Society for the Study of Xenobiotics. In 2011, Dr. Baillie became a Fellow of the American Chemical Society and, in 2012, he received the Founder's Award from the ACS Division of Chemical Toxicology. Most recently, he was the 2014 recipient of the North American Scientific Achievement Award from ISSX.

**Abstract of the talk**

# Reversible and Irreversible Covalent Drugs: Therapeutic Promise Versus Toxicological Risk

In contrast to the traditional mechanism of drug action that relies on the reversible, non-covalent interaction of a ligand with its biological target, covalent drugs (also referred to as targeted covalent inhibitors) are designed *a priori* such that the initial, reversible association is followed by the formation of a covalent bond between a judiciously positioned electrophile on the ligand and a specific nucleophilic center on the protein. Although this approach offers a variety of potential benefits, including high potency and extended duration of action, concerns over the possible toxicological consequences of protein haptenization have hindered development of the covalent mechanism in drug design. Recently, approaches to mitigate the risk of serious adverse reactions to this new class of agent have emerged, stimulating interest in the field and leading to marketing authorization of the first cadre of covalent drugs for applications in oncology. As a result, this approach is rapidly gaining acceptance as a valuable tool in drug discovery, and is poised to make a major impact on the design of enzyme inhibitors and receptor modulators for a broad range of therapeutic indications. This presentation will provide an overview of the basic concepts behind covalent drug design, and will highlight evolving strategies to minimize the risk of adverse reactions often associated with covalent protein modification.

**Michael A. Zientek (PhD)**
Associate Director,
Takeda Pharmaceuticals, USA

Michael A. Zientek is a U.S. Citizen and received his B.S. from Purdue University and MPH from the University of Michigan. Mike joined Pfizer Worldwide Research and Development as a research scientist in 1994 in a discovery enzymology role, working in the areas of cardiovascular disease and oncology where he assumed responsibilities of developing target related kinetic assays for drug discovery. In 2001, he moved from discovery biology to Pharmacokinetics, Dynamics, and Metabolism (PDM) applying his knowledge of human physiology, enzymology, enzyme kinetics and automation to the drug metabolism and pharmacokinetic disciplines. In 2016, Michael moved to Takeda Pharmaceutical with the title of associate director of DMPK, where he and his group provide mechanistic DMPK guidance to advance molecules through design efforts in discovery, optimizing and predicting human pharmacokinetic properties, preparing molecules for regulatory submission, and also progressing compounds through clinical proof of concept.

Michael's research activities have been and continue to be devoted to investigation of drug metabolizing enzymes, polymorphisms, drug-drug interactions, and human ADME of new and established xenobiotics. He has authored >30 publications in this area ranging from new tools for ADME investigations to characterization of targets therapies.

Michael has served on the steering committee of the Southern California Drug Discussion Group for the past 5 years, is currently an ISSX council member, and has been both Chair and Vice-Chair of the Gordon Research Conference (GRC) on Drug Metabolism. He has been an invited speaker or discussion leader at the GRC, American Association of Pharmaceutical Sciences (AAPS), ISSX, and is a member of several professional societies. Michael has also been a peer reviewer for a number of manuscripts for Drug Metabolism and Disposition, Clinical Pharmacokinetics, Expert Opinion in Drug Metabolism and Toxicology, and many others.

**Abstract of the talk**

# Unraveling the Mysteries of Predicting Human Pharmacokinetics of Targeted Covalent Inhibitors

Understanding the metabolic fate of any molecule is critical for prediction of human pharmacokinetics. Securing this understanding is extremely challenging for target covalent inhibitors since a number of less understood mechanisms of clearance exist for these inherently reactive molecules. These mechanisms consist of both on and off target binding (plasma proteins, hemoglobin, red blood cells), direct glutathione conjugation, and catalyzed glutathione conjugation via the glutathione S-transferase enzyme family of enzymes. During this presentation a number of these mechanisms will be discussed, their impact will be explored, and scaling methods tested via a retrospective analysis of a number of targeted covalent inhibitor using both in vitro and in vivo preclinical species translation to humans. The details of how those retrospective learning's were instituted into a successful targeted covalent inhibitor clinical candidate strategy at Pfizer will be conveyed.

**Chris Chesne (PhD)**
CEO, Biopredic International

Chris is trained as a Pharmacist and worked at Servier, a French mid-sized drug company in the DMPK department in Orleans. I made my PhD funded by Servier and hosted at the Guillouzo's laboratory at Inserm in Rennes, about the cryopreservation of hepatocytes and then he funded Biopredic, first operating as a CRO and now as a research reagent provider.

**Abstract of the talk**

# The human hepatic HepaRGtm cells and new readouts for understanding and predicting cholestatic side effects of drugs

Cholestasis is a common and serious side effects of drugs and is badly predicted with the current preclinical testing. We investigated mechanisms underlying drug-induced cholestasis using human HepaRG cells as an assay system and a set of cholestatic and non-cholestatic drugs. Bile canaliculi (BC) dynamics, Rho/myosin-light-chain (MLC) kinase pathway implication, efflux inhibition of taurocholate (a predominant BSEP substrate), and expression of the major canalicular and basolateral bile acids transporters were analyzed. We demonstrated 12 cholestatic drugs classified on the basis of reported clinical findings caused disturbances of both BC dynamics, characterized by either dilation or constriction, and alteration of the ROCK/MLCK signalling pathway while non cholestatic compounds, by contrast, have no effect. Our results show cholestatic drugs consistantly cause an early alteration of BC dynamics associated with modulation of the Rho/MLC kinases and these changes are more specific than efflux inhibition measurements alone as predictive non-clinical markers of drug-induced cholestasis.

**Michael W. Sinz (PhD)**
Senior Research Fellow,
Bristol Myers Squibb, USA

Michael W. Sinz, is a Senior Research Fellow at Bristol Myers Squibb in the department of Metabolism and Pharmacokinetics in Wallingford, CT where he manages ADME lead optimization of drug discovery assets (2001-present). Dr. Sinz previously held the position of Section Director-Pharmacokinetics and Drug Metabolism for Parke-Davis/Pfizer Global Research and Development (Ann Arbor, MI, 1991-2001). He received a B.S. degree in Chemistry (ACS) and a second B.S. degree in Biology from the University of Wisconsin-Eau Claire and a Ph.D. in Pharmacognosy/Medicinal Chemistry from the University of Minnesota. Dr. Sinz is a member of the International Society for the Study of Xenobiotics and American Chemical Society. He is also a representative on the Drug Metabolism Leadership Group of the IQ Consortium and on the organizing committee for the Land O'Lakes conference on Drug Metabolism and Pharmacokinetics (2004-present). He is editor in chief of Current Drug Metabolism, an associate editor of Drug Metabolism Letters, and on the editorial advisory board of Current Pharmacology Reports. His areas of research include in vitro and in vivo drug metabolism, clearance predictions, reaction phenotyping, and predicting drug-drug interactions. Dr. Sinz's resume includes an extensive number of peer reviewed publications, book chapters, and external presentations both locally and internationally.

**Abstract of the talk**

# Predicting Human Clearance of Drugs Metabolized by Glucuronidation Using Monkey and Human Models

Predicting human drug clearance in drug discovery and development has become common practice in many pharmaceutical companies. The desire to bring forward potential new drugs with low clearance and once-a-day dosing is an advantage for patients. As such, companies generally employ metabolic stability screening of potential new drug candidates as part of their drug discovery process. These metabolic stability assays are typically conducted in human liver microsomes supplemented with NADPH and provide suitable predictions of human drug clearance if the drug is predominately eliminated by CYP450 enzymes. In those situations where the drug is predominately cleared by mechanisms that are not mediated by CYP450 enzymes these predictions can be misleading. After CYP450-mediated oxidation, glucuronidation is the second most common form of drug metabolism and elimination. The models to evaluate and predict human drug clearance of new drug candidates that are predominately eliminated by glucuronidation are different and more complicated than those for CYP450-mediated predictions. The presentation will describe the challenges of predicting human clearance for drugs eliminated by glucuronidation, as well as the predictability of various models including both in vitro and in vivo, for example, the monkey.

**Masoud Jamei (PhD)**
Vice President,
Research and Development,
Simcyp/ Certara, UK

Masoud is the Vice President of Research and Development at Simcyp (a Certara company) where he works with a team of around 35 scientists and 15 developers focusing on the design, development and implementation of various aspects of systems pharmacology models including in vitro-in vivo extrapolation techniques, physiologically-based PK/PD models of small and large molecules and applying top-down PopPK data analysis to PBPK models in healthy volunteer and patient populations. He has been the author or co-author of over 50 manuscripts and book chapters and 110 abstracts in the field of modelling and biosimulation. He has also been an invited speaker and a session organiser/moderator at national and international meetings and also leads well-known Simcyp hands-on workshops on model-based drugs development.

He currently serves as a Vice-Chair of the Special Interest Group (SIG) on PK/PD and Systems Pharmacology of the Board of Pharmaceutical Sciences (BPS) of International Pharmaceutical Federation's (FIP) and is the Chair of the AAPS Systems Pharmacology Focus Group. In 2002 he earned a PhD in Control Systems Engineering at the University of Sheffield, UK, and carried out one year of post-doctoral research there. In 2003 he joined Simcyp

**Abstract of the talk**

# Role of IVIVE-Linked PBPK Modelling in DDI Prediction

Drug-drug interaction (DDI) remains an important issue in clinical practice and during the discovery and development of new drugs; therefore prediction of DDI risks and its major covariates is of high interest to drug developers, practitioners and regulators. In vitro in vivo extrapolation (IVIVE) a 'bottom-up' approach, in conjunction with physiologically based pharmacokinetic (PBPK) modeling in a systems pharmacology context can help to characterize potential interactions in various populations. The development of IVIVE approach has accelerated mainly due to an increase in the understanding of the multiple mechanisms involved in pharmacokinetics, DDI and the availability of appropriate in vitro systems that act as surrogates for delineating various elements of the interactions relevant to absorption, distribution, metabolism and elimination.

Over the past decade, there has been growing interest in the utility of PBPK modeling during drug development and as a result the number of submissions to regulatory bodies including PBPK modeling has rapidly been growing. Based on the PBPK review knowledgebase of the Office of Clinical Pharmacology (of FDA), there are 180 records between 2008 and 2015 addressing various clinical pharmacology issues. Of these, around 66% were related to the prediction of DDIs.

In this presentation it is intended to discuss recent advancements in application of IVIVE linked PBPK models in prediction of the potential DDIs in populations, including those who cannot be investigated in formal clinical trials for ethical reasons. In addition, the issues related to the prediction of complex DDIs involving both metabolism- and transporter-based DDIs.

**Peter W. Fan (PhD)**
Group leader,
Genentech, USA

Dr. Peter W. Fan is currently the Drug Discovery Metabolite Identification Group Leader at Genentech, a member of the Roche group, in South San Francisco, CA. Prior to that, he was a Senior Scientist in the Departmentof Pharmacokinetics and Drug Metabolism at Amgen. He received his Ph.D. in Chemical Toxicology from the Department of Medicinal Chemistry at the University of Illinois, Chicago, IL in 2000under the tutelage of Dr. Judy Bolton and was a Postdoctoral Fellow at Pharmaciain Kalamazoo, Michigan under Dr. Jeff Stevens. He has over 10 years of drug discovery experience supporting small molecule, therapeutic peptide and antibody-drug conjugate programs. His current research interests include permeability-transporter-metabolism interplay, IVIVC, and drug metabolism mechanistic studies. Peter has also produced a patent, published over 25peer-reviewed papers and is a recipient of numerous awards from Amgen, Genentech and national conferences to date.

**Abstract of the talk**

# Rate-determining and rate-limiting steps in the clearance of a potent and selective p21-activated kinase inhibitor in rat

We have developed a systematic approach in the form of a decision tree to help drug discovery scientists understandlack of *in vitro-in vivo*correlation (IVIVC) in clearance prediction by incorporating apparent permeability data generated from Mardin-Darby Canine Kidney (MDCK) cells and hepatocyte uptake data for discovery compounds. A new classification system is also introduced that can categorize and simplify the complex permeability, transport and metabolism interplay in the liver.To further illustrate this concept, a case study of a potent p21-activated kinase (PAK1) inhibitor (GNE1) is presented here. It was rapidly cleared from systemic circulation (CL = 216 mL/min/kg) in rat but its rate of elimination from whole body was a much slower process. After intravenous dose, the rate-determining step in clearance was found to be mediated by active uptake transporter, Organic cation transporter 1 (Oct1). Biliary and renal clearance of GNE1 only accounted for approximately 14 and 16% of the total clearance, respectively. Major metabolic pathway through *N*-acetylation only accounted for about 10% of the total dose. In non-cannulated rats, majority of the dose was recovered in feces as unchanged parent compound (up to 91%) overnight suggesting intestine is the major organ responsible for the elimination of GNE1 in rat.

**Subrata Deb (PhD)**
Assistant Professor,
College of Pharmacy at
Roosevelt University, USA.

Dr. Subrata Deb received his Ph.D. from The University of British Columbia, Canada, in 2009 with cytochrome P450 (CYP) enzymes as the main focus. He identified a novel CYP enzyme, named as CYP2S1, in his Ph.D. thesis work. Subsequently, his translational research in Vancouver Prostate Centre at Vancouver General Hospital highlighted the effects of drug metabolism and pharmacokinetics on prostate cancer drug efficacy and adverse effects. Dr. Deb received his M.Pharm from the Bombay College of Pharmacy, India. Currently, Dr. Deb is an Assistant Professor in the Department of Biopharmaceutical Sciences, Roosevelt University College of Pharmacy in Greater Chicago, Illinois, USA. He has published fourteenresearch papersand has more than fiftyinvited talks, conference presentations and proceedings tohis credit. Dr. Deb currently servesas apeer reviewer for multiple journals of repute including Journal of Pharmacology and Experimental Therapeutics, Biopharmaceutics & Drug Disposition, and Expert Opinion on Drug Metabolismand Toxicology, and also serves on journal Editorial boards. His areas of research expertise and interests include cytochrome P450-mediated drug interactions, pharmacogenomics, and vitamin D metabolism in prostate and breast cancers.

**Abstract of the talk**

# Interactions with vitamin D metabolism: Can they be translated to optimize vitamin D actions?

The chemopreventive and therapeutic effects of vitamin $D_3$ are exerted through 1α,25$(OH)_2D_3$, the dihydroxy metabolite of vitamin $D_3$. Inactivation of 1α,25$(OH)_2D_3$ by cytochrome P450 3A (CYP3A) enzymes can be an important determinant of its serum and tissue levels. The purpose of the present study was to assess the potential of various cancer medications (e.g. abiraterone, ketoconazole, tamoxifen, taxanes), premedications (e.g. dexamethasone, prednisone) and ginsenoside health supplements, on biotransformation of 1α,25$(OH)_2D_3$ in human and mouse liver. The ability of ginsenosides to boost vitamin D actions in prostate cancer was evaluated in vitro and in xenograft models. In vitro metabolism reactions used mouse or human hepatic microsomal protein or human recombinant CYP3A4 supersomes, various concentrations of drugs or ginsenosides, NADPH and a fixed concentration of 1α,25$(OH)_2D_3$. Formation of hydroxylated metabolites of 1α,25$(OH)_2D_3$ were analyzed by liquid chromatography-mass spectrometry method. The pharmacokinetic and pharmacodynamic implications of ginsenoside-mediated inhibition of 1α,25$(OH)_2D_3$ was investigated using experimental models of human prostate cancer. The formation of hydroxy metabolites was significantly stimulated in hepatic microsomes from mice treated with dexamethasone compared to vehicle- and prednisone-treated group. Co-incubation of 1α,25$(OH)_2D_3$ with various drugs or ginsenosides led to up to ~85-99% inhibition of formation of hydroxylated metabolites of 1α,25$(OH)_2D_3$ and therefore reduced inactivation of active vitamin $D_3$. Ginsenosides were able to boost 1α,25$(OH)_2D_3$ tissue and plasma levels and increased anticancer effects were observed in prostate cancer models. In summary, our results suggest that anticancer medications and health supplements can either exacerbate or inhibit the CYP3A-mediated inactivation of active vitamin $D_3$ leading to altered vitamin D homeostasis. The inhibitory effects of ginsenosides on 1α,25$(OH)_2D_3$ metabolism can potentially enhance the anticancer actions of vitamin D.

**Griff Humphreys (PhD)**
Executive Director,
Bristol Myers Squibb, USA.

Griff Humphreys is currently the Executive Director of the Biotransformation Department at Bristol-Myers Squibb. He received his graduate training at the University of Virginia in chemistry and completed a post-doctoral fellowship at Vanderbilt University in the Center in Molecular Toxicology. He then joined Bristol-Myers Squibb and has been with the company for 23 years. He oversees a group responsible for drug metabolism, disposition and metabolite identification studies during the candidate optimization and drug development phases. His interests include the consequences of reactive metabolite formation, development of new analytical methodologies for metabolite detection, reaction phenotyping of CYP and UGT catalyzed biotransformations, predictive metabolism and toxicology models, *in vitro-in vivo* correlations, and strategies for candidate optimization.

**Abstract of the talk**

# Importance of determining drug distribution to target

The risk of failure of a new drug during late stage development can be greatly reduced when evidence is available that the drug is distributing to the target site of action. There is further reduction of risk if it can be shown that the drug is also engaging the target with anticipated binding kinetics. Because of this, there is considerable effort throughout Pharmaceutical R&D organizations to measure these properties directly and/or to gain confidence in surrogate markers. A number of different techniques can be used to measure drug levels in target tissue with each providing different types of information as well as differing in how well they can be translated from preclinical models to the clinical setting.

The most straightforward method is to use targeted analysis via LC-MS. On the other hand, Quantitative Whole Body Autoradiography (QWBA) or Positron Emission Tomography (PET) using radiolabeled versions of a new drug are techniques that can provide detailed information on compound distribution to target and non-target tissues in preclinical species. This information can directly inform questions regarding a compound's efficacy in disease models as well as inform findings in toxicology studies. Other techniques, such as imaging mass spectrometry, can aid in the determination of the molecular level profile of species present. This presentation will detail applications and future development of these technologies as part of efforts to understand drug disposition.

**Kenneth R. Brouwer (PhD)**
Chief Scientific Officer,
Qualyst Transporter Solutions, USA

Dr. Brouwer is CSO at QualystTransporter Solutions, whichprovides solutions to transporter questions that arise during drug discovery and development in the areas ofhepatic drug transport, drug interactions, transporter regulation and hepatotoxicity. Prior to this Dr. Brouwer served as Executive Director, DMPK – PPD Discovery and Director PreClinical Development – GSK. Dr. Brouwer has over 60 publications in peer reviewed journals, and is the holder of 3 patents. Dr. Brouwer serves on the Editorial Advisory Board for the Journal of Pharmaceutical Sciences and the Applied In Vitro Toxicology journal, and is a reviewer for several additional journals. Dr. Brouwer is an adjunct faculty member in the Division of Molecular Pharmaceutics at the School of Pharmacy, University of North Carolina.

**Abstract of the talk**

# Why Basolateral Transporters Matter - Their Importance in Predicting Biliary Clearance and Hepatotoxicity

Bile acid homeostasis in the liver is tightly regulated through various pathways including synthesis, metabolism, and multiple transport proteins that remove bile acids from the blood and excrete them either into the bile by canalicular efflux transportersor back into the blood via basolateral efflux transporters. Most drugs that inhibit the efflux of bile acids can also inhibit their uptake. Many studies have identified the potential of compounds to alter the hepatobiliary disposition of bile acids through acute inhibition of their hepatic uptake and/or efflux. However, the effects of chronic exposure on transporter function have not been evaluated. The relative extent of inhibition of *both* uptake and efflux (basolateral and canalicular) determines the net effect on the biliary clearance and intracellular accumulation of bile acids. A potential inhibitor's intracellular concentration is also important since it determines the extent of transport inhibition and drives toxicity likely by elevating intracellular concentrations of bile acids. Regulation of bile acid synthesis and its role in bile acid homeostasis has been well defined; however, regulation of bile acid transport has not. We evaluated the hepatobiliary disposition of a model bile acid ($d_8$-taurocholate) and expression of bile acid synthetic enzymes, endogenous bile acids, transport proteins and regulatory factors following chronic exposure to obeticholic acid and chenodeoxycholic acid to determine the role of bile acid transport (uptake, basolateral and canalicular efflux) in bile acid homeostasis.

**Pieter Annaert (PhD)**
Professor,
Pharmaceutical Sciences,
KU Leuven, Belgium

Pieter Annaert is Professor at the department of Pharmaceutical and Pharmacological Sciences of the KULeuven (University of Leuven, Belgium). His research in the field of mechanistic pharmacokinetics focuses on hepatobiliary drug disposition, drug transporters, drug interactions, drug-induced hepatotoxicity (aka drug induced liver injury, DILI), pediatric drug development and physiologically-based pharmacokinetic (PBPK) modeling. In addition, there is special research interest in pharmacokinetic boosting, antiviral drug disposition, optimization of hepatocyte culture conditions and bioanalytical method development.

After graduation as a Pharmacist in 1994 (KULeuven), Dr. Annaert conducted doctoral research in the field of intestinal drug absorption of antiviral agents to obtain his PhD from the same university in 1998. After a 2-year postdoctoral fellowship (1999-2000) at the division of Drug Delivery and Disposition, School of Pharmacy, University of North Carolina at Chapel Hill, he held the positions of Scientist and Senior Scientist (2001-2005) at the Department of Preclinical Pharmacokinetics of Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica NV (Beerse, Belgium). Subsequently, Prof. Annaert joined the Drug Delivery and Disposition lab, at the department of Pharmaceutical and Pharmacological Sciences of KU Leuven in October 2005, where he is currently supervising a group of 4 PhD students, while involved as co-supervisor for another 4 PhD projects. In the past 10 years, he has been promotor or co-promotor of more 12 PhD researchers.Prof. Annaert has co-authored more than 100research articles and several book chapters and currently serves on the editorial boards of Drug Metabolism and Disposition and Current Therapeutic research. For many years, Prof. Annaert has been teaching courses on pharmacokinetics, drug development and drug compounding at the Bachelor, Master and PhD level. Since 2014, he is program director for the Bachelor and Master programs in Pharmaceutical Sciences at KU Leuven.He is also member of the board of the Faculty of Pharmaceutical Sciences, coordinator of the KU Leuven doctoral school of Biomedical Sciences for the Drug Design and Development theme, and chairing about 10 PhD defense committees every year.

**Abstract of the talk**

# Intrahepatic drug exposure: experimental determination and focus on the role of transporters

With the liver being a major site of drug action, toxicity and disposition, it plays a pivotal role in pharmacodynamics, drug safety and pharmacokinetics. The latter also implies its major influence on systemic drug concentrations. However, to achieve adequate and quantitative understanding of the hepatic drug disposition processes, accurate knowledge of unbound hepatic drug concentrations is critical. Although various approaches for measuring intra-hepatocyte drug levels have been proposed and applied in isolated studies, the various methods frequently suffer from disadvantages. This leads to uncertainty regarding the reliability of the data and necessitates more thorough cross-validation with other methodologies. We have recently optimized and applied two different methods for experimental determination of intracellular unbound concentrations of ritonavir, verapamil and atazanavir. The first method (for ritonavir) essentially relies on *in vitro* experiments with suspended rat and human hepatocytes pre-treated with high concentrations of ritonavir to auto-inhibit cellular disposition processes such as metabolism and transporter-mediated membrane passage. The other method was applied to verapamil and atazanavir and relies on concurrent *in vitro* metabolism experiments in hepatocytes and liver microsomes to indirectly estimate unbound intracellular concentrations. The presentation will further illustrate the utility of knowledge of intrahepatic drug exposure for a better understanding of drug clearance mechanisms. Finally, these mechanistic conclusions will be compared with results obtained in PBPK-based modelling of HIV protease inhibitors, confirming the important role of transporters in the hepatic disposition of these anti-retroviral drugs.

**Jash Unadkat (PhD)**
Professor,
University of Washington, USA

Jashvant (Jash) Unadkat, Ph.D. is a Professor of Pharmaceutics in the School of Pharmacy at the University of Washington, Seattle. He received his Bachelor's degree in Pharmacy (B.Pharm.) from the University of London (1977), his Ph.D. from the University of Manchester (1982; advisor Prof. Malcolm Rowland) and his postdoctoral training at the University of California at San Francisco (1982-85; advisor Dr. Lewis Sheiner). Dr. Unadkat's research interests are focused on elucidating the mechanisms of transport and metabolism of HIV and related drugs. In particular his laboratory has been interested in metabolism and transport of drugs during pregnancy, and transport of drugs across the placental, hepatic, intestinal and blood-brain barrier. Dr. Unadkat has published more than 180 peer-reviewed research papers. He is a fellow of AAAS, AAPS, JSSX, and the founding co-chair (1999-2001) of the focus group of AAPS on Drug Transport and Uptake. Dr. Unadkat received the AAPS Research Achievement Award in 2012. Dr. Unadkat created and leads the UW Research Affiliates Program on Transporters (UWRAPT), a cooperative effort between the UW School of Pharmacy and pharmaceutical companies. He also leads UWPKDAP, a NIDA funded program project grant on drug disposition during pregnancy. Dr. Unadkat has been an Associate Editor for the Journal of Pharmaceutical Sciences, an Editor of *AAPS Journal*, and a member of the NIH Pharmacology study section (2000-3). Dr. Unadkat is currently on the editorial board of J. Pharm. Sci. and the *AAPS Journal*. Dr. Unadkat has organized or co-organized numerous national and international conferences on the role of transporters and pregnancy in disposition of drugs.

**Abstract of the talk**

# Transport vs. metabolism: what determines the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs? Insights from the extended clearance model

Transporters are increasingly recognized as playing an important role in drug disposition. However, many drugs that are transported are also metabolized. Therefore a key question is: when will transport, metabolism or both determine the pharmacokinetics (PK) of drugs? Likewise, when will transport, metabolism or both affect tissue concentration and therefore the pharmacodynamics (PD) of drugs? These questions can be answered when transporters are included in our usual clearance models. Therefore, we have expanded the well-stirred hepatic clearance model (WSHM) to include drug transporters to create the extended clearance model (ECM). In my presentation, I will show how the ECM brings clarity to the questions posed above.

**Jasminder Sahi (PhD)**
Senior Director,
DSAR AP, Sanofi, China

JasminderSahi obtained a Ph.D. degree (Pharmacognosy, 1991) from Panjab University, India and then participated in a post-doctoral program in the Department of Physiology and Biophysics, University of Illinois at Chicago. She subsequently started her career in the Pharmaceutical Industry in the Department of Pharmacokinetics and Drug Metabolism (Parke Davis R&D), which was eventually acquired by Pfizer Global Research and Development. In 2006, she joined a new venture (CellzDirect) as the Vice President R&D. In 2012 she moved to Shanghai China, as Head of DMPK for GlaxoSmithKline Research and Development and in 2015 made the transition to Sanofi R&D leading the DMPK and Toxicology efforts in the Asia Pacific region. Jasminder's research focus is on transporters and induction of drug metabolizing enzymes, using primary hepatocytes. While her initial forays into transporter research involved ion transport to understand CFTR, at Pfizer she changed her focus to xenobiotic transporters, with the goal of improving drug disposition, delivery and safety. She has worked extensively with hepatic and renal transporters as well as the very challenging blood-brain-barrier. She is an elected member of the ISSX council, a reviewer for six journals and has authored over 40 peer-reviewed publications.

**Abstract of the talk**

# Exposure or Safety: Where do Transporter evaluations have the greater impact during drug development?

The contribution of drug transporters to the disposition and safety of small molecule therapeuticsis typically evaluated prior to taking drug candidates into the clinic. The preclinical studies are designed to help predict drug exposure (particular in organs such as the brain), drug-drug interactions (in the intestine, liver or kidneys) and potential toxicities (e.g. systemic, liver, kidneys). Since most drugs are designed to be lipophilic, diffusion into cells contributes more to exposure than active uptake by transporters ( lipophilic statins such as cerivastatin), although volume of distribution can be affected by both efflux (digoxin/P-gp) and uptake (atorvastatin/OATP1A2) transporters. The contribution of uptake transporters to tissue exposure may be more relevant in organs such as the brain, where additional physiological factors such as limited endocytosis and highly expressed tight junctions and efflux transporters limit uptake across the blood-brain barrier (zolmitriptan). Drug-drug interactions can affect both the disposition and safety profile of drug candidates, as these could limit or enhance exposure of the drug or of endogenous substrates (e.g. bile salts or bilirubin) to an organ, or into systemic circulation.Transporters are implicated in drug-induced liver injury (bosentan/BSEP in cholestasis), renal toxicities (cisplatin/MATE), disease states such as Dubin Johnson syndrome (MRP2) and in toxic drug interactions with narrow therapeutic index drugs (metformin/OCT1). The affinity of a drug to a transporter, the spatial distribution and membrane localization all contribute to this transporter-drug interplay. It is notalways easy to deconvolute the contribution of specific transporter(s) and drug metabolizing enzymes to the disposition, drug–drug interactions and toxicities of xenobiotics, as both can modulate the intracellular concentrationsof compounds, potentially altering cellular physiology, leading to adverse events.So where do transporters play a greater role – disposition or toxicity?

**Sheila Peters (PhD)**
Head, Translational Quantitative
Pharmacology, Merck Serono, Germany

Dr. Sheila Annie Petersheads the Translational Quantitative Pharmacology group at Merck Darmstadt. Her areas of expertise in DMPK includePhysiologically-based pharmacokinetic (PBPK)-Phamacodynamics(PD) modeling, and PK/PDand drug-drug interactions.In her previous position at AstraZeneca, Mölndal, shedeveloped a generic whole-body PBPK model in MATLAB® and has supported several drug discovery and early development projects across different R&D sites with innovative approaches to identifying potential limitations to drug disposition. She led the development of a pulmonary PBPK model for inhaled drugs incollaboration with Pharmaceutical Development at AstraZeneca. The work won the 2013 IMED (Innovative Medicines) Science Award at AstraZeneca for the “Design and Development of LungSim Simulation tool for Inhalation PK Modelling”.She successfully implemented Model-based drug discovery (MBDDx) strategy in RIA IMedat AstraZeneca by engaging leaders across different functions (DMPK, Bioscience, Translational Science and Clinical Pharmacology). Apart from publishing several papers in high impact journals, she also has published a book on PBPK in 2012 and co-authored a White Paper on PBPK along with other experts in industry.

**Abstract of the talk**

# Preclinical PK/PD modelling to optimize early clinical studies

Neutrophil serine proteases (NSPs) are activated by dipeptidyl peptidase 1 (DPP1) during neutrophil maturation. The effects of neutrophil turnover rate on NSP activity following DPP1 inhibition was studied in a rat pharmacokinetic/pharmacodynamics model.

Rats were treated with a DPP1 inhibitor twice daily for up to 14 days; NSP activity was measured in onset or recovery studies, and an indirect response model was fitted to the data to estimate the turnover rate of the response.

Maximum NSP inhibition was achieved after 8 days of treatment and a reduction of around 75% NSP activity was achieved at 75% in vitro DPP1 inhibition. Both the rate of inhibition and recovery of NSP activity were consistent with a neutrophil turnover rate of between 4–6 days. Using human neutrophil turnover rate, it is predicted that maximum NSP inhibition following DPP1 inhibition takes around 20 days in human.

Following inhibition of DPP1 in the rat, the NSP activity was determined by the amount of DPP1 inhibition and the turnover of neutrophils and is thus supportive of the role of neutrophil maturation in the activation of NSPs. Clinical trials to monitor the effect of a DPP1 inhibitor on NSPs should take into account the delay in maximal response on the one hand as well as the potential delay in a return to baseline NSP levels following cessation of treatment.

**Nikunj Kumar Patel**
Senior Scientist,
Simcyp, UK

Nikunj is a senior research scientist in modelling and simulations group at Simcyp. He joined Simcyp in August 2011 and worked on the development of the physiologically based - IVIVC (PB-IVIVC) module of Simcyp simulator and Pharmaceutics module of SIVA (Simcyp in Vitro (data) Analysis) platform. He is pursuing his PhD at the Jagiellonian University Medical College, Cracow with Dr Sebastian Polak and Dr. Barbara Wisniowska. He is currently project lead for the oral and dermal absorption projects at Simcyp and is member of the Cardiac Safety Simulator development team. Before joining Simcyp he spent three years at life science innovation labs of Tata Consultancy Services as a research scientist mainly working on pharmacokinetic/pharmacodynamic modelling and QSAR development for various ADMET properties. During academics he worked on using the computer aided drug design (CADD) and molecular modelling towards identifying safe and potent novel lead molecules.

**Abstract of the talk**

# Added Value of Combining PBPK and PD Models

Physiologically-based pharmacokinetic (PBPK) modeling has gained lot of attention and utilization in drug discovery and development over the last decade to predict pharmacokinetics and simulate population variability. It has been transformed from a research tool to mature technique that is incorporated within regulatory submissions and have already been shown to reduce and refine clinical trials in areas such as drug-drug interactions and special populations along with oral drug absorption predictions. However, the utilization of Physilogically based modelling is not limited to PK, it has a strong potential to simulation pharmacodynamic response including drug concentration at site of action and propagation of PK variability to PD level. Several case studies of PBPKPD modelling to simulate therapeutic response, adverse effects and virtual bio-equivalence will be discussed in this presentation.

**Eric Chun Yong CHAN**
Assistant Professor,
Department of Pharmacy,
National University of Singapore, Singapore

Eric Chan is a pharmaceutical scientist and an associate professor in the Department of Pharmacy, National University of Singapore (NUS). Eric's main research interests are (1) metabolism-driven systems biology modeling of diseases, pharmacology and toxicology and (2) xenobiotic-derived reactive metabolite research with specific focus on interaction with CYP450 enzymes, metabolic pathway regulation, protein post-translational modification and physiological-based pharmacokinetics (PBPK) modeling of drug-drug interactions.

Eric has been appointed the Dean's Chair Professorship in the Faculty of Science from 2015-2018. He was the Assistant Dean (Undergraduate Programmes) from 2011-2013 and Vice Dean (Undergraduate Studies and Student Life) from 2013-2014. He is an editorial board member of Journal of Chromatography B (Elsevier) and Journal of Proteome Research (ACS) and the grant review board member and referee panel for the Health and Medical Research Fund (HMRF) in Hong Kong. He is also the Scientific Affairs Committee member of the International Society for the Study of Xenobiotics (ISSX) and a member of the American Society for Pharmacology and Experimental Therapeutics (ASPET). Eric is a registered pharmacist under the Singapore Pharmacy Council, Ministry of Health.

Prior to joining NUS, Eric worked as Senior Manager (Market Development - Pharmaceutical and Life Sciences) at Waters Corporation and Group Leader (Analytics) at S*BIO (a pharmaceutical joint venture between the Singapore Economic Development Board and Chiron Corporation at Emeryville, California [currently Novartis]).

**Abstract of the talk**

# Drug-drug interactions between dronedarone and rivaroxaban

Physiologically-based pharmacokinetic (PBPK) modeling has gained lot of attention and utilisation in drug discovery and development over the last decade to predict pharmacokinetics and simulate population variability. It has been transformed from a research tool to mature technique that is incorporated within regulatory submissions and have already been shown to reduce and refine clinical trials in areas such as drug-drug interactions and special populations along with oral drug absorption predictions. However, the utilisation of Physilogically based modelling is not limited to PK, it has a strong potential to simulation pharmacodynamic response including drug concentration at site of action and propagation of PK variability to PD level. Several case studies of PBPKPD modelling to simulate therapeutic response, adverse effects and virtual bio-equivalence will be discussed in this presentation.

**Punit Marathe (PhD)**
Executive Director,
Bristol-Myers Squibb, USA

Punit Marathe is an Executive Director and head of Metabolism and Pharmacokinetics Department at Bristol-Myers Squibb. Dr. Marathe has extensive experience in multiple areas of Drug Metabolism and Pharmacokinetics including drug discovery, lead optimization, preclinical and clinical pharmacokinetic studies leading to product registration and life-cycle management. In her current capacity she oversees characterization of drug disposition in the drug discovery stage at four Bristol-Myers Squibb sites.

Born and brought up in Mumbai, Punit received her B.S. degree from Bombay College of Pharmacy before departing to US for higher education. Dr. Marathe received her Ph.D. degree in Pharmacokinetics from the Department of Pharmaceutics at the University of Washington followed by a postdoctoral fellowship in the Department of Medicinal Chemistry at the same university. She subsequently joined Bristol-Myers Squibb Co. where she held positions of increasing responsibility in both drug discovery and clinical development. Her current responsibilities include collaborating with drug discovery for selection, optimization and characterization of lead candidates for development. Her group conducts pharmacokinetic and metabolism studies from hit identification till nomination of the lead candidate for development. The group is also responsible for establishing PK-PD relationship for lead molecules in various disease states; projecting human pharmacokinetic profile and efficacious dose for small molecules as well as therapeutic proteins and guiding selection of doses for FIH studies.

Dr. Marathe has worked to establish a greater scientific (PK-PD-ADME) presence in India and has been involved in the co-organization of various research-oriented meetings that have fostered partnerships with India-based faculty at different research institutions. She has played a critical leadership role in the optimization of nonclinical PK study cycle times and implementation of global sourcing in support of nonclinical PK screening. Her research interests include understanding pharmacokinetic-pharmacodynamic relationships in nonclinical animal models, translation to humans and prediction of human efficacious doses.Dr. Marathe has published over 90 publications and has presented at multiple national and international conferences.

**Abstract of the talk**

# Changing the game of biologics discovery and development using PK-PD

In recent years, the role of DMPK scientists has become more complex. With increasing focus on translational medicine, we are now required to understand PK-PD relationships at all stages of drug development in order to make informed decisions. Biologics require special PK-PD considerations in addition to small molecules. These considerations can be rationalized based on key factors such as cost of goods, limitations on dose that can be formulated in a subcutaneous injection, differences in species cross-reactivity and target-mediated drug disposition. In order to understand influence of the target on the biologic as well as influence of the biologic on the target, “fit-for-purpose” bioanalytical assays have to be developed depending on the questions at each stage of biologics development. The talk will highlight several case studies including importance of “free target”, “free drug” and “intact drug” assays for establishing PK-PD relationships. Integration of all the in vitro and in vivo data across species enables translational PK-PD models for clinical dose selection and illustrates how the game of biologics discovery and development is being transformed using PK-PD.

**Sujit Nair,**
Professor,
Pharmaceutical Sciences,
Amrita University

Dr. Sujit Nair is a Professor of Pharmaceutical Sciences and Director of the Cancer Discovery Biology Laboratory at Amrita University, India. He is a member of the International Expert Panel of the National Medical Research Council, Ministry of Health, Government of Singapore. He also serves as Associate Editor of Drug Metabolism and Personalized Therapy (DMPT; de Gruyter, Germany) which is the official journal of the European Society of Pharmacogenomics and Personalized Therapy (ESPT). In addition, he ison the Editorial Board of Current Pharmacology Reports (Springer, USA) and Advances in Modern Oncology Research (Pisco Med, Singapore). He also volunteers as a peer reviewer for several international journals. Prior to joining Amrita University, Sujit trained at the Ernest Mario School of Pharmacy and Center for Cancer Research at Rutgers, The State University of New Jersey, USA. He has published over 25 manuscripts in peer-reviewed international journals (h-index 19). His current research interests include pharmacometrics, pharmacogenomics and systems pharmacology in discovery research and personalized medicine.

**Abstract of the talk**

# Pharmacometrics and systems pharmacology of immune checkpoint inhibitor nivolumab

Nivolumab, a fully human immunoglobulin G4 monoclonal antibody (mAb) that targets the programmed death-1 (PD-1) inhibitory receptor expressed on lymphocytes and dendritic cells, has been approved for metastatic melanoma, advanced squamous non-small cell lung cancer (NSCLC) and metastatic renal cell carcinoma. Key pharmacometric variables in anticancer efficacy of nivolumab such as target engagement, metabolism, systems pharmacology and clearance will be elucidated. Since ligand PD-L1 is a weak biomarker in clinical practice, appropriate patient selection methods including immunopharmacogenomics may be used to identify those patients who are most like to benefit from anti-PD-1 therapy. Indeed, the way forward to leverage maximum benefits for the cancer patient may be to synergize anti-PD-1 blockade with complementary targets in immune checkpoint pathways or other oncogenic signal transduction pathways. Of necessity, the burden of "financial toxicity" on cancer patients and families must be factored in considering nivolumab therapy. Taken together, the potential success of nivolumab strengthens the case for accelerated development of immunopharmaceuticals in oncology, and potentially non-oncology indications, by stakeholders such as clinical oncologists, pharmacists, and basic drug discovery scientists in academia and the pharmaceutical industry in a concerted fashion.

**Jae-Gook Shin**, MD, PhD
Professor of Pharmacology,
Inje University, Busan,
South Korea

Dr. Jae-Gook Shin is currently a Professor and Chair of Pharmacology and Clinical Pharmacology and Director of the Pharmacogenomics Research Center at Inje University College of Medicine, Busan, Korea. He is also the Director of the Global Center of Excellence in Clinical Trials at Inje University Busan Paik Hospital. Dr. Shin is currently serving as the Chair of the Board of Directors, of the Korean Society for Clinical Pharmacology and Therapeutics (KSCPT).

Dr. Shin received his Ph.D. in Pharmacology from Seoul National University School of Medicine in 1992, and MD degree from Inje University College of Medicine in 1986. Dr. Shin completed 2 years Clinical Pharmacology Postdoctoral training in the Division of Clinical Pharmacology at Georgetown University Medical Center, in Washington DC as a 1997 recipient of the Merck Sharp &Dohme International Fellowship in Clinical Pharmacology funded by the Merck Foundation.

Dr. Shin founded (2003) and directs the Pharmacogenomics Research Center at Inje University College of Medicine. Dr. Shin also founded (2007) and directs the Bio-Marker Research Center for Personalized Therapy (BMRC). Dr. Shin has received several awards including the Korean Federation of Science and Technology Societies' Outstanding Research in Science and Technology Award, and the Korean Society of Medical Science's 7th Pfizer Medical Research.

Dr. Shin has published over 275 papers in clinical pharmacology including Pharmacogenomics and personalized medicine, clinical PK/PD, DM/PK and drug interaction, PK/PD modeling, and other clinical pharmacology areas. He has served as an editorial board member for several renowned international publications including Clinical Pharmacology and Therapeutics(CP&T), British Journal of Clinical Pharmacology(BJCP), Pharmacogenetics and Genomics, Pharmacogenomics J, Frontiers in Pharmacogenomics, Personalized Medicine and more. Dr. Shin has served as Chair of the Organizing Committee for several national and international meetings, including the International Symposium on Pharmacogenomics and the 11th International ISSX meeting. Dr. Shin has also served many academic societies and national/regional committees such as the IUPHAR Clinical Pharmacology Council and Pharmacogenetics Committee, the Korean Association of Clinical Trial Centers, and the Korean Network of South-Eastern Regional Clinical Trial Organization. He is a member of the trustee board of directors for the Korean National Enterprise for Clinical trials.

**Abstract of the talk**

# Implementation of Genotype Guided Personalized Pharmacotherapy: Status in Korea

Pharmacogenomics (PGx) biomarkers are considered to be good predictors of individual drug responses for the personalized pharmacotherapy in the given individual patient. Although it is limited that the genetic tests for the personalized pharmacotherapy become popular in the medical practice, several pharmacogenomics biomarkers already allowed to genotype based pharmacotherapy and the validated biomarkers are listed in the drug labels. Therefore, it seems to be already in the era of personalized medicine in limited therapeutic drugs. However, it is long term process for a genetic biomarker to be applied in to clinical practice through such many steps of functional and clinical validation. In general, the biomarker for the personalized pharmacotherapy should be validated for their clinical application including following steps; from discovery of genetic biomarker, preclinical validation, clinical validation and clinical utility validation as well as analytical validation for the diagnostics.

In addition to these scientific evidence, many of other infrastructures should be established in the community for the practice of personalized medicine; i.e., regulatory approval and reimbursement, education/ training of prescribers with pharmacogenetics counsellors, ethical, legal and social considerations etc. The presentation will also touch the issues and status on the clinical implementation of pharmacogenomics knowledge in to clinical practice in Korea.

**Magnus Ingelman-Sundberg**,
Professor,
Department of Physiology and Pharmacology,
Karolinska Institutet, Sweden

Magnus Ingelman-Sundberg, PhD; BSc.Med is Professor of Molecular Toxicology since 1996 and research group leader in Pharmacogenetics at the Department of Physiology and Pharmacology , Karolinska Institutet since 2006. He has more than 420 original papers, 24 500 citations (32 000 in Google Scholar) and an h-factor of 85 (ISI) or 98 (Google Scholar). He is a member of The Nobel Assembly at Karolinska Institutet since 2008. Member of Editorial Advisory Boards of e.g. Trends in Pharmacological Sciences (Edit Board), Pharmacogenetics and Genomics, Pharmacogenomics, Drug Metabolism Reviews, Drug Metabolism and Disposition. Chairman of the Microsomes and Drug Oxidation International Advisory Committee, mdo.ki.se. Recently categorized by Thomson Reuters as one of the World's Most Influential Scientific Minds (http://sciencewatch.com/sites/sw/files/sw-article/media/worlds-most-influential-scientific-minds-2014.pdf) based on recent (2002-2012) citations and assigned "Highly Cited Researcher for 2015 by Thomson & Reuters (www.highlycited.com). His research focuses on genetics, epigenetics, polymorphism, regulation, function and toxicology of the hepatic ADME system with aims at understanding interindividual differences in drug response. Furthermore he develops novel hepatic in vitro systems for studying liver function and validation of drug targets. Further info from an Interview with Magnus Ingelman-Sundberg See: Trends Pharmacol Sci. 2015; 36:65-7.

**Abstract of the talk**

# Novel biomarkers for more effective individualized drug therapy tomorrow

There are pronounced interindividual variations in drug metabolism, drug response and incidence of adverse drug reactions. In addition to genetic variation, epigenetic and long non-coding (lncRNA) dependent regulation of these genes is important and future direction in this novel research field is outlined with respect to our understanding of interindividual differences in drug action (Ivanov et al., 2012, 2016). A novel class of drugs, so called epidrugs, are known to can intervene in the epigenetic control of gene expression for disease treatment, and many so called epidrugs are now in clinical development. In addition, disease diagnosis prognosis and drug treatment success can be monitored by epigenetic biomarkers. Regarding the genetic variation it is clear that in addition to common previously characterized variations of importance for drug response which are frequently utilized for current therapy, there are also a huge number of rare gene variants of importance for the individual response worth attention. Indeed studies in monozygotic and dizygotic twins as well as analyses of large whole genome and whole exome sequencing projects reveal that only about 50-70 % of the true interindividual variation in drug pharmacokinetics can be assigned to known mutations commonly analyzed for. The lecture will give an update in the field of current and future genomic biomarkers, epigenomic alterations during development and epigenetic mechanisms of importance for prediction of drug metabolism, drug action and ADRs focusing on the most clinically relevant examples.

**Albert P. Li**
President and CEO,
In Vitro ADMET, USA

Dr. Li has devoted his scientific career to the development and advancement of scientific concepts and in vitro technologies to accurately predict human drug properties. His research is focused on the development and application of human-based in vitro experimental models, especially primary cultured human hepatocytes and, most recently, enterocytes, in the accurate assessment of human drug properties including metabolic fate, drug-drug interactions and drug toxicity. Dr. Li was one of the first scientists to successfully cryopreserve human hepatocytes and enterocytes to retain properties of freshly isolated cells. His laboratories continue to develop innovative assays and products to address the unmet needs of the drug discovery and development industry.

Dr. Li is a frequent organizer and speaker at international conferences, and has published over 160 research articles, book chapters, and reviews, and co-edited 5 books in toxicology and drug-drug interactions. He is on the editorial board for various journals, including Current Drug Metabolism, Drug Metabolism Letters, Chemico-Biological Interactions, Journal of Toxicological Sciences, and Toxicology and Cell Biology. Dr. Li holds several U.S and international patents, including the QuickRefreeze™ process to produce highly-functional pooled cryopreserved human hepatocytes, and the Integrated Discrete Multiple Organ Co-culture (IdMOC™) system, used to co-culture multiple cell types, thus modeling the multiple organs in the human body that are interconnected by the systemic circulation.

Dr. Li is currently President, CEO and co-founder of In Vitro ADMET Laboratories LLC, Columbia, MD and Malden, MA. Previously, Dr. Li was President and CEO of Phase 1 Molecular Toxicology, Inc. in Santa Fe, New Mexico, U. S. A. (2002-2003), Chief Scientific Officer of In Vitro Technologies, Inc., Baltimore, Maryland, U. S. A. (1995-2002); Research Professor and Director of the Surgical Research Institute, Department of Surgery, St. Louis University Medical School (1993-1995); Senior Fellow and Director, Liver Biology Department, Monsanto Company (1982 – 1993); Group Leader, Cellular and Genetic Toxicology, Lovelace Inhalation Toxicology Research Institute (1979 – 1982); Assistant Professor and Research Scientist, Cancer Research and Treatment Center and Department of Radiology, University of New Mexico (1976 – 1979). Dr. Li obtained his B. Sc. (1972, Chemistry) from the University of Wisconsin, Stevens Point, and Ph. D. (1976, Biomedical Sciences) from the University of Tennessee, Oakridge Graduate School of Biomedical Sciences. His received his doctoral training and performed his dissertation research under Professor Abraham Hsie in the Biology Division of Oak Ridge National Laboratory, Oak Ridge, Tennessee.

**Abstract of the talk**

# Novel human hepatocyte and enterocyte technologies for the evaluation of human-specific drug properties in drug development

Orally-administered drugs are metabolized firstly by the intestinal epithelial cells (enterocytes), then, upon absorption, by the liver parenchymal cells (hepatocytes). For the past decades, our laboratory has successfully cryopreserved human and animal hepatocytes and has developed novel assays for the evaluation of drug metabolism, drug-drug interactions, and drug toxicity. The cryopreserved hepatocytes have high viability and can be cultured for drug metabolism, transporter uptake and efflux, P450 induction, P450 inhibition and hepatotoxicity studies. Recent novel approaches include our plated hepatocyte relay assay for slowly metabolized compounds and the ROS/ATP assay for the identification of drugs with severe hepatotoxicity. Recently, we have extended our activities towards the isolation and cryopreservation of human and animal enterocytes. The isolated enterocytes had high viability and retained drug metabolizing enzyme activities. The in vivo grape-fruit juice inhibition of enteric CYP3A activity was reproduced using the cryopreserved human enterocytes. This lecture will provide an up-to-date review on the novel approaches in the application of cryopreserved enterocytes and hepatocytes in the assessment of human drug properties.

**Larry Wienkers**
Vice President and Global Head,
PKDM, Amgen, USA

Larry C. Wienkers received his Ph.D. in Medicinal Chemistry from University of Washington in 1993 under the direction of Dr William F. Trager. He did postdoctoral work in the department of Drug Metabolism at the Upjohn Company and subsequently joined the company as a Research Scientist 1995. In 1998, Larry became Director of Drug Metabolism Enabling Technologies at Pharmacia & Upjohn and in 2002 became Executive Director of Pharmacokinetics, Dynamics and Metabolism at Pfizer. In 2004 Larry moved to Amgen and is currently Vice President of the department of Pharmacokinetics and Drug Metabolism. Larry's current research interests are focused on exploring bioactivation pathways associated with small molecule drug metabolism in order to explain the untoward side effects encountered with these compounds. In addition, he continues to investigate the application of novel in vitro metabolism techniques to understand the enzymatic basis for biotransformation of potential therapeutic agents and the prospective application of this information to predict clinically relevant drug-drug interactions. To this end, his group uses a multidisciplinary approach applying organic chemistry, biochemistry, biophysics, enzymology, analytical chemistry, and cell biology research techniques to study cytochrome P450 mechanism based inhibition of novel therapeutics.

**Abstract of the talk**

# Biochemical Aspects of Cytochrome P450 Mechanism-Based Inactivation

Cytochrome P450 (CYP) enzymes are a superfamily of mono-oxygenases which capable of carrying a diversity reaction chemistries. Although P450-catalyzed reactions are generally thought to lead to detoxication of xenobiotics, the reactions can also produce reactive intermediates that can react with cellular macromolecules leading to toxicity or that can react with the P450s that form them leading to irreversible (i.e., mechanism-based inhibition) inactivation. Mechanism-based P450 inactivation usually involves bioactivation of the xenobiotic to a reactive intermediate, which covalently adducts with an active site amino acid residue or to the heme prosthetic group.Compared to reversible inhibition, irreversible inhibition more frequently results in unfavorable Drug Drug Interactions as the inactivated P450 enzyme has to be replaced by newly synthesized protein. To this end, many drug metabolism groups within pharmaceutical companies rely upontraditionaltesting schemes to assess the magnitude of mechanism-based inactivation which is on some occasions followed by more detailed mechanistic studies to elucidate the mechanism of P450 inactivation. Ultimately, the aim of these second tier “mechanistic studies” are to obtain a deeper understanding of the steps leading to enzyme inactivation by drug candidates to yield fundamental insights which ultimately may provide strategies to circumvent the P450 inactivation/bioactivation liability.This presentation will focus on those new developments that are advancing our understanding of the molecular basis of mechanism based inhibition.

**Charles Crespi**
Business Director,
Discovery Labware,
Corning Life Sciences, USA

Charles L. Crespi received B.S. (Chemistry), M.S. and Ph.D. (Toxicology) degrees from the Massachusetts Institute of Technology. Shortly after finishing graduate school, he founded Gentest Corporation with two colleagues. Gentest commercialized toxicology tests using human cell systems. In 1988, the company refocused on products and services with the heterologously expressed xenobiotic metabolizing enzymes. The first such products were introduced in 1990. In 2001, Gentest Corporation was acquired by the Discovery Labware Unit of Becton Dickinson (BD). At BD, Charles had several roles Research and Development and General Management. In 2012, the Discovery Labware Unit was sold to Corning Life Sciences. Charles is currently the Director of New Product Realization, responsible for all Corning Life Sciences new products from ideation through to product launch.

Charles L. Crespi's research has focused on the development and characterization in vitro systems for the study of drug metabolism, drug transport and safety assessment. He has authored over 90 publications and is listed as an inventor on 12 US patents.

Charles L. Crespi is a member of the International Society for the Study of Xenobiotics, the Society of Toxicology and the American Association of Pharmaceutical Sciences. He serves as the Chair of the Steering Committee for the New England Drug Metabolism Discussion Group. He also serves as Treasurer for a local non-profit organization dedicated to the preservation of a pristine New England lake.

**Abstract of the talk**

# Analysis of the Reproducibility and Predictivity of Immortalized Hepatocyte-like Cells for CYP3A4 Induction and Hepatotoxicity

Primary human hepatocytes are the gold standard for many in vitro ADME/Tox applications. However, these materials are of limited supply and suffer from large lot-to-lot variability in key assay parameters. We have developed HepatoCells, a conditionally immortalized, human hepatocyte-like cell model. This model was derived from primary human hepatocytes immortalized with SV40 Large T antigen. Multiple lots of HepatoCells were compared to multiple lots of primary human hepatocytes for CYP3A4 induction using the Relative Induction Score (RIS) method and for cytotoxicity in conventional monolayer (2D) and spheroid (3D) culture. Data comparing the various systems will be presented. HepatoCells faithfully modeled hepatocyte responses, in addition, the lot to lot variability with HepatoCells was substantially lower than with primary human hepatocytes. We conclude that HepatoCells are a useful model for pre-clinical safety assessment.

**Abhay Sangamwar**
National Institute of Pharmaceutical
Education and Research (NIPER), India

Dr. Abhay Sangamwar studied pharmacy and did his PhD in pharmacy in 2006. He served pharmaceutical industry for 3 years and the Department of Pharmacoinformatics, NIPER, SAS Nagar from 2009 to 2014 as Assistant Professor. During this tenure he explored in silico techniques in drug binding with CYP/UGT/efflux-influx transporters/hPXR. In 2014 he joined the department of Pharmaceutics. His research focuses on mainly three areas which include prodrugs to reduce presystemic metabolism of drugs; development of co crystals and development of solid lipid hybrid nanoparticles and solid phospholipid bile salt mixed micelles in the delivery of drugs. So far, he published his work in more than 60 articles in peer reviewed, international scientific journals, 25 poster presentations and 10 oral presentations at national and international scientific meetings. One book chapter is credited to him. His awards include German Academic Exchange Service (DAAD) fellowship for research stay in Germany. Dr. Abhay is a treasurer of Society for Study on Xenobiotics (a branch of International society for study on xenobiotics, ISSX) in India. Currently, Dr. Abhay's research group consist of 3 scientists, 3 technical assistants, 3 PhD students and 15 master students.

**Abstract of the talk**

# In silico tools in understanding ligand-CYP/UGT/P-gp complex formation at the atomistic level

Substrate specificity or isoform specificity to a particular CYP/UGT and P-gp/hPXR is attributed to the favorable interactions of the enzyme with substates. Isoform specificity can be manifested in a number of ways: 1) substrate selection 2) site of metabolism and 3) catalytic rate of conversion that leads to the formation of product. Characterization of isoform specificity at an early stage helps in shifting the metabolic profile of new leads towards a particular isoform that can lead to the avoidance of unwanted /toxic metabolite formation from non-selective isoform, thus optimizing the pharmacokinetic profile of new leads. Current computational methods helps in exploring the atomic level interactions of a substrate with its isoform.

# Abstarcts

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# Dose-dependent estrogenic endocrine disruptive effect of Bisphenol-A in Sprague-Dawley Rats

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The present study was aimed to determine the dose that induces estrogenic endocrine disruptive activity upon chronic oral administration of three different doses of bisphenol-A (BPA) (10, 50, 100 Âµg /kg bw/day) in adult female Sprague-Dawley rats.

Hormones were assayed by ELISA, ovary eNOS, StAR, CYP11A1 aromatase by Western blot and biochemical parameters were estimated by spectrophotometric techniques. Histological studies of ovary and mammary tissues by hematoxylin and eosin staining.

Low dose (10 Âµg) of BPA exposure increases body weight, levels of gonadotrophic hormones such as FSH, LH, and status of lipid profile and decreases the levels of estradiol and progesterone significantly compared to control rats. Increased protein expression of eNOS and StAR, CYP11A1, Cytochrome P450 system such as aromatase protein expression in ovary and phase I detoxification agents in Microsomes of liver and mammary tissues significantly reduced in low dose treated animals. The moderate and high doses (50 and 100 Âµg) of BPA exposure increase the levels of TBARS and the status of antioxidant in liver and mammary tissue in a dose dependent manner.

The result of the study suggests that 10 Âµg/kg dose of BPA exerts more pronounced estrogen mimetic action and has the potential to disrupt estrogen synthesis and actions.

# Leaf extracts of Punica granatum alleviates experimentally-induced epilepsy in mice

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Epilepsy is a serious neurological disease characterized by transient occurrence of abnormal, excessive and/or synchronous neuronal activity in the brain, associated with various neurobiological, cognitive and psychological signs and/or symptoms]. Conventionally various chemical classes of drugs used in the management of epilepsy, recently, many newer classes of drugs such as vigabatrin, levetiracetam, topiramate, lamotrigine, zonisamide, lacosamide, rufinamide, stiripentol have been developed and they are considered to be comparatively safe. However, despite of copious efforts all the currently available drugs have one or more inherent side/adverse effects such as dizziness, mental slowing, ataxia, impaired concentration, mental confusion, sleep disturbance, Anorexia, somnolence, aggression and so on. Hence there is great scope for safe and potent drug for the management of epilepsy, in his context herbal drugs are considered to have better edge over synthetic drugs, therefore many researchers are focusing on herbal remedies to discover better and safe medicine for management of epilepsy. In this context, present study was aimed to examine the possible anticonvulsant property of leaf extracts of Punica granatum against experimental models of epilepsy in swiss albino mice. Petroleum ether (PLPG), methanolic (MLPG) and aqueous (ALPG) leaf extracts of Punica granatum were initially evaluated against 6-Hz-induced seizure model in mice, the potent extract/s were further evaluated against maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced convulsions in swiss albino mice. Further, the potent extract/s was evaluated for their effect on Gamma amino butyric acid (GABA) levels in brain homogenate to explore the possible mechanism of action. In addition, the potent extract was subjected to actophotometer test to evaluate its possible locomotor activity deficits. In 6-Hz seizure test, the MLPG has alleviated 6-Hz-induced seizures significantly and dose dependently at doses 50, 100, 200 and 400 mg/kg. In contrast, PLPG and ALPG did not show any protection, only high dose of ALPG (400 and 800 mg/kg, p.o.) showed very slight inhibition. Based on these observations only MLPG was tested in MES and PTZ models. Interestingly, the MLPG (50, 100, 200 and 800 mg/kg) has offered significant and dose dependent protection against MES and PTZ-induced seizures in mice. Further, MLPG have showed significant increase in brain GABA levels and showed minimal CNS depression in their potent anticonvulsant doses. However, highest dose MLPG (200 mg/kg, p.o.) and Diazepam (5 mg/mg, p.o.) of in all the anticonvulsant tests MLPG was found to be superior over ALPG.

These findings suggest the MLPG possess significant anticonvulsant property, further one of the possible mechanism behind the anticonvulsant activity of MLPG may be due to enhanced GABA levels in brain.

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# Biomarker Quantification: Development of fit for purpose LC-MS/MS method for determination of Methyl guanidine in mice urine

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Usage of surrogate matrix or stable-labeled standards is typically employed for quantitation of biomarkers, however high endogenous level of some biomarkers provides researcher a unique opportunity to dilute study samples with surrogate matrix and quantify them against calibration standards prepared in surrogate matrix alone. The Later approach was used to determine endogenous methyl guanidine (MG) in mice urine. MG is the product of protein catabolism, normally gets excreted in urine. Endogenous MG concentrations in urine elevates if there is reduced urine production or conversion of creatinine to MG, as proposed in patients with chronic renal failure. Hence, MG can be considered as putative biomarker for renal failure studies and determination of MG in mice urine has significance for development of mice in-vivo efficacy models. Artificial urine (devoid of endogenous MG) was used as surrogate matrix for preparation of calibration standards, while quality control standards were prepared in authentic mice urine diluted 50 fold with artificial urine. Quantitation method for MG in urine was developed on LC-MS/MS in multiple reaction monitoring using mass transitions 74.2(Q1) and 57.2, 43.2 as Q3. Chromatroghy was developed on waters, Atlantis HILIC silica column with a binary gradient mobile phase comprised of acetonitrile and 10 mM ammonium formate buffer at a flow rate of 0.5 mL/min.

Developed method was found linear from 2ng/mL to 1000 ng/mL, with R2 > 0.98. Considering mean endogenous basal levels of MG as determined in un-treated C57BL/6J mice urine, developed method can accurately quantify upto 10 fold up regulation and upto 20 fold down regulation of MG concentrations. Approach followed to quantify MG is cost effective; moreover 50 fold dilution of authentic matrix for quality control standards and study samples with artificial urine eliminates matrix effect.

A fast, robust and cost effective LC-MS/MS method was developed for determination of MG in mice urine. This is the first LC-MS/MS assay for direct quantitation of MG in mice urine samples.

# Phenotyping of aldehyde oxidase in a subset of Indian population using vanillin as a probe

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Inter individual variability in AO activity has not been well investigated. An AO genotyping study has been reported in an Italian population but no phenotyping study for AO has been reported. The objective of the study was to validate vanillin as an AO probe in vitro and subsequently phenotype a subset of Indian population for AO activity.

1) We developed an in vitro assay for estimation of AO activity, using vanillin, in partially purified guinea pig liver AO fraction. Kinetic parameters (Km, Vmax) were determined, and IC50 value was determined for inhibitor raloxifene.
2) To validate vanillin as a probe substrate, it was incubated with human liver S9 fraction, partially purified XO fraction and liver microsomes of different species (in presence and absence of specific inhibitors).
3) A phenotyping study was carried out in 100 human volunteers with 500 mg vanillin dose and collecting cumulative urine samples eight hour postdose. The samples were analyzed by HPLC. Metabolic ratios were calculated as peak area ratio of vanillic acid/vanillin. Probit analysis was carried out to determine a cut off value to distinguish between fast and slow metabolizers. Hardy- Weinberg Law was applied to determine the distribution of allele frequencies.

The Km of vanillin for AO was found to 7 ÂµM and the IC50 value for raloxifene was found to be 98.62 nM. Results of probe validation studies showed that vanillin was preferentially/exclusively metabolized by AO. The phenotyping study indicated that 73.72 % of the subjects were fast metabolizers, 24.28 % intermediate metabolizers and 2 % were slow metabolizers.

This is a first report of a phenotyping study of aldehyde oxidase in humans. It suggests the existence of genetic polymorphism of AO, at least in a subset of Indian population.

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# Deltamethrin A Xenobiotic Pesticide Compound; Shows a damaging effect on the human Peripheral Blood Mononucleocytes (PBMCs) of the Farmers employing the pesticide in fields of the Ash Sharqiyah Region of Sur in Sultanate of Oman.

Magapu solomon sudhakar and Haneen fareed attia al-jaithany. Ministry of higher education, department of applied biotechnology, Sur college of applied sciences, p.o box: 484, postal code: 411, Sur - Sultanate of Oman.

Xenobiotic chemicals are of enormous value to human society as chemicals like petrochemicals, pesticides and plastics. These compounds are major hazard to human health and environment. In this study, the effect of pesticides on human Peripheral Blood Mononucleocytes (PBMCs) isolated from the blood of farmers & non-farmers in Sur- region of Sultanate of Oman was analyzed. Deltamethrin, a xenobiotic pesticide employed by the farmers of Sur, was used in this study to observe its effect on two groups; a) Control (Non-Farmers) group which have indirect exposure to pesticide by consuming fruits and vegetables on which Deltramethrin was sprayed, and b) Farmers group which have direct exposure to pesticide while spraying the farm. With Objectives of studying;- Effect of Deltamethrin in the in-vitro culture of PBMCâ€™s with Deltamethrin, on 1) cell morphology, 2) the DNA damage in PBMCs 2) innate immunity, & 3) T-Cells counts.

Peripheral Blood Mononucleocytes (PBMCs) isolated by Ficoll-Hypaque method, from 5 farmers & 5 control groups after Institutional Ethical Committee Clearance. Isolated total antibody profile from all subjects was analyzed by SDS-PAGE experiment. Cell morphological changes under the influence of pesticide treatment were observed under the microscope. Assays carried out are: 1).Comet assay was carried out to study the degree of DNA damage of in-vitro cultured PBMCs., 2) Neutrophil assay was carried out to study the response of innate immunity for both groups by measuring the Optical Density (OD) from cultured pool of PBMCs. & 3).T-cells rosetting was carried out to detect the presence of the T-cells in the groupâ€™s individual after treating them with pesticide.

Studies showed consistent results in the above mentioned assays and cellular morphological changes. Total antibody profile in all subjects is the same. As evident, DNA damage occurred either by direct or indirect exposure of pesticide and in some subjects skewing of Neutrophil expression observed. T-Cell Assays showed steep drop in the number of T-cells as observed by the SRBC Rosetting Assay. Xenobiotic compound Deltamethrin adversely effects in the in-vitro culture of PBMCâ€™s on morphology, DNA and cell count.

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# Inhibition of P-gp mediated Rhodamine 123 efflux by ethanolic extract of Centella asiatica

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Centella asiatica (C. Asiatica) is known as longevity herb and used extensively for the treatment of anxiety and hypertension. Conventional drugs which are the substrates of P-gp, can undergo pharmacokinetic changes if co-administered with C. asiatica resulting in either reduced therapeutic effect or increased side effect profile. The aim of the research work was to investigate the P-gp inhibition potential of ethanolic extract of leaves of C. asiatica in rats using everted gut sac technique. The effect of leaf extract of C. asiatica was studied on rat intestinal P-gp using everted gut sac technique. P-gp function was evaluated by the difference of Rhodamine-123 efflux with and without the extracts.

Ethanolic extract of C. asiatica showed a concentration dependent inhibition of rhodamine- 123 efflux from the serosal to mucosal surfaces across the everted rat ileum. The IC50 of ethanolic extract of C. asiatica was found to be 60.27 Âµg/mL, respectively indicating inhibition of rat intestinal P-gp. These findings suggest the possibility of drug herb interactions at the level of gastro-intestinal absorption of drugs. Ethanolic extract of C. asiatica showed concentration dependent inhibition, as concentration of extracts increases the percentage inhibition of efflux of Rhodamine 123 also increases through P-gp and this inhibition is more significant than positive control indicating potential of pharmacokinetic drug-herb interactions when concomitantly administered with allopathic drugs

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# Pharmacokinetics and Bioavailability Assessment of a Novel Potent Antidiabetic Peptide, PSTv8 in Mice Using Liquid Chromatography Tandem Mass Spectrometry

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Pancreastatin (PST) is an endogenous diabetogenic peptide negatively regulates the insulin sensitivity and glucose homeostasis. We have discovered, characterized and investigated the PST inhibitor peptide (PST variant 8 or PSTv8) against diabetes caused by PST and a patent has been filed in India from our lab. The objective of the study is liquid chromatography coupled mass spectrometry (LC-MS/MS) method validation in mice plasma and to explore in-vivo pharmacokinetic behavior and bioavailability of PSTv8 in mice. The intraperitoneal (i.p.) and intravenous (i.v.) pharmacokinetic studies were performed on young male C57Bl/6 mice at the dose of 5 and 10 mg/kg, respectively. Samples were analyzed by using the validated LC-MS/MS method.

The intra-day assay accuracy and precision was ranged from 99.63 to 110.20% and 2.61 to 4.01%, respectively. The inter-day assay accuracy and precision was ranged from 97.01 to 110.93% and 2.90 to 7.17%, respectively. After i.v., and i.p. administration, the plasma concentrations of PSTv8 were rapidly reached to Cmax ~50000 and ~6000 ng/mL, respectively. After i.v. and i.p. administration, AUC0-âˆž was found to be ~19000 and ~4500 h*ng/mL, respectively. The percentage mean absolute bioavailability of PSTv8 for i.p was ~45%. A highly sensitive, specific and reproducible LC-MS/MS method was developed and validated for the quantification of PSTv8 in mice plasma for the first time. The validated assay of PSTv8 peptide was successfully applied to determine the plasma concentrations after i.v. and i.p. administration to mice for the first time. The high bioavailability of PSTv8 in mice was probably due to low apparent volume of distribution. The advantages of the LC-MS/MS method are cleaner solid phase extraction samples, no significant matrix effect and low sample volume (100 ÂµL). The present LC-MS/MS method can be useful for understanding the drug-drug interactions and PK-PD modeling studies.

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# A new microsome-based system to determine fm and perform reaction phenotyping:-Silensomestm

Christophe Chesne, David Steen, Yannick Parmentier, Fabrice Caradec, Corinne Pothier, Bouaita Belkacem, Fabrice Guillet. Biopredic International, Parc D'affaires De La Bretã¨Che, Bã¢Timent A4, Saint Gregoire-35760, France

Drug-drug interactions can affect the efficacy and safety of NCEs, and determining which CYP enzymes metabolize an NCE and their relative contribution to metabolism (fraction metabolized-fm) is important for candidate selection and regulatory submission. Current methods suffer from numerous deficiencies such as overestimation of individual CYP fm following the RAF evaluation, tedious, costly, and time consuming pretreatment of microsomes with chemical or antibody-based inhibitors, complex statistical analyses that can produce misleading results, or involved correlation analyses using pooled HLM demonstrating different CYP activity phenotypes. Therefore a direct method requiring no pretreatment and with straightforward interpretation of results promises to improve this process.

We treated pooled, human liver microsomes with mechanism-based inhibitors to irreversibly, potently, and specifically deactivate individual CYP enzymes. The incubation were done according to clearance conditions and we compared metabolism profiles of SilensomesTM (deactivated HLM) and their homologous controls on a CYP-by-CYP basis in order to accurately determine which CYP metabolize a compound and to evaluate the fm value by the ratio of the Cl value. After incubation of CYP3A4-SilensomesTM with CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and three CYP3A4 specific substrates, it was found that 1) The irreversible deactivation of CYP3A4 affected minimaly the other CYP-activities since less than 10% of diminution were observed 2) At least 95% of the metabolism of testrosterone, and 80% for nifedipine and midazolam were inhibited The remaining activity in presence of Nif and MDZ was inhibited further by ketoconasol representing the 3A5 activity

This ready-to-use model demonstrates excellent CYP inhibition potency and specificity, should simplify and improve determination of the contribution (fm) of different CYP enzymes to metabolism of NCEs.

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# SENS-IS: A genomic signature to assess skin sensitization

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A range of different in vitro chemistry-based like DPRA or GSH reactivity as well as cell-based methods like hCLAT or Keratinosens have been developed and allow evaluating sensitization potential of cosmetic ingredients, however these are still limited. Metabolisms as well as bioavailability of ingredients are not taken into account. 9 test compounds were analyzed according to the sens-is assay and the 61 genes were evaluated using qPCR in 2 laboratories the Agence Nationale de Securite des Medicament (ANSM) laboratory in Montpellier France and Eurosafe and results were compared to those of Immunosearch. A non irritant test substance is considered to be sensitiser if it increases the expression (compared to the solvent control) of at least 7 genes measured by qPCR in either the "SENS-IS" or the "ARE" gene sets. To take into account non-specific gene overexpression due to cell stress, the induction of more than 20 genes in the irritation gene set, classifies a result as inconclusive and the test substance is re-analysed at a lower concentration. Results were compared to the previous LLNA classification. These results assess the transferability and reproducibility of the SENS-IS protocol and its ability to correctly classified sensitizing potency in 5 classes similarly to the LLNA.

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# Biophysical and metabolic properties of striae distensae evaluated ex vivo compared to normal skin.

Coquin Youna, Fournier Manon, Oger Lucie, Dacq Hayat, Bree Francoise, Subileau Eva-Anne, Christophe Chesne. Biopredic International, Parc D'affaires De La Breteche, Batiment A4, Saint Gregoire-35760, France

When testing transcutaneous passage, company works mainly on abdominal skin, and they use skin disks that are striae distensae (SD) free. Yet it is interesting to know whether one can extrapolate the results obtain from normal skin disks to skin with SD, as it is now assumed. Moreover, 70% of women in their midlife have SD, and with the obesity rate increasing, a larger number of men and women will be concerned. The aim of this study was to determine whether the barrier property was comparable between the two skin types. Moreover, the metabolic activities, also important when studying the toxicology of a cosmetological product with a topical application, were also compared in the skin with and without SD.

Biological materials were obtained from abdominoplasty and all studies were performed ex vivo. Different parameters were compared between donorsâ€™ skin with SD and adjacent uninvolved skin to characterize: skin barrier function by measuring transepidermal water loss (TEWL), skin surface hydration using corneometry (skin capacitance), PH skin surface, and CYP1A and esterase activityNo difference was observed in skin barrier function when looking at transepithelial water lost. Other biophysical properties such as PH or hydration were also similar when compared to adjacent skin. Yet the metabolic activity of the phase I enzyme was different. Esterase activity is greater in SD compared to normal skin from the same donors. Likewise, CYP1A is more inducible in SD by 3-methylcholanthrene (3MC). When looking closer at these anatomical differences, it seems that the differences come from both the dermis and the epidermis. When the epidermis is less responsive to induction for SD, the basal level of CYP1A activity is greater in the dermis of the SD. Based on these results, the distinct metabolic features characterizing SD lesions are to be taken into consideration when making toxicological studies. These changes however do not seem to affect the skin barrier, making skin with SD acceptable material to work on, when testing transcutaneous passage.

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# Development of a predictive screening test for drugs liable to be cholestatic

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Intrahepatic cholestasis is a chronic disease resulting from an impairment of the bile flow. This can lead to the degeneration of liver tissues which may progress into cirrhosis or severe hepatic insufficiency. Indeed, intrahepatic cholestasis has been estimated to account for hospitalization in 2-5% of the cases and approaches as much as 20% in the elderly. Actually, wide variety of commonly used drugs represents to be an important cause of cholestatic liver injury with poor predictivity. Thus, our goal is to design new predictive biomarkers for the screening of drug induced-cholestasis.

We designed an assay to mimic cholestatic events using human HepaRGR hepatocytes which were shown to express detoxification function meanly bile salts trafficking to bile canalicular poles. HepaRGâ„¢ cells were treated with with molecules known to be cholestatic . The effect of these molecules on biliary canaliculi dynamics, clearance of bile salts up to bile canaliculi and alterations was measured with fluorescent probes. The cholestatic drugs induced alteration in the bile canaliculi dynamics in HepaRGâ„¢ cells. They induced a deformation of bile canaliculi and abnormal accumulation of bile acids. HepaRGâ„¢ cell line was a suitable model to reproduce cholestasis generation and mimic the cholestatic events. The newly synthesized fluorescent bile acids represent a promising biomarker to measure bile acids trafficking and clearance and predict in vivo dynamic events

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# A Systematic Approach for Clinical Pharmacokinetic Analysis - CRO Perspective

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Clinical pharmacokinetic studies are performed to examine the Absorption, Distribution, Metabolism, and Excretion (ADME) of a drug(s) under investigation in healthy volunteers and/or patients. Non compartmental analysis (NCA) of pharmacokinetic (PK) data has become a routine practice to support internal decision making and regulatory submission. Clinical PK analysis is a multimodal approach using a combination of several key steps of data analysis and PK reporting. This multimodal approach requires a great partnership between SAS (Statistical Analysis Software) programmers and a Pharmacokineticist to form a multi-disciplinary team, which engage in PK analysis and reporting.

The Clinical PK project was divided into six different phases i) Pharmacokinetic Analysis Plan (PKAP) ii) Data Merging iii) Data Cleaning iv) PK analysis v) Tables and Figures Listings (TFL) vi) SAS Transport Files (XPT). To begin with, we prepare PKAP (analogous to Statistical Analysis Plan, SAP) in accordance with clinical study protocol and sponsor specifications. Data Merging is a complex process, since data records from multiple source systems need to be in consolidated into one single record. So based on the PRS (Programming Requirement Specifications), different SDTM datasets (BA, VS, EX, DM etc.,) will be merged using SAS programme to prepare a final dataset for PK analysis. Post data merge the data cleaning process will be performed to check for missing subject/ values or BLOQ samples, inaccuracies in sample date and times or protocol deviation etc. Based on Client feedback the data will be processed for PK analysis using in Phoenix WinNonlin. TFLs will be generated within Phoenix workflow during PK analysis. Finally, XPT files will be developed using SAS.

At each phase of the project, active communication between sponsor and Cytel was key to complete the project on or before the agreed timelines. The entire Clinical PK project is completed within 12 business days.

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# Clinical Pharmacology Comparator Database - Modelling perspective

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Developing an innovative drug is too expensive and decisions are made along the development path of a compound to maximize the probability of its success. Making these decisions requires considerations and predictions of the drug product profile relative to those of key competitors in the market. As such, a thorough understanding of the data available for all the potential competitors has become one of the critical needs in the decision-making process. It is within the context of this critical drug development need, a comparator data base model is required which supports model based meta-analysis (MBMA) which helps in seeking answers for specific research questions during drug development. Through Comparator Outcome Databases (COD) we enable clients to capture summary level data for the clinical safety and efficacy outcomes. Data sources we use that facilitate quick data analysis are- publicly available data sources (PubMed, Cochrane, Trial registries, FDA Summary Basis of Approvals) and proprietary data sources (Embase, OVID, CSRs). Development of COD model is divided into 4 different steps 1. Scoping: In this first step the client requirements will be assembled and scope will be finalized to develop indication specific clinical trial outcome databases. 2. Literature mining: As per search terms defined scope of work using various data sources the abstract based literature mining will be performed. 3. Source database development: During this phase of project all the search results will be tabulated followed by review/ select references and tracking inclusions/ exclusions criteria. 4. Outcome database development: In this step specifications will be refined and extracted data will be Qcied. Selected publications will be used to capture clinical trial, treatment, patient and outcomes information and these populated information will be validated independently at each step

The comparator data base model can greatly improve the efficiency and quality of pharmaceutical drug development process.

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# Creating NONMEM datasets using SAS® program

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Pharmacokinetic (PK) is the study of what a body does with a dose of drug and Pharmacodynamic (PD) is the study what drug does to the body. Typically in traditional PK studies have usually been carried out in small numbers of subjects, often healthy volunteers. Whereas effect of compound on the target patient population is predicted using models during population PK/PD analysis. The main aim of Population pharmacokinetic studies is to identify and quantify sources of variability in drug concentration in the patient population. Nonlinear Mixed Effects Modeling (NONMEM) is a software package, just like Microsoft office which is used for population PK/PD analysis. NONMEM is a powerful tool and most commonly used for POP PK/PD modeling in clinical pharmacology research. But NONMEM software requires dataset in a very specific predefined format, which is a terrifying and time consuming part of the dataset creation process. NONMEM dataset includes various variables like study ID, subject ID, dosing records, period, PK and/or PD observational records, time dependent/independent covariates and laboratory covariates etc. The main objective is to develop automated program to create NONMEM dataset, saving scientists tremendous amount of time and decreasing the opportunities for error. We have used SAS tool for creating analysis datasets. We identified key data components of NONMEM datasets and developed standard structure for each of these components.

Standard rules were developed with regards to handling and merging different type of covariates. Identification of key components and standard data handling steps helped us to reduce program modifications between different projects. The SAS program developed for NONMEM dataset creation is proficient and time saving for POP PK/PD analysis.

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# Pharmacokinetic of piracetam

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Some of the recent clinical studies have shown that at high doses, piracetam treatment has a positive role in cognitive changes after ischemic stroke and more safe in secondary stroke prevention compared to aspirin. Thus it is important to determine the pharmacokinetic of piracetam at a higher dose of stroke.

Blood samples of 0.25 mL were collected after oral (200 mg/kg) and intravenous (75 mg/kg) administration of piracetam through right external jugular vein cannulation. Jugular vein cannulation was performed 24 h before piracetam administration. The pharmacokinetic parameters were analyzed using non-compartmental methods with PK Solver.

Plasma concentration time profile of piracetam was obtained with the absolute bioavailability of 33 %. Piracetam has a fast absorption, time of maximum concentration (Tmax )was found at 1 hr post dose with good oral exposures having maximum mean concentration in plasma (Cmax (Âµg/mL) 86 Â± 1.2) and area under the curve (AUC0-24hr (Âµg*hr/mL) 534 Â± 5.87). Whereas after intravenous administration it has high clearance and volume of distribution having CL (L/hr/kg) 0.1 Â± 0.01 and Vz_obs (L/kg) 0.8 Â± 0.10 however, half life was found to moderate with t1/2 (hr) 4.8Â± 0.19 in fasted male wistar rats. On the basis of present data we concluded that at higher doses of 200 mg/ kg p.o and 75 mg/kg i.v piracetam has good oral exposure with high clearance and volume of distribution.

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# Effect of Efavirenz on pharmacokinetics of Sitagliptin in diabetic rabbits

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The availability of potent combination antiretroviral regimens has resulted in a dramatic reduction in HIV-1 associated morbidity and mortality in the developed world. However, HIV infection and treatment has been associated with the development of insulin resistance, glucose intolerance and diabetes. The objective of the present study was to evaluate the effect of efavirenz (antiHIV drug) on pharmacokinetics of sitagliptin (antidiabetic drug) in diabetic rabbits. Alloxan-induced diabetic model in rabbits has been used in this study. After the induction of diabetes, sitagliptin (7 mg/kg/po) and efavirenz (42 mg/1.5kg/po) for 7 days. The pharmacokinetic parameters like t1/2, AUC, Clearance, Tmax and Cmax of sitagliptin with and without combination of efavirenz treatment were determined by using winnolin software.

The plasma concentration-time profiles and pharmacokinetic parameters of sitagliptin following single dose and multiple dose treatment of efavirenz were found to be similar. There was no change in the pharmacokinetic parameters in presence of efavirenz indicates that no significant ($p>0.05$) pharmacokinetic interaction.

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# MS/MS fragmentation behavior of drug or metabolite peptide adducts

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Reactive metabolites bind covalently to nucleophilic sites of cellular proteins. It is difficult to identify and quantitate such adducts in untargeted proteomic experiments as they are formed in very low concentrations. Thus, we considered that if peptide sequences of a protein that were involved in adduct formation were predicted accurately, it becomes easy to study the said adducts using targeted MS/MS peptide analysis. The objective of this study is to anticipate for the differences in MS/MS fragmentation pattern of unmodified and modified peptide adducts using multiple reaction monitoring (MRM) studies. The outcome of this study is believed to provide potential inputs in development of specific methods for quantification of adducts. To test the hypothesis and to study impact of adduct formation on the MS/MS signal, we covalently reacted human serum albumin and CYP3A4 to the electrophilic metabolites of paracetamol and ritonavir, respectively. Unmodified proteins and modified proteins, after covalent modification by reactive metabolites, were subjected to trypsin digestion at trypsin : protein 1:25 ratio. The reactive metabolites involved in adduct formation are generated invitro.

The fragment ions which are formed especially from the site of peptide modification showed a significant difference in intensity of ions formed. In case of HSA-NAPQI, b14 was the intense fragment ion observed for unmodified peptide and in modified peptide b13 is the only fragment ion observed while other are completely absent. In CYP3A4-ritonavir, y4 was the maximum intensity ion in unmodified peptide and b4 is the maximum intensity ion in modified peptide. This study evidenced the differences in MS/MS fragmentation pattern of native versus adducted peptides of two model proteins. Therefore it can be concluded that during MRM method development for quantification of low-level protein-reactive metabolites adducts, one should consider the impact of covalent modification on the intensities of MS/MS fragments, to prevent false positive results.

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# Chiral Separation and Quantification of Metabolites using SFC/QQQ

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Risperidone, an antipsychotic drug, is metabolized by different cytochrome P-450 enzymes, to predominantly form 9-hydroxylation. The 9-hydroxylation results in the formation of a chiral carbon atom yielding two enantiomers, (+)- and (-)-9-hydroxyrisperidone. To a lesser extent N-dealkylation and 7 hydroxylation may also form. Thus, sensitive and selective analytical methods are required to profile metabolite stereoisomers. To distinguish, stereoisomers, and identify trace level metabolites, baseline resolved chromatographic separation and the sensitive mass spectrometer are required. Supercritical Fluid Chromatography (SFC) provides baseline resolution using chiral columns with several advantages such as rapid and green separations over conventional normal phase separations. Here a chiral separation and sensitive quantitation of major hydroxylated metabolites of risperidone from in-vitro incubation and in-vivo PK samples using an SFC-QQQ system. Agilent 1260SFC/ 6460QQQ system with ESI source was used for the study. The electrospray source was equipped with thermal gradient focusing technology to enhance sensitivity and operated in positive mode. Protonated precursors of risperidone and its metabolites were selected for MRM based quantification from rat in-vivo samples. SFC chiral analysis using AD-3 column resulted in baseline separation of metabolite enantiomers and the drug compound within 6 minutes. The method validation criteria include method selectivity, detection limits and linearity ranges of each metabolite. Precision and accuracy were within the acceptable levels. Using this validated SFC-MS/MS method, quantitative analysis of trace metabolites were performed from biological sample volume. This faster approach can be adapted to separate stereo- regio-specific metabolites in pharmacokinetics study

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# Evaluation of an Experimental Method for Ki Determination in Transporter Assays

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Driven by the EMA Guideline on the Investigation of Drug Interactions (2012) recommendation that transporter inhibition studies are conducted to determine Ki rather than IC50 values, our primary aim was to evaluate a method to experimentally determine Ki values for prototypic inhibitors of human transporters. The second aim centered on the theory that Ki is a system-independent value, and led us to investigate the influence of the host cell background of the given transporter and the applied probe substrate on the Ki value. The third and final aim was to investigate the correlation of the Ki and IC50 values with regard to mode of inhibition (non-competitive, uncompetitive or mixed inhibition). Cellular uptake assays were performed using CHO-K1, MDCKII and HEK293 cell lines stably expressing human OCT2, while the vesicular transport assays used HEK293 and Sf9-HAM membrane vesicles containing human BCRP. The effect of verapamil (inhibitor) was examined on both OCT2-mediated tetraethylammonium (TEA) and metformin transport, while in the vesicular assay rosuvastatin was tested as inhibitor of BCRP-mediated Lucifer Yellow (LY) and estrone-3-sulfate (E3S) transport. In both assay systems the concentration dependence of probe substrate uptake was measured in the presence or absence of the selected inhibitors (applied at 5 concentrations) and Ki values were calculated by fitting a mixed model equation using nonlinear regression (GraphPad Prism, GraphPad Software, Inc. La Jolla, CA). The data indicate that in both assays systems Ki values were similar, irrespective of the host cell background. For example, inhibition of BCRP-mediated LY transport by rosuvastatin generated Ki values of 9.04 Î¼M and 13.42 Î¼M in HEK293 and Sf9-HAM membrane vesicles, respectively, and were also comparable to IC50 values. For OCT2-mediated transport, substrate-dependent differences in modes of inhibition by verapamil were observed. Verapamil inhibition of TEA uptake was found to be competitive, while verapamil inhibition of metformin transport was mixed-mode. The results indicate that Ki is system-independent, but that the mechanism of inhibition depends on the choice of probe substrate and inhibitor. However, further experiments investigating the effect of host cell line on other transporters are warranted. In addition, we recommend utilizing multiple probe substrates when conducting Ki determination experiments.

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# Addressing non-specific binding issues of highly lipophilic compounds in single vein cannulated rats for pharmacokinetic studies: Novel cannulation methodology

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Rat is commonly used to generate pharmacokinetic data for novel compounds during drug discovery. To handle a large number of compounds during lead optimization, rats with a single jugular vein cannulation are commonly utilized for intravenous pharmacokinetic studies, where same cannula is used both for dose administration and blood sampling. We demonstrate that the single cannula methodology is not suitable for all compounds, especially for highly lipophilic compounds. We propose an alternative dual cannulation technique in which two cannulas are placed in the same jugular vein, thus avoiding an additional surgery. Intravenous Pk studies were conducted in both single and dual cannulated rats for itraconazole, amiodarone (highly lipophilic compounds) and atenolol (hydrophilic compound).

For itraconazole and amiodarone, highly lipophilic compounds that bound to the material of cannula, the quantified plasma exposures were substantially higher in the single cannulated rats than those from dual cannulated rats. Area under the plasma concentration time curve differed by 79% and 74% for itraconazole and amiodarone, respectively. Clearance, volume of distribution and bioavailability were lower by 39%, 60% and 38% for itraconazole, and 46%, 34% and 42% for amiodarone, respectively, when estimated from the dual cannulated rats. In contrast, all pharmacokinetic parameters were similar between single and dual-cannulated rats for atenolol. Based on these results, we recommend the use of dual cannulated rats for intravenous pharmacokinetic studies when testing lipophilic compounds that may be prone to non-specific binding. If single cannulated rat model is selected for pharmacokinetic screening, we recommend that a bridging study be done with representative compounds of a given chemical series.

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# Comparison of permeability and efflux transport properties of permeability markers to predict the transporter mediated drug-drug interaction: In situ intestinal permeability with mesenteric blood sampling in rat model and In vitro CaCo-2 cell model

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CaCo-2 cells and in situ intestinal perfusion models are routinely used to predict drug absorption, permeability and to establish mechanism of transport in drug discovery and development. No reports are available correlating systemic permeability of marker substrates (P-gp, BCRP and MRP) of efflux drug transport pathway in situ intestinal perfusion with mesenteric blood sampling with CaCo-2 model. Transport properties of five permeability markers with different absorption mechanisms were evaluated in the in situ intestinal perfusion with mesenteric vein blood sampling in rat model and compared with the permeability data generated in human colonic cancer cell line Caco-2. Qualitative assessments demonstrated that atenolol and propranol markers of low and high permeability showed atleast 20 and 8 fold dynamic range to classify them as low and high permeable markers in our in situ and in vitro systems. Permeability of digoxin (P-gp substrate) was increased by 30 fold in presence of GF-120918 (chemical inhibitor of P-gp and BCRP protein transport pathway) in the in situ system. In the in vitro CaCo-2 system, digoxin efflux was completely inhibited (Efflux ratio â‰¤2.0) by GF-120918 and verapamil. The permeability of sulfasalazine (a substrate of BCRP transport pathway) was increased by atleast 8 fold in presence of MK-571 (chemical inhibitor of partial p-gp and BCRP transport protein) and novobiocin (chemical inhibitor of BCRP transport protein). In a standalone study a modest increase in permeability was observed with MK-571 in the in situ study. The high efflux ratio (> 90) of sulfasalazine was completely inhibited by novobiocin and in the combination of MK571+ FTC but not by MK-571 alone in the in vitro study. The obtained in situ system permeability data correlate well with the in vitro CaCo-2 system. The data suggests both the in situ intestinal perfusion and in vitro CaCo-2 system has potential to identify the substrates of multiple efflux transporters with enough dynamic sensitivity in presence of chemical inhibitors at the intestinal level. However, appropriate validation is required with low, moderate and high permeability markers, known substrates of efflux drug transport pathway substrates in presence and absence of chemical inhibitors in both the systems to predict the drug-drug interactions at intestinal level.

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# Development of fit for purpose LC-MS/MS method for determination of Anandamide in SD rat plasma for multiple pain efficacy models

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Currently, there are several bioanalytical methods available for the determination of Anandamide (AEA) in rat plasma. However, in our lab these methods resulted in poor qualitative performance (over estimation basal AEA levels in plasma) due to contamination of one of the daughter ion fragment with m/z 62.1, used in MRM transitions. In the present study, we have developed specific LC/MS/MS method for the quantification of AEA in rat plasma which is devoid of basal AEA level contamination which was observed with other published methods. Calibration curves and quality controls samples were prepared in plasma stripped with activated charcoal. AEA in plasma samples was extracted with ethyl acetate: hexane (70:30). The separation of AEA was achieved using a Zorbax XDB C18 column with a binary gradient mobile phase comprised of acetonitrile and 5 mM ammonium acetate buffer pH-3.5 at a flow rate of 0.5 mL/min. Quantitation of AEA modulation in the rat plasma samples was performed by LC-MS/MS in multiple reaction monitoring. Summation of multiple mass transitions of AEA was followed to gain better area counts, but daughter ion fragments with m/z 62.10 was found to be contaminated. Precursor ion scan of the same revealed the presence of unwanted mass with m/z-327 and 300, along with molecular ion fragment of AEA (m/z-348).Contamination from the daughter ion fragment was resolved chromatographically and samples analysis was executed successfully. Results indicate basal AEA levels in plasma are comparable with published data and are consistent in vehicle treated animals across various model evaluated, this observation also suggests that process of sample collection and bioanalysis are appropriate for the measurement of AEA in plasma. AEA was also found to be stable upto 5 months when stored at -20 Â°C. A robust and specific LC-MS/MS assay was developed for determination of AEA in rat plasma.

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# Preclinical pharmacokinetic studies of a novelanti-cancercompound, S007-1235

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CDRI's S007-1235 showed robust cancer cell-specific cytotoxicity and promising antiproliferative activity with good safety index [1]. The present work investigates its pharmacokinetics in rats and mice. S007-1235 was characterized for intestinal permeability, serum protein binding, pH dependent solubility and stability. Metabolic stability and CYP reaction phenotyping were assessed using human and rat liver microsomes and human CYP supersomes. Following the LC-MS/MS method development in various biological matrices, oral (10 mg/kg), intravenous (1 mg/kg) pharmacokinetics and tissue distribution studies were carried out in *Sprague Dawley* rats and *Balb/c* mice. S007-1235 possesses low aqueous solubility, high rat ileal permeability, moderate protein binding and was found stable insimulated gastrointestinal fluids. The estimated hepatic blood clearancevalues from rat and human liver microsomal studies were 55.75 and 9.25 ml/min/kgsuggesting it to be a high (extraction ratio, 0.79)and moderate (extraction ratio, 0.46) clearance compound in rat and human, respectively[2]. Qualitatively similar metabolites were detected in rats, mice and humanliver microsomes. Five putative metabolites [four phase I: M1 (S-oxidation), M2 (S-di-oxidation, M3 (N-de-ethylation), M4 (N-de-methylation and S-di-oxidation) and one phase II: M5 (N+-glucuronidation)] were identified in samples from *in vitro* and *in vivo* studies. Multiple CYPs metabolized S007-1235 but CYP3A4 and 2D6have major role in its metabolism both in rats and human. It exhibited a high $V_{ss}$(93.05 and 41.2 L/kg in rat and mice, [2]), high to moderate clearance (59 and 57 ml/min/kg, in rat and mice [2]) and MRT of 7-12 h.In accordanceto high$V_{ss}$, compound was distributed to lungs >intestine >liver >spleen >kidneys >heart >brain. Itpossesses low oral bioavailability (10.8%) in ratsthat might be due to poor solubility and extensive hepatic first pass metabolism. The findings provide useful insights on pharmacokinetic caveats and plusses of S007-1235 and will aid in its future development as potential anticancer drug candidate.

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# Hexavalent chromium induced oxidative stress and cytotoxicity in human blood cells and its attenuation by antioxidants

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Hexavalent chromium [Cr(VI)], widely used in various industries, induces multiple organ toxicity in humans and experimental animals. Cr(VI) is known to induce oxidative stress in target cells/ tissues. We have used taurine and 3,4 dihydroxybenzaldehyde (DHB), which are well known for their antioxidant properties, to attenuate the cytotoxicity of Cr(VI) in human erythrocytes and lymphocytes. Taurine is an organic acid present in animal tissues and several foods. DHB is a polyphenol found in green cavendish bananas.The effect of these antioxidants was studied on Cr(VI)-induced oxidative stress in human erythrocytes and lymphocytes. Erythrocytes and lymphocytes were isolated from fresh human blood and suspended in phosphate buffered saline to give 5% hematocrit. Cells were incubated with 0.5 mM potassium dichromate [K2Cr2O7; a Cr(VI) compound] either alone or in presence of taurine/ DHB, at 37 Â°C for 60 min.

Treatment of cells with K2Cr2O7 alone resulted in elevated levels of reactive oxygen species, increased lipid peroxidation and methemoglobin formation. Glutathione and total sulfhydryl content was decreased indicating the induction of oxidative stress in the cells. The plasma membrane redox system (PMRS) of human erythrocytes was also inhibited. The activities of several major antioxidant enzymes were also greatly altered. Preincubation of erythrocytes and lymphocytes with taurine and DHB attenuated the oxidative stress induced by K2Cr2O7 and also prevented inactivation of the PMRS of erythrocytes.Taurine and DHB protect erythrocytes and lymphocytes from oxidative stress produced by K2Cr2O7 under in vitro conditions. They can be potentially used to protect persons, who work in Cr utilizing industries, from the harmful/toxic effects of this metal.

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# Creatine reduces metal ions and attenuates oxidant induced hemoglobin oxidation and inactivation of erythrocyte PMRS

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Background: Creatine (Cr) is widely used by athletes as an ergogenic aid to enhance physical performance. Cr exerts beneficial effects in several diseases in which oxidative stress plays an etiological role. Studies suggest that Cr acts directly as radical species scavenger but the results are still controversial. Objectives: Excess production of reactive oxygen species results in oxidative stress condition. This can lead to inflammation, cellular injury and development of diseases. This study investigated the antioxidant potential of Cr, using erythrocytes as a model cellular system. Erythrocytes were selected since they are continuously exposed to both endogenous and exogenous sources of ROS like superoxide and hydrogen peroxide (Hâ„Oâ„). Methods: Exposure to Cr (0.25 to 10 mM) was used to determine its free radical scavenging activity and ability to reduce metal ions (Fe3+, Cu2+ and Mo6+). The effect of oxidant (H2O2) treatment on hemoglobin oxidation and erythrocytes plasma membrane redox system (PMRS) and ascorbate free radical (AFR) reductase activity was also evaluated in the presence and absence of Cr.

Results and discussion: Treatment of erythrocytes with 0.05 mM H2O2 increased hemoglobin oxidation to give methemoglobin. It also inhibited the PMRS and AFR reductase which maintain the redox environment of the cell. However, these changes were attenuated in the presence of Cr. Treatment with Cr alone upregulated both PMRS and AFR reductase above control values. Under an acellular setting Cr could reduce metal ions and also quench free radicals. Conclusion: Cr treatment can attenuate oxidant induced damage to cells and represents a promising therapeutic approach for reducing oxidative damage to cells/tissues induced by various xenobiotics.

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# Investigation of effect of Biochanin A on the pharmacokinetics Profile of Docetaxel using validated LC-MS/MS method

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Docetaxel (DTX) widely used as potent anticancer agent, is a substrate for ATP-binding cassette transporters such as P-glycoprotein and its metabolism is mainly catalysed by CYP3A. Biochanin A (BCA) is consumed worldwide as dietary supplements for relieving postmenopausal symptoms. The aim of this study was to investigate the effect of biochanin A (BCA) on the pharmacokinetics of DTX in female SD rats.

A rapid, selective and validated method was developed over the concentration range 1-500 ng/mL using liquid chromatography couple with tandem mass spectrometry (LC-MS/MS) for estimation of DTX in rat plasma. The DTX was administered orally (20 mg/kg) and intravenously (2 mg/kg) without or with oral BCA (100 mg/kg) after one week in female SD rats. As BCA is an inhibitor of CYP 3A and P-gp it was expected to increase the bioavailability of DTX, as a known substrate of CYP3A4/Pgp. Plasma samples were processed with liquid-liquid extraction and analysed by using validated LC-MS/MS method.

After compared with the control group (treated with DTX alone), BCA pre-treated animals did not showed significantly difference area under the plasma concentration-time curve from time zero to time infinity (AUC0-â^ž) and maximum DTX concentrations (Cmax).

If the results of this study are further confirmed by clinical trials, Docetaxel dosages should not be need to adjusted to avoid potential drug interaction when Docetaxel is used clinically in combination with BCA and BCA-containing dietary supplements.

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# A Novel Methodology to Determine NCE Enzyme Kinetics and Inhibition by Monitoring Parent Disappearance

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The determination of enzyme kinetic parameters, Km and Vmax, for cytochrome P450-catalyzed reactions is an important aspect in drug discovery and development. The in vitro approach of determining Km values are useful in early drug discovery efforts to identify those compounds with low Km values and, hence exhibit a greater probability of exhibiting supra proportional dose-exposure relationships in the clinic. Conventional determinations of Km values are made by assessingthe rate of product(metabolite) formation at several substrate concentrations.It is a Challenge to determine Vmax and Km when no single prominent metabolite is formed. Monitoring parent depletion is one option to measure Vmax and Km. In this study we assessed the accuracy of Km and Vmax determinations using a substrate-depletionapproach with those determined using the conventional product-formation approach, using recombinant human cytochrome P450 (CYP) enzymes.

For midazolam, using parent disappearance, the Km and Vmax were found to be 0.5ÂµM and 6.9nmol/min/nmol 3A4, respectively, using recombinant CYPs and were within two-fold to the parameters determined by the product formation method. For atazanavir, using parent disappearance, the Km and Vmax values were found to be 0.8ÂµM and 6.9nmol/min/nmol 3A4, respectively, and were also within two fold to the parameters determined using product formation.

In conclusion, the current study showed the substrate-depletion approach can be used to estimate Km and Vmax using recombinant CYPs. Estimation of these parameters during early discovery will aid in the understanding of dosages at which non-linearity may occur. This methodology is especially suited for compounds which form many metabolites, or where no metabolite standards are available.

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# Comprehensive evaluation of liver microsomal cytochrome P450 3A (CYP3A) inhibition: comparison of cynomolgus monkey and human

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1. Members of the cytochrome P450 3A (CYP3A) subfamily metabolize numerous compounds and serve as the loci of drug-drug interactions (DDIs). Because of high amino acid sequence identity with human CYP3A, the cynomolgus monkey has been proposed as a model species to support DDI risk assessment.

2. Therefore, the objective of this study was to evaluate 35 known inhibitors of human CYP3A using human (HLM) and cynomolgus monkey (CLM) liver microsomes. Midazolam was employed as substrate to generate IC50 values (concentration of inhibitor rendering 50% inhibition) in the absence and presence of a preincubation (30 mins) with NADPH.

3. In the absence of preincubation, theIC50 values generated with CLM were similar to those obtained with HLM (86% within 2-fold; 100% within 3-fold difference). However, significant differences (up to 48-fold) inpreincubation IC50were observed with 17% of the compounds (raloxifene, bergamottin, nicardipine, mibefradil, ritonavir, and diltiazem).

4. Our results indicate that in most cases the cynomolgus monkey can be a viable DDI model. However, significant species differences in time-dependent CYP3A inhibition can be observed for some compounds. In the case of raloxifene, such a difference can be ascribed to a specific CYP3A4 amino acid residue.

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# Characterisation of Trastuzumab using the Q Exactive Plus Benchtop Orbitrap mass spectrometer

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Monoclonal antibodies (mAbs) are an increasingly used modality in several therapeutic areas given their high sensitivity and selectivity. High resolution mass spectrometry (HRMS) is an essential technology in structural characterisation of these mAbs. The objective of current work was to use Trastuzumab (TRZ) as a tool molecule to establish a LC/MS-based mAb characterization workflow. Intact mass analysis (including light and heavy chain analysis), elucidation of glycoforms and protein sequence coverage were determined for TRZ using UHPLC and Q Exactive Plus mass spectrometer.

TRZ (CANMAb, Biocon, India) was procured locally. The bioanalytical workflow utilized a Shimadmzu Nexera X2 UHPLC coupled with Thermo Q-Exactive Plus mass spectrometer. Chromatographic separation was achieved with Zorbax 300SB-C8 column (Agilent, 300 A0, 4.6 X 100, 5Âµm). The data acquisition was accomplished using Xcalibur. Protein Deconvolution and PepFinder softwares were utilized for intact and peptide mass analysis, respectively. With optimized chromatography and HRMS parameters (S-lens RF value, capillary temperature, AGC target and resolution (17500)) the intact mass of TRZ was found to be 148057 Da (G0F/G0F). After reduction and alkylation, light and heavy chain masses were found to be 23437.635 Da and 50764.477 Da (G0F), respectively. Glycoforms viz. G0/G0F, G0F/ G0F, G1F/ G0F, G1F/G1F or G2F/G0F, and G1F/G2F were identified and further confirmed with heavy chain analysis. Optimised digestion protocol showed sequence coverage of 89.3% of light chain and 79.2 % of heavy chain. TRZ is a suitable tool molecule to establish mAB characterisation workflow in a bioanalytical lab. Results obtained in our lab are in concordance with those is reported in literature.

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# Quantitative bioanalytical approaches for estimation of Trastuzumab in a biological matrix

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Quantitative estimation of monoclonal antibodies (mAbs) in biological matrices such as plasma is essential for the assessment of its pharmacokinetic (PK) and pharmacodynamic characteristics. Traditionally, ligand binding assays (LBAs) are the first choice for quantitation given their sensitivity; however, LC/MS based assays are increasingly used an orthogonal quantification technology given their selectivity and ability to combine structure modifications along with quantification information. The objective of the current work was to develop direct plasma digestion (DI) and Immunocapture (IC)-LC/MS bioanalytical methods for estimation of Trastuzumab (TRZ) in mouse PK plasma samples and compare the results with traditional ELISA method. TRZ (CANMAb, Biocon, India) was procured locally. In-silico digestion of TRZ using Skyline provided list of probable tryptic peptides. A sensitive and reproducible TRZ peptide was selected for LCMS method based on trypsin digestion. The IC method used streptavidin magnetic beads to which capture antibody was tagged (goat anti-human Fc antibody). Method used 40 ÂµL plasma sample (calibrants/study samples) to which tagged magnetic beads were added, followed by incubation, wash and on-bead digestion. The DI method involved denaturation, reduction and alkylation steps before trypsin digestion. Digested samples were analysed using Waters UPLC coupled with API-5500 mass spectrometer. Developed methods and commercially available ELISA kit was used to analyse mouse PK (10 mpk, IV) study samples. DI, IC & ELISA methods showed LOQ of 391, 20 & 80 ng/ml, respectively. TRZ exposures (& PK parameters) measured by DI, IC and ELISA methods were comparable. The CL was 0.0072 ml/min/kg, the Vss 0.09 L/kg and the t1/2 165h as determined by IC-LC/MS.

For quantitative bioanalysis of mAbs, LC-MS methods provide suitable alternative / act as orthogonal technique to LBA methodology.

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# Simultaneous and Sensitive determination of vanillin and vanillic acid in Guinea pig plasma by offline derivatization LC-MS/MS: Assay development and Validation

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Aldehyde oxidase (AO) is a cytosolic enzyme and is ubiquitous. AO is a complex molybdoflavoprotein that belongs to a family of structurally related molybdenum-containing enzymes. Vanillin is used as a positive control for evaluation of AO activity by various laboratories. HPLC-UV methods have been reported for estimation of these analytes in biological matrices but limitations lies with the LLOQ and non-selectivity. Independent LC-MS/MS based methods are also reported for vanillin and vanillin acid with limited LLOQ (5 ng/mL for vanillin and 10 ng/mL for vanillic acid). We report here an offline derivatization of vanillin and vanillic acid by dansyl chloride in guinea pig plasma followed with LC-MS/MS simultaneous measurements in ESI positive ion mode.

Vanillin and vanillic acid in plasma was derivatized using dansyl chloride and separated on a C18 UPLC column (Waters BEH C18, 2.1 X 50mm, 1.7ÂµM) and detected on a QqQ mass spectrometer (AB Sciex API 4000) by monitoring MRM transitions (vanillin-386.2/156.2 and vanillic acid-402.2/171.2). Salicylic acid was used as an internal standard. Method was validated for precision, accuracy, matrix effect, recovery, stability of derivatized product and reproducibility. An LLOQ of 0.4 ng/mL is achieved for both vanillin and vanillic acid in plasma which is about 10-25 times more sensitive compared to published methods. Three precision and accuracy results from method validation were within Â±20% of their nominal concentrations. Derivatized product was subjected to bench-top stability (25Â°C) and was found to be stable for 6 hrs. Derivatized product was subjected to auto-sampler stability (10Â°C) and was found to be stable for 12 hrs. Method is robust and reproducible.

A simple offline dansyl chloride derivatization is used for simultaneous measurement of Vanillin and Vanillic acid using UPLC-MS/MS. Higher sensitivity of this method helped us to adopt this for the evaluation of vanillin as a substrate of Aldehyde oxidase in in-vitro and in-vivo studies.

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# Relative quantitation of five human drug metabolizing enzymes -Carboxylesterases 1, Aldehyde Oxidase1 (AOX1) and Sulfotransferases (1A1, 1A2 and 1E1- using a surrogate peptide approach in a novel Q-TOF platform.

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The expression levels of the drug metabolizing enzymes are very crucial in understanding the disposition of the xenobiotics. In the present work we have determined the relative levels of five DME's using a UPLC-Q-TOF mass spectrometer. Since protein standards availability of the endogenous proteins are difficult to procure, a surrogate peptide approach was chosen to quantify the endogenous levels of DME's. We also semi-automated the unique peptide search utilizing NCBI Blast, R and Strawberry Pearl to reduce the manual work of selecting the peptide against the various background. A typical approach to quantify proteins is to use using high resolution mass spectrometer for identification and followed by quantitation using triple quadrupoles; in the results presented here, we have used Q-TOF as a platform to combine both the workflow into one reducing the time of operation and reducing inter-instrument variability's. Human liver cytosol and Human S-9 was purchased from BD Biosciences, Gibco, Xenotech and CelsisIn Vitro Inc. The synthetic peptide standard was obtained from ABI scientific (Sterling, VA, USA). In order to determine the human AOX1, CES1 (Carboxylesterases 1) and SULT's (Sulfotransferases (1A1, 1A2 and 1E1) levels an efficient, simple digestion method was developed. The semiautomatic workflow of the BLAST search has showed its utility in searching the peptides with the predefined selection criteria. The levels of SULT's and AO was found to be more in the cytosolic fraction then S-9, in the case of esterases the liver S-9 fraction was found to be richer fraction. The Q-TOF platform with the all ion approach has proved to be a good quantitative tool. The developed approach is useful in identifying the unique peptide and quantitation of the DME's. Agilent's 6550 Q-TOF is a useful platform to quantify endogenous proteins in complex matrix.

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# A novel liquid chromatography tandem mass spectrometry method for the estimation of bilirubin glucuronides and its application to in vitro enzyme assays

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Bilirubin is a toxic waste product of metabolism, eliminated mainly through UGT1A1 mediated conjugation to mono- and di-glucuronides. Due to the low Km value of bilirubin glucuronidation, the sensitivity obtained with UV/visible light detection is not sufficient to accurately calculate UGT1A1 inhibition properties of new chemical entities at low bilirubin concentrations. In addition, bilirubin, as well as its metabolites, are unstable during sample preparation and bioanalysis. The objective of the current work to develop an LC-MS/MS method for the quick and accurate quantitation of low levels of bilirubin glucuronides in in vitro incubations.

In vitro incubations were conducted with bilirubin in HLM and hrUGT1A1. The samples were analyzed for bilirubin glucuronides using novel LC-MS/MS method. UV-Visible/MS correction approach was used to determine the concentrations of bilirubin glucuronides in the absence of synthetic standards. Enzyme kinetic parameters for the total glucuronide formation was determined using Michaelis Menten model by Graph Pad Prism. The metabolites were quantified using a qualitative/ quantitative approach utilizing UV to MS correction, thereby eliminating the need for synthetic standards. The method was sensitive enough to quantify mono- and di-glucuronides as low as 3 nM from in vitro incubations, and kinetics data was determined for total glucuronide formation. The Km and Vmax values for total bilirubin glucuronide formations were determined to be 0.05 Â± 0.01 ÂµM and 181.9 Â± 5.3 pmol/min/mg-protein, respectively, in human recombinant UGT1A1, and 0.23 Â± 0.05 ÂµM and 875 Â± 45 pmol/min/mg protein in human liver microsomes (HLM).

In summary, for the first time we have reported a LC-MS/MS based method for quantitation of bilirubin and its glucuronides from in vitro incubations.

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# Complementary in vitro tools to investigate renal drug transport

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Renal excretion is an important pathway for the elimination of endogenous and xenobiotic substances. A wide range of efflux and uptake transporters are expressed in the renal epithelial cells to regulate the excretion and the reabsorption of various kinds of organic anions, cations, peptides and nucleosides. Primary proximal tubule cell (PTC) monolayer assay was established by Dr Colin Brown (Newcastle University, UK). PTCs were isolated from human kidney less than 18 hours ex vivo and cultured on transwell inserts. The expression and functionality of kidney transporters is maintained in this model, therefore it is unique in vitro tool for investigating renal drug handling, potential nephrotoxicity and transporter mediated drug-drug interactions. To identify individual transporter interaction and investigate the underlying mechanisms of renal elimination of compounds, the following double transfected cell lines have been developed in accordance with their substrate specificity: MDCKII-OAT1/BCRP, MDCKII-OAT3/BCRP, MDCKII-OCT2/MATE1 and MDCKII-OCT2/MATE2-K. Transport of selected drugs and physiologically relevant endogenous substrates such as metformin, methotrexate, PAH, urate, TEA and E3S have been investigated with both PTC and appropriate double transfectant monolayers. Unlike other primary renal cell models, PTC monolayer assay maintains the full complement and expression level of endogenous renal transporters, resulting in a more physiologically relevant, and therefore predictive, model of drug handling in the clinical setting. Results in double transfected cell lines provided further evidence of transporter specific vectorial transport of the selected substrates. While the PTC model serves as a holistic approach to study renal handling of drug molecules and endogenous substrates, double transfected monolayer assays could complete the results gained in PTC model with functional characteristics, such as substrate specificity and transport mechanisms involved in the renal elimination. Our results highlight the complementary nature of the two models.

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# A novel dried blood spot platform for LC-MS/MS analysis of fipronil and its metabolites in human and rat blood: an approach to facilitate cost effective bio-monitoring and toxicokinetic studies.

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The rampant use of insecticides has caused havoc to human health. Development of a robust detection method for these chemicals is need of the hour. Fipronil is used to control household insects like cockroach, beetles, fleas, ticks, rootworm etc. Metabolism studies in rats and humans have shown that fipronil gets converted primarily into fipronil sulfone, a more persistent metabolite and on exposure of sunlight fipronil degrades in fipronil desulfinyl. Both fipronil sulfone and fipronil desulfinyl are more toxic than fipronil. In present study, we have developed and validated a high throughput LC-MS/MS method for the quantification of fipronil and its metabolites using micro volume dried blood spot technique.

A simple protein precipitation technique was used for extraction of all analytes from DBS card. The chromatographic separations were carried out by using Waters Atlantis C18 column (4.6 Ã— 50 mm, 5.0 Î¼m) with a short run time of 2 min. Acetonitrile and acetic acid (0.1% v/v) in a ratio of 70:30 (v/v) was used as mobile phase. Linearity were in the range of 0.1 to 100ng/ml and limit of detection (LOD) of the method was 0.01, 0.01 and 0.03 ng/ml for fipronil, fipronil sulfone and fipronil desulfinyl, respectively. All validation parameters such as precision, accuracy, recovery, matrix effect and stability met the acceptance criteria as per regulatory guidelines. Mass spectrometer was operated in negative ionization mode for all the analytes. Fipronil, fipronil sulfone and fipronil desulfinyl were monitored using MRM transitions of 434.9/329.8, 450.9/415.0, and 387.0/351, respectively. The method was successfully applied for the toxicokinetic study of fipronil desulfinyl in rats. DBS technique has significant advantage in reduction of number of rodents used and refinement in sample collection procedure for toxicokinetic studies. Minimal invasive blood sampling approach (eg. heal and finger prick) and ease of storage and transport makes DBS an ideal for biomonitoring studies.

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