

**OIII-A-3**

**ACOUSTIC RHINOMETRY IS A SENSITIVE OBJECTIVE MEASURE FOR NASAL CONGESTION IN PATIENTS WITH SEASONAL ALLERGIC RHINITIS EXPOSED TO RAGWEED POLLEN IN AN ENVIRONMENTAL EXPOSURE CHAMBER.** S. McCue, A. M. Salapatek, D. Botetzayas, C. Au Yeung, M. Ducharme, P. Patel; Allied Research International, Mississauga, ON, Canada

**BACKGROUND:** Seasonal allergic rhinitis (SAR) trials performed in an environmental exposure chamber (EEC) have improved symptoms variability due to controlled pollen exposure. Further improvement of study outcomes could be made by utilization of objective measures of nasal congestion. ACR is a non-invasive diagnostic assessment of nasal geometry sending wide-band noise signals into the nose and analyzing the reflected sound. In a clinical trial setting, ACR was conducted before and after ragweed exposure to assess its usefulness as an objective measure of nasal congestion.

**METHODS:** Retrospective analyses of clinical trial data from 228 ragweed-allergic patients were performed. In all cases, subjects had mean ACR recordings ( $\geq 3$  measurements made for left and right nostrils): Minimal Cross-sectional Areas (MCA) and Volumes (Vol) (recorded at MCA1, Vol1: 0-2.2cm and MCA2, Vol2: 2.2-5.4cm from the nasal opening). ACR assessments were made before EEC entry and after 90 minutes of ragweed pollen exposure in the EEC. MCA and Vol were compared using paired t-test, significant at  $p < 0.01$ .

**RESULTS:** Intra-patient ( $\leq 0.05\%$ ) or inter-patient ( $\leq 0.10\%$ ) variances were low. Minimal MCA and Vol declined significantly ( $p < 0.001$ ) from before (MCA:  $0.47 \pm 0.17 \text{ cm}^2$ ; Vol:  $1.91 \pm 0.43 \text{ cm}^3$ ) compared to after (MCA:  $0.39 \pm 0.18 \text{ cm}^2$ ; Vol:  $1.73 \pm 0.45 \text{ cm}^3$ ) pollen exposure. The change in MCA and Vol after pollen exposure was greater in MCA2 ( $0.11 \text{ cm}^2$ ) and Vol2 ( $0.75 \text{ cm}^3$ ) than MCA1 ( $0.06 \text{ cm}^2$ ) and Vol1 ( $0.08 \text{ cm}^3$ ).

**CONCLUSION:** These data demonstrate that patient exposure to pollen in an EEC resulted in increased congestion particularly in distal nasal turbinates (region of MCA2 and Vol2). This work indicates that ACR is a sensitive and effective objective measure for nasal congestion. Conduct of rhinitis studies with an objective marker of nasal congestion such as ACR is useful in determining clinical efficacy of new therapies in the treatment of rhinitis and will be proposed for future studies.

**OIII-A-4**

**SEMI-PHYSIOLOGICAL POPULATION PHARMACODYNAMIC MODEL FOR CHEMOTHERAPY-INDUCED NEUTROPENIA AND THROMBOCYTOPENIA IN CANCER PATIENTS.** C. M. Ng, C. Takimoto, M. Beeram, C. Lin, A. Patnaik; Cancer Therapy and Research Center, San Antonio, TX

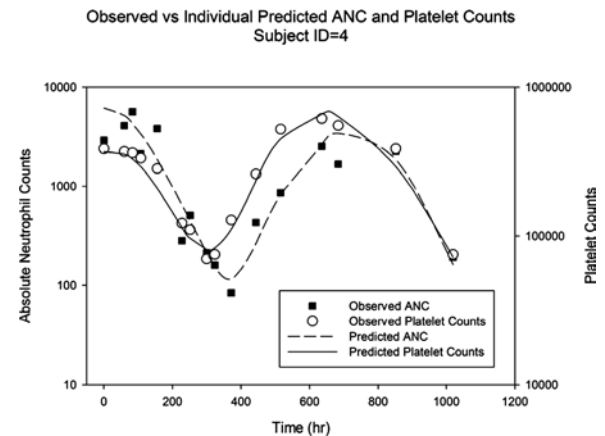
**BACKGROUND:** Neutropenia and thrombocytopenia are dose-limiting hematological toxicities for many anticancer drugs. The aim was to develop a population pharmacodynamic (PD) model that can simultaneously describe the time course of the absolute neutrophil and platelet count changes in cancer patients receiving chemotherapy.

**METHODS:** PKPD data from 31 subjects who received troxacitabine (TCB)/cisplatin therapy was used for model development. Monte-Carlo Parametric Expectation Maximization algorithm was used to develop the model and obtain population parameter estimates.

**RESULTS:** A three-compartment linear PK model was used to describe the TCB concentration-time profile. The final PD model consisted of 1) a drug-sensitive uncommitted progenitor cell compartment that differentiates into committed drug-sensitive neutrophil and platelet progenitor cells, and 2) transit compartments that represent cell maturation stages in bone marrow. The model included a feedback mechanism for rebound phenomena and hemostasis. The TCB affected the proliferation of sensitive progenitor cells through an inhibitory Emax model. The PD model well described the time-course of TCB-induced neutropenia and thrombocytopenia. The model predicted that neutrophil progenitor cells were more sensitive to TCB therapy compared

to platelet progenitor cells, and supported the findings that Grade 3/4 neutropenia is observed more frequent than thrombocytopenia in TCB-treated patients from early clinical studies.

**CONCLUSION:** To our knowledge, this is the first reported population PD model that can simultaneously describe both the intensity and duration of chemotherapy-induced thrombocytopenia and neutropenia in cancer patients. This model may be valuable for selecting treatment regimens that minimize chemotherapy-limiting hematological toxicities.



**PIII-01**

**THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF THE ANTI-TRYPANOSOMAL DRUG MELARSO-PROL: ADAPTATION OF AN *IN VITRO* SYSTEM MODELING PHARMACOKINETICS TO STUDY THE PHARMACODYNAMICS OF ANTI-TRYPANOSOMAL DRUGS.** R. P. Bakshi, J. I. Lee, F. M. Hamzeh, C. W. Hendrix, T. A. Shapiro; Johns Hopkins University, Baltimore, MD

**BACKGROUND:** African sleeping sickness is a deadly disease caused by the protozoan parasite *Trypanosoma brucei*. The toxic organoarsenical melarsoprol is the only drug that can cure all forms of human disease. Current treatment strategies require extended dosing regimens which were painstakingly developed through trial and error in clinical studies without the benefit of understanding the pharmacokinetic-pharmacodynamic (PK-PD) relationship of the drug. Systems for studying PK-PD relationships of melarsoprol and other anti-parasitic drugs *in vitro* have not been developed yet may greatly facilitate more rational development of these compounds.

**METHODS:** Commercially available bioreactors for *in vitro* PK-PD studies of anti-bacterial and anti-viral drug evaluation do not support the growth of protozoan parasites. We describe a novel *in vitro* PK-PD modeling system that supports the growth of *Trypanosoma brucei*, and its use in determining the PK-PD relationship of melarsoprol. In this system, we simulate concentration-time profiles of melarsoprol and measure the anti-parasitic effect on trypanosomes.

**RESULTS:** Testing three different  $AUC_{0-24}$  values for melarsoprol ( $0.96 \mu\text{M-h}$ ,  $1.68 \mu\text{M-h}$ ,  $2.16 \mu\text{M-h}$ ) delivered as a bolus compared to continuous infusion revealed that the bolus dose was more effective than continuous infusion for all 3  $AUC_{0-24}$  exposures: bolus/infusion % kill of 2, 1.5, and 1.3, respectively. This indicates that melarsoprol demonstrates concentration-dependent killing.

**CONCLUSION:** These results have implications for optimizing melarsoprol dosing *in vivo*. The modified PK-PD system we developed will allow testing of varied dosing regimens and associated efficacy of candidate anti-trypanosomal compounds early in the development process, inform rational dosing for human studies, and facilitate the development of new drugs for sleeping sickness.

### PIII-02

MULTIPLE DOSE PHASE 1 INVESTIGATION OF INTRAVENOUS ARTESUNATE IN HEALTHY VOLUNTEER SUBJECTS. L. R. Cantilena,<sup>1</sup> G. A. Saviolakis,<sup>1</sup> K. J. Leary,<sup>1</sup> R. S. Miller,<sup>2</sup> P. J. Weina;<sup>2</sup> <sup>1</sup>Uniformed Services University, Bethesda, MD, <sup>2</sup>Walter Reed Army Institute of Research, Silver Spring, MD

**BACKGROUND:** Only quinine and quinidine are currently available for parenteral use for the treatment of severe malaria worldwide and in the United States. The adverse event profile for both of these agents is significant and the efficacy of quinine is declining. Artemisinins are antimalarial sesquiterpenes from the plant *Artemisia annua* and have documented efficacy against malaria infection. Until recently no GMP formulation for intravenous artesunate (ART) has been available for systematic study in the US. The present study was conducted to establish the safety of a new GMP ART formulation given on the anticipated dosing schedule of once daily for 3 consecutive days.

**METHODS:** An inpatient, IV, double blinded, placebo-controlled phase 1 investigation with ART given once daily for 3 consecutive days was conducted in healthy male (23) and female (1) volunteers between 18 and 55 years of age. Three cohorts (2, 4 or 8 mg/kg ART) of 8 subjects (6 received ART and 2 received placebo) were studied. Serial ECG, hemodynamic and clinical observations were performed throughout.

**RESULTS:** No dose limiting toxicity was found for ART at the doses studied. No subject discontinued for an AE or treatment related issue. A dose related decrease in reticulocyte count was noted that peaked 5 to 7 days after dosing and returned to normal by study day 15 in most cases. This was not consistently associated with a decrease in Hgb or Hct. There were no other clinically significant laboratory abnormalities detected. No deleterious hemodynamic or electrocardiographic effects were seen. A reversible sensation of altered taste, lasting < 30 min, was seen with higher doses of ART. All remaining AEs were generally mild and all were reversible.

**CONCLUSION:** Once daily dosing for 3 consecutive days with this formulation of ART was generally well tolerated in healthy volunteers at the doses studied. Confirmatory efficacy testing with the GMP formulation is underway.

### PIII-03

THREE-DIMENSIONAL SPECT/CT CURVE FITTING ALGORITHM WITH LOCAL DENSITY DISTRIBUTION COMPARED TO DIRECT METHODS FOR QUANTIFICATION OF THE DISTRIBUTION OF RECTALLY-APPLIED HIV-SIZED PARTICLES. Y. J. Cao,<sup>1</sup> B. S. Caffo,<sup>2</sup> E. J. Fuchs,<sup>1</sup> L. A. Lee,<sup>1</sup> T. L. Parsons,<sup>1</sup> R. P. Bakshi,<sup>1</sup> W. A. Khan,<sup>1</sup> R. Wahl,<sup>3</sup> C. W. Hendrix<sup>1</sup>; <sup>1</sup>Department of Medicine, Johns Hopkins University, Baltimore, MD, <sup>2</sup>Department of Biostatistics, Johns Hopkins University, Baltimore, MD, <sup>3</sup>Department of Radiology, Johns Hopkins University, Baltimore, MD

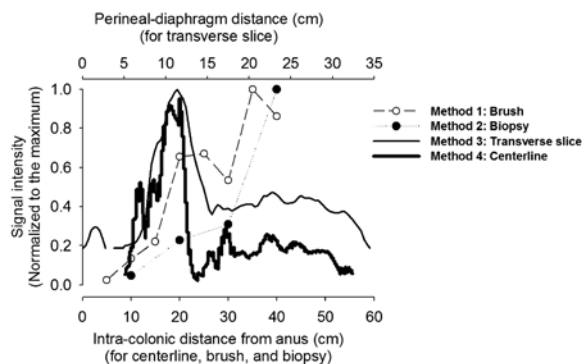
**BACKGROUND:** Despite the advanced stage of clinical development of candidate microbicides for HIV prevention, such as costly Phase III trials, of which several have already failed, little is known about the distribution of the HIV after intercourse. This study compares several novel methods to quantify the distribution of HIV-sized particles within the distal gastrointestinal (GI) tract.

**METHODS:** Three subjects received microbicide gel containing 0.5 mCi <sup>99m</sup>Tc-sulfur colloid (HIV-sized particle) via simulated anal sex. Subjects had SPECT/CT taken 4 hours after dosing, followed by sigmoidoscopy. Four methods were used for the quantitation of HIV-sized particle distribution: brush; biopsy; transverse slices of SPECT; colon centerline based on 3-dimensional SPECT data. The last method involves principal curve fitting for the identification of a centerline, around which the signal density was calculated at different radii.

**RESULTS:** Figure 1 shows the data for the first subject. With Method 1 and 2, only a few discrete locations could be sampled and it was hard to interpolate the density at other locations. Method 3 provided overall estimation at a 2-dimensional (2-D) transverse plane but did not allow the estimation of the 3-D pathway of strongest signals. The density gradient generated with Method 4 (the centerline with gradient averaged around 0.21 cm in radius) indicated the particles at

a specific space and most closely follow the knowledge upon visual examination of the SPECT/CT fusion image. Similar patterns were seen for the other two subjects.

**CONCLUSION:** The distribution gradient of HIV-sized particles administered via simulated anal sex can be semi-automatically quantified (Method 4). This enables the estimation of anatomically correct density-time-distance relationship within the GI tract to inform rational microbicide development.



### PIII-04

IN VITRO INHIBITION OF CYTOCHROME P450S (CYP) ENZYMES BY THE ANTIFUNGAL DRUG VORICONAZOLE AND PREDICTION OF DRUG INTERACTIONS IN VIVO. S. Jeong,<sup>1</sup> B. A. Ward,<sup>2</sup> P. D. Nguyen,<sup>2</sup> Z. Desta<sup>2</sup>; <sup>1</sup>Chonnam National University, Gwangju, Republic of Korea, <sup>2</sup>Indiana University, Indianapolis, IN

**BACKGROUND:** Voriconazole, a second-generation triazole, exhibits potent activity against a broad spectrum of clinically significant fungal pathogens, but drug interactions with it are of major concern. To allow prediction of these interactions, we quantified the inhibitory potency of voriconazole on a panel of CYP isoforms.

**METHODS:** Isoform specific probe substrates were incubated with HLMs (or expressed CYPs) in the presence and absence of multiple voriconazole concentrations to estimate IC<sub>50</sub> values. The potential for mechanism-based inactivation was tested by preincubation voriconazole for different times with HLMs and NADPH before the probe substrate was added. Dixon plots were constructed in HLMs to estimate K<sub>i</sub> values. In vivo prediction from the in vitro data was made using the  $AUC_{\text{inhibited}}/AUC_{\text{uninhibited}} = 1 + (I/K_i)$  and average voriconazole C<sub>max</sub> and C<sub>min</sub> of 9 μM and 3 μM respectively.

**RESULTS:** Voriconazole was shown to be a potent inhibitor of CYP2B6, CYP2C9, CYP2C19 and CYP3A (IC<sub>50</sub>s <5 μM). Voriconazole (up to 100 μM) had no inhibitory effect on CYP1A2, CYP2A6, CYP2C8 and CYP2D6 in coinubation or preincubation experiments (inhibition by <20%). Detailed inhibition experiments in HLMs show that voriconazole is a highly potent competitive (CYP2B6, CYP2C9 and CYP2C19) and a noncompetitive (CYP3A) inhibitor (Table). We predict a significant effect of voriconazole on the pharmacokinetics of drug metabolized by these enzymes (Table).

**CONCLUSION:** Voriconazole would be anticipated to markedly slow the clearance of drugs predominantly metabolized by CYP2B6, CYP2C9, CYP2C19 and CYP3A.

TABLE. In vitro inhibitory potency of voriconazole (Vori) and estimated in vivo drug interactions

CYP isoforms	CYP2B6	CYP2C9	CYP2C19	CYP3A	
In vitro K <sub>i</sub> values (μM)	1.19	2.79	3.16	2.97	
Predicted (AUC <sub>i</sub> /AUC)	At C <sub>max</sub> of Vori	8.6	4.2	3.8	4.0
	At C <sub>min</sub> of Vori	3.5	2.1	1.9	2.0

**PIII-05**

MAPPING THE RELATIONSHIP BETWEEN CLINICAL RESPONSE AND AN ANTIMICROBIAL DRUG EXPOSURE/SENSITIVITY INDEX USING A NEURAL NETWORK. D. E. Mager,<sup>1</sup> M. H. Adelman,<sup>2</sup> J. J. Schentag<sup>2</sup>; <sup>1</sup>University at Buffalo, SUNY, Buffalo, NY, <sup>2</sup>CPL Associates LLC, Buffalo, NY

**BACKGROUND:** The area under the inhibitory curve (AUC<sub>24</sub> = AUC<sub>24</sub>/MIC) is an important pharmacokinetic/pharmacodynamic (PK/PD) index associated with antimicrobial activity and clinical outcome, particularly for concentration-dependent agents. However, characterizing the relationship between the rates of bacterial eradication and clinical improvement remains a formidable challenge. The purpose of this study was to develop a neural network (NN) model that correlates subsequent pneumonia score (PS) values, a measurement of clinical status, with current culture results and PS and AUC<sub>24</sub> calculations.

**METHODS:** Data were obtained from 3 clinical trials of patients presenting with severe pneumonia (n=81). Serial cultures, AUC<sub>24</sub>, and PS values were extracted, where PS is a clinical response composite of 10 clinical parameters (score range 10-40) previously developed in our lab. A back-propagation NN was developed to anticipate subsequent (next day) PS values. Model inputs included: 1) day since the patient entered the trial, 2) current (present day) culture results (positive or negative) and AUC<sub>24</sub> values for each identified organism, and 3) current PS values. Network training was conducted using a scaled conjugate gradient algorithm.

**RESULTS:** The final NN reasonably described the temporal patterns of PS values (r<sup>2</sup>=0.74) without specification of a structural PK/PD model. Despite inter-patient variability in response-time profiles, the NN model captured overall trends as well as individual divergence from population profiles.

**CONCLUSION:** The relationship between rates of clinical outcome and organism eradication is complex and is challenging to characterize with traditional numerical methods. Non-deterministic modeling with NNs may be useful for predicting clinical outcomes of antimicrobial pharmacotherapy from readily available patient and organism characteristics (PS and culture results) and the AUC<sub>24</sub> PK/PD index.

**PIII-06**

ABSOLUTE ORAL BIOAVAILABILITY AND BIOEQUIVALENCE OF TELBIVUDINE IN HEALTHY SUBJECTS. X. Zhou,<sup>1</sup> B. A. Fielman Constance,<sup>1</sup> K. Kleber,<sup>1</sup> D. Frank,<sup>1</sup> J. Ke,<sup>2</sup> C. C. Chao,<sup>1</sup> N. A. Brown<sup>1</sup>; <sup>1</sup>Idenix Pharmaceuticals, Inc., Cambridge, MA, <sup>2</sup>Novartis Pharmaceuticals Corp., East Hanover, NJ

**BACKGROUND:** Telbivudine has been approved in many countries/regions as a QD 600mg film-coated tablet for the treatment of chronic hepatitis B. A 20mg/mL oral solution is being developed for patients requiring dose adjustment, and a 200mg tablet formulation was used in the phase III GLOBE registration trial. This study investigates the absolute bioavailability (BA, F) of telbivudine after PO and IV dosing and to establish bioequivalence (BE) among the oral formulations.

**METHODS:** Two open-label, single-dose, crossover studies were conducted in healthy subjects. The BA study enrolled 12 subjects randomized into 2 equal groups. Each received telbivudine 15mg IV and 200mg PO via a 2-way crossover design. The BE study enrolled 24 subjects randomized into 3 equal groups. Each received telbivudine 600mg as the 3 oral formulations via a 3-way crossover design. Blood and/or urine were collected through 120h after dosing and assayed using validated LC/MS/MS methods.

**RESULTS:** In the BA study, mean cumulative urinary excretion was 44.8% of the 200mg oral dose and 83.3% of the 15mg IV dose. F was 52% based on urinary or plasma data. In the BE study, plasma PK parameters of telbivudine 600mg were comparable for the 3 oral formulations: mean C<sub>max</sub> and AUC<sub>t</sub> were 3.1, 3.3, and 3.5µg/mL and 28.4, 30.1, and 30.8µg\*<sup>h</sup>/mL for the 200mg tablet, 600mg tablet, and oral solution, respectively. The 90% CI of the geometric mean ratios (600mg tablet and oral solution vs. 200mg tablet, and oral solution vs. 600mg tablet) of exposure parameters all fell within the 80%-125%

critical range for BE. The median T<sub>max</sub> (3h) was identical for all formulations.

**CONCLUSION:** Oral telbivudine is well absorbed, with a high bioavailability yielding consistent exposure in a QD regimen. The 200mg tablet, 600mg film-coated tablet, and oral solution are all bioequivalent, supporting QD dosing with a single 600mg tablet or the oral solution when dose adjustment is required.

**PIII-07**

EVALUATION OF LIMITED SAMPLING METHODS FOR ORAL BUSULFAN PHARMACOKINETIC MONITORING IN ADULT PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION. P. Bouchard,<sup>1</sup> S. Bilodeau,<sup>1</sup> K. Alain,<sup>1</sup> B. Vadnais,<sup>1</sup> M. Franco,<sup>1</sup> V. Michaud,<sup>2</sup> J. Turgeon<sup>2</sup>; <sup>1</sup>Hôpital Maisonneuve-Rosemont, Montréal, QC, Canada, <sup>2</sup>Research Center, Centre hospitalier de l'Université de Montréal, Montréal, QC, Canada

**BACKGROUND:** Busulfan is one of the most commonly used drug in preparative regimens for hematopoietic stem cell transplantation. Area under the concentration-time curve (AUC) is monitored to ensure efficacy and avoid toxicity. A number of limited sampling models (LSM) have been proposed for busulfan AUC monitoring as 10 blood samples are usually performed. Our objective was to evaluate the available LSM and develop new LSM to determine the AUC achieved after an oral dose of busulfan in adult patients.

**METHODS:** Pharmacokinetic profiles were obtained from charts of patients who received busulfan between June 2003 and March 2007. From the literature, 9 LSM were identified and 9 new LSM were developed. LSM were required to have between 2 and 5 samples. AUC was calculated with the trapezoidal method and extrapolated to infinity. For each LSM and each profile, AUC was compared to the reference method with Pearson's correlation, Student's t-test for mean difference for paired observations and Bland and Altman graphical method. To further determine the security of LSM, we considered the incidence of difference between LSM and reference exceeding 10 %.

**RESULTS:**

Evaluation of limited sampling methods (LSM) for oral busulfan in adult patients

LSM	Number of samples	Mean of LSM AUC/reference AUC	Pearson's correlation coefficient	% of LSM AUC exceeding 10 % difference with reference AUC	Mean difference (%) between LSM and reference (Student's t-test)	p-value for mean difference (Student's t-test)
Schuler et al.	2	1,128	0,498	47,4	-13,01	<0,001
Bullock et al.	2	1,098	0,734	22,3	-10,86	<0,001
Schuler et al.	3	1,040	0,670	32,1	-4,00	0,034
Bullock et al.	3	1,009	0,876	12,2	-1,12	0,219
Bullock et al.	4	0,986	0,924	3,3	1,25	0,047
New LSM 4-3	4	0,994	0,938	7,3	0,35	0,562
Bullock et al.	5	1,000	0,936	2,6	-0,12	0,844
New LSM 5-2	5	0,991	0,928	3,9	0,71	0,270
New LSM 5-4	5	1,000	0,929	1,3	-0,18	0,777

We analysed 153 profiles. The last 3 LSM presented in the table provided adequate safety and efficacy. These methods had 5 samples and did not produce statistically significant mean difference between LSM and reference. Bland and Altman graphical method demonstrated constant agreement between LSM and reference AUC. The 10 % margin was crossed in rare occasions.

**CONCLUSION:** It would be safe and effective to use one of the 3 LSM identified to determine the AUC after the first or second dose of busulfan. Using a LSM would result in a significant reduction in nursing workload as well as analysis time and cost.

**PIII-08**

TEMPORAL INFLUENCE OF ALCOHOL DETOXIFICATION AND SMOKING ON PLASMA DOPAMINE AND R/S-SALSOLINOL IN ALCOHOLICS. S. Brar,<sup>1</sup> V. Ramchandani,<sup>2</sup> J. Hersh,<sup>2</sup> J. Lee,<sup>2</sup> H. Kim,<sup>2</sup> D. T. George,<sup>2</sup> D. Herion,<sup>2</sup> J. Venitz<sup>1</sup>; <sup>1</sup>Virginia Commonwealth University, Richmond, VA, <sup>2</sup>National Institute on Alcohol Abuse and Alcoholism-NIH, Bethesda, MD

**BACKGROUND:** The purpose of this clinical investigation was to evaluate (R/S)-salsolinol levels in alcohol-dependent patients undergoing detoxification. Salsolinol (1-methyl-1,2,3,4-tetrahydro-6,7-dihydroxyisoquinoline, SAL), an acetaldehyde and dopamine (DA) condensation product, is currently being examined as a potential alcoholism biomarker.

**METHODS:** Alcoholics undergoing inpatient detoxification were evaluated over 3-weeks in which serial R/S-SAL and DA plasma concentrations were measured. The observational study is longitudinal in design and attempts to describe the natural progression of R/S-SAL during detoxification. Patients were selected based on diagnosis of alcoholism and lack of other substance abuse, besides smoking. Demographics and information on smoking and alcohol use were investigated as possible covariates of R/S-SAL and DA exposures.

**RESULTS:** Analysis of data from 6 patients showed that R- and S-SAL concentrations persisted and, on average, declined throughout the detoxification period. A significant change from baseline was observed with all patients (>23% change,  $p < 0.05$ ). Although a moderate correlation ( $r > 0.6$ ) was observed for both enantiomers and DA, levels of DA remained relatively constant throughout the period. R- and S-SAL sensitivity (defined: change in SAL conc./change in DA conc.) infers that smoking has an influence on SAL exposures for a given DA exposure. Ratio of R/S-SAL composition appeared to be influenced by smoking with smokers having a higher ratio than nonsmokers on average.

**CONCLUSION:** In this small sample size study, significant inter-patient variability in R/S- salsolinol concentrations was observed. Detoxification and smoking status in these patients appear to relate to SAL exposures. Further covariate exploration (i.e., effects of drinking history, diet and withdrawal severity) on R/S-SAL exposure is warranted for evaluation of SAL as a putative biomarker for alcohol dependence.

**PIII-09**

RISK OF FETAL ALCOHOL EXPOSURE AMONG NEWBORNS IN A HIGH RISK UNIT. Y. I. Goh,<sup>1</sup> J. R. Hutson,<sup>1</sup> H. Roukema,<sup>2</sup> G. Koren<sup>1</sup>; <sup>1</sup>The Hospital for Sick Children, Toronto, ON, Canada, <sup>2</sup>St. Joseph Health Care London, London, ON, Canada

**BACKGROUND:** Fatty acid ethyl esters (FAEEs) are sensitive and specific markers for prenatal alcohol exposure found in meconium. A previous study reported a 2.3% prevalence of FAEE positive meconium in Grey-Bruce, Ontario. However, not all residents deliver within the Grey-Bruce region. High risk maternal-fetal conditions are transferred to tertiary healthcare in London, Ontario. The objective of our study was to measure the prevalence of FAEE positive meconium of Grey-Bruce babies delivered in tertiary healthcare setting. We hypothesized that there will be higher positive rates in tertiary versus primary healthcare settings due to alcohol involvement or association with high risk pregnancies.

**METHODS:** Babies born to Grey-Bruce residents delivering at St. Joseph's Health Care London were identified. Mothers were informed of the anonymous prevalence study and were provided specimen bags and instructions on meconium collection. Mothers declining to participate indicated so by marking a designated box on the bag. All specimen bags were placed in a -20°C freezer. The meconium was transported on dry ice to The Hospital for Sick Children where FAEEs were quantified using gas chromatography-mass spectrometry.

**RESULTS:** Forty-six meconium specimens were collected from August 1, 2006 - August 1, 2007. Twelve specimens (26%) tested

positive for FAEEs. This translated to a 10-fold higher rate than babies born in primary healthcare settings ( $p < 0.0001$ ).

**CONCLUSION:** Referral to high risk tertiary unit confers a 10-fold risk for in utero alcohol exposure. From the current data it is unclear whether alcohol exposure is a cause for a high risk delivery and transfer or whether it is a marker of another risk factor. Nevertheless, efforts should be directed towards implementing screens for infants born in these environments and instituting appropriate follow-up programs.

**PIII-10**

GAMMA-HYDROXYBUTYRATE (GHB) EFFECTS UPON DRIVING A CAR. D. A. Dempsey,<sup>1</sup> M. Meyers,<sup>1</sup> D. Fiorentino,<sup>2</sup> C. A. Haller,<sup>3</sup> N. L. Benowitz,<sup>1</sup> N. Murphy<sup>4</sup>; <sup>1</sup>University of California, San Francisco, San Francisco, CA, <sup>2</sup>Southern California Research Institute, Van Nuys, CA, <sup>3</sup>Amgen Inc., South San Francisco, CA, <sup>4</sup>IWK Regional Poison Centre, Halifax, NS, Canada

**BACKGROUND:** GHB (Xyrem®), used for the treatment of narcolepsy, is also a drug of abuse implicated as a "date rape" drug. There are GHB case reports of impaired driving. The aim of this study was to document the effect of GHB upon driving using a driving simulator validated in alcohol driving studies ([www.nhtsa.dot.gov/people/injury/research/pub/impaired\\_driving/BAC/impairment.pdf](http://www.nhtsa.dot.gov/people/injury/research/pub/impaired_driving/BAC/impairment.pdf))

**METHODS:** Sixteen healthy consented subjects (8 men) with scant exposure to GHB completed a 3 day, 2 arm blinded, randomized, cross over study of 50 mg/kg GHB versus placebo (P). Day 1 was for practice and mastery of the driving simulator. Days 2 and 3 were study days. After a baseline driving session, subjects received P or GHB, followed by driving sessions at 1, 3 and 6 hrs post dosing. The driving simulator consists of 3 LCD monitors (110° viewing angle), steering wheel, accelerator, brake pedals, turn signals, and horn. The 18-20 minute driving session includes several traffic scenarios, with peripheral visual cues prompting driver actions. P and GHB driving scores were compared using paired T Tests. Concurrent blood samples for GHB levels were collected; these data and their relationship to driving scores will be presented.

**RESULTS:** There were no significant (sig.) differences between the GHB and P sessions at baseline, or at 3 and 6 hrs post dosing. At 1 hour, subjects in the GHB arm had sig. more collisions (8.25 vs 2,  $p = 0.0003$ ) and off road accidents (1.75 vs 0.2,  $p = 0.01$ ). They also had sig. poorer performances for response time ( $p = 0.05$ ), speed ( $p = 0.04$ ), speed deviations (0.003), lane position deviations (0.003), and median time to collisions (0.01). Self rating for ability to drive was also sig. different ( $p < 0.00001$ ).

**CONCLUSION:** This is the first study to objectively document the detrimental effects of GHB upon the ability to drive at doses used to treat narcolepsy which may be less than doses likely to occur with abuse. We were able to demonstrate rapid reversal of these effects by 3 hours post dosing.

**PIII-11**

IMPROVEMENTS IN LONG-TERM MORTALITY AFTER MYOCARDIAL INFARCTION AND INCREASED USE OF CARDIOVASCULAR DRUGS AFTER DISCHARGE: A 10-YEAR TREND ANALYSIS. S. Setoguchi, R. J. Glynn, J. Avorn, M. A. Mittleman, R. Levin, W. C. Winkelmayr; Brigham and Women's Hospital and Harvard Medical School, Boston, MA

**BACKGROUND:** Objective: Assess the relationship between increasing use of cardiovascular medications and trends in long-term prognosis after MI in the elderly. Background: Statins, beta-blockers (BB), angiotensin converting enzyme-inhibitors (ACEI) and angiotensin-II-receptor blockers (ARB) have been increasingly used over the past decade after myocardial infarction (MI). However, little is known about the relationship between increasing use of these medications and improvements in prognosis after MI.

**METHODS:** Using data from pharmacy assistance programs and Medicare in two states (1995-2004), we identified patients with MI who survived  $\geq 30$  days after discharge. We assessed age, gender, race, comorbidities, and coronary interventions during the MI hospitalization, and recorded filled prescriptions for statin, BB, ACEI/ARB or antiplatelet

agents within 30 days after discharge. All patients were tracked until they died, or until the end of eligibility/study period. We built multivariate Cox regression models to assess trends in the long-term mortality and the contribution to increasing medication use after MI.

**RESULTS:** Of 21,484 patients identified, 12,142 died during 74,982 person-years of follow-up. After adjusting for demographics and comorbidities, we found that mortality after MI decreased significantly from 1995 to 2004 (HR for annual trend: 0.97; 95% CI 0.97-0.98), a 3% reduction in mortality each year. Adjusting for the use of statins, BB, ACEI/ARB and antiplatelet drugs after discharge completely eliminated the association between time trend and mortality (HR 1.00; 95% CI 0.99-1.01).

**CONCLUSION:** The observed improvement in long-term mortality in elderly patients with MI may be mainly due to increased use of cardiovascular medications after discharge.

**PIII-12**

Withdrawn

**PIII-13**

APPLICATION OF CLINICAL PHARMACOLOGY IN PHARMACOECONOMICS: ECONOMIC EVALUATION OF HMG-COA REDUCTASE INHIBITORS USING PRAGMATIC HEAD-TO-HEAD RANDOMIZED CONTROLLED TRIALS WITH UP-TITRATION OF DRUG DOSES TO OBTAIN THERAPEUTIC TARGETS FOR LDL-CHOLESTEROL. P. Farahani; Centre for Evaluation of Medicines - St. Joseph's Hospital, McMaster University, Hamilton, ON, Canada

**BACKGROUND:** Economic evolution of therapeutics should reflect the situation that is occurring in clinical settings. Pragmatic head-to-head randomized controlled trials (RCTs) can potentially be a better estimate of the real world compared to placebo-RCTs. Head-to-head RCTs of statins, which applied up-titration of drug doses to obtain therapeutic targets for LDL-C during an adequate span of time, are appropriate to obtain a better estimate for economic benefits of statins. **OBJECTIVE:** To estimate the cost-effectiveness outcomes of rosuvastatin (ROS), atorvastatin (ATR) and simvastatin (SIM) for therapeutically equivalent starting doses using pragmatic head-to-head RCTs.

**METHODS:** The summary data for lipid profile parameters was extracted from two published head-to-head RCTs. These RCTs compared ROS (starting dose 5 mg) with ATR (starting dose 10 mg) [Am Heart J 2002;144:1044-51] and ROS (starting dose 5 mg) with SIM (starting dose 20 mg) [Am Heart J 2002;144:1036-43] with up-titration doses to obtain LDL-C goals for the patients with hypercholesterolemia during a period of 52 weeks. Costs of the drugs including monitoring costs for up-titration were considered for the analysis.

**RESULTS:** The annual costs (Canadian dollars) including monitoring costs for ROS, ATR and SIM were estimated to be \$ 558.14, \$ 714.03 and \$ 561.82, respectively. Weighted average of LDL-C reduction (as primary target) and TC / HDL-C reduction (as secondary target) for ROS, ATR and SIM were estimated to be [44%, 42% and 37%] and [34%, 32% and 30%], respectively. ICERs were calculated for LDL-C reduction and TC/HDL-C reduction, respectively as follows: [ROS vs. ATR] -39.90 and -52.14, [ROS vs. SIM] -0.80 and -1.39.

**CONCLUSION:** Resulted ICERs from both pragmatic RCTs demonstrated that ROS can be more economically efficient than ATR and SIM for the reduction of both primary and secondary clinical targets during a one year period of hypercholesterolemia treatment in clinical settings.

**PIII-14**

APPLICATION OF CLINICAL PHARMACOLOGY IN PHARMACOECONOMICS: COST-EFFECTIVENESS ANALYSIS OF THERAPEUTICS USING CLINICALLY EQUIVALENT DOSES: AN EXAMPLE FROM HMG-COA REDUCTASE INHIBITORS (STATINS). P. Farahani; Centre for Evaluation of Medicines - St. Joseph's Hospital, McMaster University, Hamilton, ON, Canada

**BACKGROUND:** Economic evolution of therapeutics can accurately support decision-making in resource allocation in healthcare if principals of clinical pharmacology are considered for the models. Therapeutically equivalent doses should be incorporated into the economic analysis to reflect the clinical comparability of therapeutics. **Objective:** To determine the cost effective outcomes for clinically equivalent doses of rosuvastatin, atorvastatin and simvastatin using lipid profile parameters and LDL-C goal attainment.

**METHODS:** Clinical efficacy data were obtained from STELLAR Study<sup>1</sup> (Statin Therapies for Elevated Lipid Levels Compared Across Doses to Rosuvastatin), which was a multi-centre randomized, parallel group, open label trial. Lipid profile parameters including LDL Cholesterol (LDL-C), HDL Cholesterol (HDL-C), Total Cholesterol (TC), TC to HDL-C Ratio, Triglycerides (TG), Apolipoprotein B (apoB) and apoB to apoA-I ratio were used as efficacy measures. Rosuvastatin 10 mg = atorvastatin 20 mg = simvastatin 40 mg were considered as therapeutically equivalent doses<sup>2</sup>.

**RESULTS:** Results from economic analysis are summarized below.

Parameters	Rosuvastatin 10 mg	Atorvastatin 20 mg	Simvastatin 40 mg	ICERs for Rosuvastatin Versus Atorvastatin	ICERs for Rosuvastatin Versus Simvastatin
Annual Cost (Canadian dollars)	496.40	759.20	514.65		
% LDL-C Reduction	45.87	42.57	38.81	-79.63	-2.58
% TC / HDL-C	37.36	34.43	31.16	-89.69	-2.94
%TC Reduction	32.89	31.76	27.87	-232.56	-3.63
%HDL-C Increase	7.75	4.89	5.31	-91.88	-7.47
% TG Reduction	20.06	22.62	14.93	469.28	-3.55
% apoB Reduction	36.77	35.16	30.89	-163.22	-3.10
% apoB / apoA-I Reduction	40.99	38.24	34.97	-95.56	-5.58
Proportion of patients at LDL-C goal (Canadian)	0.85	0.78	0.66	-3754.28	-96.05

**CONCLUSION:** Rosuvastatin dominated atorvastatin and simvastatin for almost all lipid profile parameters measures and LDL-C goal attainment. Therefore, rosuvastatin is more cost-effective compared with atorvastatin and simvastatin.

References:

- 1-Jones PH, Davidson MH *et al.* Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR\* Trial). Am.J Cardiol. 2003;92:152-60.
- 2-Kendrach MG. Approximate equivalent rosuvastatin doses for temporary statin interchange programs. Ann.Pharmacother. 2004;38:1286-92.

**PIII-15**

GLUCOSAMINE USE WITH WARFARIN IS ASSOCIATED WITH ELEVATED INTERNATIONAL NORMALIZED RATIO (INR) VALUE. J. F. Knudsen, G. Sokol; Food & Drug Administration, Silver Spring, MD

**BACKGROUND:** Glucosamine (GL) is a dietary supplement touted for joint health. We describe a patient with an elevated INR after concomitant use of GL and warfarin. Our objective was to identify reports of this association and the potential for increased bleeding.

**METHODS:** The design was a pharmacovigilance survey of spontaneously reported adverse events in warfarin-treated patients

who took GL or a combination product containing it. The Food and Drug Administration's Adverse Events Reporting System (AERS) database was searched using applicable search terms. PubMed was searched for reports of an interaction between GL and warfarin. Data mining scores associated with reports in the AERS database were examined. The setting was that of a government-affiliated drug evaluation center.

**RESULTS:** We identified 81 U.S. cases from AERS that co-reported warfarin use with GL or a combination product; 61 cases were eliminated due to confounding by multiple drug use and/or insufficient clinical data for a reliable assessment. Sex delineation: 10 men, 9 women, 1 unknown. Ages varied from 33 to 89 yrs (median 62 yrs). The majority had taken warfarin chronically when the increased INR was noticed. INR values increased 2- to 3-fold from baseline, returning to normal after GL was stopped or warfarin was lowered. There was a report of a subdural hematoma with permanent vegetative state and cases of intraventricular bleeding and excessive bruising. Vitamin K was required in some patients. A summary by Yue et al. (WHO Uppsala) delineated 21 cases (39-99 yrs) with increased INR, the event resolved in 17 cases upon GL discontinuation. Data mining score (drug-event combination) EB05 >1, indicating 95% confidence that a drug-event combination is reported at a higher-than-expected rate, does not prove causality or an increased relative risk.

**CONCLUSION:** The aggregate data suggest that concomitant use of GL may potentiate warfarin's effects on the INR and increase risk of bleeding.

### PIII-16

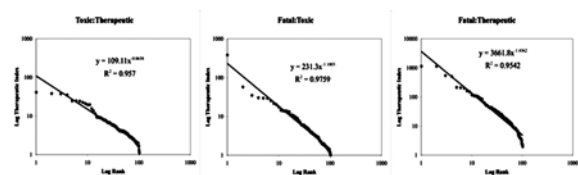
POWER LAW DISTRIBUTION OF THERAPEUTIC, TOXIC, AND FATAL CONCENTRATIONS OF MEDICATIONS. D. A. Frattarelli, MD; Henry Ford Health System, Dearborn, MI

**BACKGROUND:** Power law distributions are unique patterns which arise from complex systems. It has previously been described at the population level that adverse drug reactions (ADRs) follow a power law distribution when the incidence of reaction type is plotted as a function of its severity. To better understand the reasons for this observation, therapeutic indices were examined to see if this same pattern was present.

**METHODS:** A published database of the therapeutic, toxic, and fatal concentrations of 800 compounds was used as the source of data. Only those compounds which were therapeutic medications (as opposed to toxins), which were currently in use in the US, and which had complete data for all three concentrations were included (n=100). For values presented as a range, means were calculated and used in the analysis. The ratios of toxic:therapeutic, fatal:toxic, and fatal:therapeutic concentrations were calculated and ranked in descending order. These were plotted on log-log axes and fit to equations which described their distribution.

**RESULTS:** As shown in the figure below, all data were best described by a power law, which correlated well with the observed data (Pearson's correlations of 0.874, 0.937, and 0.890, respectively,  $p < 0.005$  for all). Slopes for all sets were similar.

**CONCLUSION:** Therapeutic indices for fatal and toxic reactions to pharmacotherapy follow a power law distribution, as has been described in population studies. As this type of distribution is characteristic of complex adaptive systems, these observations give further support to the theory that ADRs are the result of and are well conceptualized as failures resultant from perturbations of complex adaptive systems. Further studies which look at the interaction of multiple agents in a system, rather than single dominant genetic factors, may better explain the majority of ADRs.



### PIII-17

Withdrawn

### PIII-18

SINGLE DOSE ESCALATION PHASE I STUDY TO EVALUATE THE PHARMACOKINETICS (PK) OF SILYMARIN (LEGALON®) AND THE EFFECT OF FOOD IN PATIENTS WITH CHRONIC HEPATITIS C (HCV). T. A. Soule,<sup>1</sup> S. J. Schriber,<sup>1</sup> Z. Wen,<sup>1</sup> A. Wahed,<sup>2</sup> P. C. Smith,<sup>1</sup> M. W. Fried,<sup>1</sup> R. Reddy,<sup>3</sup> V. J. Navarro,<sup>4</sup> N. H. Afdhal,<sup>5</sup> S. H. Belle,<sup>2</sup> J. Berman,<sup>6</sup> Q. Y. Liu,<sup>6</sup> E. Doo,<sup>7</sup> R. L. Hawke<sup>1</sup>; <sup>1</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC, <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, <sup>3</sup>University of Pennsylvania, Philadelphia, PA, <sup>4</sup>Thomas Jefferson University, Philadelphia, PA, <sup>5</sup>Beth Israel Deaconess Medical Center, Boston, MA, <sup>6</sup>National Center for Complementary and Alternative Medicine, Bethesda, MD, <sup>7</sup>National Institute for Digestive Disease and Kidney, National Institutes of Health, Bethesda, MD

**AIMS:** We have previously shown silymarin (SM) exposures are 3 to 5-fold higher in patients with liver disease compared to healthy subjects (Clin Pharmacol Ther, 2007; 81:S97). To optimize dose selection for planned Phase II studies with Legalon® (LG), dose-exposure relationships and the effect of food on SM disposition were determined using higher than customary single oral doses of LG in non-cirrhotic HCV patients.

**METHODS:** Subjects (N=8 per dose) were randomized 3:1 to receive SM at doses of 140, 280, or 560 mg or placebo. The effect of food on SM PK was determined at 280 mg using a cross-over design with a 7 day washout. 14 blood samples were obtained over 48 hrs and plasma concentrations of 6 major flavonolignans (FG) were quantified by LC/MS before (Free) and after (Free + deconjugated FG) enzymatic cleavage. AUC, C<sub>max</sub>, T<sub>max</sub>, CL/F, Vd, and t<sub>1/2</sub> for each FG were calculated using a noncompartmental approach (WinNonlin®4.1). For dose-exposure relationships, plasma concentrations of 6 FG were summed at each time point and PK analysis was performed on Sum FG concentration-time data for each subject.

**RESULTS:** Dose proportional 2.5- and 2.3-fold increases were seen in Sum AUCs for Free FG between 140 to 280 mg and 280 to 560 mg, respectively. Median (25<sup>th</sup> and 75<sup>th</sup> percentiles) for differences in AUC<sub>0-48h</sub> for Sum Free SM and Sum Free + deconjugated SM between fed and fasted states were -1.8 (-80.3, 66.5) and -1177.9 (-3228.7, 1590.1), respectively. Administration of SM with food delayed time to C<sub>max</sub> for Free FG, but did not significantly affect exposures.

PK Results for Sum Free & Free + deconjugated SM FG (Median (25<sup>th</sup> % -ile, 75<sup>th</sup> % -ile))

PK Measures	Dose (mg)			
	140	280 (Fed)	280 (Fasted)	560
<b>Sum Free SM</b>				
AUC <sub>0-48h</sub> (ng*hr/ml)	148 (115, 182)	361 (161, 465)	288 (220, 442)	754 (632, 842)
C <sub>max</sub> (ng/ml)	60 (33, 73)	113 (67, 169)	154 (102, 328)	299 (239, 388)
T <sub>max</sub> (hr)	1.1 (0.6, 2.0)	2.0 (2.0, 4.0)	0.8 (0.5, 1.5)	1.5 (1.0, 4.0)
<b>Sum Free + deconjugated SM</b>				
AUC <sub>0-48h</sub> (ng*hr/ml)	6690 (3229, 7090)	11210 (7867, 12499)	12029 (7166, 15557)	46735 (19386, 52390)
C <sub>max</sub> (ng/ml)	893 (530, 1050)	1264 (999, 1513)	1773 (726, 2196)	4471 (2656, 5624)
T <sub>max</sub> (hr)	1.8 (1.5, 2.0)	2.0 (2.0, 4.0)	1.5 (1.0, 2.0)	4.0 (2.0, 4.0)

**CONCLUSION:** Dose-proportional increases in exposures for Free SM FG were seen up to 560 mg. Administration of SM with food did not affect SM exposures.

### PIII-19

PHARMACOLOGICAL PROFILE OF CP-945,598, A POTENT AND SELECTIVE CANNABINOID CB<sub>1</sub> RECEPTOR ANTAGONIST. J. R. Hadcock, D. A. Griffith, D. O. Scott, P. A. Carpino, P. DaSilva-Jardines, R. Day, J. Dibrino, R. L. Dow, D. Dutcher, M. W. Fichtner, D. Gautreau, P. A. Iredale, J. S. Lizano, R. E. O'Connor, C. R. Rose, S. Sakya, M. A. Sands, A. G. Swick, D. Tess, K. M. Ward, S. C. Black; Pfizer Inc, Groton, CT

**BACKGROUND:** Cannabinoid CB<sub>1</sub> receptor antagonists exhibit pharmacological properties favorable for the treatment of obesity, alcohol abuse and dependence, and other related disorders. We report the discovery and pharmacology of a novel cannabinoid CB<sub>1</sub> receptor antagonist, CP-945,598 (1-[9-(4-Chlorophenyl)-8-(2-chlorophenyl)-9H-purin-6-yl]-4-ethylaminopiperidine-4-carboxylic acid amine hydrochloride).

**METHODS:** Binding efficacy was determined with a radioligand binding assay using [<sup>3</sup>H]-SR141716A; functional potency and intrinsic efficacy were determined using a GTPγ[<sup>35</sup>S] assay. The effect of CP-945,598 on the cannabinoid CB<sub>1</sub> agonist-induced tetrad of behaviors (hypolocomotor activity and catalepsy [10.0, and 17.8 mg/kg, SC], and analgesia, and hypothermia [1.0, 5.6, 10.0, and 17.8 mg/kg, SC]) was assessed in male ICR mice administered the synthetic cannabinoid agonist CP-55,940 (0.78 mg/kg, SC).

**RESULTS:** CP-945,598 exhibited sub-nanomolar potency at human CB<sub>1</sub> receptors in both binding (K<sub>i</sub>=0.75 nM) and functional assays (K<sub>i</sub>=0.2 nM), and low affinity for CB<sub>2</sub> receptors (K<sub>i</sub>=7600 nM). *In vitro*, CP-945,598 behaved as a competitive antagonist and exhibited no agonist activity when tested in the absence of the agonist CP-55,940. CP-945,598 did not exhibit significant binding activity for any other receptors, ion channels, and uptake sites. Within the cannabinoid tetrad of behaviors, CP-945,598 potently and selectively dose-dependently reversed CP-55,940-induced hypolocomotor activity, analgesia, hypothermia, and catalepsy. *In vivo*, CP-945,598 did not exhibit any activity in the tetrad when administered in the absence of an agonist challenge.

**CONCLUSION:** CP-945,598 is a highly potent and selective cannabinoid CB<sub>1</sub> receptor antagonist. CP-945,598 inhibits both basal and cannabinoid agonist-mediated CB<sub>1</sub> receptor signaling *in vitro* and *in vivo* and exhibits >1,000 fold selectivity for CB<sub>1</sub> vs CB<sub>2</sub> receptors.

### PIII-20

THE POTENT AND SELECTIVE CANNABINOID CB<sub>1</sub> RECEPTOR ANTAGONIST CP-945,598 EXHIBITS DOSE-DEPENDENT ANORECTIC ACTIVITY AND INCREASED ENERGY EXPENDITURE IN RATS. J. R. Hadcock, D. A. Griffith, D. O. Scott, P. A. Carpino, R. L. Dow, D. Hargrove, P. A. Iredale, D. Kelly-Sullivan, K. A. Martin, N. A. Nardone, A. G. Swick, S. C. Black; Pfizer, Groton, CT

**BACKGROUND:** Cannabinoid CB<sub>1</sub> receptors play a role in regulating energy balance and provide a promising target for the pharmacotherapy of obesity. We report the effect of a novel CB<sub>1</sub> receptor antagonist, CP-945,598 (1-[9-(4-Chlorophenyl)-8-(2-chlorophenyl)-9H-purin-6-yl]-4-ethylaminopiperidine-4-carboxylic acid amine hydrochloride), on feeding behavior and energy expenditure in rats.

**METHODS:** The effects of oral administration of CP-945,598 vs vehicle (0.5% methyl cellulose) on consumption of standard laboratory chow was assessed in a spontaneous nocturnal food intake assay (3 and 10 mg/kg, po) and fast-induced re-feeding assay (3, 10, and 30 mg/kg, po) in individually housed, male, Sprague-Dawley rats (250–300 g). Whole body oxygen consumption was measured with an indirect calorimeter in pre-fed rats (300–380 g) administered CP-945,598 (10 and 30 mg/kg, po).

**RESULTS:** CP-945,598 dose-dependently reduced nocturnal feeding. The reductions in food intake were significant vs vehicle at 2, 3, and 8 hours post access to chow in rats administered 3 mg/kg CP-945,598, and at 0.5 and 2–10 hours post access to chow in rats administered 10 mg/kg CP-945,598. Following an overnight fast, CP-945,598 significantly reduced food intake vs vehicle at 0.5, 1, and 2 hours post access to chow

in rats administered 10 and 30 mg/kg CP-945,598, and at 1 and 2 hours post access to chow in rats administered 3 mg/kg CP-945,598. Compared with baseline levels, CP-945,598 increased oxygen consumption by 16% (10 mg/kg) and 18% (30 mg/kg) 2–3 hours post-dosing (both doses: p<0.05 vs vehicle); the respiratory quotient decreased by 6% for both doses 3 hours post-dosing, suggesting increased fat oxidation.

**CONCLUSION:** CP-945,598 exhibits dose-dependent anorectic activity in two models of feeding behavior and increases energy expenditure and fat oxidation in rats. These data support the development of the novel CB<sub>1</sub> receptor antagonist, CP-945,598, as a therapeutic agent for obesity.

### PIII-21

GUANYLYL CYCLASE C (GCC) INHIBITS COLON CANCER CELL METASTASIS THROUGH MATRIX METALLOPROTEINASE 9 (MMP-9). D. S. Zuzga,<sup>1</sup> W. J. Lubbe,<sup>1</sup> A. Berger,<sup>1</sup> Z. Y. Zhou,<sup>2</sup> S. Schulz,<sup>1</sup> S. A. Waldman,<sup>1</sup> G. M. Pitari,<sup>1</sup> <sup>1</sup>Thomas Jefferson, Philadelphia, PA, <sup>2</sup>University of Pennsylvania, Philadelphia, PA

**BACKGROUND:** GCC, the intestinal epithelial cell receptor for diarrheagenic bacterial heat-stable enterotoxins (STs), and MMP-9, a collagenase mediating extracellular matrix remodeling, are functionally opposing regulators of colorectal tumor initiation and progression. Since intestinal tumorigenesis is universally associated with disruption of GCC, but augmented MMP-9, signaling, the impact of ligand-dependent activation of GCC on MMP-9 signaling was examined.

**METHODS:** The pathological significance of tumor epithelial cell MMP-9 was evaluated in matched normal and tumor cell populations from patients by RT-PCR. Regulation of MMP-9 signaling by ligand activation of GCC in human colon carcinoma cells was examined *in vitro* by cell spreading and invasion and *in vivo* employing murine models of metastasis. The role of MMP-9 in processes regulated by GCC was defined employing tumor cells over-expressing MMP-9, purified active MMP-9 and MMP-9 inhibitors.

**RESULTS:** Over-expression of MMP-9 mRNA in cancer, compared to normal adjacent epithelial, cells significantly correlated with pericolic lymph node metastasis in patients. Notably, induction of GCC signaling by ST in cancer cells inhibited the secretion of MMP-9, suppressed cell spreading and invasion, and opposed hematogenous and peritoneal metastases in a MMP-9-dependent fashion.

**CONCLUSION:** GCC-mediated inhibition of tumor epithelial cell MMP-9 represents a novel therapeutic target for treating patients with colon cancer metastasis.

### PIII-22

A COMPUTATIONAL BIOLOGY APPROACH TO LIFECYCLE MANAGEMENT AND DEVELOPMENT OF A CD59-BASED DIAGNOSTIC PRODUCT. L. T. Herren, PhD, N. Kabrun, PhD, B. L. Handelin, PhD, H. J. Gomez, MD, PhD; DNAPrint Pharmaceuticals, Inc, Sarasota, FL

**BACKGROUND:** The development of sensitive and reliable monitoring tests to identify diabetic patients who are at increased risk of developing vascular complications is a public health priority. CD59, a GPI-linked protein expressed on the cell membrane of many cell types, regulates the terminal step of complement-mediated inflammation. Research has demonstrated that CD59 can be glycosylated on vascular endothelial cells and that this glycation disrupts the normal activity of CD59, resulting in localized and persistent vascular damage.<sup>1</sup> We are developing a new clinical diagnostic test to assess glycation of CD59 in diabetes patients; here we report on how a computational approach to modeling the pathophysiology of diabetic vascular disease is enabling more effective preclinical and clinical development.

**METHODS:** Computational synthesis and modeling of biological systems illuminates the complex interactions among biological elements. The first step is constructing a detailed map of the biological process or domain by identifying, synthesizing, and organizing published data/knowledge into a comprehensive graphical representation. The resulting structured knowledge provides a blueprint for the

implementation of a dynamic computer simulation. A multi-level map was constructed of the process of CD59 glycation and the role of CD59 in the initiation and progression of microvascular damage.

**RESULTS:** Developing the map of CD59 glycation and function was a key step in preclinical assay development. The maps provided guidance in the design of preclinical and clinical studies including comparing how glycated CD59 is related to other commercial and emerging markers of diabetic complications.

**CONCLUSION:** Computational biology offers promise to improve the development of new diagnostic indices. The development of structured maps is the first step in leveraging the power of this approach in diagnostic development programs.

<sup>1</sup>Acosta et al. *Curr Pharm Des*, 10, 203 (2004)

### PIII-23

PLACEBO RESPONSE MODEL OF WEIGHT LOSS IN 1238 PATIENTS FROM WELL-CONTROLLED, RANDOMIZED STUDIES DESIGNED TO INVESTIGATE WEIGHT LOSS DRUG CANDIDATES. J. A. Wald, Y. Li, R. O'Connor-Semmes, P. P. Hastie, M. A. Young, M. A. Bush, T. J. Schibler; GlaxoSmithKline, Research Triangle Park, NC

**BACKGROUND:** Multi-center Phase 1/2 studies to evaluate weight loss products are frequently complicated by variability between subjects and lack of control in the magnitude of placebo response, despite efforts to control it. The aim is to create model-based tools to improve phase 2 study designs with better insight and control of placebo response and subject variability.

**METHODS:** A database of 8 weight loss studies was assembled including 1238 placebo treated patients. Eighteen study-related and patient-specific covariates that might explain individual patient placebo weight loss response were examined in the aggregated database. Study length varied between 30 days and 1 year. Data up to 120 days was included in the model to span the usual time-frame for early stage clinical studies. A mixed-effects model with asymptotic change from baseline (CFB) was developed to handle weight loss or gain as a function of time and covariates. Analysis was conducted in S-Plus (Insightful Corporation).

**RESULTS:** The median CFB weight was 3 kg. The T1/2 for CFB was 34 days. Study related variables were, in general, poor predictors of placebo response; covariates that reached statistical significance were frequently of low absolute magnitude and thus had little clinical relevance. Statistically significant effects on CFB and rate of CFB were found for patient-specific covariates such as Hip:Waist ratios, non-HDL cholesterol:HDL ratios, smoking history, menopausal status, sex, and baseline weight.

**CONCLUSION:** In general, the covariate effects were subtle. In addition, inconsistent data collection across the different studies did not permit evaluation of each possible covariate on the entire placebo patient population. The analyses indicate factors thought important in weight loss may not be influential for placebo response in multi-center, multi-country studies.

### PIII-24

MODEL-BASED APPROACH TO STUDY FASTING PLASMA GLUCOSE DURING WASHOUT OF PRIOR ANTIDIABETIC THERAPY. J. A. Wald, M. A. Bush, M. A. Young, R. O'Connor-Semmes, P. P. Hastie, T. J. Schibler; GlaxoSmithKline, Research Triangle Park, NC

**BACKGROUND:** Phase I/II studies investigating treatment for Type 2 Diabetes Mellitus often involve washout of prior anti-diabetic (AD) therapy. Length of washout varies from study to study. Analysis of efficacy for AD therapy involves baseline correction and is complicated by time-dependent baseline glucose during washout. The aim is to develop a model to characterize the time course of fasting plasma glucose (FPG) following washout of prior AD therapy.

**METHODS:** Data were pooled from 9 clinical studies comprising 238 subjects randomized to receive placebo. This modeling effort

focused on short-term (Phase I/II studies), therefore FPG data to 6 weeks following initiation of washout were included in the analysis database. The analysis included subjects who were treatment naïve (38%), receiving prior monotherapy metformin (MET - 28%), monotherapy sulfonylurea (SU - 26%), or combination therapy with MET and a SU (8%). An exponential model was fit to FPG for treatment-naïve subjects and subjects washed out of prior anti-diabetic therapy. The model was parameterized to assess effects of prior treatment on baseline FPG (BSL), asymptotic change from baseline in FPG ( $\Delta$ FPG), and rate of change in FPG (Kdb).

**RESULTS:** Prior therapy was a significant covariate on BSL and  $\Delta$ FPG. Estimated BSL was higher for treatment-naïve subjects (~169 mg/dL) compared to subjects receiving prior MET or SU (~154 mg/dL). Estimates of  $\Delta$ BSL for SU, MET and naïve subjects were 50, 30, and -2.4 mg/dL, respectively. Prior therapy was not a significant covariate for Kdb. The common estimate of Kdb across prior therapy was 0.117 day<sup>-1</sup>.

**CONCLUSION:** The current model indicates that  $\Delta$ FPG during washout is dependent on prior therapy. The time to reach approximate steady-state for FPG following washout is ~30 days for all prior treatments. Simulations based on the current model may be used to optimize washout duration, enrollment criteria, and analysis plans for studies employing a washout strategy.

### PIII-25

SNP DISCOVERY AND FUNCTIONAL ASSESSMENT OF VARIATION IN THE *UDP-GLUCURONOSYLTRANSFERASE (UGT2B7)* GENE. F. Innocenti,<sup>1</sup> W. Liu,<sup>1</sup> D. Fackenthal,<sup>1</sup> J. Ramirez,<sup>1</sup> P. Chen,<sup>1</sup> X. Ye,<sup>1</sup> X. Wu,<sup>1</sup> W. Zhang,<sup>1</sup> S. Mirkov,<sup>1</sup> S. Das,<sup>1</sup> E. Cook Jr.,<sup>2</sup> M. J. Ratain<sup>1</sup>; <sup>1</sup>University of Chicago, Chicago, IL, <sup>2</sup>University of Illinois at Chicago, Chicago, IL

**BACKGROUND:** UGT2B7 plays a central role in the liver-mediated biotransformation of endogenous and exogenous compounds. The genetic basis of interindividual variability in UGT2B7 function is unknown. This study aimed to discover novel gene variants of functional significance.

**METHODS:** Caucasian human livers (n=54) were used. *UGT2B7* was resequenced in 12 samples (6 highest and 6 lowest for the formation of morphine-3-glucuronide, M3G). Haplotype-tagging single nucleotide polymorphisms (tSNPs) were genotyped in the entire sample set. Samples were phenotyped for mRNA expression.

**RESULTS:** Ten tSNPs were identified and their haplotypes were inferred. Haplotype 4 (frequency of 0.12) was associated with an increase in enzyme activity and gene expression. The 1/4 and 4/6 diplotypes had higher M3G formation compared to 1/1 (p<0.05) and 2/3 (p<0.01) diplotypes. Diplotypes containing haplotype 4 resulted in a significant 45% average increase in the formation of M3G compared to diplotypes without haplotype 4 (p=0.002). There was also an association between haplotype 4 and increased mRNA expression. IVS1+985A>G, 735A>G and 1062C>T are the putative functional variants of haplotype 4. We also identified two mRNA splicing variants (UGT2B7v1 and UGT2B7v2) splicing out exon 1 and sharing exons 2 to 6 with the involvement of additional 5' exons. UGT2B7v1 was detected in all livers tested, but UGT2B7v2 was present at much lower levels compared to UGT2B7v1.

**CONCLUSION:** *UGT2B7* haplotype 4 is functional and its effects on the biotransformation of UGT2B7 substrates should be tested in controlled clinical trials. Biochemical studies should investigate the functional role of the newly discovered mRNA splicing variants.

**PIII-26**

DUPLEX PYROSEQUENCING ASSAY OF THE 338A>G AND 521T>C *SLCO1B1* POLYMORPHISMS IN THREE ASIAN POPULATION. E. Kim,<sup>1</sup> D. Cho,<sup>1</sup> H. Shin,<sup>1</sup> S. Lee,<sup>1</sup> J. Shon,<sup>1</sup> J. Shin,<sup>1</sup> S. Shin<sup>2</sup>; <sup>1</sup>Inje University College of Medicine, Busan, Republic of Korea, <sup>2</sup>Seoul National University College of Medicine, Seoul, Republic of Korea

**BACKGROUND:** We developed Haaaaa method for detecting important *SLCO1B1* polymorphisms and compared the haplotype frequencies in three Asian populations.

**METHODS:** We designed a duplex pyrosequencing assay to detect simultaneously the 338A>G and 521T>C variants of *SLCO1B1*; this method can identify *SLCO1B1*\*1b, *SLCO1B1*\*5, and *SLCO1B1*\*15. The method was validated by direct sequencing of 96 Korean subjects. In addition, duplex genotyping and the monoplex method were compared and validated with 469 Korean subjects. To characterize the haplotype frequencies based on the two polymorphisms, we genotyped 106 Chinese and 104 Vietnamese subjects, as well as Korean subjects, using the new method.

**RESULTS:** The results showed 100% concordance among the monoplex and duplex pyrosequencing assays and direct sequencing method. The allele frequencies were similar in the three Asian populations: the most common allele was *SLCO1B1*\*1b, while *SLCO1B1*\*5 was rare or absent. The frequencies of functional *SLCO1B1*\*15 alleles differed statistically between Chinese (8.2%) and Korean (14.0%) and Vietnamese (16.3%) (p<0.05,  $\chi^2$ -test).

**CONCLUSION:** The duplex pyrosequencing assay appears to be an accurate, rapid, and cost-effective genotyping method to detect major *SLCO1B1* important alleles in Asian populations.

**PIII-27**

CO-REGULATION OF HEPATIC UDP-GLUCURONOSYLTRANSFERASE 1As BY TRANSCRIPTION FACTORS. S. Mirkov, J. Ramirez, W. Liu, M. J. Ratain; University of Chicago, Chicago, IL

**BACKGROUND:** A large portion of UDP-glucuronosyltransferase 1A (*UGT1A*) phenotypic variability is not explained by genetic variation. The expression of *UGT1A* is *trans*-regulated by several transcription factors (TFs), including *GCCR*, *AhR*, *SPI*, *HNF1 $\alpha$*  and *HNF3 $\alpha$* . We hypothesize that variation in *TF* expression contributes to the residual variability in constitutive *UGT1A* mRNA levels. The aim of this study was to test the correlation between the expression of *TFs* and hepatic *UGT1A* genes in Caucasians.

**METHODS:** mRNA levels of *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6*, *UGT1A9*, *GCCR*, *AhR*, *SPI*, *HNF1 $\alpha$*  and *HNF3 $\alpha$*  were measured in 169 Caucasian livers using real-time PCR. Quantitation was normalized to the endogenous control *18S*. Pearson correlations were performed.

**RESULTS:** The variability (%CV) in mRNA levels of the *UGTs* and *TFs* ranged from 140%-164% and 75%-112%, respectively. The *UGT* mRNA levels were related to each other (median  $r^2=0.48$ ; range, 0.17-0.61; p<0.0001). The highest correlations were observed between (1) *UGT1A3* and *UGT1A4* ( $r^2=0.61$ , p<0.0001), (2) *UGT1A6* and *UGT1A9* ( $r^2=0.58$ , p<0.0001) and (3) *UGT1A1* and *UGT1A4* ( $r^2=0.56$ ), *UGT1A6* ( $r^2=0.49$ ) and *UGT1A9* ( $r^2=0.55$ ) (p<0.0001). The mRNA levels of the *TFs* correlated with those of each *UGT*: *GCCR* (median  $r^2=0.26$ ; range, 0.17-0.30; p<0.0001), *AhR* (median  $r^2=0.24$ ; range, 0.21-0.28; p<0.0001), *SPI* (median  $r^2=0.22$ ; range, 0.07-0.25; p<0.0001), *HNF3 $\alpha$*  (median  $r^2=0.18$ ; range, 0.08-0.20; p<0.0001), and *HNF1 $\alpha$*  (median  $r^2=0.14$ ; range, 0.09-0.25; p<0.0001). Among the *TFs*, the highest correlations were found between (1) *GCCR* and *AhR* ( $r^2=0.61$ ), *SPI* ( $r^2=0.53$ ) and *HNF3 $\alpha$*  ( $r^2=0.51$ ) (p<0.0001) and (2) *SPI* and *HNF3 $\alpha$*  ( $r^2=0.57$ , p<0.0001).

**CONCLUSION:** The data suggest that the hepatic *UGT1A* genes share common regulatory pathways and their mRNA expression is moderately regulated by multiple *TFs*. Regulatory cross-talk may also exist between *GCCR*, *AhR*, *SPI*, *HNF1 $\alpha$*  and *HNF3 $\alpha$* .

**PIII-28**

IDENTIFICATION OF MICRORNAS THAT TARGET CYP2D6. A. Ramamoorthy, D. A. Flockhart, T. C. Skaar; Indiana University School of Medicine, Indianapolis, IN

**BACKGROUND/ AIMS:** Variability in CYP2D6 metabolic activity contributes to the pharmacodynamic variability of several drugs. Although part of this variability is due to CYP2D6 genetic variants and drug-interactions, the cause of much of the CYP2D6 variability remains unknown. To better understand this variability, we hypothesized that CYP2D6 expression and activity may be regulated by endogenous microRNAs. These microRNAs (miRNA) are small, non-coding RNAs. They bind to the 3'UTRs of target gene mRNAs and regulate gene expression either by causing mRNA degradation or by blocking translation.

**METHODS:** We performed *in silico* analysis to identify miRNAs that are predicted to target the 3'-UTR of the human CYP2D6; our analysis included 6 different bioinformatic algorithms. Two miRNAs were analyzed by quantitative RT-PCR to determine if they were expressed in RNA samples from two livers and one primary hepatocytes culture preparation.

**RESULTS:** The bioinformatic analysis identified a total of 31 miRNAs that were predicted to target the 3'-UTR of the CYP2D6 mRNA (see Table). Two of the algorithms predicted that the CYP2D6 mRNA was a target of both miR-493-5p and miR-137. The expression of both of these miRNAs was detected in human livers and in hepatocytes. Laboratory experiments are currently underway to test the effect of these miRNAs on CYP2D6 expression.

**Bioinformatic Algorithms**

Micro RNA Registry	PicTar	miRanda	Target Scan	Target ScanS	RNA22	Over lap between at least 2 algorithms
29	None	None	3	None	1	2 (6%)

**CONCLUSION:** Our results indicate that CYP2D6 is a likely target of multiple miRNAs. These results indicate a novel mechanism that is likely to regulate CYP2D6 expression.

**PIII-29**

QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION ANALYSIS OF MRP-2 EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS. P. Suchada,<sup>1</sup> D. A. Brazeau,<sup>2</sup> R. C. Venuto,<sup>3</sup> K. M. Tornatore<sup>4</sup>; <sup>1</sup>Department of Pharmacy Practice, Buffalo, NY, <sup>2</sup>Department of Pharmaceutical Sciences, Buffalo, NY, <sup>3</sup>Department of Medicine, School of Medicine and Biomedical Sciences, Buffalo, NY, <sup>4</sup>Department of Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, Buffalo, NY

**BACKGROUND:** Limited data is available in regards to assessment of multi-drug resistance protein 2 (MRP-2) expression in peripheral blood mononuclear cells (PBMCs). We describe the development of quantitative polymerase chain reaction (QPCR) technique to quantify MRP-2 mRNA expression using as little as 200 ul of sample.

**METHODS:** PBMCs were collected from renal transplant recipients who were receiving chronic maintenance immunosuppression of cyclosporine and mycophenolic acid. Total RNA was extracted followed by reverse transcription (100 ng per sample) and all samples were spiked with a known concentration of Alien® RNA as an internal normalization control. The QPCR technique was used to determine MRP-2 gene expression in PBMCs. The PCR product was cloned into a plasmid vector (PCR-TOPO) to confirm the sequence. The cloned PCR product was also used to establish standard curves of known concentrations for the quantitation of gene expression.

**RESULTS:** The cloned MRP-2 used to establish the standard curve was linear ( $r^2 = 0.996$ ) over six orders of magnitude (copy number ranging from 10e2 to 10e8). Average PCR efficiencies (99.5%) of samples and standards were equivalent allowing for absolute estimates of copy number. Initial analysis of samples indicated 30-60 fold differences in MRP2 values (estimated copy number ranging from 320 to 20,000 copies/100 ng of total RNA).

**CONCLUSION:** Real time RT-PCR with Alien® RNA normalization is an effective method to analyze gene expression of MRP-2 in PBMCs.

### PIII-30

THE INFLUENCE OF SEX, RACE/ETHNICITY AND CYP 2B6 GENOTYPE ON BUPROPION (B) METABOLISM. K. Ilic,<sup>1</sup> G. Hogeland,<sup>1</sup> N. Rezk,<sup>1</sup> N. White,<sup>1</sup> J. Lamba,<sup>2</sup> C. Lee,<sup>1</sup> A. D. Kashuba,<sup>1</sup> E. G. Schuetz,<sup>2</sup> C. M. Lindley,<sup>1</sup> M. L. Chen,<sup>3</sup> R. L. Hawke<sup>1</sup>; <sup>1</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC, <sup>2</sup>St. Jude Children's Research Hospital, Memphis, TN, <sup>3</sup>Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD

**AIM:** We have previously reported significant ethnic and sex differences in CYP2B6 genotype-phenotype associations in human liver (JPET 2003;307:906). The aim of this study was to confirm these findings *in vivo* using hydroxybupropion (HB) plasma concentration as a phenotypic measure of CYP2B6 activity.

**METHODS:** 85 healthy volunteers 18-50 yrs old having 4 grandparents of the same race/ethnicity were divided into 4 groups: Caucasian (CF), African American (AAF) and Latina (LF) females, and Caucasian males (CM). CYP2B6 phenotype was estimated by a plasma HB concentration obtained 28 (2.8) hours after a 150mg dose of slow release B. B and HB were measured using HPLC/MS. CYP2B6 SNPs at intron 3 (15582C>T), exon 4 (516G>T;15631G>T), and exon 5 (785A>G; 18053A>G) were genotyped by PCR. Statistical analyses were performed using STATA. Data are presented as mean (SD).

**RESULTS:** Similar allelic frequencies in intron 3, exon 4, and exon 5 were observed for Caucasians; African Americans; and Latinas (48%, 44%, 48%; 24%, 64%, 64%; and 60%, 60%, 60%, respectively). A difference in HB was observed between CM and CF (169 vs 230; p<0.01). No differences in HB were observed between the three female ethnic groups (p=0.8). Although no statistically significant differences in HB were noted when comparing individuals with specific variant (VAR) alleles to those with only reference (REF) alleles (p>0.1; see table below), multivariate regression analysis yielded an overall association between variant alleles at all three loci and lower HB concentrations (reflecting lower CYP2B6 activity; p<0.0004).

HB Concentration for All Subjects (ng/mL)

	Intron 3	Exon 4	Exon 5
REF	224 (112)	236 (93)	230 (92)
VAR	206 (91)	199 (110)	205 (112)

**CONCLUSION:** Women with CYP2B6 variant alleles in intron 3, exon 4, and exon 5 may have reduced hepatic CYP2B6 activity.

### PIII-31

ANGIOTENSINOGEN HAPLOTYPE AND POTASSIUM RESPONSE TO SPIRONOLACTONE. L. H. Cavallari, V. L. Groo, M. A. Viana, J. R. Camp, T. D. Stamos; University of Illinois at Chicago, Chicago, IL

**BACKGROUND:** Hyperkalemia is a serious adverse effect of aldosterone antagonist in heart failure. The angiotensinogen (AGT) -6G/A and 235Met/Thr gene polymorphisms have been associated with aldosterone levels and ACE inhibitor effects in hypertension. We sought to determine whether AGT genotype influences aldosterone levels and potassium (K<sup>+</sup>) response to spironolactone in heart failure.

**METHODS:** In this prospective cohort study, 55 spironolactone-naïve patients (37 blacks and 18 whites) followed in a heart failure clinic were started on spironolactone 12.5 mg/day, titrated to 25 mg/day in one week if tolerated. Blood was collected at baseline for genotyping, electrolytes, and aldosterone levels and again one week after spironolactone dose titration for repeat electrolytes. Aldosterone was measured by radioimmunoassay, and genotypes were determined by PCR and RFLP.

**RESULTS:** The -6G and 235Met allele frequencies were 0.24 and 0.25, respectively, and the 2 polymorphisms formed the -6A/235Thr and -6G/235Met diplotypes. At baseline, drug therapy, renal function, and median (interquartile range) aldosterone levels [101 (68-134) vs 81 (61-175) pg/ml] were similar between -6A/235Thr homozygotes (n=34) and -6G/235Met allele carriers (n=21), respectively,

and median K<sup>+</sup> level was 4.3 mEq/L in each group. However, there were more blacks (88% vs 33%; p<0.01) and tended to be fewer diabetics (26% vs 52%; p=0.05) in the -6A/235Thr homozygote group. One week after spironolactone titration to a median dose of 25 mg/day in each group, K<sup>+</sup> was 4.2 (4.1-4.7) mEq/L in -6A/235Thr allele homozygotes and 4.8 (4.3-5.2) mEq/L in -6G/235Met allele carriers; p=0.01; however, this difference was no longer significant when controlling for differences in race and diabetes prevalence between groups.

**CONCLUSION:** Our data do not support a role for the AGT gene in contributing to aldosterone concentrations or K<sup>+</sup> response to spironolactone in heart failure.

### PIII-32

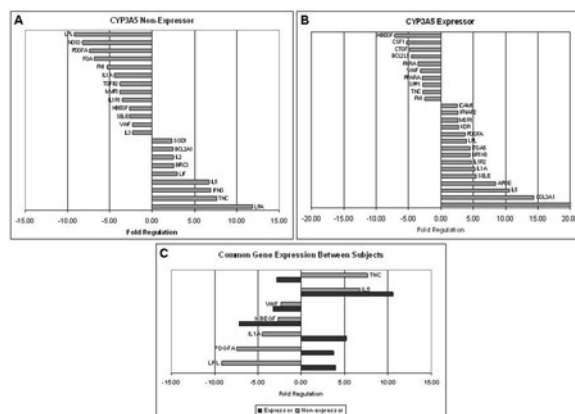
LEUKOCYTE EXPRESSION OF ATHEROSCLEROSIS-RELATED GENES IN RESPONSE TO ATORVASTATIN DIFFERS BY CYP3A5 GENOTYPE. J. D. Duarte, G. J. Welder, I. Zineh; University of Florida College of Pharmacy and Center for Pharmacogenomics, Gainesville, FL

**BACKGROUND:** HMG-CoA reductase inhibitors (statins) are standard of care in cardiovascular disease, in part due to anti-inflammatory effects. However, variability in response exists and polymorphic drug metabolism may contribute. Atorvastatin is metabolized by CYP3A5. Polymorphisms in the CYP3A5 gene may contribute to variability in the systemic anti-inflammatory effects of statins. Thus, in a pilot experiment we investigated whether changes in *in vivo* leukocyte expression of atherosclerosis-related genes after atorvastatin treatment differs by CYP3A5 genotype.

**METHODS:** Circulating leukocytes were collected from two healthy women selected on CYP3A5 genotype and matched on age before and after treatment with atorvastatin 80 mg daily for 8 weeks. One participant was a CYP3A5 expressor (CYP3A5\*1 carrier) and one was a non-expressor (CYP3A5\*3/\*3 genotype). Leukocyte gene expression analysis was performed using an RT-PCR array for genes involved in atherosclerosis (SuperArray Bioscience Corp., MD). Fold changes in expression in response to atorvastatin were calculated by the 2<sup>-ΔΔCt</sup> method.

**RESULTS:** Atorvastatin effects on gene expression differed by CYP3A5 genotype (Figure 1). Atorvastatin caused ≥ 2-fold change in 22 genes in the CYP3A5 non-expressor, and 25 genes in the expressor (Figure 1A and 1B). The ratio of down- to up-regulated genes was high in the CYP3A5 non-expressor (60:40), while the converse was true for the CYP3A5 expressor (40:60). For genes commonly modulated in both patients, there was a stark difference in the magnitude and/or direction of the change (Figure 1C).

**CONCLUSION:** CYP3A5 genotype may influence the anti-inflammatory effect of atorvastatin in humans. These preliminary findings indicate the need for further investigation into the clinical effect of CYP3A5 on atorvastatin therapy using various phenotypes.



**PIII-33**

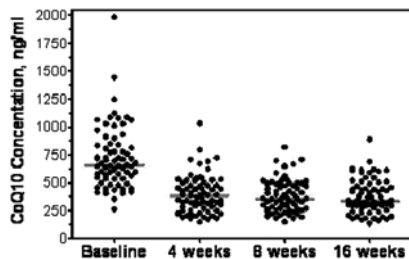
CHANGES IN COENZYME Q10 (COQ10) FOLLOWING HIGH-DOSE ATORVASTATIN THERAPY. O. J. Enogieru, M. Pacanowski, R. Frye, I. Zineh; University of Florida College of Pharmacy, Gainesville, FL

**BACKGROUND:** HMG-CoA reductase inhibitors (statins) are commonly used to treat dyslipidemia. Statins are generally well tolerated but have been associated with potentially life-threatening skeletal muscle toxicity. The mechanism of these myopathic syndromes has been hypothesized to be due to a reduction in coenzyme Q10 (CoQ10), an endogenously synthesized provitamin involved in ATP production. We tested the effect of high-dose atorvastatin on CoQ10 plasma concentrations.

**METHODS:** Subjects were eligible if they were at least 18 years old, normocholesterolemic, without cardiovascular disease or contraindications to statins. Subjects received atorvastatin 80 mg daily for 16 weeks. CoQ10 concentrations in plasma were determined by liquid chromatography tandem mass spectrometry at baseline and every 4 weeks. Lipids were measured at baseline, 8 weeks, and 16 weeks. We tested changes in three endpoints: CoQ10, CoQ/total cholesterol (TC), and CoQ/LDL changes by ANOVA. Correlation between CoQ10 and cholesterol changes was evaluated using Spearman's correlation.

**RESULTS:** Data were analyzed for 72 patients (31±12 years old, 63% female, 72% white). Average plasma CoQ10 decreased from 722±278 ng/ml at baseline to 398±168, 381±141, and 372±159 ng/ml after 4, 8, and 16 weeks, respectively (Figure; P<0.001). The ratio of plasma CoQ10/TC changed by -16±24% and -17±29% at 8 and 16 weeks (P<0.001), while the ratios of CoQ10/LDL increased by 32±46% and 28±54% at these time points (P<0.001). CoQ10 changes were weakly correlated with cholesterol changes at 8 weeks (TC r=0.10 LDL r=0.13) and 16 weeks (TC r=0.39; LDL r=0.29).

**CONCLUSION:** Atorvastatin 80 mg over 16 weeks significantly modulates CoQ10 concentrations. The relevance of these findings to statin-induced myopathy should be further explored.



**PIII-34**

MOLECULAR REMODELING OF CALCIUM CHANNELS IN HUMAN CARDIAC MYOCYTES AFFECTED BY DILATED CARDIOMYOPATHY. N. Gronich, MD,<sup>1</sup> Y. Zhang, MD, PhD,<sup>2</sup> A. Kumar, PhD,<sup>2</sup> W. Hucker,<sup>3</sup> W. Wood III,<sup>2</sup> K. Becker,<sup>2</sup> I. Efimov,<sup>3</sup> N. Soldatov<sup>2</sup>; <sup>1</sup>ClinPRAT fellowship, NIA, NIH, Baltimore, MD, <sup>2</sup>NIA, NIH, Baltimore, MD, <sup>3</sup>Washington University, St. Louis, MO

**BACKGROUND:** The Ca<sub>v</sub>1.2 calcium channel subunits are subject to genetic and splice variations that generate multiple molecular isoforms. Approximately 50 other cardiac ion channels, including voltage-gated Na<sub>v</sub> sodium and K<sub>v</sub> potassium channels, cyclic nucleotide gated channels, and trp channels are subject to alternative splicing and/or variation in expression levels. Precise identification of molecular species that constitute the array of ion channels in major cell types of the heart is crucial for understanding mechanisms shaping electrical excitability of cardiac tissues as well as their changes in cardiovascular diseases.

**METHODS:** To address the most important aspects linking molecular complexity of channel-gated signaling and cardiovascular pathophysiology, we developed an approach that combines laser-cap-

ture microdissection of human cardiac myocytes, proportional RNA amplification, ion channel splice microarray on custom-designed chips, long-range RT-PCR, quantification by real time PCR, and functional expression analysis by patch clamp in Ca<sup>2+</sup> channel-free cells. Human cardiac tissue was obtained from eight anonymous cardiac transplant recipients and donors (MedStar Protocol #2003-071, P.I. D.R. Abernethy, MD, PhD, in collaboration with I.R. Efimov, PhD, Washington University).

**RESULTS:** We found that left ventricle myocytes from donors with dilated cardiomyopathy displayed alternative splicing of Ca<sub>v</sub>1.2 subunits that generated subsets of channels with lower Ca<sup>2+</sup> current density, slower kinetics of inactivation and isoforms lacking sensitivity to PKA/PKC-dependent regulation.

**CONCLUSION:** Dilated cardiomyopathy results in large molecular and functional remodeling of key channel proteins that define shape and duration of cardiac action potentials. These changes may contribute to the pathogenesis of cardiomyopathy, affect response to known therapeutic interventions and help define new useful therapeutic targets.

**PIII-35**

PLASMA MEMBRANE TRANSPORTERS IN HUMAN MUSCULAR CELLS AND THEIR POTENTIAL ROLE IN STATIN-INDUCED TOXICITY. H. Ligeret,<sup>1</sup> F. Bélanger,<sup>1</sup> M. Phillips,<sup>2</sup> J. Tardif,<sup>3</sup> J. Turgeon<sup>1</sup>; <sup>1</sup>Centre de Recherche Hotel Dieu du CHUM, Montreal, QC, Canada, <sup>2</sup>Montreal Heart Institute, Montreal, QC, Canada, <sup>3</sup>Montreal Heart Institute, Montreal, QC, Canada

**BACKGROUND:** Atherosclerosis represents the major cause of death in developed countries. HMG CoA reductase inhibitors (statins) are currently the most potent class of drugs to reduce serum cholesterol levels. Unfortunately, serious muscle toxicity remains a major complication during chronic treatment with statins. The aim of this study was to identify transporters that regulate intracellular concentrations of statins in human skeletal muscle cells (SKMC).

**METHODS:** Primary human SKMC cells were cultured at 37°C in basal medium and in differentiation medium 24h before experiment (PromoCell). Total RNA was extracted using the RNeasy Mini Kit (Qiagen). cDNA was synthesized by RT-PCR using SuperScript III (Invitrogen). 40 ng cDNA and appropriate primers were used for real time PCR performed with the Rotor Gene Thermocycler (Montreal-Biotech).

**RESULTS:** The level of expression of 24 transporters located on the plasma membrane of SKMC cells was determined and compared under different conditions. In unstimulated cells, more than 60% total expression of transporters tested were represented by, first, MCT-4 (a lactic acid transporter) and, second, ABCA-1 (a cholesterol transporter). MCT-4 is a relevant transporter to study since statins such as lovastatin acid are substrates of this transporter in bovine kidney cells and have been shown to inhibit lactic acid uptake in pig kidney cells. Moreover, statins are known to increase expression levels of ABCA-1 in HepG2 cells and to decrease its expression in macrophages. Preliminary studies in our laboratory have shown that 10 µM fluvastatin increases ABCA-1 mRNA levels in SKMC cells by 2.4 fold.

**CONCLUSION:** Transporters such as MCT-4 and ABCA-1 expressed in human SKMC can regulate the intracellular concentrations of statins which may also modulate the expression of these transporters. These transporters may represent key determinants of underlying mechanisms associated with statin-induced muscle toxicity.

**PIII-36**

MADD VARIANTS AND RISK OF CARDIOVASCULAR EVENTS IN THE INTERNATIONAL VERAPAMIL SR/TRANDOLAPRIL STUDY - GENETIC SUBSTUDY (INVEST-GENES). M. A. Martin, E. T. Price, M. Pacanowski, R. M. Cooper-DeHoff, C. J. Pepine, J. A. Johnson, I. Zineh; University of Florida, Gainesville, FL

**BACKGROUND:** Apoptosis pathways are implicated in cardiovascular disease (CVD). MAP kinase-activating death domain protein (MADD) is involved in apoptosis signaling. We tested whether a

common *MADD* gene polymorphism is associated with variable CVD event rates in elderly patients with hypertension and coronary disease, and if this risk is modified by antihypertensive drugs in the International Verapamil SR/Trandolapril Study (INVEST).

**METHODS:** INVEST participants were randomized to either atenolol or verapamil-SR-based treatment algorithms for blood pressure control. We conducted a case-control study within INVEST, where subjects who experienced a primary outcome (all-cause death, nonfatal myocardial infarction or nonfatal stroke) were matched based on age, race and gender to event-free control subjects (258 cases and 774 controls). We genotyped *MADD* -Chr11:47250597- G>T polymorphism (rs11039159) and analyzed its effect and interaction with antihypertensive treatment on the primary outcome in each racial group using logistic regression.

**RESULTS:** The minor allele frequency was 39% in whites, 13% in blacks, and 29% in Hispanics. The T/T genotype was protective against the primary outcome in whites (adjusted OR=0.532, p=0.036). This effect was seen in white patients in the verapamil SR treatment strategy (adjusted OR=0.413, p=0.034), but not in atenolol-treatment strategy patients (adjusted OR=0.639, p=0.313). There were no genetic or pharmacogenetic associations in the black or Hispanic patients.

**CONCLUSION:** *MADD* is a polymorphic gene with potential implications in CVD. The observed main effects and pharmacogenetic associations observed in whites warrant further analysis.

### PIII-37

NOS3 POLYMORPHISMS DO NOT ASSOCIATE WITH ADVERSE CLINICAL OUTCOMES IN ELDERLY HYPERTENSIVES WITH CORONARY ARTERY DISEASE: ANALYSIS OF THE INTERNATIONAL VERAPAMIL SR-TRANDOLAPRIL STUDY - GENETIC SUBSTUDY (INVEST-GENES). M. A. Pacanowski, I. Zineh, G. J. Welder, J. Adicks, R. M. Cooper-DeHoff, C. J. Pepine, J. A. Johnson; University of Florida, Gainesville, FL

**BACKGROUND:** Nitric oxide synthase gene (NOS3) polymorphisms confer variable cardiovascular (CV) risk. However, several studies demonstrated no genetic associations with CV risk in hypertensive patients. To further investigate this observation, we examined whether two widely studied NOS3 polymorphisms (-786 T/C and E298D) were associated with adverse CV outcomes in elderly patients with treated hypertension and established coronary artery disease.

**METHODS:** In the INVEST, patients were randomly assigned to an atenolol- or verapamil SR-based antihypertensive strategy. The primary outcome was the first occurrence of all-cause death, nonfatal myocardial infarction, or nonfatal stroke. Genotyping was performed on a nested case-control population consisting of 258 cases and 774 controls, matched by age, sex, and race. Adjusted odds ratios (OR) for the associations between NOS3 polymorphisms and the outcomes (primary and individual) were calculated using logistic regression, with and without stratification by race. Drug \* gene interaction terms were tested to probe for modification of genotype effects by drug therapy.

**RESULTS:** The frequencies of the -786 T and 298 D alleles were 0.33 and 0.29, respectively. The primary outcome did not differ by -786 T/C (OR: CT 1.20, p=0.9; TT 1.38, p=0.3) or E298D (OR: ED 1.28, p=0.5; DD 1.29, p=0.6) genotype. Neither genotype was associated with death, MI, or stroke. The results did not differ when stratified by race. Treatment strategy did not modify genotype associations (pinteraction=0.3 for -786 T/C, pinteraction=0.7 for E298D).

**CONCLUSION:** Consistent with other studies, NOS3 variants seem to have neutral effects on clinical endpoints in individuals with established hypertension, and this was not modified by antihypertensive drug therapy. Hypertension may mask the adverse genetic effects previously demonstrated in non-hypertensive populations.

### PIII-38

DIABETES RISK ASSOCIATED WITH BETA 1-ADRENERGIC RECEPTOR POLYMORPHISMS DIFFERS BY ANTIHYPERTENSIVE DRUG THERAPY: RESULTS FROM THE INTERNATIONAL VERAPAMIL SR/ TRANDOLAPRIL STUDY - GENETIC SUBSTUDY (INVEST-GENES). M. A. Pacanowski,<sup>1</sup> R. M. Cooper-DeHoff,<sup>1</sup> Y. Gong,<sup>1</sup> N. J. Schork,<sup>2</sup> C. J. Pepine,<sup>1</sup> J. A. Johnson<sup>1</sup>; <sup>1</sup>University of Florida, Gainesville, FL, <sup>2</sup>University of California, San Diego, San Diego, CA

**BACKGROUND:**  $\beta$ -blockers and diuretics increase diabetes (DM) risk, while calcium channel blockers are neutral and ACE inhibitors are protective. Adrenergic receptors regulate glucose metabolism, and adrenergic receptor gene variants have been associated with metabolic disturbances. We hypothesized that antihypertensive regimens including a  $\beta$ -blocker modify the risk of new-onset DM associated with  $\beta$ 1-adrenergic receptor gene (ADRB1) variants.

**METHODS:** Hypertensive patients with coronary artery disease were randomly assigned to an atenolol- or verapamil SR-based antihypertensive strategy. Hydrochlorothiazide and/ or trandolapril were added as needed. ADRB1 variants (S49G and R389G) were genotyped in patients that developed new-onset DM (n=430) and a group of never-diabetic controls matched by age, sex, and race (n=424). Adjusted logistic regression was performed to calculate odds ratios (OR) associated with ADRB1 variants. The ADRB1 interaction with atenolol exposure was evaluated in stratified analysis and by testing gene-drug interaction terms.

**RESULTS:** The S49G and R389G variants were not associated with DM risk in the overall population or within any race groups. However, the R389G variant was associated with a lower risk of DM in atenolol-treated patients (R/G vs. R/R OR=0.92 p=0.19; G/G vs R/R OR=0.43 p=0.04), but not in verapamil SR-treated patients (R/G vs. R/R OR=0.79 p=0.23; G/G vs R/R OR=1.08, p=0.53), resulting in a significant interaction between drug exposure and G389 homozygote status (p=0.03). Similar trends were noted within each racial group. The association with the S49G variant did not differ by drug exposure.

**CONCLUSION:** The risk for DM associated with ADRB1 variants differed according to antihypertensive drug therapy, such that the G389 allele may be protective in atenolol-treated patients. Thus, genetic variation in ADRB1 may influence the risk of developing DM in the course of treatment for hypertension.

### PIII-39

P-GLYCOPROTEIN (*MDR1*) EXPRESSION MODULATES  $IC_{50}$  OF DOMPERIDONE FOR BLOCK OF THE RAPID COMPONENT OF THE DELAYED RECTIFIER POTASSIUM CURRENT ( $I_{KR}$ ) IN A HEK293 CELL LINE STABLY EXPRESSING HERG. I. Plante, R. Hreiche, F. Bélanger, J. Turgeon; CHUM Research Center, Montreal, QC, Canada

**BACKGROUND:** The efflux transporter P-glycoprotein (P-gp; MDR1) is expressed in various tissues including the human heart. Inter-subject variability in the expression of this transporter modulates intracellular concentrations of drugs and could explain a great deal of intersubject variability observed in drug response/toxicity. We have shown recently that HERG, which encodes  $I_{Kr}$ , co-immunoprecipitates with MDR1 in membrane protein extracts of human heart ventricular samples. We also demonstrated the co-localization of MDR1 and HERG at the cellular surface of cardiac myocytes. The objective of our studies was to assess block of  $I_{Kr}$  by domperidone, a P-gp substrate, in cells coexpressing HERG and MDR1.

**METHODS:** Cells from a HERG-transfected HEK 293 stable cell line (Craig January) were cultured in MEM medium and transiently transfected with recombinant pIRES2MDR1-AcGFP1 vector using lipofectamine (Gibco BRL). Fluorescent cells were voltage-clamped in the whole-cell configuration 48 to 72 hours after transfection. At 23, 30 and 37°C to assure functionality of MDR1.

**RESULTS:** Block of  $I_{Kr}$  by 100 nM domperidone was consistently less in cells co-expressing MDR1.

**Block, by 100 nM domperidone, of  $I_{Kr}$  in HERG and HERG+MDR1 cells at three different temperatures.**

Temperature	% of block of $I_{Kr}$ (mean±SEM)	% of block of $I_{Kr}$ (mean±SEM)	% of block of $I_{Kr}$ (mean±SEM)
	HERG cells	HERG+MDR1 cells	p-value
23°C	78.12±2.41 (n = 5)	44.85±2.54 (n = 4)	<0.001
30°C	56.44±7.57 (n = 5)	38.40 ± 3.66 (n = 7)	0.04
37°C	56.55±5.45 (n = 4)	31.33 ± 2.23 (n = 4)	0.007

These results were reproduced in HERG (48.06% block) and HERG+MDR1 (29.83% block) cells (n=100) (p=0.01) exposed in an unpaired fashion to buffer alone or domperidone alone, in order to eliminate the confounding effects of potential run-down of  $I_{Kr}$ .

**CONCLUSION:** These observations demonstrate that P-glycoprotein may modulate drug effects in the heart. Furthermore, they provide new information of the potential role of membrane transporters in our understanding of risk factors to drug-induced Long QT syndrome.

**PIII-40**

EFFECTS OF GENETIC VARIANTS OF ORGANIC CATION TRANSPORTER 2 (OCT2) ON THE PHARMACOKINETICS OF METFORMIN IN HEALTHY SUBJECTS. I. Song, H. Shin, E. Shim, I. Jung, W. Kim, J. Shon, J. Shin; Inje Univ. College of Medicine, Busan, Republic of Korea

**BACKGROUND:** Metformin is a substrate for organic cation transporter 2(OCT2), encoded from SLC22A2, and showed inter-individual variations in its pharmacokinetics and efficacy. Especially, contribution of genetic component has been suggested in the renal excretion of metformin.

**METHODS:** To investigate the genetic contribution to the renal excretion, metformin uptake were measured in vitro systems overexpressing OCT2 wildtype and variants (c.596C>T(T199I), c.602C>T(T201M), and c.808G>T(A270S). In in vivo clinical study, PK parameters were compared among subjects with wild type and variant groups, after 500mg metformin was administered to 26 healthy volunteers with different SLC22A2 genotypes: group1, wildtype(n=9); group2, 596CT and 602CT hetero(n=5); group3, 808GT hetero(n=6); group4, 808TT homo(n=6).

**RESULTS:** The uptake of metformin in oocytes expressing OCT2 wildtype showed much greater increase compared with those in OCT1, and significantly decreased in OCT2 variants. The intrinsic clearance of metformin was decreased 68.6%, 60.1%, and 39.6% in OCT2-T199I, T201M, A270S expressing cells, respectively. These results suggested that genetic variants in OCT2 may change the pharmacokinetics of metformin by decreasing transport activity. In the clinical study, AUC values of metformin were increased in groups2,3,4 compared with group1.  $C_{max}$ , Cl/F and Vd/F values were decreased in group2 and group4, while other parameters such as  $T_{max}$  and  $K_e$  were not changed among different genotypes. Renal clearance and active secretion clearance were decreased in group2,3, and 4, compared with those in group1, suggesting that decreased transport function of OCT2 variants resulted in decreased renal excretion and, consequently, increased plasma concentration of this substrate.

**CONCLUSION:** The genetic variants in OCT2(T199I,T201M, and A270S) may be controlling factors responsible for the inter-individual variations in the renal clearance of metformin.

**PIII-41**

ATORVASTATIN REGULATES GLOBAL INFLAMMATORY AND ANTI-INFLAMMATORY GENE EXPRESSION IN HUMAN ENDOTHELIAL CELLS. G. J. Welder, I. Zineh; University of Florida College of Pharmacy and Center for Pharmacogenomics, Gainesville, FL

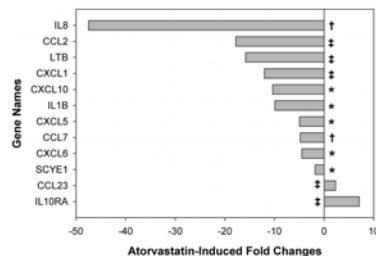
**BACKGROUND:** Endothelial inflammation plays a major role in the development of cardiovascular disease (CVD). Endothelial inflammatory processes are complex and involve differential expression of genes that encode cytokines, chemokines, and their respective receptors. HMG-CoA reductase inhibitors (statins) have been shown to exhibit anti-inflammatory properties, however their simultaneous impact on the aforementioned mediators of inflammation is unknown. We investigated the effect of atorvastatin on the expression of 84 inflammatory and anti-inflammatory genes in human endothelial cells (HUVECs).

**METHODS:** HUVECs were treated with atorvastatin calcium 10µM or control solvent (DMSO) for 24 hours (N=2-3). RNA was isolated and 500ng was reverse transcribed. Focused, pathway-driven, PCR-based gene expression was performed per protocol (Human Inflammatory Cytokine and Receptor RT<sup>2</sup> Profiler PCR Array; Super-Array Bioscience Corp., Frederick, MD). A t-test was used for comparison with a P≤0.05 considered significant. Fold changes were determined by the 2<sup>-ΔΔCt</sup> method.

**RESULTS:** Atorvastatin down-regulated the gene expression of five CXC chemokines (CXCL6, CXCL5, CXCL10, CXCL1, and IL8) and two CC chemokines (CCL7 and CCL2). The expression of two cytokine genes (SCYE1 and IL1B) and a member of the tumor necrosis super family (LTB), whose receptor has been recently implicated in regulating cholesterol homeostasis, were also down-regulated. Atorvastatin up-regulated the gene expression of a CC chemokine, CCL23, and the anti-inflammatory IL10RA. Data shown in the Figure (\*P<0.05, ‡P<0.01, and †P<0.001).

**CONCLUSION:** Atorvastatin’s ability to simultaneously regulate endothelial gene expression of multiple chemokines, cytokines, and their receptors could contribute to its pleiotropic effects and should be further explored.

Gene Regulation of Atorvastatin on Human Endothelial Cells



**PIII-42**

AVERAGE BIOEQUIVALENCE TEST USING NON-PARAMETRIC METHODS; EXPERIENCES ON BOOTSTRAP BIOEQUIVALENCE. S. Han, MD, B. Ahn, MD, D. Yim, MD, PhD; The Catholic University of Korea, Seoul, Republic of Korea

**BACKGROUND:** Ninety percent confidence intervals (CI) obtained from average bioequivalence (ABE) tests are based upon the assumption that log-transformed AUC and Cmax are normally distributed. To compare the CI with those obtained from non-parametric methods, we performed repeated estimation of bootstrapped datasets SAS and NONMEM.

**METHODS:** Three original datasets of AUC and Cmax (2 bioequivalence and 1 bioinequivalence results) were used for this study. Bootstrap resampling was repeated for 1,000 times using MACRO function of SAS for each original dataset. ABE tests (PROC MIXED, SAS) were performed for these 1,000 resampled datasets to find the distribution of formulation effect values. The formulation

effects were also estimated using NONMEM (Ver.6) for the same resampled datasets. Medians and 90 percentiles of formulation effects thereafter were compared with the 90% CI of the original datasets.

**RESULTS:** We could not find any significant differences in the formulation effect estimates between SAS and NONMEM. In contrast with the original 90% CI's, the 90% CI's from 1,000 resampled datasets were narrower for all of the three original datasets. The shapes of histograms and density curves of bootstrapped log(AUC) and log(Cmax) were similar to those of normal distribution.

**CONCLUSION:** Although the current 80-125% rule on the 90% CI is widely used, the narrower 90% CI's obtained from repeated tests on the resampled datasets presented herein deserve further research for its application to regulatory process.

### PIII-43

A NOVEL ERYTHROPOIETIN FUSION PROTEIN PT-401 WITH ENHANCED BIOLOGICAL ACTIVITY. J. Jeong,<sup>1</sup> K. L. Davis,<sup>1</sup> A. L. Socha,<sup>1</sup> H. J. Gomez,<sup>2</sup> A. J. Sytkowski,<sup>1</sup>; <sup>1</sup>Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, <sup>2</sup>DNAPrint Pharmaceuticals, Inc., Sarasota, FL

**BACKGROUND/AIMS:** Recombinant human erythropoietin (rhEPO, epoetin) is used widely for treatment of anemia due to chronic kidney disease, cancer and other conditions. Alternative erythropoiesis stimulating agents (ESAs) and modified versions of rhEPO have been and continue to be developed to overcome the relative short *in vivo* half-life of rhEPO. We previously described the production and initial characterization of PT-401, a recombinant fusion protein comprising two EPO domains. We now report that PT-401 interacts specifically with the human EPO receptor (EpoR). Importantly, PT-401 exhibits enhanced *in vivo* activity in mice compared to rhEPO.

**METHODS:** PT-401 was obtained from cell culture supernatant of stably transfected Chinese hamster ovary (CHO) cells. Immunoblotting with the monoclonal anti-EPO antibody (AE7A5) was used to detect PT-401. Binding experiments of PT-401 to the EpoR were performed using radioiodinated PT-401 and BaF3 cells stably expressing the EpoR.

**RESULTS:** CHO cell clones producing PT-401 with the greatest extent of glycosylation, as indicated by SDS-PAGE and isoelectric focusing, were selected. PT-401 was purified by sequential column chromatography to near homogeneity as determined by SDS-PAGE. Receptor binding studies demonstrated the specific binding of PT-401 to the EpoR in a saturable manner, but with unique thermodynamic characteristics that were distinct from rhEPO. Subcutaneous injections of mice with PT-401 (300 IU/kg or 100 IU/kg) on days 1, 3 and 5 increased their mean hematocrit at day 7 to levels significantly higher than that seen with rhEPO. Importantly, an increase in mean hematocrit was achieved at day 7 with only a single dose (day 1) of PT-401 (100 IU/kg) while an equivalent unit dose of rhEPO had no effect.

**CONCLUSIONS:** PT-401, a novel EPO fusion protein, exhibits an *in vivo* biological activity superior to that of rhEPO, implying the therapeutic convenience of lower dosages and less frequent administration.

### PIII-44

IMPACT OF THE 1998 RENAL GUIDANCE ON RECENT NEW DRUG APPLICATION SUBMISSIONS. S. Huang,<sup>1</sup> S. Abraham,<sup>1</sup> S. Apparaju,<sup>1</sup> A. Atkinson, Jr.,<sup>2</sup> G. Burckart,<sup>2</sup> C. Lee,<sup>2</sup> K. Roy,<sup>1</sup> J. Strong,<sup>3</sup> S. Xiao,<sup>4</sup> T. Wu,<sup>1</sup> L. Zhang,<sup>1</sup> Y. Zhang,<sup>1</sup> L. Lesko<sup>1</sup>; <sup>1</sup>Office of Clinical Pharmacology, Office of Translational Sciences, CDER, FDA, Silver Spring, MD, <sup>2</sup>Sabbatical at the FDA, Silver Spring, MD, <sup>3</sup>Office of Pharmaceutical Sciences, CDER, FDA, Silver Spring, MD, <sup>4</sup>Office of New Drugs, CDER, FDA, Silver Spring, MD

**BACKGROUND:** "The Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function- Study Design, Data Analysis and Impact on Dosing and Labeling" was issued by the FDA in

1998. The purpose of this presentation is to assess the impact of this guidance on regulatory submissions and to determine the necessity for guidance update.

**METHODS:** Various efforts are undertaken by the Renal Guidance Working Group including a survey of new molecular entities approved over the past 5 years (2003-2007). The impact of the 1998 renal guidance is assessed by comparing these results against those from a survey conducted in 1996-1997 (Ibrahim, *et. al.*, J Clin Pharm, 40:31-38, 2000).

**RESULTS:** A total of 95 new molecular entity drugs are included in the survey of which 53 are orally administered.

Percent	Current (2003-2007, Oral Drugs)	Previous (Oct 1996-Sept 1997)
Renal Impairment Study	77% (41/53)	56% (40/71)
Full Study Design	72% (23/41)	25% (10/40)
Hemodialysis	41% (17/41)	15% (6/40)
Dosage Adjustment based on PK changes	39% (16/41)	28% (11/40)

**CONCLUSION:** Preliminary results from the survey indicated that 1) the 1998 guidance had an impact on the determination of need to conduct a renal impairment study, study design and labeling, 2) more studies are needed for hemodialysis patients, and 3) the effect of renal impairment on drug metabolism and transport needs to be understood better.

### PIII-45

COMPARATIVE STUDY OF DIHYDROARTEMISININ AND ARTESUNATE SAFETY IN HEALTHY THAI VOLUNTEERS. S. Kongpatanakul, MD,<sup>1</sup> S. Chatsiricharoenkul, MD,<sup>1</sup> A. Khuhapinart, MD, PhD,<sup>1</sup> S. Atipas, MD,<sup>1</sup> J. Kaewkungwal, PhD<sup>2</sup>; <sup>1</sup>Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, <sup>2</sup>Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

**BACKGROUND:** Artemisinin derivatives including artesunate and dihydroartemisinin (DHA) have been used for treatment of malaria. As part of new drug development initiatives in Thailand, a new tablet formulation of DHA has been developed. Our initial phase I bioequivalence study indicated that the new and reference DHA formulations were well tolerated; however, a significance decrease in hemoglobin (0.7-0.9 g/dl) was detected after a single 200 mg oral dose. To explore further, a clinical study with an emphasis on hematological parameters was conducted.

**METHODS:** A single-center, randomized, single-blind, cross-over clinical study was conducted in 24 healthy Thai volunteers with a dosage of 300 mg daily for 2 days. Artesunate was used as a comparator. Clinical adverse events were monitored. Laboratory assessment (CBC, RBC morphology, reticulocyte count, Coombs'test, total and direct bilirubin, LDH, AST, ALT, and hemoglobinuria) was performed on study days 0 prior to drug administration and days 2, 5, and 7 post drug administration.

**RESULTS:** Eighteen volunteers (10 males and 8 females) completed both rounds of the study. All adverse events were mild. Nausea and vomiting were common and occurred only in female volunteers. A statistically significant decrease in hemoglobin was detected (0.5 g/dl at study day 7,  $P < 0.05$ ). Decreases in laboratory values below the normal limits were observed in some volunteers for reticulocyte and WBC counts. Other changes in laboratory values were minor. Transient, mild bone marrow suppression was evidenced by: 1) reduction of reticulocytes, 2) leukopenia, and 3) a minor drop of platelet counts.

**CONCLUSION:** The present study confirmed our previous finding on a significant decrease in hemoglobin. Bone marrow suppression might be one of the causes. Considering the absence of clinically significant anemia (though decrease in hemoglobin) and its similarity in drug response profiles to artesunate, the development of the DHA should pursue.

**PIII-46**

THE USE OF COGNITIVE FUNCTION TESTING TO IDENTIFY POTENTIAL COGNITION ENHANCERS IN PHASE I: CASE HISTORIES OF TRANSLATIONAL MEDICINE. S. T. Satek,<sup>1</sup> K. A. Wesnes<sup>2</sup>; <sup>1</sup>Cognitive Drug Research, Ltd., Chicago, IL, <sup>2</sup>Cognitive Drug Research, Ltd., Goring-on-Thames, United Kingdom

**BACKGROUND:** Drugs under development as cognition enhancers can be evaluated in Phase I for potential efficacy using tests of cognitive function. Case studies will be presented showing the utility of such evaluation in identifying potential candidates for subsequent development.

**METHODS:** The Cognitive Drug Research (CDR) computerized assessment system has been widely used in worldwide Phase I research and has an extensive bibliography for identifying cognitive enhancements in volunteers and various patient populations. It assesses core domains of function including attention, information processing, working memory and episodic memory. The individual tests are brief, fully automated and can be repeatedly administered over hours, days or weeks. This enables the testing to be seamlessly incorporated into safety and tolerability Phase I trials without compromising the primary objectives of such work. Its use in such studies, as well as pharmacodynamic models (scopolamine model of dementia, sleep deprivation model) will be described.

**RESULTS:** Three case histories will be provided showing how the inclusion of testing in safety trials accurately predicted subsequent efficacy in Alzheimer's disease (AD) patients with S-12024, Adult ADHD with NS2359 and Age-Associated Memory Impairment and Mild Cognitive Impairment with TC1734/AZD3480. In the scopolamine model, ZT-1 showed comparable utility to donepezil, and has since been found effective in AD. Finally, modafinil was found to be effective in reversing sleep deprivation induced cognitive deficits in volunteers, and subsequent work has shown the r-isomer to be highly effective in narcolepsy and shift work sleep disorder.

**CONCLUSION:** Cognitive testing in Phase I with an appropriately sensitive and validated tests can provide information which is predictive to patients, and thus satisfies the requirements for being a practical, appropriate, valid and sensitive technique in translational medicine.

**PIII-47**

EFFECT OF AROMATASE INHIBITORS, LETROZOLE AND EXEMESTANE, ON PLATELET COUNT AND AGGREGATION IN WOMEN WITH BREAST CANCER. J. Miao, Y. Kreutz, A. T. Nguyen, S. M. Lemler, Z. Desta, D. A. Flockhart, Y. Jin; West China Hospital, Sichuan University, Chengdu, P.R. China; The division of clinical pharmacology of IUPUI, Indianapolis, IN

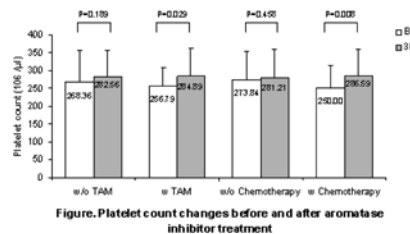
**BACKGROUND:** Increased cardiovascular risk is of clinical concern during chronic treatment with aromatase inhibitors in postmenopausal women with breast cancer. We tested whether aromatase inhibitors influence platelet count and aggregation.

**METHODS:** Postmenopausal women with estrogen receptor positive breast cancer were randomized to receive letrozole (2.5 mg/day) or exemestane (25mg/day). Blood samples were collect before and 3 months after initiating treatment. Platelet counts were measured by COULTER<sup>®</sup>AC.T10 Analyzer. Platelet aggregation was assessed by light transmission aggregometry after stimulation with adenosine diphosphate (ADP), arachidonic acid (AA), and collagen. Statistical analysis was performed using the SPSS 15.0.

**RESULTS:** A total of 67 patients (40 on letrozole and 27 on exemestane) completed the 3 months follow-up for both platelet count and aggregation. The mean platelet counts increased from 263.52×10<sup>6</sup> /μl to 283.54×10<sup>6</sup> /μl (P=0.015). The increase was most apparent in patients with previous tamoxifen or chemotherapy treatment (Figure). Letrozole treatment significantly increased platelet count (from 260.03×10<sup>6</sup> /μl to 283.48×10<sup>6</sup> /μl, P=0.016), while exemestane did not (from 268.70×10<sup>6</sup> /μl to 283.63×10<sup>6</sup> /μl, P=0.312). When platelet aggregation was assessed, 41 showed cyclooxygenase-1 (COX-1) inhibition (AA induced platelet aggregation less than 5%) at baseline, 39 showed COX-1 inhibition at month 3; and 20 subjects showed no consistent COX-1 inhibition during

the first 3 month. In the remaining 47 patients, no statistically significant changes were observed in ADP, AA and collagen induced aggregation before and after aromatase inhibitors treatment.

**CONCLUSION:** Aromatase inhibitor treatment lead to an increase in platelet count without any significant change in platelet aggregation.



**PIII-48**

ASSOCIATION OF ESTROGEN RECEPTOR HAPLOTYPE WITH AROMATASE INHIBITOR-INDUCED PLATELET CHANGES. J. Miao, Y. Kreutz, A. T. Nguyen, S. M. Lemler, Z. Desta, D. A. Flockhart, Y. Jin; West China Hospital, Sichuan University, Chengdu, P.R. China; The division of clinical pharmacology of IUPUI, Indianapolis, IN

**BACKGROUND:** Estrogen regulates platelet function via platelet estrogen receptors. Therefore, depletion of estrogen by aromatase inhibitors may increase cardiovascular risk. We tested the hypothesis that polymorphisms in estrogen receptor genes may influence platelet function after aromatase inhibitor treatment.

**METHODS:** Postmenopausal women with estrogen receptor(ER) positive breast cancer were randomized to receive exemestane (25mg/day) or letrozole (2.5 mg/day). Blood samples were collect before and 3 months after initiating treatment. Platelet counts were measured using a COULTER<sup>®</sup>AC.T10 Analyzer. Platelet aggregation was assessed by light transmission aggregometry after stimulation with adenosine diphosphate (ADP), arachidonic acid (AA), and collagen. Polymorphisms in ER genes: ESR1-03 (ERα PvuII), ESR1-39 (ERα XbaI) and ESR2-02 were determined by TaqMan<sup>™</sup> Assays. EAR1 haplotype was estimated using the PHASE II.

**RESULTS:** Four common haplotypes [T-A (47.9%), C-G (34.8%), C-A (15.9%), T-G (1.0%)] were observed based on the ESR1-03 and ESR1-39 genotypes in 67 patients. While mean platelet count increased in the entire cohort, this increase was most evident in women who carried 1 or none T-A allele (21.62±59.98×10<sup>6</sup>μl, P=0.011), or in subjects who carried at least 1 C-G allele (24.54±57.14×10<sup>6</sup>μl, P=0.01). ESR2-02 genotypes were not associated with changes in platelet counts. Associations between ESR genotype and changes in platelet aggregations were evaluated in subjects who used consistently cyclooxygenase-1 inhibitors (n=47). No associations between polymorphisms in either ESR genes and changes in platelet aggregation were observed.

**CONCLUSION:** ESR1 genetic polymorphisms may influence aromatase inhibitor-induced increases in platelet count and may alter the cardiovascular risk of these medications in selected patients as a result.

**PIII-49**

INCLUSION OF SUBPOPULATIONS IN EARLY PHASE CLINICAL TRIALS SUBMITTED TO THE FDA: A REVIEW OF NMEs APPROVED IN 2006. E. Pinnow, A. Parekh, P. Sharma, N. Gevorkian, K. Uhl; FDA, Rockville, MD

**BACKGROUND:** Historically subpopulations such as women & the elderly have been excluded from clinical trials. This is particularly evident in Phase I trials. The goal of this study was to determine the overall participation of women & elderly subjects in Phase I trials & the dose ranges studied in these populations.

**METHODS:** Clinical trials submitted to the FDA for New Molecular Entities (NMEs) for adult, non-sex specific indications in 2006

were reviewed. Electronic data available on Phase I studies were evaluated for proposed indications, sex & age of participants & doses tested. Therapeutic doses were obtained from the approved labeling.

**RESULTS:** FDA approved 18 NMEs for non-sex-specific indications in 2006. Data for 194 Phase I studies of 16 NMEs were reviewed, 1 was not available electronically & 1 provided only summary demographic data. Overall 29.5% (1836/6231) of study participants were women, 3.6% were  $\geq 65$ . 95/194 (49.0%) studies included safety/tolerability testing above the highest approved dose. In trials that included a dose above the highest recommended dose, 31.4% of participants were women, 3.6% were elderly. 65 (33.5%) trials were exclusively male & 129 (66.5%) also enrolled females. 21 (10.8%) enrolled participants  $\geq$  age 65. Similar percentages of studies, overall & those including a dose above the highest recommended dose, included women & elderly participants.

**CONCLUSION:** Women & elderly subjects are underrepresented in Phase I trials. In early phase dose escalation safety/tolerability trials where drug exposure exceeds the highest therapeutic dose, the importance of including women and elderly subjects should be considered, because of potential differences in response and as they will be exposed postmarketing.

#### Number and Percent of Trials Including Female and Elderly Participants

	All Trials (n=194)	Dose above highest recommended dose (n=95)
Trials including female participants	129 (66.5%)	64 (67.4%)
Trials including elderly ( $\geq 65$ ) participants	21 (10.8%)	10 (10.5%)

### PIII-50

BREAKING THE RULES: MISINTERPRETATION OF DOSE-RESPONSE RELATIONSHIPS IN CLINICAL TRIALS. L. B. Pearce, A. N. Pitman, A. G. Greenburg; Biopure Corporation, Cambridge, MA

**BACKGROUND:** Identification and analysis of the dose and response relationships is central to the assessment of safety and efficacy in clinical trials. The observation of a strong positive correlation between dose and outcome is commonly viewed as evidence supporting a causal relationship. This report describes the situation where highly significant correlations between dose and outcome do not represent true dose-response relationships.

**METHODS:** The dose response relationships for test drug (HBOC-201), a hemoglobin-based oxygen carrier, and control therapy, transfusion with packed red blood cells (RBC), were examined in two single blind, randomized and controlled phase III clinical trials (HEM-0115 and HEM-0114) investigating the treatment of acute surgical anemia in the perioperative elective surgical setting. Patients were randomized to treatment with HBOC-201 (N=350 or 83) and RBC (N= 338 or 77) and the extent of blood avoidance and safety were evaluated in studies HEM-0115 and HEM-0114, respectively.

**RESULTS:** Regression analysis using a simple general linear model revealed a significant correlation between dose of HBOC-201 and safety outcome measured in terms of the number of adverse events per patient (AE/pt) in study HEM-0115:  $AE/pt = 5.25(\pm 0.61) + 0.77(\pm 0.12)[HBOC]$ ,  $p < 0.0001$ . A similar relationship for dose of the control treatment:  $AE/pt = 3.43(\pm 0.48) + 0.81(\pm 0.13)[RBC]$  with  $p < 0.0001$ . As confirmation, highly significant ( $p < 0.0001$ ) correlations for HBOC-201:  $AE/pt = 4.48(\pm 1.90) + 0.78(\pm 0.34)[HBOC]$  and dose of RBC:  $AE/pt = 2.57(\pm 0.94) + 0.83(\pm 0.16)[RBC]$  were observed in study HEM-0114.

**CONCLUSION:** Randomization of drug therapy to dose is preferable but not always possible and when not, dose response relationships can be confounded which predisposes to misinterpretation. The inaccuracy of the assessment will be greatest in the situation where dose is determined by clinical need and clinical need is the primary predictor of risk (AE/pt).

### PIII-51

A FLEXIBLE OUTCOME SCORING SYSTEM FOR CLINICAL TRIALS. A. N. Pitman, L. B. Pearce; Biopure Corporation, Cambridge, MA

**BACKGROUND:** Quantification of the safety outcome is central to the evaluation of results of clinical trials. Even though the search for safety signals could be conducted on the level of particular AEs, the most statistically desirable approach to overall risk assessment is assignment of a risk score for every subject in the trial. The best known method is assignment of risk scores by a panel of blinded reviewers. The latter is very time consuming, costly and may introduce sufficient bias.

**METHODS:** An outcome scoring system (OSS) was developed as a flexible method to create a morbidity/mortality score for outcome assessment incorporating the following major characteristics: objective and unbiased application to all patients, based on expert medical opinion, completeness without double counting of events, and adjustment for risk tolerability tailored to the clinical situation. The key element of this OSS is combination of primary scores assigned to multiple covariates to provide a final score expressed in mortality terms using a symmetrical asymptotical function with additive properties. The specific numerical details and sequence of major steps with possible variations will be presented.

**RESULTS:** This methodology provides a quantitative continuous score in mortality equivalents for every subject in any given trial. Clinical contextualization is incorporated during the numerical scoring and applied to all patients equally without the risk of bias. Scoring is unique for individual trials depending on risk tolerability with retention of the major properties described above.

**CONCLUSION:** This method is more precise than scaled scoring, more objective, and logistically more feasible than post-study by-patient blinded reviews. This approach is independent of trial size which opens the opportunity for numerous applications (Pearce et al. abstract). This is particularly suited for the assessment of controversial oversized trials with multiple outcomes of interest.

### PIII-52

APPLICATION OF A UNIQUE SCORING SYSTEM FOR NUMERICAL DETERMINATION OF A BENEFIT RISK RATIO IN CLINICAL TRIALS. L. B. Pearce,<sup>1</sup> A. Pitman,<sup>1</sup> A. G. Greenburg,<sup>1</sup> D. A. Freilich,<sup>2</sup> L. Kaplan<sup>3</sup>; <sup>1</sup>Biopure Corporation, Cambridge, MA, <sup>2</sup>Naval Medical Research Center, Silver Spring, MD, <sup>3</sup>Yale University School of Medicine, New Haven, CT

**BACKGROUND:** Quantification of a true benefit:risk ratio (BRR) can prove exceedingly difficult when the units of measure of benefit and risk are not equivalent and/or include  $>2$  contributing factors. When the numerical assessment of the BRR is difficult, decisions may be based on expert opinion - a method lacking objectivity. In contrast, this report describes the application of a new general methodology for determination of a projected BRR for a clinical trial in the setting of life threatening trauma under waiver of informed consent.

**METHODS:** The relative severity of adverse events (AEs) were scored on a percentage scale by 12 trauma/critical care physicians (for an expected hypothetical mortality of 30%, 60% and 90%). Net risk in mortality equivalents was estimated based on the results of a phase III clinical trial with more sensitive reporting of AEs in a low mortality surgical setting. Scores for individual patients were calculated by the recursive method and mean scores for subsets of interest were derived. Benefit was estimated as a 15% decrease in mortality from preclinical findings. The BRR was calculated as the ratio of deltas for mean scores for both treatment arms for a projected mortality of 58%. Data are means  $\pm$  SEM.

**RESULTS:** The population risk scores (mortality equivalents) from the phase III study intent to treat analysis were  $0.050 \pm 0.007$  (N = 350) and  $0.035 \pm 0.007$  (N=338) and for the worse case analysis were  $0.083 \pm 0.010$  (N = 139) and  $0.053 \pm 0.016$  (N=107) for treatment (HBOC-201) and control (RBCs) arms, respectively. The resulting BRR ranged from 5 to 3. The robustness of a projected favorable BRR was established against variations of assumptions for risk and benefit.

**CONCLUSION:** Application of this methodology for objective numerical non-biased assessment of BRR may allow rational comparison of benefit:risk ratios across both proposed and completed clinical trials. This methodology may help establish a new rational standard in clinical trial evaluation.

### PIII-53

A PILOT STUDY TO VALIDATE THE PATIENTS SUPPORT SYSTEM, RTIME<sup>®</sup>, AS A RELIABLE TOOL FOR CLOSE-MONITORING OF CLINICAL RESEARCHES. A. Inano,<sup>1</sup> I. Horikawa,<sup>2</sup> E. Hatakeyama,<sup>3</sup> T. Sumiyoshi,<sup>4</sup> I. Oshiba,<sup>3</sup> T. Amamoto,<sup>3</sup> W. Sato,<sup>4</sup> M. Masuda,<sup>4</sup> T. Morimoto,<sup>5</sup> K. Ohashi<sup>5</sup>; <sup>1</sup>NPO HEART Hokuriku Clinical Research Supporting Center, Kanazawa, Japan, <sup>2</sup>Horikawa Clinic, Nonouchi, Japan, <sup>3</sup>NEUES Corp., Tokyo, Japan, <sup>4</sup>Rtime Co.Ltd., Tokyo, Japan, <sup>5</sup>Oita University, Oita, Japan

**BACKGROUND:** Clinical researches require accuracy and confidence of subjective information obtained from study participants or patients because research endpoints thoroughly depend on that information. Resources of subjective information are often limited to patient's diaries, but most of them are paper-based records. Ensuring confidence of subjective information is still a significant challenge in clinical researches. In this context, we have developed a patient support system, Rtime<sup>®</sup>, which utilizes e-mails, data communication and application functions of cell phones. In order to seek the validity and user-friendliness of Rtime<sup>®</sup>, we followed outpatients with seasonal allergy the symptoms of which are severe enough for H-1 antagonist treatments.

**METHODS:** After informed consent procedures, participants' cell phone e-mail addresses were registered to Rtime<sup>®</sup> system. The system automatically generated and sent e-mails requesting all participants to respond to the questionnaire at 19:00 every day. The questionnaire included patient's global assessment of symptoms, compliance check and Epworth Sleepiness Scale (ESS) scoring. All the data were recorded in a main server with access time and response time was also evaluated.

**RESULTS:** 40 patients with average age of 34.5 were enrolled. Of total 3280 generated e-mails, 3112 were received their responses and about 80% was returned within 2 hours after the first sending. The identical ESS score at visit and at Rtime<sup>®</sup> was 52.4%. Seasonal transition of medication compliance was successfully monitored as well as ESS and patient's global assessment.

**CONCLUSION:** Rtime<sup>®</sup> enabled us to monitor pharmacologically relevant parameters timely and easily. Its user-friendliness may contribute to data accuracy. It could be an effective tool of therapeutic monitoring for a large number of patients.

### PIII-54

DIFFERENTIAL EFFECTS OF CO-ADMINISTRATION OF RACEMIC KETOCONAZOLE AND LEVDEXKETOCONAZOLE ON THE PHARMACOKINETIC PROFILE OF ATORVASTATIN. S. Boudriau, PhD,<sup>1</sup> R. Demnati, MD, PhD,<sup>1</sup> D. Swearingen, MD,<sup>2</sup> T. Stewart, PhD,<sup>3</sup> M. K. Di Marco, MSc, PhD,<sup>1</sup> B. Welles, MD, MBA<sup>3</sup>; <sup>1</sup>MDS Pharma Services, St.Laurent, QC, Canada, <sup>2</sup>MDS Pharma Services, Phoenix, AZ, <sup>3</sup>DiObex, San Francisco, CA

**BACKGROUND:** Ketoconazole (KTZ) is an equimolar mixture of the 2S,4R and 2R,4S enantiomers. Levdexketoconazole (LDKTZ), the 2S,4R enantiomer, is being developed as a novel cortisol synthesis inhibitor to treat hyperglycemia in type 2 diabetic patients. LDKTZ, may also improve hyperlipidemia and hypertension. While the IC<sub>50</sub> toward CYP3A4 of both enantiomers is comparable, LDKTZ has a 12-fold higher IC<sub>50</sub> toward CYP7A. CYP7A suppression can lead to functional cholestasis and accumulation of xenobiotics; thus LDKTZ may be safer than KTZ if administered with CYP3A4 substrate such as Atorvastatin (ATV). This study's aim was to assess the impact of co-administration of LDKTZ or KTZ on the pharmacokinetics (PK) of ATV.

**METHODS:** 24 healthy adults were randomized in a 3-way placebo-controlled cross-over study receiving 7 days of dosing with either LDKTZ 400 mg; KTZ 400 mg or Placebo 400 mg; each treatment period was separated by a 12-day washout. PK was determined on day 5 when a single 80 mg dose of ATV was co-administered.

**RESULTS:** ATV exposure (AUC) increased by ~3-fold with ATV+LDKTZ or ATV+KTZ but Cmax was not significantly affected with LDKTZ. Tmax was delayed by 1.6h with ATV+ LDKTZ compared to 0.6h with ATV+KTZ. The AUC and Cmax of 2-OH-ATV decreased by 76 and 95% vs 69 and 94%, respectively when either LDKTZ or KTZ were co-administered with ATV. Levels of 4-OH-ATV were close to the limit of detection. Cortisol exposure (AUC) was lowest during the LDKTZ treatment. The number of subjects reporting adverse events was similar across all 3 treatments, however, incidence of headache, back pain and nausea reported with LDKTZ and KTZ treatments was higher compared with placebo.

**CONCLUSION:** Despite comparable inhibition of CYP3A4, the increased AUC of ATV+LDKTZ was less than ATV+KTZ and Cmax was within 90%CI for ATV+LDKTZ. The increase in total exposure to pharmacologically active ATV related compounds (parent ATV, 2-OH ATV, 4-OH ATV) was significantly less for LDKTZ than for KTZ.

### PIII-55

PHARMACOKINETICS AND PHARMACODYNAMICS OF LC15-0444, A NOVEL DPP IV INHIBITOR, AFTER SINGLE ORAL ADMINISTRATION IN HEALTHY SUBJECTS. Y. J. Choi,<sup>1</sup> K. S. Lim,<sup>1</sup> K. P. Kim,<sup>1</sup> J. Y. Cho,<sup>1</sup> K. S. Yu,<sup>1</sup> S. G. Shin,<sup>1</sup> H. J. Yim,<sup>2</sup> O. H. Kwon,<sup>2</sup> J. I. Lee,<sup>2</sup> D. K. Kim,<sup>3</sup> I. J. Jang<sup>1</sup>; <sup>1</sup>Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea, <sup>2</sup>Research & Development Park, LG Life Sciences, Ltd., Daejeon, Republic of Korea, <sup>3</sup>Clinical Development, LG Life Sciences, Ltd., Seoul, Republic of Korea

**BACKGROUND:** LC15-0444 is an orally active and selective inhibitor of dipeptidyl peptidase IV (DPP IV) for treatment of type 2 diabetes. The aim of this study was to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of single ascending doses of LC15-0444 in healthy male subjects.

**METHODS:** A dose block-randomized, double-blind, placebo-controlled, single ascending dose study was performed in 6 groups with 10 subjects (8 for active; 2 for placebo) per group; each group received 25, 50, 100, 200, 400, or 600 mg of the study drug. Blood and urine samples were collected up to 72 h post-dose. An additional food effect study was performed in a 100 mg dose group.

**RESULTS:** Individual plasma LC15-0444 concentration-time profiles showed characteristics of two compartment disposition. LC15-0444 was well absorbed with a mean elimination half life of 16.7 ~ 21.3 h. No dose-dependent increase in elimination half life was observed. Mean apparent volume of distribution and oral clearance by dose groups ranged 1086 ~ 2166 L and 45.4 ~ 83.6 L/h, respectively. LC15-0444 revealed mean fraction of unchanged drug excreted in urine ranging 0.21 ~ 0.34 and renal clearance averaging 15.5 ~ 23.6 L/h. Dose-normalized area under the plasma concentration-time curve (AUC) confirmed the dose-linearity over the range of 50 mg to 400 mg. Single doses of LC15-0444 inhibited 80% of DPP IV activity for more than 24 hours in dose groups of 100 mg or higher. While high-fat diet decreased maximum plasma concentrations of LC15-0444 by 38% compared to a standard diet, AUC was not significantly influenced by food.

**CONCLUSION:** Single oral doses of LC15-0444 were safe and well tolerated up to 600 mg. This study provided evidence of pharmacologic activity for LC15-0444 in humans. LC15-0444 possesses PK and PD characteristics that support a once-daily dosing regimen.

### PIII-56

MODEL FEASIBILITY ASSESSMENT AS A DRIVER FOR MODEL BASED DRUG DEVELOPMENT. B. B. Cirincione,<sup>1</sup> E. Blase,<sup>2</sup> M. Cummings,<sup>1</sup> M. S. Fineman,<sup>2</sup> T. H. Grasela<sup>1</sup>; <sup>1</sup>Cognigen Corp., Buffalo, NY, <sup>2</sup>Amylin Pharmaceuticals Incorporated, San Diego, CA

**BACKGROUND:** Complex pharmacometric analyses raise concerns about cost, time, and reliability of model building process (MBP). The goal was to use a model feasibility assessment (MFA) process to improve the performance characteristics of MBP.

**METHODS:** Literature review provided a basis for proposed mechanistic model of exenatide effects in type 2 diabetes. A study index database (SID) detailing design characteristics, interventions, and comparators of available studies was assembled and used to generate informatics to facilitate data pooling. Cross-study endpoint databases (CSED) for each endpoint were assembled and used to generate exploratory analyses (EA) of posited model relationships. A gap analysis (GA) performed during the assembly of SID and CSED identified issues regarding study design alignment, data adequacy, and the types and timing of interventions and endpoint measurements that impacted MBP.

**RESULTS:** 38 studies were reviewed and were included in MBP. EA aided in determining functional form, providing initial parameter estimates; and specifying data programming requirements. GA was critical in choosing data for MBP and generating design recommendations for future studies. The informatics generated during GA and the discussions to resolve discrepancies enhanced data assembly and accelerated model-building efforts.

**CONCLUSION:** MFA provides systematic approach to facilitate data selection and pooling and improve the performance characteristics of MBP so that results are available for decision-making.

### PIII-57

**MULTIPLE INHIBITORS: CLINICAL STUDIES MAY BE NECESSARY.** C. J. Collins, R. H. Levy; University of Washington, Seattle, WA

**BACKGROUND:** The profound increase in exposure observed with some substrates in the presence of multiple inhibitors is of interest due to our inability to predict the magnitude of this increase. Previously, we used the equations of Chou and Talalay (1981) to evaluate the pharmacokinetic behavior of substrates metabolized by CYP2D6 or CYP2C19 with a minor CYP3A4 pathway. In these special cases, exposure in poor metabolizers administered a CYP3A4 inhibitor could be predicted because the lack of CYP2C19 activity and CYP3A4 inhibition exhibited independence of processes. This may not be the case for concomitantly administered inhibitors.

**METHODS:** The University of Washington Metabolism and Transport Database was mined to identify all studies where drug interaction studies with a single substrate were performed with two inhibitors separately and combined. Predicted AUC was calculated based on the fractional decrease of each inhibitor using the equations of Chou and Talalay.

**RESULTS:** Three studies meeting the above criteria were identified involving loperamide, repaglinide and pioglitazone as substrates. The inhibitors in these studies were gemfibrozil and itraconazole. For loperamide and repaglinide, an independence model underpredicted the observed AUC by 70% and 53% suggesting that the effect of the two inhibitors was synergistic. For pioglitazone, applying an independence model yielded a predicted AUC 11% less than the observed AUC.

**CONCLUSION:** At the present time it appears that there is no method to predict changes in substrate exposure with multiple inhibitors. Therefore clinical studies will be needed to elucidate concomitant inhibition.

#### Predicted and Observed AUC in the Presence of Gemfibrozil and Itraconazole

Substrate	FDCL		Predicted AUC Itra + Gem	Observed AUC Itra + Gem
	Itraconazole	Gemfibrozil		
Loperamide	0.74	0.54	92.62 ng/mL * h	142 ng/mL * h
Pioglitazone	0.08	0.69	18.08 mg/L * h	20.05 mg/L * h
Repaglinide	0.29	0.88	89.8 ng/mL * h	152.5 ng/mL * h

### PIII-58

**AN OPEN LABEL STUDY TO DETERMINE HOW DIFFERENT DURATIONS OF KETOCONAZOLE (KET) DOSING INHIBIT CYTOCHROME P450 3A4 (CYP3A4) AS ASSESSED BY MIDAZOLAM (MDZ) PHARMACOKINETICS (PK).** E. Friedman, A. Maes, Y. Xu, P. Larson, S. Li, J. Chodakewitz, J. A. Wagner, S. Stoch; Merck & Co., Inc., Rahway, NJ

**BACKGROUND/AIMS:** KET interaction studies are frequently performed to determine a given compound's sensitivity to CYP3A4 metabolism. In these studies, KET, a strong CYP3A4 inhibitor, is often administered for 5 days prior to co-dosing with the potential CYP3A4 substrate. The present study compared the effects of single and multiple doses of KET on the single dose PK of MDZ, a sensitive CYP3A4 substrate.

**METHODS:** In this open-label, 4-period, crossover study, 12 healthy adults received 2-mg MDZ alone and with 400-mg KET after either 1, 2, or 5 days of dosing with 400-mg KET. Blood samples for MDZ PK were collected in each treatment period.

**RESULTS:** As summarized in the table below, no clinically meaningful differences, defined by inclusion of the 90% CI for the geometric mean ratio within prespecified similarity bounds of (0.5, 2.00), were observed for MDZ AUC<sub>(0-∞)</sub> following 2-mg MDZ dosed on Day 1 or 2 of once daily dosing with 400-mg KET versus 2-mg MDZ dosed on Day 5 of once daily dosing with 400-mg KET. However, the comparison of MDZ AUC<sub>(0-∞)</sub> after Day 1 versus Day 5 KET and MDZ co-dosing reached statistical significance (p=0.002) as did the Day 1 versus Day 2 comparison (p=0.007).

**CONCLUSIONS:** Single dose MDZ PK is clinically similar after dosing 2-mg MDZ on Day 1 or 2 of 400-mg KET multiple dosing as compared to dosing of 2-mg MDZ on Day 5 of 400-mg KET multiple dosing.

Treatment	N	LS-Mean (ng•hr/ml)	SD <sup>†</sup> (ng•hr/ml)	GMR <sup>‡</sup>
400-mg KET + 2-mg MDZ <sub>Day5</sub>	10	265.9	82.9	13.96
400-mg KET + 2-mg MDZ <sub>Day2</sub>	10	250.3	105.6	13.14
400-mg KET + 2-mg MDZ <sub>Day1</sub>	10	195.9	76.7	10.28
2-mg MDZ	11	19.0	10.6	--
Treatment Comparison	GMR	90% CI <sup>§</sup> for GMR	Between-Treatment p-Value	
KET + MDZ <sub>Day5</sub> / KET + MDZ <sub>Day2</sub>	1.06	(0.92, 1.23)	>0.200	
KET + MDZ <sub>Day5</sub> / KET + MDZ <sub>Day1</sub>	1.36	(1.17, 1.57)	0.002	
KET + MDZ <sub>Day2</sub> / KET + MDZ <sub>Day1</sub>	1.28	(1.11, 1.47)	0.007	

LS Mean= Least-squares Mean back transformed from log scale;

<sup>†</sup> SD=Standard Deviation

<sup>‡</sup> GMR=LS-Mean Ratio (KET + MDZ / MDZ Alone)

<sup>§</sup> CI=Confidence Interval

### PIII-59

**DETERMINATION OF PROOF OF CONCEPT DOSE AND DOSING REGIMEN WITH POPULATION PHARMACOKINETICS AND PET RECEPTOR OCCUPANCY MODELING AND SIMULATIONS.** P. Panorchan, X. Li, S. Bi, S. M. Sanabria, E. Hostetler, D. Burns, I. De Lepeleire, D. Mozley, W. Cho; Merck and Co., West Point, PA

**BACKGROUND:** Selection of Phase II, proof-of-concept (POC) dose and dosing regimen based on Phase I pharmacokinetics (PK), safety and positron emission tomography (PET) receptor occupancy data for the development of Drug X.

**METHODS:** Pharmacokinetic data were available from single and multiple dose escalation studies in healthy subjects (n=51). PET receptor occupancy (RO) data were obtained from single and multiple dose PET studies of drug X (n=9; two scans per subject). Population PK/PD modeling was performed using NONMEM. Simulations of different dose and dosing regimens were conducted via Trial Simulator. To project the expected PK/PD outcomes and variability of different dosing regimens, steady state PK/PD responses of 1000 patients were simulated for each dosing scenario. Additionally, to assess the range of potential outcomes for a given POC study, 100 replicates of 50 patients per dose (tentative sample size for the POC study) were conducted.

**RESULTS:** A two-compartment, first-order absorption and elimination, mixed-effect pharmacokinetics model adequately characterized the concentration-time profiles and pharmacokinetics (AUC,  $C_{max}$  and  $C_{trough}$ ) of drug X. A simple  $E_{max}$  model was adequate in relating the plasma concentration to RO. Exposures, peak and trough concentrations were simulated and evaluated based on margins relative to the maximum tolerated dose (MTD), percentage of population above target RO, and exploratory efficacy data. An optimal dosing scheme of 50 mg three times daily was identified, which is projected to yield approximately 90% of patients above target RO while providing reasonable margins relative to the MTD.

**CONCLUSION:** A population PK/PD model was developed for drug X. PK/PD modeling and simulations with PET receptor occupancy data provides a powerful approach to quantitatively make decisions for dose and dosing regimen selection. A dosing scheme of 50 mg three times daily was chosen for the POC study.

**PIII-60**

PHARMACOKINETIC MODELING OF A NOVEL TESTOSTERONE FORMULATION IN HYPOGANADAL SUBJECTS. P. Tremblay,<sup>1</sup> M. Tanguay,<sup>1</sup> C. Mattern<sup>2</sup>; <sup>1</sup>Anapharm, Québec, QC, Canada, <sup>2</sup>Mattern Pharmaceuticals AG, Stans, Switzerland

**BACKGROUND:** Nasobol<sup>®</sup> is a testosterone (T) nasal gel formulation that is intended to provide replacement therapy in hypogonadal (HG) males. The nasal mucosa offers an alternative route of administration with high permeability and ease of administration. The aim of this simulation was to determine the Nasobol<sup>®</sup> dosing regimens in moderately HG patients that would provide T exposure similar to what would be observed in a healthy population.

**METHODS:** Determine the model of endogenous T rhythm in HG and normal subjects. For HG men, a cosine function was fit to the data of 80 HG subjects using WinNonlin (v5.0). For healthy adults, the model parameters were taken from Diver (2003, 2006). Determine an appropriate PK model for Nasobol. The complete PK model was constructed from two parts: (1) the HG endogenous T model and (2) the Nasobol<sup>®</sup> PK model. The two parts were simultaneously fit to Phase I data using WNL. Simulations of dosing regimens in HG subjects. Dosing regimens were simulated to optimize the T time within normal range (TWNR) (300 to 1000 ng/dL). Model parameters of 100 healthy subjects and 100 HG subjects treated with Nasobol<sup>®</sup> were simulated with SAS (v8.02) and analyzed in WNL. Regimens of two (7.6 mg bid) or two and a half (7.6 mg, 3.8 mg, 7.6 mg) daily doses were simulated.

**RESULTS:** The selected PK model consisted in a HG T baseline model plus a one-compartment model for the Nasobol<sup>®</sup> component. The table below displays the simulation results.

Treatment	Summary stats	$C_{max}$ (ng/dL)	$C_{min}$ (ng/dL)	$C_{avg}$ (ng/dL)	AUC <sub>0-24h</sub> (h·ng/dL)	TWNR (%)
Healthy Subjects	Mean	547.93	294.88	421.39	10113.48	87.12
	SD	58.30	58.22	58.24	1397.87	14.49
Nasobol (bid = 7.6 mg & 7.6 mg)	Mean	637.60	246.83	384.77	9234.44	75.19
	SD	65.17	18.80	30.44	730.58	11.16
Nasobol (tid = 7.6 mg, 3.8 mg & 7.6 mg)	Mean	664.12	266.81	415.30	9967.30	84.40
	SD	71.33	15.44	39.39	945.33	11.39

**CONCLUSION:** The bid regimen should provide T in range close to the normal physiological values. If for some subjects the TWNR is too short, the tid regimen should be able to palliate this limitation. In addition, Nasobol<sup>®</sup> may reproduce a T pulsatile circadian pattern not observed with currently available formulations.

**PIII-61**

IDENTIFIABILITY (ID) AND FALSE RATE (FR) OF TWO ACTIVE COMPONENT (TAC) EXPOSURE-RESPONSE RELATIONS (ERR) IN MODELING TAC SYSTEMS (POTENT PARENT/METABOLITES OR COMBINED THERAPIES). A. J. Xiao, B. Birmingham; AstraZeneca LP, Wilmington, DE

**BACKGROUND:** When multiple components are potent (parent/metabolites or combined agents), it is important but difficult to quantitatively evaluate ERR to better assess therapeutic outcomes. This research was to investigate the ID and FR of using TAC ERR in clinical data analysis in practice.

**METHODS:** 108 scenarios were simulated using SAS 8.2 with a TAC ERR in the same mechanism of action:  $E = [E_{max1} * (Cp1/EC501)^{n1} + E_{max2} * (Cp2/EC502)^{n2}] / [1 + (Cp1/EC501)^{n1} + (Cp2/EC502)^{n2}]$  where E represents response rate;  $E_{maxi}$ ,  $EC50i$ ,  $n_i$  and  $Cp_i$  represent  $E_{max}$ ,  $EC50$ , Hill coefficient  $n$  and exposure  $Cp$  for the active component  $i$  ( $=1$  and  $2$ ). The simulated 108 scenarios were:  $E_{max1}/E_{max2}=0.2, 1$  and  $5$ ;  $EC501/EC502=0.1, 1$  and  $10$ ;  $n1/n2=0.3, 1$  and  $3$ ; and  $Cp2/Cp1=0.25, 0.75, 1.3$  and non-correlated, with typical variabilities. 1000 subjects were used in each simulation with 4 data points per subject. Altogether, 1080 NONMEM (v.6.0) programs were created to estimate parameters, with 10 repeats for each scenario with initial parameter guesses completely randomized around the expected values with a 20% variance. In addition, 1080 programs with one active component (OAC) were also simulated to assess the rate of false ID of the ERR for the other component.

**RESULTS:** All primary parameters for the TAC ERR were well estimated for a TAC system with good duplications for all scenarios (100%, i.e., no missing ID), although interindividual variability estimates could be less accurate if the exposures were highly correlated. TAC ERR failed to identify the inactive component for a OAC system in all cases (100%, i.e., no false ID). As a contrast, When a OAC ERR was used to fit a TAC system, the parameters were well-estimated only when one component was far more potent than the other.

**CONCLUSION:** TAC ERR can be well characterized for a TAC system, potentially with no false ID for representative informative data, whereas OAC ERR may mis-specify a TAC system.

**PIII-62**

ROLE OF CUT-OFF NUMBER (CON) FOR SUBPOPULATION (SP) IN EVALUATION OF DRUG-DRUG INTERACTION (DDI) IN POPULATION PHARMACOKINETIC (PK) ANALYSES. A. J. Xiao; AstraZeneca LP, Wilmington, DE

**BACKGROUND:** Different CONs have been reported in population PK analysis in the literature. This research was to investigate the role of setting CONs in evaluation of DDIs under different scenarios.

**METHODS:** A total of 57500 data sets were simulated using SAS 8.2 for a one-compartmental model (1CMT) with a DDI proportional to clearance (CL) in various SP sizes (2-9, 10, 20 and 30% of the total population (TP) of 100 or 600) under 25 different scenarios (at low, moderate or high CL, DDI and inter-individual variability (IIV) and their standard errors (SE)). 20% of TP were assumed to have dense PK sampling (13 data points following a single oral dose) and the remaining 80% having sparse PK sampling (3 data points in 3 different visits at steady state following a once daily oral dosing regimen: randomly around the peak, around the trough and around  $0.4 * T_{max}$ ). 100 replicate data sets were simulated for each scenario. Each data set was then modeled with a 1CMT via NONMEM V for identifying (ID) DDI in CL.

**RESULTS:** Except for the cases of large DDI with small SE and small IIV with large SE in TP=600, where increases in SP increased both accuracy and precision of the DDI estimate but slightly decreased the power of ID the DDI, SP size did not largely affect DDI evaluation. In comparison, the magnitude of DDI and IIV and their SEs had stronger effect on ID DDI: increases in the magnitude of DDI or IIV at high SE increased the power; increases in the magnitude of DDI or decreases in IIV and IIV SE increased accuracy of the DDI estimate; increases in IIV or decreases in IIV SE increased precision of the DDI estimate. CL/SE did not impact ID DDI.

**CONCLUSION:** While it may enhance a more robust estimate for a less significant DDI from those more commonly concomitantly used drugs, setting a CON might miss chances of ID potentially significant DDIs for those less commonly concomitantly used drugs.

### PIII-63

THE POTENTIAL IMPACT OF UNCERTAINTY IN ACCURACY OF PLASMA FREE FRACTION OF HIGHLY PROTEIN BOUND DRUG CANDIDATES ON PREDICTED VALUES OF MINIMUM EFFICACIOUS DOSE (MED) AND THERAPEUTIC INDEX (TI) IN MAN. R. X. Fang; Pfizer Inc, Chesterfield, MO

**BACKGROUND:** There are widely divided perspectives on the potential impact of plasma free fraction ( $f_u$ ) determination on development risk assessment and decision-making in drug research and development. Simulation was conducted to illustrate the effect associated with uncertainty in  $f_u$  accuracy.

**METHODS:** MED in man was predicted by observing a desired effect at trough of steady state effect-time curve simulated using direct  $E_{max}$  PD model with unbound  $EC_{50}$  (5 nM) translated from rat and one compartment PK model with clearance (hCL) value scaled from rat based on unbound allometric single species scaling (UASSS) and in vitro human liver microsomes (HLM scaling), respectively. Human volume of distribution was scaled from rat UASSS. The values of predicted TI (pTI) in man were calculated by dividing unbound AUC at no adverse effect level in man translated from dog by unbound AUC expected for pMED. The effect of variation of  $f_u$  in rat, dog, and human on predicted MED (pMED) and pTI were simulated by changing  $f_u$  between 0.01 and 0.001. Propagation of errors of  $f_u$  measurements to pMED and pTI was assessed by Monte Carlo simulation.

**RESULTS:** Change in human  $f_u$  had marginal effect on both pMED and pTI. Decreasing dog  $f_u$  had no effect on pMED but caused a proportional decrease in pTI. Decreasing rat  $f_u$  caused an exponential increase in pMED and a proportional increase in pTI when hCL is predicted based on UASSS. However, when hCL is predicted based on HLM scaling, the same change resulted in a decreases in pMED along with a greater increase in pTI. The above trends of  $f_u$  effect also apply to MED and TI predicted based on indirect  $E_{max}$  link model. A 10% (CV) error of  $f_u$  at mean=0.1 cross species resulted in 90% CI of 13-17 for pMED (initial value (iv)=15) and 12-19 for pTI (iv=15), when hCL was predicted by UASSS.

**CONCLUSION:** Under free drug hypothesis, development risks assessed by pMED and pTI could increase substantially when  $f_u$  values in animals are too low to measure for highly bound drug candidates.

### PIII-64

CHARACTERIZING ABATACEPT EXPOSURE IN JUVENILE AND ADULT ARTHRITIS PATIENTS USING A UNIFIED POPULATION EXPOSURE MODEL. Z. Zhou, L. K. Tay, M. Pfister, A. Roy; Bristol-Myers Squibb Company, Princeton, NJ

**BACKGROUND:** Abatacept, a recombinant soluble fusion protein that is approved in several countries including the US and EU for the treatment of adult rheumatoid arthritis (RA), is currently being developed for juvenile rheumatoid arthritis (JRA) and juvenile idiopathic arthritis (JIA). Abatacept acts by selectively inhibiting the CD80/CD86:CD28 co-stimulatory signal required for T cell activation,

thereby reducing autoimmune responses in RA and JIA/JRA. The objective of this analysis was to characterize the exposure of JRA/JIA patients to abatacept, by extending the existing abatacept population pharmacokinetic (P-PK) model for adult RA patients.

**METHODS:** A unified P-PK model for abatacept in adult RA and JRA/JIA patients was developed with 4235 samples from 574 patients (of which 2087 samples were from 186 JRA/JIA patients). The P-PK model was developed in NONMEM, and included an assessment of the following covariates: body weight (baseline and time-varying), age, gender, glomerular filtration rate, concomitant methotrexate use, etc. The model was evaluated by checking its predictive performance.

**RESULTS:** Abatacept concentration-time data in both RA and JRA/JIA patients were well described by a linear 2-compartment model, with baseline body weight as the only statistically significant and clinically relevant covariate. Based on the unified model, a 70 kg subject is expected to have a clearance (CL) of 0.521 L/day, a central volume (VC) of 3.07 L, an intercompartmental clearance (Q) of 0.509 L/day, and a peripheral volume of 4.09 L. Increases in abatacept CL, VC, and VP with body weight were described using a power covariate model, and were less than proportional to body weight.

**CONCLUSION:** The pharmacokinetics of abatacept in JRA/JIA patients was found to be similar to that in adult RA patients, after accounting for the effect of body weight. Abatacept clearance and distribution volumes (central and peripheral) were found to increase with baseline body weight.

### PIII-65

SIMULATION TO SUPPORT DEVELOPMENT OF ZIAGEN SCORED TABLET: SIMPLIFIED DOSING INITIATIVE FOR PEDIATRIC HIV INFECTION IN RESOURCE-POOR SETTINGS. I. H. Song,<sup>1</sup> G. Yuen,<sup>1</sup> W. Snowden,<sup>2</sup> S. Weller<sup>1</sup>; <sup>1</sup>GlaxoSmithKline, RTP, NC, <sup>2</sup>GlaxoSmithKline, Greenford, United Kingdom

**BACKGROUND:** Due to limitations of liquid formulations (e.g. storage requirements, transport issues, and dosage volumes), solid oral dosage forms is beneficial for pediatrics for improving access to antiretroviral treatment in resource-poor settings (e.g., Sub-Saharan Africa, South and Southeast Asia). As part of a broader initiative to help address these issues, a scored tablet of Ziagen (abacavir/ABC, 300mg) has been developed for use by children able to swallow solid dosage forms. Pharmacokinetic (PK) modeling and simulations were performed to support dosage recommendations for the scored tablet.

**METHODS:** Dosage considerations for the scored tablet were based on selection of body weight ranges for which half and full tablet regimens would provide daily ABC doses from -10% to +40% of that from the approved 8mg/kg twice-daily (BID) dose of the marketed solution formulation. Systemic ABC exposures from these regimens were determined by Monte Carlo simulations based on a published population PK model and re-analysis of historical data, and compared with historical PK data at approved doses in adults and children.

**RESULTS:** The proposed scored tablet doses are one half tablet BID for weight from 14-<21kg, one half tablet in the morning and one full tablet in the evening for weight from 21-<30kg, and one full tablet BID for weight  $\geq$ 30kg. The geometric means of simulated daily AUC and  $C_{max}$  values from the proposed scored tablet doses were within the expected range (16-24% and 7-31% greater, respectively) of historical pediatric control values. Simulated  $C_{max}$  values were lower than those from historical adult controls at the approved 600mg once daily regimen for which safety has been established.

**CONCLUSION:** A weight-range based, BID dosing regimen of scored ZIAGEN tablets is recommended for children  $\geq$ 14kg (not to exceed 300mg BID) in combination with other antiretroviral agents for the treatment of HIV.

PIII-66

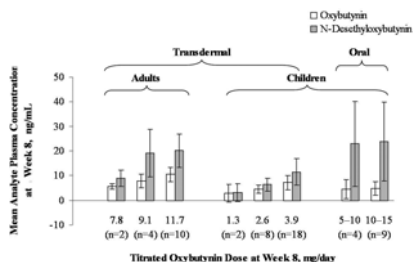
PHARMACOKINETICS AND SAFETY OF TRANSDERMAL OXYBUTYNYN FOR TREATMENT OF NEUROGENIC BLADDER IN ADULTS AND CHILDREN. L. A. Hill,<sup>1</sup> P. C. Cartwright,<sup>2</sup> M. J. Kennedy<sup>3</sup>; <sup>1</sup>Watson Laboratories, Inc., Salt Lake City, UT, <sup>2</sup>Department of Urology, University of Utah, Salt Lake City, UT, <sup>3</sup>Carolinas Medical Center, Charlotte, NC

**BACKGROUND:** Neurogenic overactive bladder (NGB) can be treated with orally administered oxybutynin (OXY-oral), but use is limited by anticholinergic adverse effects attributable to the metabolite *N*-desethyloxybutynin (DEO). Pharmacokinetics (PK) and tolerability of transdermally delivered oxybutynin (OXY-TDS) were examined in 2 multicenter, open-label, dose titration studies in adults and children with NGB.

**METHODS:** Study 1 (N = 24) included adults ≥18 years old with NGB resulting from spinal cord injury (SCI); treatment was OXY-TDS (3.9-11.7 mg/day). Children with NGB (N = 56) aged 6-15 years were recruited for study 2 and randomized 3:1 to treatment with OXY-TDS (1.3-3.9 mg/day) or OXY-oral (≤15 mg/day). In each study, the dose of OXY-TDS could be adjusted after 2 weeks, based on symptoms. In study 2, this dose was maintained to study end (week 14). In study 1, the dose could be titrated every 2 weeks until study end (week 8). Plasma concentrations of oxybutynin (OXY) and DEO were measured at week 8.

**RESULTS:** In study 1, most patients (mean age, 41.9 y) were male (88%) and African American (58%). In study 2, 51% of patients (mean age, 10.4 y) were male and 74% were Caucasian. Mean OXY plasma concentrations were higher at greater doses (Figure). The DEO/OXY ratio was lower with OXY-TDS than with OXY-oral (Figure). Anticholinergic adverse events were observed infrequently during treatment with OXY-TDS and OXY-oral and did not exhibit dose-dependence (Table).

**CONCLUSION:** Patients with NGB who were treated with OXY-TDS had low ratios of DEO to OXY in plasma and experienced a low incidence of anticholinergic adverse events.



Anticholinergic Adverse Events (none with 1.3 and 2.6 mg/day OXY-TDS and ≤5 mg/day OXY-oral)

Treatment	OXY-TDS	OXY-TDS	OXY-TDS	OXY-TDS	OXY-TDS	OXY-oral	OXY-oral
Study	1	1	1	1	2	2	2
Dose, mg/day (n)	3.9 (6)	7.8 (24)	9.1 (20)	11.7 (11)	3.9 (22)	5-10 (6)	10-15 (11)
Adverse Event, n (%)							
Constipation	...	1 (4.2)	...	...	1 (4.5)	1 (16.7)	...
Dizziness	...	...	...	...	...	1 (16.7)	1 (9.1)
Dry mouth	2 (33.3)	...	...	...	...	...	...
Photophobia	...	...	...	1 (9.1)	...	...	...
Somnolence	...	...	...	...	1 (4.5)	...	...
Vision abnormal	...	...	2 (10.0)	...	...	...	...

PIII-67

MECHANISM BASED PHARMACOKINETIC/PHARMACODYNAMIC MODEL OF PARATHYROID HORMONE-CALCIUM HOMEOSTASIS IN RATS AND HUMANS. A. K. Abraham,<sup>1</sup> D. E. Mager,<sup>1</sup> X. Gao,<sup>2</sup> T. Maurer<sup>3</sup>; <sup>1</sup>Department of Pharmaceutical Sciences, University at Buffalo, State University of New York, Amherst, NY, <sup>2</sup>Clinical Pharmacology, Pfizer Global Research and Development, Groton, CT, <sup>3</sup>Pharmacokinetics, Pharmacodynamics and Metabolism Department, Pfizer Global Research and Development, Groton, CT

**AIMS:** To develop a mechanism based pharmacokinetic/pharmacodynamic (PK/PD) model that describes ionized calcium dependent regulation of parathyroid hormone (PTH) in rats and humans.

**METHODS:** Literature data for ionized calcium (Ca<sup>2+</sup>) and PTH concentrations in rats (normal and aged males) and humans (males and females) were used for model development. Depletion of Ca<sup>2+</sup> was modeled using an indirect response (IDR) model and was driven by chelating agent PK. A precursor pool IDR model was used to fit PTH concentrations. Experimental data for subcutaneous (SC) PTH PK was modeled using a dual-absorption input into the PTH compartment of the precursor pool model. Simultaneous estimation of model parameters in rats and humans was conducted using a maximum likelihood algorithm (ADAPT II, BMSR, Los Angeles). To compare the relative response to decreases in Ca<sup>2+</sup>, simulations of similar chelating agent dosing regimens was conducted for both species.

**RESULTS:** Lowering of Ca<sup>2+</sup> provided a stimulus for greater PTH secretion with a steep dose response relationship. For both rats and humans, a similar structural model well described the data. Change in receptor occupancy of the calcium sensing receptor was used to stimulate PTH responses in the model. Extent of PTH stimulation was baseline dependent in rats [S<sub>max,Rats</sub> = 34.8 x PTH<sub>0</sub>] and humans [S<sub>max,humans</sub> = 392 / PTH<sub>0</sub>]. The equilibrium dissociation constant for Ca<sup>2+</sup> was fixed to an *in vitro* estimate (K<sub>D</sub> = 1.2 mmol/L). Analysis of model scalability in response to Ca<sup>2+</sup> perturbations showed that the extent of PTH stimulation achieved in normal male rats was roughly twice that of humans.

**CONCLUSION:** Ca<sup>2+</sup> and PTH concentrations in the body are tightly regulated through feedback mechanisms. A comprehensive mechanism based model has been developed and is capable of predicting PTH stimulation in response to altered ionized calcium in rats and humans. The model provides the basis for scalability of predictions from rats to humans.

PIII-68

MAGNESIUM AND ALUMINUM HYDROXIDES PLUS SIMETHICONE, FAMOTIDINE, OR OMEPRAZOLE DO NOT SIGNIFICANTLY AFFECT THE PHARMACOKINETICS OF SAXAGLIPTIN IN HEALTHY SUBJECTS. D. W. Boulton, D. Adams, L. Li, C. G. Patel, B. J. Komoroski, D. Whigan, E. U. Frevert, A. Goyal, D. M. Kornhauser; Bristol-Myers Squibb Co., Princeton, NJ

**BACKGROUND:** Saxagliptin is a potent DPP-4 inhibitor being developed for the treatment of type 2 diabetes. Magnesium and aluminum hydroxides plus simethicone (MAS), famotidine (FAM) and omeprazole (OMZ) are medicines that alter gastric pH which may be co-administered with saxagliptin. The aim of this study was to determine the effect of these medicines on the pharmacokinetics (PK) of saxagliptin and its active metabolite.

**METHODS:** This open-label, randomized, unbalanced, 3-way crossover study was conducted in 14 healthy subjects in a clinical pharmacology unit. On separate occasions subjects received single oral doses of 10 mg saxagliptin alone and co-administered with oral doses of 30 mL MAS, 40 mg FAM (dosed 3 h earlier), or 40 mg OMZ dosed to steady-state (5 QD doses). The study had 99% power to detect a PK interaction. Lack of a PK interaction was to be concluded if the 90% confidence interval (CI) for the population geometric mean C<sub>max</sub> and AUC ratios for saxagliptin given in combination to alone were within 0.80 and 1.25.

**RESULTS:** 15 subjects completed the study. When saxagliptin was co-administered with MAS or FAM compared to saxagliptin alone, the point estimates [90% CI] for saxagliptin C<sub>max</sub> showed an effect (0.74 [0.65, 0.84] and 1.14 [1.00, 1.30], respectively), however, the

90% CIs for AUC showed no effect (0.96 [0.93, 1.00] and 1.02 [0.98, 1.07], respectively). The effects on saxagliptin C<sub>max</sub> are not considered to be clinically meaningful. When saxagliptin was coadministered with OMZ, the point estimates [90% CI] for saxagliptin C<sub>max</sub> and AUC showed no effect (0.98 [0.86, 1.11] and 1.12 [1.08, 1.17], respectively). In all cases, the PK of saxagliptin's metabolite generally paralleled those of the parent.

**CONCLUSION:** Co-administration of MAS, FAM and OMZ did not meaningfully alter the PK of saxagliptin or its active metabolite. No separation of dosing or dosage adjustment is needed when saxagliptin is used with these medicines.

### PIII-69

NO PHARMACOKINETIC INTERACTION BETWEEN SAXAGLIPTIN AND DIGOXIN IN HEALTHY SUBJECTS. D. W. Boulton, L. Li, C. G. Patel, B. J. Komoroski, D. Whigan, E. U. Frevert, D. M. Kornhauser; Bristol-Myers Squibb Co., Princeton, NJ

**BACKGROUND:** Saxagliptin is a potent DPP-4 inhibitor being developed for the treatment of type 2 diabetes. Digoxin is a cardiac glycoside that is a probe of P-gp activity. The aims of this study were to determine the effect of saxagliptin on the pharmacokinetics (PK) of digoxin, and the effect of digoxin on the PK of saxagliptin.

**METHODS:** This open-label, randomized, 3-period, 3-treatment, crossover study was conducted in 14 healthy subjects in a clinical pharmacology unit. In Period 1 (P1), each subject received a single oral dose of 10 mg saxagliptin (Treatment (TRT) A). In P2, subjects received loading doses of oral digoxin on Days 1 & 2 and then on Days 3-7 0.25 mg digoxin daily either alone (TRT B) or in combination with 10 mg saxagliptin daily (TRT C). In P3, subjects received the treatment (B or C) they did not receive in P2. The study had ≥93% power to detect a PK interaction between saxagliptin and digoxin. Lack of a PK interaction was to be concluded if the 90% confidence interval (CI) for the population geometric mean C<sub>max</sub> and AUC ratios for each drug given in combination to each alone were within 0.80 and 1.25.

**RESULTS:** When digoxin was co-administered with saxagliptin compared with digoxin alone, the point estimate (90% CI) for digoxin C<sub>max</sub> and AUC were 1.09 (1.00, 1.19) and 1.06 (1.02, 1.11) respectively. The corresponding values for saxagliptin C<sub>max</sub> and AUC when saxagliptin was co-administered with digoxin compared with saxagliptin alone were 0.99 (0.87, 1.12) and 1.05 (0.99, 1.11), respectively. The results for both C<sub>max</sub> and AUC satisfied the pre-specified criteria for concluding absence of effect on the pharmacokinetics of both digoxin and saxagliptin.

**CONCLUSION:** Co-administration of digoxin, a P-gp substrate, did not alter the steady-state PK of saxagliptin and co-administration of saxagliptin did not alter the steady-state PK of digoxin. No dosage adjustment is needed for either saxagliptin or digoxin when they are co-administered.

### PIII-70

TARANABANT EXHIBITS NO CLINICALLY MEANINGFUL EFFECT ON THE PHARMACOKINETIC PROFILE OF DIGOXIN. A. E. Denker,<sup>1</sup> L. Vessey,<sup>2</sup> X. S. Li,<sup>3</sup> J. Yuan,<sup>4</sup> N. G. Agrawal,<sup>3</sup> S. Dunbar,<sup>4</sup> J. A. Wagner<sup>4</sup>; <sup>1</sup>Merck & Co., Inc., Boston, MA, <sup>2</sup>Merck & Co., Inc., Seattle, WA, <sup>3</sup>Merck & Co., Inc., West Point, PA, <sup>4</sup>Merck & Co., Inc., Rahway, NJ

**BACKGROUND:** Taranabant is a structurally distinct cannabinoid-1 receptor (CB-1R) inverse agonist being developed for treatment of obesity. Digoxin is a cardiac glycoside compound with a narrow therapeutic index indicated for treatment of heart failure and arrhythmias. This study evaluated the effect of multiple dose administration of taranabant on the single dose pharmacokinetic (PK) profile of digoxin.

**METHODS:** This was a 2-period, open label, fixed sequence study in which 12 healthy male or female subjects were administered a single oral dose of digoxin 0.5 mg in the first period, and 19 once-daily doses of taranabant 6 mg with a single dose of digoxin 0.5 mg concomitantly administered with the 15<sup>th</sup> dose of taranabant in the second. Blood samples were collected for digoxin plasma concentrations predose and at selected timepoints up to 120 hours following digoxin administration in each period. There was a 10 day washout between periods.

**RESULTS:** The geometric mean ratio (GMR) and corresponding 90% confidence interval (CI) for digoxin AUC<sub>0-∞</sub> and C<sub>max</sub> following administration of a single oral dose of 0.5 mg digoxin concomitant with multiple doses of 6 mg taranabant dosed to approximate steady-state, versus a single dose of 0.5 mg digoxin administered alone is 0.91 (0.83, 0.99) and 1.23 (1.09, 1.40), respectively. Multiple doses of 6 mg taranabant with a single oral dose of 0.5 mg digoxin were generally well tolerated.

**CONCLUSIONS:** Multiple doses of 6 mg taranabant does not have a clinically meaningful effect on the PK of a single oral dose of digoxin, as determined by the GMR and 90% CI for digoxin AUC<sub>0-∞</sub> being within the prespecified comparability interval of (0.8, 1.25).

### PIII-71

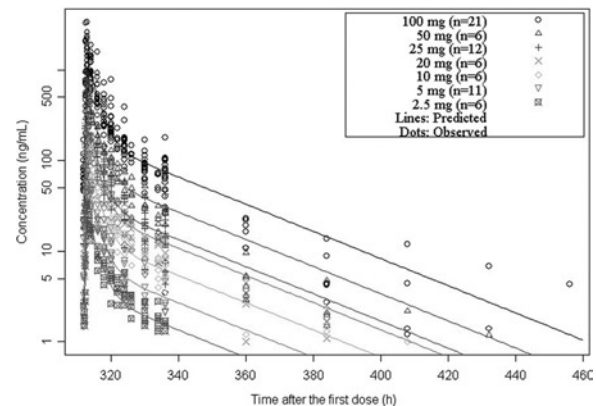
POPULATION PHARMACOKINETIC ANALYSIS OF DAPAGLIFLOZIN IN HEALTHY AND SUBJECTS WITH TYPE 2 DIABETES MELLITUS. Y. Feng, L. Zhang, B. Komoroski, M. Pfister; Bristol-Myers Squibb Co., Princeton, NJ

**BACKGROUND:** Dapagliflozin is a novel, selective inhibitor of the sodium-glucose co-transporter 2 (SGLT2). The analysis aims to characterize the exposure levels of dapagliflozin in healthy subjects and subjects with type 2 diabetes mellitus (T2DM).

**METHODS:** Two double-blind, placebo-controlled, randomized, multiple-dose studies evaluated dapagliflozin 2.5 mg to 100 mg once per day for 2 weeks in 30 healthy subjects and 38 subjects with T2DM. A total of 2039 dapagliflozin concentrations were available for analysis. Population pharmacokinetic (PK) analysis was performed by using NONMEM. Effects of subjects' age, gender, body weight, disease status, and creatinine clearance (CrCL) on PK parameters were tested. Covariate effects greater than 20% were considered to be clinically relevant.

**RESULTS:** The dapagliflozin PK data appeared to be adequately described by a two-compartment model with first order absorption and proportional residual error (Figure and Table). The clearance estimate is comparable between healthy (20.2 ± 2.3 L/h) and T2DM subjects (19.1 ± 6.0 L/h). None of tested covariates had more than 20% effect on dapagliflozin PK parameters.

**CONCLUSION:** Population PK results indicate that dapagliflozin PK is comparable in healthy and T2DM subjects. No clinically relevant covariates were identified in the analysis.



Parameters [Units]	Dapagliflozin Population PK Parameter Estimate	95% Confidence Interval
<b>Fixed Effects</b>		
CL [L/h]	19.5	17.7 - 21.3
V2 [L]	82.7	76.3 - 89.1
KA [1/h]	1.83	1.51 - 2.15
Q [L/h]	11.2	9.20 - 13.2
V3 [L]	194	152 - 236
<b>Random Effects: Inter-individual variability (IIV)</b>		
IIV_CL	0.0549	0.0286 - 0.0812
IIV_V2	0.0522	0.0275 - 0.0769
IIV_KA	0.175	0.0233 - 0.327

**PIII-72**

THE PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF AN ORALLY ACTIVE GONADOTROPIN-RELEASING HORMONE (GNRH) ANTAGONIST (NBI-56418) WITH ONCE DAILY AND SIPPING DOSE REGIMENS IN HEALTHY PREMENOPAUSAL WOMEN. J. Grundy,<sup>1</sup> A. Nicholls,<sup>1</sup> A. Copa,<sup>2</sup> S. Struthers,<sup>1</sup> R. Jimenez,<sup>1</sup> R. Luo,<sup>1</sup> M. Shapiro,<sup>1</sup> T. Kaileh,<sup>1</sup> H. Bozigian,<sup>1</sup> C. O'Brien<sup>1</sup>; <sup>1</sup>Neurocrine Biosciences, Inc., San Diego, CA, <sup>2</sup>PRACS Institute, Ltd., Fargo, ND

**BACKGROUND:** NBI-56418 is an orally active, non-peptide, GnRH antagonist currently being investigated for the management of pain associated with endometriosis. The clinical study objectives were to determine and compare the PK properties and PD effects, as well as safety and tolerability, of two different NBI-56418 oral dosing treatments (once daily and 'sipping' regimens) in healthy premenopausal women.

**METHODS:** A total of 14 subjects (age 18-40 years) were equally divided between two treatment groups: NBI-56418 200 mg (oral solution) given once daily, or NBI-56418 200 mg (oral solution) given as a daily sipping regimen (divided into 9 doses at two-hourly intervals). Study drug was administered starting in the morning for 3 consecutive days (study days 1-3), beginning at any time from the second to seventh day (inclusive) after the onset of menses. Serial blood samples were collected on study days 3-4 for assessing plasma PK (NBI-56418) and on study days 1-6 for assessing serum PD [lutening hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2)].

**RESULTS:** NBI-56418 was well tolerated for both dose regimens. Selected calculated mean (SD) PK and PD parameters are shown in the following table.

PK or PD Parameters (Day 3)	200 mg QD (N = 7)	200 mg sipping (N = 7)
AUC <sub>0-24</sub> (ng·h/mL)	1740 (738)	739 (216)
C <sub>max</sub> (ng/mL)	787 (388)	46.8 (13.8)
C <sub>12h</sub> (ng/mL)	7.0 (3.4)	23.3 (4.7)
C <sub>24h</sub> (ng/mL)	1.7 (1.1)	11.0 (4.4)
T <sub>max</sub> (h)	0.50 (0.50-1.07) <sup>a</sup>	9.9 (0.5-17.0) <sup>a</sup>
t <sub>1/2</sub> (h)	3.7 (2.0)	3.7 (0.7)
LH C <sub>avg</sub> (mIU/mL) <sup>b</sup>	4.0 (0.59-10.1) <sup>a</sup>	0.76 (0.52-3.2) <sup>a</sup>
FSH C <sub>avg</sub> (mIU/mL) <sup>b</sup>	6.2 (1.7-8.9) <sup>a</sup>	4.1 (2.3-5.4) <sup>a</sup>
E2 C <sub>avg</sub> (pg/mL) <sup>b</sup>	19.8 (14.7-110) <sup>a</sup>	6.8 (5.1-81.5) <sup>a</sup>

<sup>a</sup>Median (range); <sup>b</sup>C<sub>avg</sub>=AUC<sub>0-24</sub>/24.

Despite lower mean peak and total NBI-56418 plasma exposure, the distributed doses of the sipping regimen produced lower median LH, FSH and E2 serum exposure levels.

**CONCLUSION:** Both NBI-56418 regimens suppressed LH, FSH, and E2 serum levels. Greater suppression of the evaluated hormones by the sipping regimen is likely related to longer maintenance of NBI-56418 plasma concentrations above a threshold or minimally effective plasma concentration throughout each dosing day.

**PIII-73**

PHARMACOKINETICS AND PHARMACODYNAMICS OF VILDAGLIPTIN IN HEALTHY CHINESE VOLUNTEERS. Y. L. He,<sup>1</sup> Q. Yin,<sup>2</sup> F. Deckert,<sup>3</sup> M. Ligueros-Saylan,<sup>4</sup> M. Wang,<sup>4</sup> J. Jiang,<sup>5</sup> D. Liu,<sup>5</sup> L. Kjems,<sup>1</sup> W. P. Dole,<sup>1</sup> P. Hu<sup>5</sup>; <sup>1</sup>Novartis, Cambridge, MA, <sup>2</sup>Novartis, Beijing, China, <sup>3</sup>Novartis, Basel, Switzerland, <sup>4</sup>Novartis, East Hanover, NJ, <sup>5</sup>Peking Union Medical College Hospital, Beijing, China

**BACKGROUND:** Vildagliptin is an orally active, potent and selective inhibitor of dipeptidyl peptidase IV (DPP-4) that improves glycaemic control in patients with type 2 diabetes.

**METHODS:** This was a randomized, double-blind, parallel and placebo-controlled study in a total of 60 healthy Chinese subjects. Single- and multiple-dose PK, PD, safety and tolerability of vildagliptin were assessed following oral dose of vildagliptin at 25, 50, 100 or 200 mg qd, or 50 mg bid.

**RESULTS:** Vildagliptin was rapidly absorbed (t<sub>max</sub> 1.5-2.0 hours) at all doses and eliminated with a t<sub>1/2</sub> of approximately 2 hr. Consistent with the short elimination t<sub>1/2</sub>, no accumulation of vildagliptin was observed at steady-state (accumulation factors were 1.00-1.05 over 25-200 mg). Exposure to vildagliptin (AUC and C<sub>max</sub>) increased in an approximately dose-proportional fashion (β: 1.00-1.16). Vildagliptin resulted in rapid and almost complete (>95%) inhibition of DPP-4 activity for at least 4 h after all doses, and the duration of DPP-4 inhibition was dose-dependent. The IC<sub>50</sub> for DPP-4 inhibition estimated in Chinese subjects was 1.38±0.082 ng/mL, which is very similar to that observed in non-Chinese subjects. DPP-4 inhibition was accompanied by increases in post prandial plasma active GLP-1 levels of up to 2-3-fold compared with placebo. Glucose and insulin levels were not affected by vildagliptin in healthy Chinese subjects, consistent with its glucose-dependent mode of action. Vildagliptin was generally safe and well tolerated at doses up to 200 mg.

**CONCLUSION:** Vildagliptin 25-200 mg qd exhibits approximate dose-proportional PK with no evidence of accumulation at steady-state in healthy Chinese subjects. Vildagliptin is a highly potent DPP-4 inhibitor as reflected by the low IC<sub>50</sub>. The onset of DPP-4 inhibition was rapid and the duration of DPP-4 inhibition was dose-dependent. Vildagliptin exhibits similar PK/PD profiles in Chinese subjects as compared to non-Chinese subjects.

**PIII-74**

USE OF MODELING & SIMULATION (M&S) IN THE DESIGN OF A DRUG INTERACTION STUDY OF ALVIMOPAN (ALV). B. Johnson, V. D. Schmith, M. A. Young; Glaxo Smith Kline, RTP, NC

**BACKGROUND:** ALV is an investigational, peripherally acting mu-opioid receptor antagonist without anti-analgesic effects in development for management of post-operative ileus (POI) & opioid-induced bowel dysfunction (OBD). The pharmacokinetics (PK) of its active metabolite (MET), which is formed by gut microflora, vary extensively (CV>100%) between & within subjects. The purpose of the present M&S was to determine the feasibility/optimal design for a drug interaction study evaluating ALV & MET PK before, during & after administration of a short course of antibiotics (ATB).

**METHODS:** M&S was conducted using a population PK model for MET (1) with various sample sizes (n=20-100) and assumed various magnitudes of an interaction (20%, 50%, & 99%) to assess whether an effect of ATB on MET PK could be detected. The ATB was assumed to affect rate and extent of MET formation. The mean time to maximum ATB effect and time to recovery were based on minimal data from the literature. Each scenario was replicated 100 times. Additional M&S evaluated (a) whether early discontinuation of subjects with low MET concentrations would increase precision and (b) to select the most appropriate dose of ALV.

**RESULTS:** M&S showed that despite large variation in MET PK, an antibiotic study appears feasible with a reasonable number of subjects (n=40). This study would likely detect a 50% reduction (but not a 20% reduction) in MET exposure. Prescreening subjects for MET concentrations does not improve probability of success or efficiency of the study. A dose of 6 mg was chosen to optimize MET producers. The study was conducted successfully and results are presented elsewhere (2).

**CONCLUSION:** M&S determined feasibility, improved efficiency, and optimized the design of this drug interaction study.

<sup>1</sup> Foss, Fisher, Schmith *Clin Pharmacol Ther* 2007 In press.

<sup>2</sup> Vasist et al., ASCPT Annual Meeting 2008

### PIII-75

EFFECT OF HEPATIC IMPAIRMENT ON THE PHARMACOKINETICS OF TAK-390MR (MODIFIED RELEASE). R. D. Lee, J. Wu, M. Vakily, D. Mulford; TAP Pharmaceutical Products Inc, Lake Forest, IL

**BACKGROUND:** TAK-390MR is a proton pump inhibitor under development that employs a modified release formulation of TAK-390. TAK-390 is extensively metabolized by the liver. This study evaluates the effect of hepatic impairment on pharmacokinetics (PK) of TAK-390 and its metabolites.

**METHOD:** 12 subjects with moderate hepatic impairment (Child-Pugh B) and 12 healthy subjects, matched by gender, weight, age and smoking status, were administered a single 60 mg oral dose of TAK-390MR. Blood samples were obtained at pre-dose and up to 24 hours post-dose. Plasma concentrations of TAK-390 and its 5-hydroxy and sulfone metabolites were determined using a validated LC-MS/MS assay. PK parameters were estimated using standard noncompartmental methods. Plasma protein binding of TAK-390 was determined by ultrafiltration. The difference in select PK parameters for TAK-390 and its metabolites between the 2 hepatic function groups was assessed by ANOVA.

#### RESULTS:

#### Mean PK Parameters ( $\pm$ SD)

PK Parameters	Normal Hepatic Function	Moderate Hepatic Impairment
<b>TAK-390</b>		
$C_{max}$ (ng/mL)	912 $\pm$ 496	1315 $\pm$ 665
Unbound $C_{max}$ (ng/mL)	17.9 $\pm$ 9.09	27.3 $\pm$ 12.2*
$AUC_{\infty}$ (ng-h/mL)	7563 $\pm$ 9794	16306 $\pm$ 9209**
Unbound $AUC_{\infty}$ (ng-h/mL)	150 $\pm$ 193	351 $\pm$ 206**
$f_u$ (%)	1.99 $\pm$ 0.155	2.13 $\pm$ 0.396
<b>5-Hydroxy TAK-390</b>		
$C_{max}$ (ng/mL)	36.3 $\pm$ 20.1	10.1 $\pm$ 9.01**
$AUC_t$ (ng-h/mL)	190 $\pm$ 109	77.3 $\pm$ 45.0
$AUC_t$ Ratio	0.0478 $\pm$ 0.0245	0.00880 $\pm$ 0.00936**
<b>TAK-390 Sulfone</b>		
$C_{max}$ (ng/mL)	11.4 $\pm$ 13.5	54.6 $\pm$ 64.6***
$AUC_t$ (ng-h/mL)	96.2 $\pm$ 232	841 $\pm$ 964***
$AUC_t$ Ratio	0.00669 $\pm$ 0.00922	0.0529 $\pm$ 0.0430***

\*, \*\*, \*\*\*:  $p < 0.05$ , 0.01 and 0.001, respectively, compared to Normal Hepatic Function

Mean TAK-390  $C_{max}$  and  $C_{max,u}$  values were about 1.5-times higher, and mean TAK-390  $AUC_{\infty}$  and  $AUC_{\infty,u}$  values were approximately 2-times greater in subjects with moderate hepatic impairment compared to those with normal hepatic function. Mean percent unbound values for TAK-390 in plasma between the 2 liver function groups were similar. The exposure of these inactive metabolites in plasma compared to TAK-390 was minimal ( $\leq 5\%$ ).

**CONCLUSION:** The differences in PK parameters between subjects with moderate hepatic impairment and healthy subjects administered TAK-390MR 60 mg were not considered clinically significant. No dosage adjustment for TAK-390MR doses up to 60 mg is likely to be necessary for subjects with mild or moderate hepatic impairment.

### PIII-76

PHARMACOKINETICS AND SAFETY OF CKD-501, A NOVEL PPAR ALPHA/GAMMA DUAL AGONIST, AFTER ORAL ADMINISTRATION IN HEALTHY FEMALE SUBJECTS. H. E. Lee,<sup>1</sup> J. W. Kim,<sup>1</sup> J. R. Kim,<sup>1</sup> K. S. Lim,<sup>1</sup> B. H. Kim,<sup>1</sup> S. K. Ahn,<sup>2</sup> C. Kim,<sup>3</sup> K. S. Yu,<sup>1</sup> S. G. Shin,<sup>1</sup> I. J. Jang<sup>1</sup>; <sup>1</sup>Seoul National University Hospital, Seoul, Republic of Korea, <sup>2</sup>Chong Kun Dang Research Institute, Seoul, Republic of Korea, <sup>3</sup>Chong Kun Dang Clinical Research Team, Seoul, Republic of Korea

**BACKGROUND:** CKD-501 is a peroxisome proliferator-activated receptor (PPAR) alpha/gamma dual agonist and designed to treat both type II diabetes mellitus and dyslipidemia. We studied the pharmacokinetics of CKD-501 after oral administration in healthy female subjects, and compared the results with those in the previous clinical trial with healthy male subjects.

**METHODS:** A randomized, placebo-controlled, dose-rising, parallel group study was conducted at the Clinical Trials Center. Total 22 female subjects were administered with 2 or 4 mg or placebo of the study drug. Serial blood samples were collected after oral administration for 60 h. Plasma concentrations of CKD-501 were analyzed by liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters were determined by noncompartmental methods.

**RESULTS:** Maximum plasma concentration ( $C_{max}$ ) in the 2 and 4 mg dose groups were 214.8 $\pm$ 56.4 ug/L and 310.0 $\pm$ 47.8 ug/L, respectively. Area under curve (AUC) for the 2 and 4 mg groups were 2251.3 $\pm$ 721.2 ug $\cdot$ h/L and 6942.6 $\pm$ 1778.9 ug $\cdot$ h/L each. Statistical analyses of  $C_{max}$  and AUC by dose indicated that relationships were not dose-proportional.

The female to male ratios of  $C_{max}$  and AUC in the 2 mg group were 1.23 (95% confidence interval, 0.89-1.69) and 1.11 (0.73-1.68) respectively. These values in the 4 mg were 1.28 (1.01-1.63) and 2.36 (1.60-3.47) each. There was no sex difference in the number of subjects experiencing adverse events in the 4 mg group.

**CONCLUSION:** Linear pharmacokinetic profiles of CKD-501 were not shown in females. Pharmacokinetic differences between males and females were observed in the 4 mg dose group. However, safety characteristics in women did not differ from those in men.

### PIII-77

PHARMACOKINETIC AND PHARMACODYNAMIC PROFILES OF LC15-0444, A NOVEL DPP IV INHIBITOR, AFTER MULTIPLE ORAL ADMINISTRATION IN HEALTHY SUBJECTS. K. H. Shin,<sup>1</sup> K. S. Lim,<sup>1</sup> K. P. Kim,<sup>1</sup> J. Y. Cho,<sup>1</sup> K. S. Yu,<sup>1</sup> S. G. Shin,<sup>1</sup> H. J. Yim,<sup>2</sup> O. H. Kwon,<sup>2</sup> J. Lee,<sup>2</sup> D. K. Kim,<sup>3</sup> I. J. Jang<sup>1</sup>; <sup>1</sup>Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea, <sup>2</sup>Research & Development Park, LG Life Sciences, Ltd., Daejeon, Republic of Korea, <sup>3</sup>Clinical Development, LG Life Sciences, Ltd, Seoul, Republic of Korea

**BACKGROUND:** LC15-0444 is a selective and competitive inhibitor of dipeptidyl peptidase IV (DPP IV) for the treatment of diabetes. The purpose of this study was to investigate the pharmacokinetic and pharmacodynamic profiles after multiple ascending doses of LC15-0444 in healthy male subjects.

**METHODS:** A dose block-randomized, double-blind, placebo-controlled, dose-escalation, parallel group study was performed in 3 groups with 10 subjects (8 for active; 2 for placebo) per group; each group received 200 mg, 400 mg, or 600 mg of the study drug once daily for 10 days, respectively. Blood and urine samples were collected up to 24 hours after the first dosing and up to 72 hours after the last dosing.

**RESULTS:** LC15-0444 concentration-time profiles showed characteristics of two-compartment disposition. No dose- or time-dependent change in elimination half-life was observed. Mean terminal elimination half-life ranged 16.6 to 20.1 h by dose groups. Mean apparent volume of distribution and mean total oral clearance ranged 1079 L  $\sim$  1319 L and 43.0 L/h  $\sim$  45.8 L/h. Mean renal clearance and fraction of unchanged drug excreted in urine showed values of 18.0 L/h  $\sim$  20.1 L/h and 0.45  $\sim$  0.54. At steady state, mean accumulation indices by

dose groups were between 1.22 and 1.31. There were no statistically significant differences in log-transformed and dose-normalized  $C_{max,ss}$  and  $AUC_{\tau,ss}$  among the three dose groups, and the 95% confidence interval for slope of linear regression included 1.0 for both parameters. More than 80% inhibition of DPP IV activity from baseline was sustained for over 24 hours in all dose groups.

**CONCLUSION:** Multiple oral doses of LC15-0444 were safe and well tolerated up to 600 mg. Linear pharmacokinetic characteristics were observed for the entire range of dose groups studied. Inhibition of plasma DPP IV activity by LC15-0444 persisted for more than 24 hours for all dose groups, supporting a once-daily dosing regimen.

### PIII-78

EFFECT OF AGE AND GENDER ON THE PHARMACOKINETICS OF A SINGLE ORAL DOSE OF TAK-390MR (MODIFIED RELEASE). M. Vakily, W. Zhang, J. Wu, D. Mulford; TAP Pharmaceutical Products Inc, Lake Forest, IL

**BACKGROUND:** TAK-390MR is a proton pump inhibitor that employs a modified release technology designed to provide prolonged plasma concentration of TAK-390 following once daily oral dosing. Because age and gender may affect the pharmacokinetics (PK) of drugs, this study assessed PK of TAK-390MR in healthy male and female subjects aged 18 - 40 years or 65 - 80 years.

**METHODS:** Phase 1, open-label, parallel-group study. Males and females aged 18-40 years or 65-80 years in general good health were enrolled. Subjects were administered a single oral dose of TAK-390MR 60 mg. Blood samples were drawn at predetermined pre- and post-dose time points and plasma concentrations of TAK-390 and its metabolites were determined. PK parameters were estimated using noncompartmental methods. A 2-way ANOVA model was used to investigate the effects of age and gender on the PK parameters.

**RESULTS:**

#### Mean PK Parameters (CV%)

Group (N=24)	$t_{max}$ (h)	$C_{max}$ (ng/mL)	$AUC_{\infty}$ (h·ng/mL)	$t_{1/2z}$ (h)
Male (n=12)	5.6 (48)	1306 (38)	7483 (44)	1.72 (64)
Female (n=12)	5.3 (41)	1703 (46)	10685 (71)	1.87 (54)
Young (18-40 y) (n=12)	4.5 (42)	1472 (48)	7749 (52)	1.50 (45)
Elderly (65-80 y) (n=12)	6.4* (40)	1538 (43)	10419 (70)	2.23† (54)

\* Statistically significantly different ( $p \leq 0.05$ ) from young subjects † Statistically significantly different ( $p \leq 0.05$ ) from young subjects based on  $\lambda_z$  Harmonic Mean

There was no statistical difference of TAK-390 systemic exposure between males and females, and between young and elderly. The observed differences in  $C_{max}$  and  $AUC_{\infty}$  were primarily due to 1 female elderly subject who had very high  $C_{max}$  and  $AUC_{\infty}$  (2740 ng/mL and 30602 h·ng/mL). Plasma protein binding of TAK-390 in male, female, young, and elderly subjects was similar (approximately 97%). Overall, the circulating metabolites concentrations were lower than those observed for TAK-390.

**CONCLUSIONS:** In this study, age and gender did not have a clinically important effect on the exposure of TAK-390 following a single oral dose of TAK-390MR 60-mg; therefore no adjustment in dose of TAK-390MR is required in the different age and gender groups.

### PIII-79

PHARMACOKINETICS OF TAK-390MR (MODIFIED-RELEASE) 30, 60, AND 90 MG IN SUBJECTS WITH SYMPTOMATIC, NON-EROSIVE GASTROESOPHAGEAL REFLUX DISEASE (GERD). W. Zhang, J. Wu, M. Vakily; TAP Pharmaceutical Products Inc, Lake Forest, IL

**BACKGROUND:** TAK-390MR is a proton pump inhibitor that employs a modified release technology designed to provide prolonged plasma concentration of TAK-390 following once daily oral dosing. This study evaluated pharmacokinetics (PK) of multiple oral doses (30, 60, and 90 mg) of TAK-390MR in subjects with symptomatic non-erosive GERD.

**METHODS:** Phase 1, single-center, randomized, open-label, parallel-group study. Male and female subjects aged  $\geq 18$  years with non-erosive GERD were enrolled and randomized in 1:1:1 ratio to 3 treatment groups (TAK-390MR 30, 60, or 90 mg). Subjects received their assigned treatment once daily (QD) orally for 8 consecutive days (at home on days 1 to 4 and during confinement on days 5 to 8). Antacid (Gelusil) tablets were used as needed as a rescue medicine. Blood samples were drawn on day 5 and day 8 at predetermined time points and plasma concentrations of TAK-390 were determined by a validated LC-MS/MS method. PK parameters were estimated using standard noncompartmental methods. Intra- and inter-subject variability was estimated using a mixed model.

**RESULTS:** 36 subjects were enrolled, 2 subjects were prematurely discontinued; 34 subjects were included in PK analyses.

#### Mean PK Parameters (CV%) for TAK-390MR Doses

TAK-390MR Dose (mg)	N	$t_{max}$ (h)	$C_{max}$ (ng/mL)	$AUC_{24}$ (ng·h/mL)
<b>Day 5</b>				
30	10	3.7 (63)	796 (70)	4732 (108)
60	12	5.1 (41)	1466 (42)	11428 (93)
90	12	3.3 (68)	1971 (62)	12799 (96)
<b>Day 8</b>				
30	10	4.0 (57)	662 (68)	4913 (113)
60	12	4.6 (43)	1550 (41)	12097 (97)
90	12	3.2 (55)	2233 (64)	13687 (100)

The plasma TAK-390 concentration-time profiles displayed MR characteristics. Higher  $C_{max}$  and AUC values were observed as doses increased. The estimates of intra-subject variability for dose-normalized  $C_{max}$  and AUC were 31% and 22%, respectively and the intersubject variability estimates for dose-normalized  $C_{max}$  and AUC were 49% and 105%, respectively.

**CONCLUSIONS:** The plasma TAK-390 concentration vs. time profiles displayed MR characteristics in GERD patients, similar to those observed in healthy subjects in previous studies.

### PIII-80

EFFECTS OF CIPROFLOXACIN ON THE PHARMACOKINETICS (PK) OF ALVIMOPAN (ALV) & ITS ACTIVE METABOLITE (MET). L. Vasist,<sup>1</sup> V. D. Schmith,<sup>1</sup> D. Veareer,<sup>2</sup> D. Kelleher,<sup>1</sup> M. A. Young,<sup>1</sup> V. Ameen,<sup>1</sup> G. Dukes<sup>1</sup>; <sup>1</sup>Glaxo Smith Kline, RTP, NC, <sup>2</sup>Glaxo Smith Kline, Harlow, United Kingdom

**BACKGROUND:** ALV is an investigational, peripherally acting mu-opioid receptor antagonist without anti-analgesic effects in development for management of post-operative ileus (POI) & opioid-induced bowel dysfunction (OBD). MET, formed by gut microflora, was substantially reduced or eliminated with preoperative oral antibiotic administration in POI patients (1). The present study was designed to characterize the PK of ALV & MET before, during & after

concomitant administration of a short course of antibiotics, more typical of potential administration in OBD patients.

**METHODS:** This study was an open-label, sequential, drug interaction study in 45 healthy male & female subjects. Each subject received ALV 6 mg BID for 30 days, with concomitant administration of ciprofloxacin (CIP) 500 mg BID on days 11-20. Serial blood samples were collected throughout treatment. Stool consistency and frequency, and safety were assessed.

**RESULTS:** The average concentration of MET decreased by 99% during ALV + CIP compared to ALV alone. This decrease occurred within 4 days with recovery beginning by 10 days post-ALV+CIP. CIP admin decreased ALV C<sub>max</sub> by 24%, with little or no effect on trough concentrations & AUC. ALV was well tolerated, with no stool pattern changes.

**CONCLUSION:** This study confirms that MET is formed by gut microflora and recovery occurs with re-colonization. Individual differences in gut microflora may account for inter-subject variability in MET PK; thus, clinical relevance of co-administration of CIP on MET PK may vary between OBD patients. Decreased ALV C<sub>max</sub> following CIP is either a function of intra-subject variability or a small interaction with no clinical relevance.

<sup>1</sup> Foss, Fisher, Schmith *Clin Pharmacol Ther* 2007, In press.

### PIII-81

ABT-335 DOES NOT HAVE A CLINICALLY SIGNIFICANT PHARMACOKINETIC INTERACTION WITH ROSUVASTATIN IN HUMANS. T. Zhu, M. T. Kelly, B. S. Hosmane, T. O. Chira, D. J. Sleep, K. X. Wan, R. S. Pradhan; Abbott Laboratories, Abbott Park, IL

**BACKGROUND:** ABT-335, a novel fibrate, is under clinical development for the management of dyslipidemia. ABT-335 and rosuvastatin (R) have different mechanisms of actions and exert complementary PD effects on lipids. Phase 3 development of the combination of ABT-335 and R is ongoing. A Phase 1 study assessed the PK interaction between the two drugs.

**METHODS:** The multiple-dose, open-label, 3-period, randomized, crossover study was conducted at a single clinical research center. Eighteen healthy adults (16M/2F, 13 white, 5 black) of mean (range) age 40 (21-55) years, mean weight 83 (67-101) kg and mean height 178 (164-190) cm received 40 mg R monotherapy, 135 mg ABT-335 monotherapy, and the two drugs in combination QD for 10 days in a randomized fashion. Blood samples were collected prior to dosing on Days 1, 5, 7, 8, 9, and 10, and up to 120 hours after Day 10 dosing for determination of fenofibric acid (FA) and R concentrations in plasma. PK of FA and R for the combination regimen were compared to those for each monotherapy regimen. Safety and tolerability were also assessed.

**RESULTS:** FA and R levels reached steady state on Day 8. Co-administering 40 mg R had no significant effect on the steady-state C<sub>max</sub>, C<sub>min</sub> or AUC<sub>24</sub> of FA (p>0.05). Co-administering ABT-335 had no significant effect on the steady-state C<sub>min</sub> or AUC<sub>24</sub> of R (p>0.05) but increased C<sub>max</sub> by 20% (90%CI, 12-28%). All 3 regimens were generally well tolerated with no clinically significant changes in clinical laboratory values, vital signs, or ECGs during the study. All adverse events were mild. In a previous study when fenofibrate, the ester form of FA, and R were co-administered at lower doses, a 20% increase in C<sub>max</sub> was also observed and concluded not clinically significant by the FDA.

**CONCLUSION:** This study used the highest dose approved for R and the full clinical dose of ABT-335, and demonstrated no significant PK interaction between the two drugs.

### PIII-82

DETERMINATION OF PLASMA LEVELS OF ROSUVASTATIN IN PATIENTS PRESENTING WITH DRUG-RELATED MYOTOXICITY. J. Turgeon,<sup>1</sup> S. Poirier Larabie,<sup>1</sup> A. Furtos,<sup>2</sup> N. Laplante,<sup>3</sup> D. Gaudet,<sup>4</sup> M. Santur ,<sup>4</sup> M. Phillips,<sup>3</sup> J. C. Tardif<sup>3</sup>; <sup>1</sup>Research Center, Centre Hospitalier de l'Universit  de Montr al, Montreal, QC, Canada, <sup>2</sup>Department of Chemistry, Universit  de Montr al, Montreal, QC, Canada, <sup>3</sup>Research Center, Montreal Heart Institute, Montreal, QC, Canada, <sup>4</sup>Research Center, Centre Hospitalier de la Sagamie, Chicoutimi, QC, Canada

**BACKGROUND:** HMG CoA reductase inhibitors (statins) are among the most potent drugs used clinically to reduce serum cholesterol levels. Unfortunately, this class of drugs may be associated with serious muscle toxicity. The aim of our study was to develop a sensitive assay to monitor plasma and tissue levels of rosuvastatin in patients presenting with drug-induced muscle toxicity.

**METHODS:** Plasma samples from 104 patients presenting with documented muscle toxicity during treatment with rosuvastatin were analysed by LC-MS-MS (Quantum Ultra AM). Samples were extracted at an acidic pH by liquid-liquid extraction (70% diethylether / 30% dichloromethane) following the addition of deuterated rosuvastatin as the internal standard. Organic extracts were evaporated to dryness and reconstituted in the mobile phase consisting of 60% acetonitrile / 40% ammonium formate 5 mM pH 3. Separation was achieved on a Phenomenex Luna Phenyl-hexyl column and ions m/z 482.2 and 258.088 were monitored.

**RESULTS:** The assay developed showed linearity between 0.3 ng/mL to 38.4 ng/mL with an r<sup>2</sup>=0.996. The detection limit was 0.1 ng/mL with a limit of quantification of 0.3 ng/mL. Samples obtained in patients varied within this range of concentrations; dilution of samples was needed only on rare occasions. Population pharmacokinetic analyses are performed to correlate levels of rosuvastatin observed to that predicted at the time of manifested toxicity. This information is combined with results from a panel of genetic markers (1536 genetic variants) associated with determinants of drug disposition (CYP450s, ABC and SCL transporters, mostly).

**CONCLUSION:** We have developed a reproducible and sensitive assay for the determination of rosuvastatin in plasma and biological samples (muscle biopsies). This methodology shall help us better understand mechanism of drug toxicity by measuring levels of drugs in the blood as well as in the effector compartment at the site of toxicity.

### PIII-83

DRUG INTERACTIONS BETWEEN THE IMMUNOSUPPRESSANT SIROLIMUS AND THE CHOLESTEROL-LOWERING AGENT EZETIMIBE IN HEALTHY SUBJECTS. S. Oswald, L. Borgwardt, T. Giessmann, C. Modess, W. Siegmund; University of Greifswald, Department of Clinical Pharmacology, Greifswald, Germany

**BACKGROUND:** Hypercholesterolemia is a frequent finding in organ transplant recipients receiving immunosuppressant drugs such as sirolimus (SIR). To prevent increased cardiovascular morbidity and mortality, co-medication with lipid-lowering statins is recommended as a first-line therapy. However, mono-therapy with statins is limited in many patients by insufficient cholesterol-lowering efficacy, serious adverse drug reactions and drug interactions. These patients may profit from combinations with the cholesterol absorption inhibitor ezetimibe (EZE). However, drug interactions with SIR may occur because both drugs are substrates of the efflux transporter P-glycoprotein (P-gp). Therefore, our clinical study in healthy subjects was initiated to evaluate whether interactions between SIR and EZE may be clinically relevant.

**METHODS:** Disposition of SIR (5 mg, po) and EZE (10 mg, po) alone and in combination was studied in a randomized, three-period, cross-over study in 24 healthy subjects (6 females, 18 males, age 21-34, body mass index 19.7-29.1) with at least 14 days wash-out. Whole

blood and serum samples were collected for 144 h and urine and feces for 5 and 10 days, respectively. EZE, its glucuronide and SIR were quantified using validated LC-MS/MS methods (LOQ: SIR 1 ng/ml, EZE 0.05 ng/ml).

**RESULTS:** EZE co-medication neither affected the maximum serum concentrations (14.6±4.23 ng/ml vs. 16.1±5.29 ng/ml,  $p=0.181$ ) nor the AUC values of SIR (174±111 ng\*h/ml vs. 182±98.2 ng\*h/ml,  $p=0.668$ ). Furthermore,  $t_{max}$  and  $t_{1/2}$  of SIR were not significantly influenced by the co-medication. The AUC of EZE and its glucuronide were slightly elevated which in turn resulted in a significantly increased urinary excretion of the glucuronide.

**CONCLUSION:** Cholesterol-lowering treatment with EZE is not expected to alter pharmacokinetics of the immunosuppressant SIR. The influence of SIR on disposition of EZE seems not to be of clinical relevance.

### PIII-84

LACK OF DRUG-DRUG INTERACTIONS AND FOOD EFFECTS WITH THE NOVEL, CARDIOSELECTIVE, VASODILATORY  $\beta$ -BLOCKER, NEBIVOLOL. J. M. Martin; Forest Research Institute, Jersey City, NJ

**BACKGROUND:** Hypertensive patients often receive multiple anti-hypertensive medications. Nebivolol is a novel, cardioselective  $\beta$ -blocker with nitric-oxide-mediated vasodilatory properties. As well as glucuronidation to active glucuronides, nebivolol is metabolized via the polymorphic cytochrome P450 (CYP)2D6 pathway, resulting in two metabolizer phenotypes: extensive (EM) and poor metabolizers (PM=7% of Caucasians). Drug-drug interactions (DDIs) and the effects of food on nebivolol pharmacokinetics (PK) and bioavailability were investigated.

**METHODS:** Oral nebivolol (2.5-20mg) PK and bioavailability were investigated in healthy volunteers (EM/PM) under fasting and fed conditions. DDIs of nebivolol (10mg) with spironolactone (25mg), hydrochlorothiazide (25mg), warfarin (10mg), digoxin (0.25mg), losartan (50mg), ramipril (5mg), furosemide (40mg) and fluoxetine (20mg) were also assessed

**RESULTS:** All doses of nebivolol were well tolerated; food had no effect on bioavailability regardless of CYP2D6 genotype. Co-administration of fluoxetine, which inhibits CYP2D6, confirmed the reliance of nebivolol on the CYP2D6 pathway for elimination and produced elevated plasma nebivolol levels, but with a similar safety profile to nebivolol alone. No effects were seen on the PK of either drug when nebivolol was co-administered with other common antihypertensive medications (furosemide, ramipril, losartan, hydrochlorothiazide and spironolactone). Concurrent administration of nebivolol and digoxin did not alter digoxin PK; warfarin anti-coagulant activity and PK were also unaffected by nebivolol co-administration. Nebivolol was safe and well tolerated, with no cardiovascular abnormalities, or changes in laboratory parameters.

**CONCLUSION:** Nebivolol is safe and well tolerated when given with other classes of medications often used in hypertensive patients, and may be a useful addition to the antihypertensive therapeutic repertoire.

### PIII-85

IN-VITRO PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF PHYSOSTIGMINE (PHYS) IN HUMAN WHOLE BLOOD, PLASMA AND RED BLOOD CELLS (RBC). D. S. Lee, J. Venitz; Virginia Commonwealth University, School of Pharmacy, Dept. of Pharmaceutics, PK/PD Lab., Richmond, VA

**BACKGROUND/AIMS:** PHYS is a prototypical cholinesterase (ChE) inhibitor that has shown a rapid clearance in previous human PK studies, suggesting extrahepatic metabolism, possibly due to degradation by blood and tissue cholinesterases (ChE). The aim of this study was to determine the PK and PD of PHYS in human whole blood, plasma and RBCs.

**METHODS:** PHYS was added to the whole blood, plasma and RBCs collected from young healthy adults and incubated at 37°C with various PHYS starting concentrations. Serial samples were collected to measure PHYS plasma concentrations using a validated HPLC-FD method and ChE activity by Ellman's colorimetric method in plasma and RBCs.

**RESULTS:** The clearance of PHYS in whole blood was concentration-dependent and followed apparent Michaelis-Menten kinetics with a  $K_m$  of 47 nM and  $v_{max}$  of 0.015 nmol/min for each ml of plasma. RBCs and plasma was found to contribute approximately 40% and 60%, respectively, to the total *in-vitro* clearance. The PHYS-induced inhibition of RBC acetyl-ChE (AChE) and plasma butyryl-ChE (BuChE) was rapid, concentration-dependent, nearly complete and reversible. Using an inhibitory  $I_{max}$  model, PHYS was an apparent competitive inhibitor with  $K_i$  values of 1.3 nM for both enzymes and  $I_{max}$  values of 93% and 98% for BuChE and AChE, respectively. At clinically relevant concentrations of 18 nM, AChE and BuChE were inhibited by 15% and 20%, respectively.

**CONCLUSIONS:** In this *in-vitro* study, PHYS was rapidly degraded in blood, however, this accounted for only approximately 7% of the reported *in-vivo* total body clearance, suggesting hydrolysis by tissue ChE may be pivotal for PHYS metabolism. AChE and BuChE were inhibited non-selectively and reversibly by PHYS. At clinically relevant plasma concentrations, PHYS hydrolysis followed apparent first-order PK, and AChE and BuChE were inhibited to a similar extent as observed in *in-vivo* studies.

### PIII-86

POPULATION PHARMACOKINETIC/PHARMACODYNAMIC (PK/PD) MODELING FOR AN ANTISENSE OLIGONUCLEOTIDE, TARGETING HUMAN APOB, IN SUBJECTS WITH MILD HYPERCHOLESTEROLEMIA. R. Z. Yu,<sup>1</sup> R. S. Geary,<sup>1</sup> J. Flaim,<sup>1</sup> D. Tribble,<sup>1</sup> M. Wedel,<sup>1</sup> J. Kastelein<sup>2</sup>; <sup>1</sup>ISIS Pharmaceuticals, Inc, Carlsbad, CA, <sup>2</sup>University of Amsterdam, Amsterdam, The Netherlands

**BACKGROUND:** To develop a semi mechanistic population PK/PD model of an antisense apoB inhibitor, ISIS 301012, to establish the PK and PD relationship and to quantify the degree of inter- and intra-subject variability.

**METHODS:** Results from Phase 1 and Phase 2a clinical trial data of ISIS 301012 in subjects with mild hypercholesterolemia were used for the population PK/PD analysis using NONMEM<sup>®</sup>. PK and PD data were obtained from 53 subjects receiving ISIS 301012 as 2-hr intravenous infusion and/or subcutaneous injections at weekly doses ranged from 50 mg to 400 mg with or without loading doses for a duration of 4 weeks or 13 weeks. Plasma concentrations of ISIS 301012 and LDL-C in serum were measured at various time points during treatment and up to 6 months after treatment was completed.

**RESULTS:** Plasma ISIS 301012 concentration-time data were described with a 2-compartment model and first order elimination. Typical estimates of clearance, distribution volumes of central ( $V_2$ ) and deep ( $V_3$ ) were 5.8 L/hr, 19.8 L and 1490 L, respectively. Inter-subject variability was 39% for CL, 89% for  $V_2$  and 86% for  $V_3$ . Preliminary analysis did not show any covariate influence. Serum LDL-C was fitted to an indirect response model in an effect compartment with multiple transit compartments reaching the observation compartment, using sequential fitting method. The typical values of  $I_{max}$ ,  $IC_{50}$  and  $K_{eq}$  were 103%, 208 ng/mL, and 0.000608/hr, respectively. Simulations using this PK/PD model showed good agreement with the observations obtained from other Phase 2 trials.

**CONCLUSIONS:** The semi mechanistic population PK/PD model described the time course of ISIS 301012 plasma concentrations and serum LDL-C reduction profiles appropriately. Consequently, this model may be useful to predict the time-course of plasma ISIS 301012 concentrations and serum LDL-C reductions in future clinical studies.

### PIII-87

CHARACTERIZATION OF A THREE-DRUG NON-LINEAR MIXTURE RESPONSE SURFACE MODEL. Y. F. Brun,<sup>1</sup> W. R. Greco,<sup>2</sup> D. B. White<sup>3</sup>; <sup>1</sup>Novartis Pharmaceuticals, East Hanover, NJ, <sup>2</sup>Roswell Park Cancer Institute, Buffalo, NY, <sup>3</sup>University of Toledo, Toledo, OH

**BACKGROUND:** Our group recently developed a response-surface modeling paradigm (White *et al.*, *Curr. Drug Metab.* 2, 399-409, 2003) and tested its application on both mixtures of anticancer agents and antifungals. This new model is a Hill-type equation, with the slope and potency parameters being functions of the normalized drug ratios, using polynomial expressions. Response surface methods allow one to model and interpret all of the information present in the full concentration-effect data set, to visualize local regions of synergy, additivity and antagonism, and even to quantify the degree of synergy or antagonism, both globally, and across local regions of the response surface.

**METHODS:** We study the effect of changes in the different parameters of the polynomial expressions for 2-drug and 3-drug mixtures, on the geometrical shapes of several 2-dimensional representations of the 3-dimensional concentration-effect response surface, by changing one parameter at a time, all other parameters being equal to zero or fixed. We also simulate a previously published response-surface paradigm by Minto *et al.*, (*Anesthesiology*, 92:1603-1616, 2000) which also uses polynomial expressions (albeit different ones from the White model) to characterize the slope and potency parameters as function of the normalized drug ratios, to compare and contrast the two models.

**RESULTS:** This allows a better comprehension of the model, its abilities, and its limits, thereby making the use of the characterized model easier and more relevant afterwards.

**CONCLUSION:** The White model showed good flexibility and allowed for simulation of relevant shapes; it also had some definite improvements over the Minto model. Supported in part by NIH RR10742 (WRG), and an educational grant from Novartis through the University at Buffalo, SUNY (YFB).

### PIII-88

EVALUATION OF SAFETY, TOLERABILITY, AND MULTIPLE-DOSE PHARMACOKINETICS OF ISTRADefylline IN PARKINSON'S DISEASE PATIENTS. N. Rao,<sup>1</sup> T. Uchimura,<sup>2</sup> A. Mori<sup>2</sup>; <sup>1</sup>Kyowa Pharmaceutical, Inc., Princeton, NJ, <sup>2</sup>Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan

**BACKGROUND:** Istradefylline, an adenosine A<sub>2A</sub> receptor antagonist, is in development for the treatment of Parkinson's disease as adjunctive therapy to levodopa/carbidopa. The objective of this study was to characterize the safety, tolerability, and pharmacokinetics (PK) of multiple ascending doses of istradefylline in patients with Parkinson's disease treated with levodopa/carbidopa. A secondary objective was to compare PK in patients and healthy subjects.

**METHODS:** The study was conducted in an open-label, sequential, ascending-dose fashion (60 and 80 mg/day for 14 days) in 10 patients (5 subjects/group). Patients had to meet the UK Parkinson's Disease Society Brain Bank diagnostic criteria, took between 500 and 1550 mg/day levodopa, and were on stable doses of levodopa for at least 7 days before dosing. Serial plasma samples collected after single and multiple doses of istradefylline were analyzed for istradefylline using a validated HPLC-UV assay. PK analysis was conducted using non-compartmental methods. Safety assessments included vital signs, 12-lead ECG, physical examination, blood chemistry, hematology, and urinalysis. Adverse events (AEs) were assessed throughout the study. Dose escalation was allowed only after review of safety data by a safety review committee.

**RESULTS:** No dose-limiting AEs were reported. All treatment-emergent AEs were mild and reversible. Fewer AEs were reported in the 80 mg/day group (6 AEs in 2 patients) than in the 60 mg/day group (14 AEs in 4 patients). Istradefylline exhibited dose-proportional

increases in AUC and C<sub>max</sub> after single and multiple doses. T<sub>1/2</sub> (range ~64-69 h) and accumulation ratio (range ~3-4) were dose independent. Cross-study comparison with PK data obtained at 60 mg and 80 mg/day in healthy subjects indicated similar PK.

**CONCLUSION:** Istradefylline is safe and well tolerated and exhibits predictable PK. Steady state PK in Parkinson's disease patients is comparable to that in healthy subjects.

### PIII-89

EVALUATION OF SAFETY, TOLERABILITY, AND MULTIPLE-DOSE PHARMACOKINETICS OF ISTRADefylline IN HEALTHY SUBJECTS. N. Rao,<sup>1</sup> T. Uchimura,<sup>2</sup> A. Mori<sup>2</sup>; <sup>1</sup>Kyowa Pharmaceutical, Inc., Princeton, NJ, <sup>2</sup>Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan

**BACKGROUND:** Istradefylline, an adenosine A<sub>2A</sub> receptor antagonist, is in development for the treatment of Parkinson's disease. The objective of this study was to characterize the safety, tolerability and pharmacokinetics (PK) of multiple ascending doses of istradefylline in healthy, adult, male subjects.

**METHODS:** The study was conducted in a placebo-controlled, randomized, blinded, sequential ascending-dose fashion (40, 60, 80, 120, and 160 mg/day for 14 days) in 10 healthy, adult male subjects in each group (8 active, 2 placebo). Serial plasma samples collected after single and multiple doses of istradefylline were analyzed for istradefylline using a validated HPLC-UV assay. PK analysis was conducted using non-compartmental methods. Safety assessments included vital signs, 12-lead ECG, physical examination, blood chemistry, hematology, and urinalysis. Adverse events (AEs) were assessed throughout the study. Dose escalation was allowed only after review of safety data by a safety review committee.

**RESULTS:** No dose-limiting AEs were reported. All treatment-emergent AEs were mild and reversible. Istradefylline exhibited dose-proportional increases in AUC and C<sub>max</sub>. Steady state C<sub>1/F</sub> (range ~4.1-6.0 L/h), apparent V<sub>d,ss</sub>/F (range ~448-557 L), T<sub>1/2</sub> (range ~67-95 h), and accumulation ratio (range ~3-5) were dose independent.

**CONCLUSION:** Istradefylline is safe and well tolerated and exhibits predictable PK at doses up to 160 mg/day.

### PIII-90

IMPACT OF PXR ON PLACENTAL ABC DRUG TRANSPORTERS. S. Gahir, M. Piquette-Miller; University of Toronto, Toronto, ON, Canada

**BACKGROUND:** Numerous studies have established the importance of the Pregnane X Receptor (PXR) in the hepatic and intestinal regulation of several drug transporters. However, the involvement of PXR in the regulation of placental drug transporters has not been established. As the placental expression of the ABC drug efflux transporters plays a particularly important role in fetal safety, our aim was to elucidate the role of PXR in the regulation of placental ABC efflux drug transporters.

**METHODS:** Basal levels of placental transporters were measured in the PXR wildtype (WT) and knockout (KO) mice. Placental tissue samples were collected on gestation day (GD) 17 and 10. The mRNA levels of Mdr1a, Mrp1-3 and Bcrp were quantified using real time PCR (RT-PCR). The impact of PXR activation on placental expression was examined in WT mice treated with Pregnane-16 $\alpha$ -carbonitrile (PCN, 50mg/kg ip, daily) from GD 13 to 17. Placental tissue from PCN treated and vehicle treated controls were collected on GD 17.

**RESULTS:** The mRNA levels of Mdr1a, Mrp1-3 and Bcrp were significantly higher in the placenta of the KO mice in contrast to the WT samples on gestational day 17. Tracking the transporters levels over time, mRNA levels of Mrp3 and Mdr1a decreased over the course of gestation in the WT mice while an induction was seen in the KOs. Upon activation by PCN, no significant difference was observed in the

mRNA levels of transporters in the treatment and the control groups of the WT mice.

**CONCLUSION:** Since changes in the expression of placental transporters can significantly impact the fetal drug exposure, it vital to understand the role of PXR in regulating drug transporters at the maternal-fetal interface. Our results indicate PCN mediated activation of PXR does not induce placental transporters. On the other hand, basal expression changes from GD 10 to GD 17 may suggest either a possible inhibitory role for PXR or over compensation by other closely related nuclear receptors.

### PIII-91

SELECTION OF OPTIMAL DATA DISTRIBUTIONS AND ACCOUNTING FOR TIME VARIANCE IN BAYESIAN POPULATION PHARMACOKINETIC MODELS. S. J. Kathman; GlaxoSmithKline, Research Triangle Park, NC

**BACKGROUND:** When modeling population pharmacokinetic data, consideration should be given to the underlying distribution of the data and to uncertainty in sampling time relative to dosing. The normal and log-normal distributions are the most commonly used, but other choices are readily available. Intravenous infusions of a drug often require more precise timing of the sample taken at termination of the infusion, and uncertainty in the timing of this event should be considered as well. The goal of this study is to illustrate the importance of these considerations using data from an anti-mitotic oncology drug.

**METHODS:** Escalating doses of an anti-mitotic drug were administered as 1-hour intravenous infusions on Days 1, 8 and 15 of a 28-day treatment cycle in 30 patients. Serial blood samples were collected on Days 1 and 15. A pre-dose sample was collected on Day 8. The data were modeled assuming several different underlying distributions for the data: normal, log-normal, gamma, student's t, and log-t. The data were also modeled accounting for uncertainty in the post-infusion concentration, allowing for error in either the time of sampling or the duration of infusion between subjects. The models were compared graphically as well as using different versions of the deviance information criteria, and the expected value of a loss function comparing predicted values to observed values. The models were fit using Bayesian methods, which readily allow for the necessary adjustments.

**RESULTS:** Of the distributions considered, the best fits were obtained when using a student's t and a log-t distribution. The gamma distribution also outperformed the log-normal distribution. A substantial improvement was also obtained when accounting for uncertainty in the post-infusion sample times.

**CONCLUSION:** Population pharmacokinetic models may be improved by considering different underlying distributions for the data and by making adjustments for uncertainty in sampling time.

### PIII-92

TEXT MINING ON PHARMACOKINETICS PARAMETERS. Z. Wang, S. Kim, L. Li; Indiana University, Indianapolis, IN

**BACKGROUND:** Pharmacokinetics (PK) parameters from either non-compartmental analysis or compartment model analysis have been routinely published in literatures, such as AUC and CL. These published data are extremely valuable in establishing a drug's compartment or non-compartment models. Hence, it forms the essential basis of simulation and modeling in the Critical Path Initiative (FDA, 2004). Therefore, mining these PK parameters from published literature is a crucial step in establishing models for prediction and simulation.

**METHODS:** The most comprehensive PK parameter database we can find is named DiDB (<http://druginteractioninfo.org>), with data manually retrieved and input. We used drug Midazolam as the benchmark and retrieved its PK data from DiDB as gold standard. We designed a template library to retrieve pharmacokinetics relevant articles, and performed text mining to extract candidate PK numerical data. We also developed a meta-analysis approach to filter outliers from the retrieved PK data.

**RESULTS:** The comparison between our mined PK data and DiDB data is given in [table 1](#).

PK parameter	# of Gold Standard	# of Mined Data
AUC	1	4
Clearance	4	34

Table 1 Comparison of information content from DiDB and text mining. The number represents the number of publications. The PK parameters are for healthy human subjects, single dose Midazolam (PO and IV) based. This text mining method gives more information than that from DiDB database.

The meta-analysis model was tested on Midazolam clearance data, as shown in [figure 1](#).

**CONCLUSION:** The data mining results are very encouraging based on Midazolam example. Our method is able to find 4 to 8 fold more relevant published PK parameters comparing to the current database, and follow-up meta-analysis can efficiently filter out outliers. This approach provides us a valuable PK parameter resource for pharmaceutical researchers.

