

### OIII-A-IV

PHARMACOKINETIC MODEL OF PROCHLORPERAZINE AEROSOL IN VOLUNTEERS. D. A. Spyker, PhD, MD, M. J. Avram, PhD, T. K. Henthorn, MD, W. Houghton, MD, J. V. Cassella, PhD, Alexza, Pharmaceuticals, Inc., Director, Mary Beth Donnelley Clinical Pharmacology Core Facility of the Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Professor and Chair, Department of Anesthesiology, University of Colorado Health Sciences Center, Alexza Pharmaceuticals, Chicago, IL.

**BACKGROUND/AIMS:** A thermally-generated aerosol (TGA) system can deliver excipient-free drug reliably to the alveoli, resulting in rapid systemic drug absorption. We developed a pharmacokinetic (PK) model of prochlorperazine (PCZ) administered as a TGA and as intravenous (IV) infusions to healthy volunteers and determined absolute TGA bioavailability.

**METHODS:** PCZ disposition was determined three times in each of 8 healthy adult volunteers (6 males, 2 females, mean  $\pm$  SD age  $31.5 \pm 9.2$  yr and weight  $73.4 \pm 7.5$  kg) in this IRB-approved 3-period cross-over study. Venous blood samples were collected 19 times from 1 min to 24 h after drug administration via a 2 min IV infusion (10 mg), a 5 sec IV infusion (0.5 mg), and a TGA delivered in a single breath (0.625 mg coated dose via Staccato<sup>®</sup> Prochlorperazine for Inhalation, Alexza Pharmaceuticals, Palo Alto, CA). Plasma PCZ concentrations were measured using liquid chromatography-tandem mass spectrometry. IV and TGA PK were characterized simultaneously by the same 3-compartment model with independently chosen multiple absorption delays to describe the observed drug concentration profiles.

**RESULTS:** TGA administration of PCZ produced venous plasma drug concentrations similar to those produced by rapid IV administration. The common PK model described both TGA and IV PCZ disposition well. The VSS,  $1335 \pm 612$  L, CLE,  $1.68 \pm 0.43$  L/min, and  $t_{1/2\beta}$ ,  $9.63 \pm 3.04$  hr, were similar to those reported by others for single IV doses. Model-predicted peak concentrations were  $1.62 \pm 0.71$  ng/ml for the TGA dose and  $0.99 \pm 0.70$  ng/ml for the 5 sec IV dose and predicted times to peak concentration were  $2.25 \pm 1.28$  min for the TGA dose and  $3.00 \pm 1.31$  min for the 5 sec IV dose. The geometric mean [90% CI] bioavailability of the TGA based on coated dose was  $0.949 [0.693, 1.30]$  ( $N = 8$ ), similar to results for non-compartmental methods.

**CONCLUSION:** Single breath TGA of PCZ resulted in IV-like PK in terms of speed, extent, and reliability of absorption. Pulmonary administration via properly designed TGA may offer a viable alternative to rapid IV administration for drugs requiring fast, predictable production of effective plasma concentrations.

#### REFERENCES:

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### PIII-1

IDENTIFICATION AND CHARACTERIZATION OF CYTOCHROME P4502D6\*56B (*CYP2D6\*56B*), A NOVEL ALLELE ASSOCIATED WITH THE POOR METABOLIZER PHENOTYPE. A. Gaedigk, MS, PhD, J. D. Eklund, BS, R. E. Pearce, PhD, J. S. Leeder, PharmD, PhD, S. W. Alander, MD, L. D. Bradford, PhD, M. J. Kennedy, PharmD, The Children's Mercy Hospital, Morehouse School of Medicine, University of Louisville, Kansas City, MO.

**BACKGROUND:** A 5 year old African American female presented with a *CYP2D6\*4x2/\*10* genotype that was discordant with her phenotype towards the probe drug dextromethorphan. The poor metabolizer phenotype was confirmed in a repeat assessment suggesting that the *CYP2D6\*10* allele carries a novel debilitating sequence variation(s).

**METHODS:** Both alleles were cloned, entirely sequenced and compared to other *CYP2D6\*4* and *\*10* sequences previously analyzed. Subsequently, 59 *CYP2D6\*10* alleles present among

813 subjects ( $n = 346$  Caucasians,  $n = 425$  African American,  $n = 39$  Asians and  $n = 3$  unknown) were retested by newly designed PCR-RFLP assays to determine the frequency of the novel allele.

**RESULTS:** A C > T SNP was discovered in exon 7 on the allele initially genotyped as *CYP2D6\*10*. This SNP (GenBank accession M33388, position 3201) changes the amino acid codon for Arg344 into a TGA stop-codon. Consequently, translation is prematurely terminated resulting in a truncated protein product. The novel allele was designated *CYP2D6\*56B* by the nomenclature committee. While this variant was not detected in *CYP2D6\*10* in alleles of Caucasian or Asian individuals, we discovered two, including the index case, among the 426 African American individuals tested. The frequency of *CYP2D6\*56B* was 0.23% in this population. Sequence analysis confirmed the second allele as a novel *CYP2D6\*4* sub-variant as it lacks four SNPs compared to other *CYP2D6\*4* sequences.

**CONCLUSIONS:** The heterogeneity of the *CYP2D6* gene locus in African Americans is further emphasized by the discovery of yet another non-functional allelic variant. 3201C > T was first found in a Caucasian on a *CYP2D6\*2* backbone (linkage disequilibrium with *CYP2D6\*2* SNPs) and termed *CYP2D6\*56*, while 3201C > T occurred on a *CYP2D6\*10* backbone (linkage with *CYP2D6\*10* SNPs) in our case. Due to the shared detrimental SNP these alleles were termed *CYP2D6\*56A* and *\*56B*, respectively. Genotype analysis in absence of 3201C > T testing leads to false-positive *CYP2D6\*2* and *\*10* assignments depending on which backbone 3201C > T is located. Regardless, lack of 3201C > T testing leads to an incorrect phenotype prediction. Albeit infrequent, additional testing of *CYP2D6\*2* and *CYP\*10* alleles for the *CYP2D6\*56*-defining SNP will likely improve the phenotype prediction for individuals of African descent.

### PIII-2

THE ACTIVITY SCORE (AS): FACILITATING *CYP2D6* PHENOTYPE PREDICTION FROM GENOTYPE DATA. A. Gaedigk, MSc, PhD, S. D. Simon, PhD, R. E. Pearce, PhD, M. J. Kennedy, PharmD, L. D. Bradford, PhD, J. S. Leeder, PharmD, PhD, The Children's Mercy Hospital, University of Louisville, Morehouse School of Medicine, Kansas City, MO.

**BACKGROUND:** To date 60 *CYP2D6* allelic variants have been defined and the resulting plethora of genotypes causes a wide range of activity in vivo. Inferring phenotype from genotype is increasingly challenging and poses a central question in translational research where genotyping is utilized as a (diagnostic) tool for phenotype prediction. To simplify genotype interpretation and improve phenotype categorization we explored the utility of an 'activity score' (AS) system.

**METHODS:** A value was assigned to each allele approximating its in vivo or in vitro activity as described in the literature and deduced from own data (0 = null, 0.5 and 0.75 = reduced, 1 = functional, 2 = duplication). The sum of both alleles defined the AS of a genotype. 678 subjects (mostly Caucasian and African American) were included into a multiple linear regression model to determine whether AS accurately predicted phenotype (dextromethorphan metabolic ratio,  $\log(\text{DM}/\text{DX})$ ).

**RESULTS:** 99 different genotypes were observed and reduced to 9 AS groups (0, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 and >2). Linear regression with race, age and genotype as co-variables revealed a significant contribution of genotype to the variability in  $\log(\text{DM}/\text{DX})$  ( $r^2 = 0.63$ ). AS alone was nearly as predictive with  $r^2 = 0.58$ . Mean  $\log(\text{DM}/\text{DX})$  declined with increasing AS with the exception of the group with an AS of 1.25. This group consisted of 18 African Americans and 1 Caucasian; all carried reduced function alleles in various combinations.

**CONCLUSIONS:** The AS system is a simple and user-friendly tool that reliably 'translates' genotype into phenotype as demonstrated using the probe drug DM. Categorization into 9 AS groups instead of the 4 historical metabolizer groups (PM, IM, EM, UM) especially caters to genotypes with reduced function. Considerable variability of

log(DM/DX) among the AS groups, and the higher than expected mean for AS = 1.25, suggests that additional factors such as novel/ untested *CYP2D6* alleles, other genes/variants, host or environmental factors influence DM metabolism. Refinement of the AS system (assignment of additional values to better reflect the activity encoded by a given allele and/or the addition of other genetic variables) and its evaluation for other probe drugs may ultimately lead to an improved phenotype prediction from genotype data.

### PIII-3

GLUTATHIONE S-TRANSFERASE T1 AND M1 PHARMACOGENOMICS. A. M. Moyer, O. E. Salavaggione, MD, S. J. Hebringer, I. Moon, M. A. Hildebrandt, B. W. Eckloff, D. J. Schaid, PhD, E. D. Wieben, PhD, R. M. Weinshilboum, MD, Mayo Clinic College of Medicine, Rochester, MN.

**BACKGROUND/AIMS:** The glutathione S-transferases (GSTs) are enzymes that catalyze glutathione conjugation of reactive electrophiles. The *GSTT1* and *GSTM1* genes are polymorphically deleted, which has been the focus of many epidemiologic studies. However, the full range of genetic polymorphisms in these two genes has not been explored. We set out to systematically identify polymorphisms in *GSTT1* and *GSTM1* using a genotype-to-phenotype strategy, followed by functional characterization of common polymorphisms and studies of mechanisms by which they might alter function.

**METHODS:** *GSTT1* and *GSTM1* copy number assays were performed with 100 DNA samples from each of four ethnic groups. Resequencing was performed using DNA samples with at least one copy of *GSTT1* or *GSTM1*. Functional studies of SNPs and characterization of molecular mechanisms by which variant DNA sequences might alter enzyme function were performed.

**RESULTS:** *GSTT1* and *GSTM1* deletion allele frequencies varied among ethnic groups, ranging from 33.5% in Caucasian Americans to 73.5% in Han Chinese-Americans (HCA) for *GSTT1* and from 50.5% in African Americans (AA) to 78.0% in HCA for *GSTM1*. *GSTM1* duplication was also observed. *GSTT1* deletion data correlated with mRNA microarray expression in the same lymphoblastoid cells from which DNA had been obtained for resequencing. The 18 SNPs in *GSTT1* included three ns cSNPs and one indel, while the 51 *GSTM1* SNPs included two ns cSNPs. Two of the ns cSNPs in *GSTT1* resulted in significant decreases in levels of immunoreactive protein, to 56 ± 1% and 12 ± 3% of WT, while those in *GSTM1* resulted in modest increases. Reporter gene assays showed that one *GSTT1* 5'-flanking region (FR) SNP, with a 32% allele frequency in the AA population, resulted in an increase in transcription in JEG-3 cells to 351 ± 2% of WT. One *GSTM1* 5'-FR haplotype construct resulted in an increase in transcription in JEG-3 cells to 129 ± 6% of WT and in HEK 293T/17 cells to 131 ± 7% of WT. Three additional *GSTM1* promoter haplotypes significantly decreased transcription in HEK 293T/17 cells to 75 ± 4%, 85 ± 2%, and 73 ± 3% of the WT promoter activity.

**CONCLUSION:** These studies raise the possibility of functionally significant pharmacogenomic variation in these two genes that may contribute to variation in response to drugs as diverse as acetaminophen and antineoplastic agents.

### PIII-4

GENETIC POLYMORPHISMS IN ESTROGEN RECEPTORS ARE ASSOCIATED WITH CHANGES IN THYROID FUNCTION AFTER TAMOXIFEN TREATMENT. A. T. Nguyen, BSc, CCRP, T. Skaar, PhD, S. Philips, BSc, MSc, R. McClintock, P. Hu, MD, PhD, A. Storniolo, MD, D. Hayes, MD, V. Stearns, MD, D. A. Flockhart, MD, PhD, Y. Jin, MD, Indiana University, University of Michigan, John Hopkins University, Indianapolis, IN.

**BACKGROUND/AIMS:** Hypothyroidism is a common comorbid condition in women with breast cancer. Tamoxifen treatment has been associated with changes in thyroid function. We hypothesize that the changes in thyroid function after tamoxifen treatment are associated with genetic polymorphisms in estrogen receptors.

**METHODS:** To test the effect of tamoxifen treatment on thyroid function, we performed a nested case-control study by 1:2 match of hypothyroid with euthyroid subjects. Serum thyroid binding globulin (TBG), thyroid-stimulating hormone (TSH) and free thyroxine (T4) concentrations were measured with enzyme immunoassay (EIA). Common polymorphisms in *ESR1* were determined using restrictive fragment length polymorphism, and in *ESR2* using a TaqMan™ assay.

**RESULTS:** In 237 subjects who participated in a prospective trial to examine pharmacogenetic associations with tamoxifen effects, we identified 37 subjects who were taking a levothyroxine supplement. Serum samples from 78 subjects were selected for analysis, including 22 subjects with hypothyroidism and 56 subjects as matching controls. After tamoxifen treatment, there were significant increases in TBG concentration (22.3 ± 4.5 µg/ml to 27.0 ± 4.8 µg/ml,  $P < 0.001$ ), and TSH (from 1.99 ± 1.7 mIU to 2.50 ± 2.0 mIU,  $P = 0.006$ ); while serum free T4 remained unchanged (from 2.3 ± 2.1 µg/dl to 2.2 ± 1.7 µg/dl,  $P = 0.42$ ). When the changes in TBG, TSH and free T4 during tamoxifen treatment were examined, there were no significant differences between hypothyroid and euthyroid subjects (data not shown). Changes in TBG concentrations were associated with *ESR1* PvuII (CC = 3.8 ± 3.2; CT = 3.4 ± 3.2; TT = 7.8 ± 3.3,  $p = 0.001$ ) and *ESR2-02* genotype (AA = 0.17 ± 1.5; AG = 5.0 ± 2.9; GG = 6.3 ± 3.7,  $p = < 0.001$ ); changes in TSH concentrations were associated with *ESR1* PvuII genotype only (CC = 0.21 ± .95; CT = 0.03 ± 1.1; TT = 1.48 ± 2.4,  $p = .004$ ); while changes in serum free T4 concentration were not associated with any *ESR* polymorphisms (data not shown).

**CONCLUSION:** More than 15% of subjects with breast cancer were on levothyroxine treatment. Tamoxifen treatment led to a decrease in thyroid function regardless of the baseline thyroid status. *ESR1* PvuII genotype was associated with changes in serum TSH concentration, suggesting that these effects may be mediated by this estrogen receptor.

### PIII-5

NOVEL FUNCTIONAL IMPAIRED SINGLE NUCLEOTIDE POLYMORPHISM OF NA<sup>+</sup>-TAUROCHOLATE COTRANSPORTING POLYPEPTIDE (NTCP) IN KOREA POPULATION. W. Pan, MD, H. Kang, MS, S. Lee, PhD, H. Shin, PhD, W. Kim, MS, J. Shin, MD, PhD, Department of Pharmacology and Pharmacogenomics Research Center, Busan, Republic of Korea.

**BACKGROUND:** Na<sup>+</sup>-taurocholate cotransporting polypeptide (NTCP, *SLC10A1*) is the key transporter responsible for hepatic uptake of bile acids from portal circulation. This study was designed to identify common *SLC10A1* genetic variants and determine their *in vitro* functional change.

**METHODS:** Genetic variants in exons and upstream domain of the gene were detected by direct sequencing in 50 Korean subjects. The novel nonsynonymous was further screened by pyrosequencing in Chinese and Vietnamese population. Transfected MDCK cells of human NTCP were used for *in vitro* uptake study. [<sup>3</sup>H] Taurocholic acid and [<sup>3</sup>H] estrone sulfate were selected as probe substrates.

**RESULTS:** Sequence analysis revealed 2 *SLC10A1* upstream SNPs and 4 code region SNPs, including a novel nonsynonymous SNP G190A (Ala64Thr) with 1% allele frequency in Korean ( $n = 150$ ). However, this SNP was not found in 360 Chinese and 152 Vietnamese subjects. The alleles frequency of reported C800T (Ser267Phe) SNP were found 5%, 7.8% and 9.2% in Korean, Chinese and Vietnamese subjects respectively. Ser267Phe variant, which has been reported to exhibit near complete loss of function for bile acid uptake, fully normal transport function for estrone sulfate. Otherwise, Ala64Thr variant showed decreased uptake activity of both [<sup>3</sup>H] Taurocholic acid and [<sup>3</sup>H] estrone sulfate substrates when compared with wild type though they had same expression level. However, both variants (Ala64Thr, Ser267Phe) and wild type showed the similar response to the known NTCP inhibitor (taurocholic acid, bromosulfalein and cyclosporin A).

**CONCLUSION:** Novel nonsynonymous SNP was found in SLCO1A1 gene, which showed ethnic specificity, suggesting that this position may be part of a region in the transporter critical and specific for bile acid substrate recognition. Our study indicates functionally important polymorphisms in NTCP exist and that the likelihood of being carriers of such polymorphisms is dependent on ethnicity.

### PIII-6

**FUNCTIONAL ACTIVITY OF A GENETIC VARIANT IN THE ESTROGEN RECEPTOR BETA TATA BOX.** S. Philips, A. Richter, S. Oesterreich, J. Rae, N. Perumal, D. Flockhart, T. Skaar, Indiana University, Baylor College of Medicine, University of Michigan, Indianapolis, IN.

**BACKGROUND:** The phenotypic responses to estrogen receptor targeting drug therapies can be highly variable. Baseline and drug induced responses in these phenotypes (e.g. bone mineral density and serum lipids) have been associated with germline genetic polymorphisms in the estrogen receptors (ERs). We hypothesized that genetic variations in the ER $\beta$  promoter may account for part of the variability. The aim of this study was to identify genetic polymorphisms in the promoter of the ER $\beta$  gene and determine their functional significance.

**METHODS:** Using a bioinformatic approach, we identified a 1.6 kb region of the ER $\beta$  gene that was highly conserved and contained multiple transcription factor binding sites. This region contains part of the promoter, exon 0N and intron 1. To identify genetic variants in this region, we resequenced this 1.6 kb fragment in 50 African-American and 50 Caucasian subjects from the Coriell diversity panel. To test the functional effect of the single nucleotide polymorphism (SNP), we cloned a fragment of the promoter containing either the wild type or variant form of the TATA box into the pGL3 luciferase reporter plasmid. These were transfected into LnCAP cells and assayed to determine their transcriptional activity.

**RESULTS:** A total of 6 SNP's were identified in the ER $\beta$  gene, 5 in the promoter and 1 in intron 1. One of these was predicted to alter the function of the TATA box. Its allelic frequency was 2% in African Americans and 0% in Caucasians. In the in vitro luciferase reporter assays, the TATA box variant promoter had approximately 50% less transcriptional activity than the wild type.

**CONCLUSION:** We identified a SNP in the TATA box of the ER $\beta$  promoter that has decreased in vitro transcriptional activity. This genetic variant may contribute to the interindividual variability in ER $\beta$  expression and result in altered responses to estrogen and antiestrogens.

### PIII-7

**THE UGT1A9 INTRONIC I399 POLYMORPHISM IS NOT ASSOCIATED WITH UGT1A9 ACTIVITY OR mRNA EXPRESSION.** J. Ramirez, MS, W. Liu, A. A. Desai, S. Mirkov, P. Chen, S. Das, F. Innocenti, M. J. Ratain, University of Chicago, Chicago, IL.

**BACKGROUND/AIMS:** Interindividual variability in the glucuronidation of xenobiotics metabolized by UDP-glucuronosyltransferase 1A9 (UGT1A9) suggests the presence of functional UGT1A9 variants. The data on the function of the well described  $-118T_{9>10}$  variant are contradictory. A recent study identified an intronic variant ( $I399C > T$ ) that was associated with increased UGT1A9 protein expression and activity, and shown to be in low linkage disequilibrium (LD) with other UGT1A9 variants (Girard, DMD, 2006). Variants  $-275T > A$  and  $-2152C > T$  have also been correlated with increased enzyme activity. The aim of this study was to investigate whether these four polymorphisms contribute to the variability in UGT1A9 activity and mRNA levels in Caucasian human livers.

**METHODS:** UGT1A9 genotypes ( $-118T_{9>10}$ ,  $I399C > T$ ,  $-275T > A$  and  $-2152C > T$ ) and UGT1A9 activity (i.e., flavopiridol glucuronidation) were determined in 48 Caucasian human livers. mRNA levels were determined by RT-PCR in 37 of the livers. For confirming the allele frequencies and LD pattern, samples from 60 unrelated Caucasians belonging to the HapMap Project were also genotyped.

**RESULTS:** The allele frequencies of the  $-118T_{9>10}$ ,  $I399C > T$ ,  $-275T > A$  and  $-2152C > T$  variants were 0.39, 0.39, 0.02 and 0.02 in the livers. The  $I399C > T$  variant was in complete LD with  $-118T_{9>10}$  (linked alleles: C and 9T, respectively). Complete LD between these two variants was also found in the HapMap samples (frequencies of  $-118T_{9>10}$  and  $I399C > T = 0.38$ ).  $I399C > T$  and  $-118T_{9>10}$  correlated with neither UGT1A9 activity nor mRNA levels ( $p > 0.05$ , ANOVA). Due to the low frequencies of the  $-275T > A$  and  $-2152C > T$  variants, an effect on UGT1A9 phenotype could not be studied.

**CONCLUSION:** Our data demonstrate that 1)  $I399C > T$  and  $-118T_{9>10}$  do not explain interindividual variation in hepatic UGT1A9 activity and mRNA expression in the donor liver samples we studied, and 2) these two variants appear to be in complete LD in Caucasians.

### PIII-8

**RNA INTERFERENCE (RNAI) SCREEN FOR MODIFIERS OF DRUG ACTION: IDENTIFICATION OF SENSITIZERS FOR A CYTOSTATIC DRUG CANDIDATE.** C. Sachse, B. Bader, K. Regener, C. Frenzel, S. Kluge, C. Holz, M. Hannus, A. Walsh, S. Precht, C. Merz, B. Weiss, B. Kreft, G. Siemeister, C. Echeverri, D. Mumberg, Cenix BioScience GmbH, Schering AG, Dresden, Germany.

**BACKGROUND:** In recent years, high-throughput applications of RNA interference (RNAi) have become powerful tools for target discovery. In the present study, RNAi was employed successfully in a drug modifier context, to screen for targets whose inhibition enhances the cytostatic effects of the drug candidate ZK-A. Such targets are therefore strong candidates for developing ZK-A sensitizer compounds.

**METHODS:** An RNAi screen was set up targeting 264 selected genes expressed in a cultured human cell line, with each gene targeted by three individual siRNAs. Three experimental sets were performed in parallel: one with siRNAs plus ZK-A at a level around its IC50, one with siRNAs plus ZK-A at a level below its IC50, and one control set with siRNAs plus DMSO. At 48 h and 72 h after siRNA transfection, cells were fixed and subjected to a High Content microscopy-based assay, wherein proliferation, apoptotic index, mitotic index and other proprietary cytological parameters were simultaneously quantified.

**RESULTS:** First, a number of targets were identified whose silencing through RNAi resulted in inhibition of proliferation and/or induction of apoptosis, thus representing potential new targets for anti-tumor drug development. Secondly, a number of targets were revealed whose silencing by RNAi was found to modify the drug effect: sensitizer targets displayed an enhancement of the anti-proliferative drug effect, whereas suppressor targets resulted in a weakening of the drug effect. The latter targets indicate contra-indicated combination treatments, and may also represent candidate biomarkers for identifying non-responders. First round hit candidates were confirmed in a secondary screening round including qRT-PCR: for confirmation, at least two distinct siRNAs per target had to show a consistent functional effect over the two screening passes, together with target silencing as measured by qRT-PCR. Follow-up validation studies in additional cell lines are currently underway.

**CONCLUSION:** The very clear and consistent data of the present study indicate that RNAi drug modifier screens can add significant value to the drug development process, by revealing targets for possible combination therapies, and, potentially, also for enabling Pharmacogenomics-driven individualized drug therapies in the future.

### PIII-9

PHARMACOGENOMICS OF PROTEASOME INHIBITION. L. Wang, MD, PhD, L. Pelleymounter, I. Moon, S. Kumar, R. Weinsilboum, P. Greipp, Mayo Clinic, Rochester, MN.

**BACKGROUND:** The proteasome is a multienzyme complex that contains three catalytically active  $\beta$  subunits. More than 80% of proteins in the cell are degraded through the proteasome. The proteasome is a regulator of tumorigenesis and, as a result, is a target for drug therapy. Bortezomib, an inhibitor of the  $\beta 5$  subunit, is used to treat refractory multiple myeloma. Therefore, sequence variation in genes encoding proteasome subunits might influence drug response and disease pathophysiology. In this study, we resequenced the three active  $\beta$ -subunits and performed functional studies with nonsynonymous cSNPs identified in the  $\beta 5$ -subunit.

**METHODS:** The 3 active  $\beta$ -subunits (encoded by *PSMB1*, *PSMB2* and *PSMB5*) were resequenced using 240 ethnically defined DNA samples from Coriell lymphoblastoid cells and *PSMB5* was also resequenced using bone marrow and/or germline DNA isolated from multiple myeloma patients treated with bortezomib. Proteolytic activity was determined with proteasomes isolated from HeLa cells transfected with WT and the 4 *PSMB5* variant allozyme constructs, as well as Coriell lymphoblastoid cells containing the 4 *PSMB5* nonsynonymous cSNPs with Suc-LLVY-amc as substrate. Inhibition by the proteasome inhibitor MG262 was also determined with these proteasomes and cells. Finally, Coriell cell lines with the 4 *PSMB5* nonsynonymous cSNPs were also used to perform cytotoxicity studies with MG262 at various concentrations for 48 hours, and 50% growth inhibition was calculated for each cell line.

**RESULTS:** We identified 27 SNPs, including 2 nonsynonymous cSNPs in *PSMB1*, 40 SNPs in *PSMB2* and 24 SNPs in *PSMB5*, including 4 nonsynonymous cSNPs. We also identified 3 additional novel SNPs in *PSMB5*, including one nonsynonymous cSNP in DNA from multiple myeloma patients treated with bortezomib. Although the functional studies showed no significant differences with regard to substrate kinetics or IC50 values for inhibition between WT and the 4 *PSMB5* nonsynonymous cSNPs identified by resequencing the Coriell DNA samples.

**CONCLUSION:** These studies represent a step toward understanding how sequence variation in genes encoding proteasome subunits might influence proteasome function and/or response to proteasome inhibition therapy.

### PIII-10

OCT2 PROMOTER POLYMORPHISM AND FUNCTIONAL CONSEQUENCES. Z. Wang, O. Yin, M. Chow, School of Pharmacy, The Chinese University of Hong Kong, Shatin, Hong Kong.

**BACKGROUND/AIMS:** The human organic cationic transporter 2 (OCT2) plays an important role in the renal clearance of many drugs. A number of single nucleotide polymorphisms (SNPs) in OCT2 gene coding region have been identified recently. However, the promoter polymorphisms of OCT2 are not well characterized at present. Thus the present study was designed to evaluate OCT2 promoter polymorphisms and the potential functional consequences in a Chinese population.

**METHODS:** To determine SNPs, direct sequencing of the proximal promoter region of OCT2 gene was performed using genomic DNA from 56 healthy Chinese subjects. Haplotypes were estimated by Bayesian algorithm based method. To determine the OCT2 variant promoter transcriptional activity, promoter fragment containing the identified variants was prepared by PCR and cloned into pGL3-basic vector. Reporter gene constructs were transiently transfected in HEK293T cells.

**RESULTS:** 5 SNPs were identified in OCT2 promoter region: -1430 T > C, -1351 A > G, -1283 T > C, ins TTCA at -109 to -1102, and del AAG at -780 to -782. Haplotype analysis revealed 5 haplotypes, with frequencies ranging from 2.68% to 38.4%. Haplotype III, characterized by the presence of -1283 T > C variant, exhibited significantly lower luciferase activity (26.7%,  $p < 0.05$ ) in

comparison to the wild-type promoter (haplotype I). Slightly lower luciferase activities were also observed for haplotypes II, IV and V, but the differences were not statistically significant when compared to the wild-type promoter.

**CONCLUSION:** Our study demonstrates the existence of genetic polymorphisms in OCT2 promoter in Chinese population. Specifically, a promoter variant, -1283 T > C, is associated with an altered promoter activity in vitro. Further studies are warranted to investigate the mechanisms of such effect and its clinical relevance.

### PIII-11

IDENTIFICATION OF NULL ALLELIC VARIANT OF CYP2C8 IN A KOREAN POPULATION. C. Yeo, MD, D. Cho, MD, H. Jung, MS, S. Lee, PhD, J. Shon, MD, PhD, J. Shin, MD, PhD, Department of Pharmacology & Pharmacogenomics Research Center, Busan, Republic of Korea.

**BACKGROUND:** The human cytochrome P450(CYP) 2C8 is a major hepatic P450, constituting about 7% of total microsomal CYP content in the liver and is responsible for the metabolism of a wide range of drugs such as paclitaxel, cerivastatin, amiodarone and rosiglitazone. In the present study, the genotype profile of CYP2C8 was analyzed in a Korean population. Frequency in multi-ethnic population and in vivo functionality of novel null allelic CYP2C8 variant were evaluated.

**METHODS:** Whole blood samples from 50 unrelated Korean subjects were genotyped for 3 kb of 5' upstream region, all exon-intron boundaries, exons, and UTR regions of *CYP2C8* gene by direct sequencing. Genotyping of CYP2C8 has been addressed only for null allelic variant, CYP2C8\**M* using pyrosequencing in the 447 Koreans, 93 African-Americans, 100 Caucasians, 348 Chinese and 100 Vietnamese. Then, in-vivo single PK study of CYP2C8 probe, rosiglitazone (4 mg), was conducted in 7 healthy subjects with CYP2C8\*1/\*1 and 2 with CYP2C8\*1/\**M*.

**RESULTS:** We identified a novel null allelic variant (CYP2C8\**M*) in Koreans, Chinese and Vietnamese, but not founded in Caucasian and African-american population enrolled in this study. The allelic frequency of this novel SNP was 0.3% in 447 Koreans. After single oral dose of rosiglitazone, subjects with heterozygous mutation of CYP2C8\**M* seemed likely to be higher plasma concentration of rosiglitazone than those with wild genotype. The AUC of rosiglitazone in 2 subjects with CYP2C8\**M* (2334 and 2350 ng\*hr/ml) showed higher value than that in those with CYP2C8\*1/\*1 ( $N = 7$ ,  $1414 \pm 311$  ng\*hr/ml).

**CONCLUSION:** The null allelic variant, CYP2C8\**M*, was identified in Asian races, but its frequency was rare ( $\leq 0.3\%$ ). Subjects with heterozygous mutation of CYP2C8\**M* showed the higher oral bioavailability of rosiglitazone than those with wild genotype. The subject with CYP2C8\**M*/\**M* mutant genotype would be expected to demonstrate the marked defective catalyzing activity of CYP2C8 substrates and might lead to clinically relevant results after taking those.

\*CYP2C8\**M* is designated as CYP2C8\*11 by CYP nomenclature committee. SNP information will be released after publication.

### PIII-12

RELATIONSHIP BETWEEN CYP 2A6 GENETIC POLYMORPHISM, AS A MARKER OF NICOTINE METABOLISM, AND ULCERATIVE COLITIS. S. Zevin, MD, G. Alterescu, MD, D. Rachmilewitz, MD, Shaare Zedek Medical Center, Jerusalem, Israel.

**BACKGROUND/AIM:** Ulcerative colitis (UC) is a very common and difficult to treat disease. Non-smokers have a relative risk of 2.9 of developing UC compared with smokers. Smokers have a later onset and a milder form of UC. Nicotine is the component of cigarette smoke responsible for the favorable effects in UC. Nicotine is metabolized to cotinine by the enzyme CYP2A6. Polymorphisms in CYP2A6 gene have been described. Subjects who are homozygotes

for CYP2A6\*4 are completely deficient in cotinine formation, while homozygotes for CYP2A6\*1A have a high capacity to produce cotinine. Polymorphisms in the gene encoding the alpha 3 subtype nicotinic cholinergic receptor (CHRNA3) have also been described. To compare the frequency of CYP 2A6 and CHRNA3 polymorphisms among smokers and non-smokers with UC, and to examine their effect on disease severity.

**METHODS:** 69 patients with UC were recruited from the IBD clinic in Shaare Zedek Medical Center. Information regarding the age of onset, disease activity and treatment was obtained from questionnaires completed by the subjects. CYP2A6 \*1A,\*4A and CHRNA3 polymorphisms were determined by PCR and restriction enzyme analysis.

**RESULTS:** 9% of the patients were current smokers, 30% ex smokers and 61% nonsmokers. 63% of smokers and ex smokers were homozygotes for CYP 2A6 \*1A (extensive metabolizers of nicotine) and 4% were homozygotes for CYP 2A6 \*4A (poor metabolizers), whereas among nonsmokers 66% were poor metabolizers ( $p < 0.0001$ ). There was no significant effect of CYP 2A6 or CHRNA3 genotype on UC activity.

**CONCLUSION:** We found a very high proportion of poor nicotine metabolizers among nonsmoking patients with UC and a very low proportion among current and ex smokers. Therefore we could not determine the effect of poor metabolizer genotype (CYP 2A6\*4A homozygotes) on disease activity in smokers with UC. However, it may be possible to identify UC patients who are poor metabolizers of nicotine who may benefit from nicotine or nicotine-like pharmacological treatment.

### PIII-13

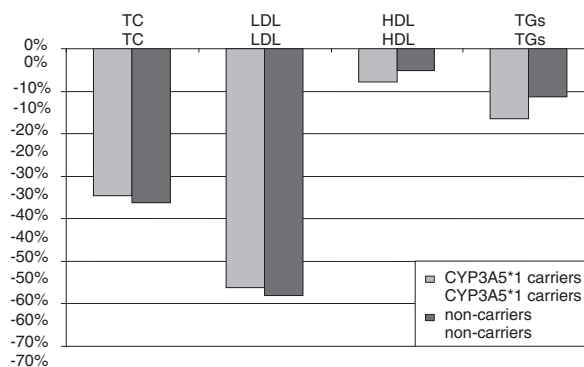
EFFECTS OF CYP3A4 AND CYP3A5 POLYMORPHISMS ON CHOLESTEROL RESPONSES TO ATORVASTATIN. T. Y. Langae, PhD, MSPH, B. Burkley, MSc, Y. Gong, PhD, I. Zineh, PharmD, University of Florida, Gainesville, FL.

**BACKGROUND/AIMS:** Atorvastatin is metabolized primarily by the CYP3A family of drug metabolizing enzymes. The CYP3A4\*1B and CYP3A5\*3 and \*6 polymorphisms result in low expression of CYP3A4 and CYP3A5 proteins. Contrarily, CYP3A\*1 carriers express CYP3A proteins. We hypothesized that CYP3A expressors might exhibit higher metabolism of atorvastatin resulting in attenuated lipid-lowering responses compared to those with homozygote variant genotypes.

**METHODS:** Eight-week treatment responses by genotype were assessed for 68 individuals without cardiovascular disease treated with atorvastatin 80 mg daily. Genotyping for CYP3A4\*1B, CYP3A5\*3, and CYP3A5\*6 polymorphisms was performed by PCR and followed by pyrosequencing. Lipid profiles were determined by our university hospital clinical laboratory. Analyses were done by general linear model with significance set at  $p < 0.05$ .

**RESULTS:** Baseline total cholesterol (TC), LDL, HDL, and triglycerides (TGs) for the whole population were  $182.04 \pm 40.81$ ,  $99.60 \pm 32.69$ ,  $62.57 \pm 18.04$ , and  $99.28 \pm 54.32$  mg/dl, respectively. Overall, atorvastatin 80 mg resulted in average changes of  $-33 \pm 10.81\%$ ,  $-54.92 \pm 13.84\%$ ,  $-0.46 \pm 19.03\%$  and  $-17.53 \pm 37.78\%$ , respectively. As shown in the figure, there were no significant differences in drug response between CYP3A5\*1 carriers ( $N = 13$ ) vs. non-carriers ( $N = 55$ ;  $p > 0.7$ ). Likewise, no significant differences were found between CYP3A4\*1 carriers ( $N = 65$ ) and non-carriers ( $N = 2$ ; data not shown;  $p > 0.7$ ).

**CONCLUSIONS:** We did not observe any associations between atorvastatin lipid lowering responses and CYP3A4 and CYP3A5 polymorphisms. The lack of an association may, in part, be due to use of the highest available dose of atorvastatin. Whether or not use of maximum doses of statins overcomes the contribution of drug metabolizing enzyme polymorphisms to variable drug response, should be further evaluated.



### PIII-14

CURRENT STATUS OF GENOTYPING METHODS AND GENOTYPING DATA THAT SHOULD BE INCLUDED IN FDA A VOLUNTARY GENOMIC DATA SUBMISSION. M. M. Chan, PhD, S. U. Yasuda, MS, PharmD, R. Farkas, MD, PhD, F. Goodsaid, PhD, M. Kim, PharmD, E. Lacana, PhD, N. Rahman, PhD, K. Thummel, PhD, S. Huang, PhD, FDA, University of Washington, Silver Spring, MD.

**BACKGROUND/AIMS:** The aim of this work was to identify 1) methods useful for genomic data submissions, 2) genes with currently available tests, and 3) key information to support clinical and clinical pharmacology evaluation in a voluntary genomic data submission (VGDS).

**METHODS:** Literature and current pharmacogenomics texts were reviewed to summarize currently available genotyping methods. A list of FDA cleared genotype tests was generated as was a list of genotype tests described either in approved drug labeling or in the literature. Key information desirable in a pharmacogenomic data submission was identified.

**RESULTS:** Many methods are available for characterizing DNA variation, with new procedures rapidly being developed. In this presentation, various approaches for detecting known differences in a DNA sequence will be described and a list of genes with FDA approved or "laboratory developed" tests available as a service will be provided. For the metabolic enzyme group, the majority of laboratory developed genotyping tests are for CYP2D6, CYP2C9, and CYP2C19, followed by TPMT and UGT1A1; the predominant method used is multiplexed PCR.

To support clinical or clinical pharmacology evaluation, key information in a genotyping report should include: 1) description of assay platform or methodology; 2) description of study samples, including demographics and sample size justification for genotype/clinical phenotype correlation, and adequate coverage or justification for ethnic/racial group inclusion; 3) summary of alleles measured and correlation with metabolic status designation; 4) description of how extensive metabolizer (EM), poor metabolizer (PM), intermediate metabolizer (IM), or ultra rapid metabolizer (UM) are determined for metabolizing enzymes; 5) data on the relationship between gene variant and encoded protein activity for new genes; and 6) discussion as to whether the assay was performed in a CLIA certified or research lab. Several test reports will be listed as examples.

**CONCLUSIONS:** A variety of methods are available for genotyping that will support clinical and clinical pharmacology evaluation of a VGDS. Key information outlined here should provide consistency in reporting and help in evaluation and interpretation of VGDS data.

**PIII-15**

**CORRELATION OF ETHNICITY AND VKORC1 AND CYP2C9 GENOTYPES IN PREDICTING WARFARIN MAINTENANCE DOSE.** C. A. Haller, MD, P. Wang, PhD, A. Smith, MT (ASCP), A. Wu, PhD, University of California, San Francisco, San Francisco, CA.

**BACKGROUND:** Polymorphisms in genes for cytochrome P450 (CYP) 2C9 and vitamin K epoxide reductase complex subunit 1 (VKORC1) are associated with variable anticoagulant response to warfarin. For VKORC1, 10 single nucleotide polymorphisms (SNPs) are identified, and warfarin dose variability in Caucasians is linked to 5 of 9 haplotypes, with H1/H2 associated with low dose, and H7-H9 with high doses of warfarin. Associations in other ethnicities and influences of other VKORC1 haplotypes on warfarin dose have not been examined.

**METHODS:** We extracted and genotyped DNA for CYP2C9 and VKORC1 from 123 patients on warfarin at San Francisco General Hospital to characterize the frequency of VKORC1 haplotypes and 2C9 alleles in this ethnically-diverse population, using 2 new commercial platforms: a SNP analysis of the -1639 promoter region (Invader, Third Wave Technologies) and H1-H9 haplotypes using a novel combined CYP2C9/VKORC1 chip (Infiniti, Autogenomics). We compared maintenance warfarin doses in 34 patients with predicted doses from a published algorithm by Sconce et al that includes adjustments for age, height, 2C9 and VKORC1 genotypes.

**RESULTS:** Results (Table) show that Hispanics are similar to Caucasians in distribution of VKORC1 haplotypes, while Asians and African Americans have higher prevalences of H1/H2 and H3-H6 haplotypes, respectively. Mean ( $\pm$ SD) outpatient warfarin doses for patients with wild-type CYP2C9 were highest for individuals homozygous for H7-H9 (5.9  $\pm$  3.0 mg) and H3-H6 (6.1  $\pm$  2.2 mg) and lowest for homozygous H1/H2 (2.3  $\pm$  0.8 mg) VKORC1 haplotypes. CYP2C9 genotypes correlated less strongly with warfarin doses. Predicted warfarin doses compared to actual doses using the Sconce algorithm showed a correlation coefficient of 0.61, which improved to 0.76 when Caucasians and Hispanics were excluded.

**CONCLUSIONS:** VKORC1 H3-H6 haplotypes are associated with high maintenance warfarin doses, similar to H7-H9 haplotypes. Hispanic patients have similar VKORC1 haplotype distributions as Caucasians. The Sconce algorithm may have ethnic-based differences in strength of correlation.

Ethnicity	No. Alleles	VKORC1 Haplotype (%)			CYP2C9 Genotype (%)		
		H1/H2	H3-H6	H7-H9	*1	*2	*3
Asian	54	80%	5%	15%	89%	0	11%
Caucasian	80	40%	4%	55%	55%	36%	9%
African American	80	10%	31%	58%	95%	2.5%	2.5%
Hispanic	32	41%	6%	53%	88%	0	12%

**PIII-16**

**PHARMACOLOGY OF A C. AURANTIUM AND CAFFEINE-CONTAINING THERMOGENIC DIETARY SUPPLEMENT.** C. A. Haller, MD, P. Jacob III, PhD, M. J. Duan, N. L. Benowitz, MD, University of California, San Francisco, San Francisco, CA.

**BACKGROUND:** Thermogenic dietary supplements (DS) promoted to enhance weight loss and athletic performance often contain herbal sympathomimetics such as *C. aurantium* (synephrine) and caffeine. Recent evidence indicates these DS may have cardiovascular effects similar to banned ephedra products. The pharmacology of these DS has not been well characterized, particularly in the setting of exercise.

**METHODS:** Ten healthy adults took 1 dose of DS (Ripped Fuel Extreme Cut<sup>®</sup> with 20 mg synephrine and 304 mg caffeine by LC-MS/

MS analysis) under resting conditions and 1 hour prior to moderately intense exercise (30 min on cycle ergometer at 75–80% HR<sub>max</sub>) in a 3-arm, double blind, placebo-controlled crossover study. Plasma synephrine and caffeine concentrations were measured over 12 hours, and vital signs, serum electrolytes, oxygen consumption, and perceived exercise exertion were monitored.

**RESULTS:** Seven men and 3 women completed the study without significant adverse effects. Synephrine and caffeine PKs were unaffected by exercise. Mean  $\pm$  SD kinetic parameters for synephrine were T<sub>1/2</sub> 2.6  $\pm$  0.8 hr, T<sub>max</sub> 2.1  $\pm$  0.7 hr, C<sub>max</sub> 1.8  $\pm$  0.8 ng/ml, AUC<sub>0-∞</sub> 428  $\pm$  173 ng·min/ml, CL/F 60.0  $\pm$  32.5 L/min; and for caffeine were T<sub>1/2</sub> 6.0  $\pm$  1.9 hr, T<sub>max</sub> 2.4  $\pm$  0.8 hr, C<sub>max</sub> 5.9  $\pm$  2.0 mcg/ml, AUC<sub>0-∞</sub> 58.9  $\pm$  32.6 mcg·min/ml, CL/F 102.6  $\pm$  36.4 ml/min. Post-exercise diastolic BP was higher after DS (peak mean 71.7  $\pm$  8.7 mm Hg) than PLC (63.0  $\pm$  4.9 mm Hg) (p = 0.007). There were no substantial treatment-related differences in post-exercise HR, SBP, or temp. Post-prandial plasma glucose increased to 121.0  $\pm$  31.6 mg/dl with DS and exercise vs. 103.7  $\pm$  25.5 mg/dl with PLC and exercise (p = 0.004). No differences in exercise-related oxygen consumption, or changes in serum lactate, insulin, or potassium were observed between DS and PLC. Exercise was rated less difficult with DS than PLC (p = 0.001).

**CONCLUSIONS:** DS increased blood pressure and plasma glucose post-exercise, which could be detrimental in hypertensive or diabetic patients taking these products to lose weight. Exercise was perceived as less strenuous after DS, presumably due to the stimulant effects of caffeine.

**PIII-17**

**A WARFARIN DOSING MODEL IN ASIANS USING SINGLE NUCLEOTIDE POLYMORPHISMS IN VITAMIN K EPOXIDE REDUCTASE COMPLEX AND CYTOCHROME P450 2C9.** L. Tham, MSc, PharmD, B. Goh, MD, A. N. Nafziger, MD, MHS, J. Guo, MSc, L. Wang, MSc, R. Soong, PhD, C. Wong, MD, S. Lee, MD, National University Hospital, Ordway Research Institute, Drug Development Center, Oncology Research Institute, National University of Singapore, Singapore, Singapore.

**BACKGROUND/AIMS:** Due to the unique lack of genetic diversity despite the multi-ethnicity in our population, we hypothesize that single nucleotide polymorphisms in cytochrome P450 2C9 (CYP2C9\*3) and vitamin K epoxide reductase complex subunit 1 (VKORC1) at position 381 to infer VKORC1 haplotype in combination with demographic factors can accurately predict warfarin doses. The aims of this study were to derive a pharmacogenetic-based dosing algorithm using retrospective information, and to validate it through a data-splitting method in a separate cohort of equal size.

**METHODS:** 215 records of warfarin patients recruited in a VKORC1 genotyping study were used to perform this analysis. Data-splitting was employed to create a learning dataset and a separate validation dataset. Univariate analyses for individual predictors, including age, weight, gender, serum albumin concentration, ethnic groups, International Normalized Ratio (INR), CYP2C9 and VKORC1-381 genotypes were conducted to select variables with p values < 0.1 for further inclusion into the multivariate regression analysis. In the final model, only predictors reaching a statistical significance of p < 0.05 were retained. All statistical and regression analyses were performed using SPSS version 13.0.

**RESULTS:** Data from 107 subjects on maintenance warfarin with INR stabilized between 2 and 3 were used to derive the final model, an exponential function of age, weight, CYP2C9\*3 allele and VKORC1-381 CC and TC genotypes, and accounted for 60.2% of the variability in daily warfarin dose requirement. The model was validated in a separate cohort of 108 subjects and showed a mean under-estimation of 0.23  $\pm$  1.21 mg/day.

**CONCLUSION:** Warfarin dose requirements in Asians can be accurately predicted using a combination of patient demographics and a simplified genotyping approach for single variants in CYP2C9 and VKORC1.

### PIII-18

CLINICALLY SIGNIFICANT INTERACTION BETWEEN ORALLY ADMINISTERED TEDISAMIL AND VERAPAMIL. J. Burggraaf, MD PhD, A. D. van Haarst, PhD, H. Weimann, PhD, M. J. Kemme, MD PhD, J. J. Bosch, MD, R. C. Schoemaker, PhD, A. F. Cohen, MD PhD, Centre for Human Drug Research, Solvay Pharmaceuticals, Leiden, The Netherlands.

**AIM:** Tedisamil is a K-channel blocker antiarrhythmic drug, being developed for the treatment of angina pectoris and atrial fibrillation and flutter. Tedisamil treatment may implicate co-administration with frequently prescribed Ca-channel blocker antiarrhythmics, such as verapamil. Therefore a study was performed to assess pharmacokinetic and pharmacodynamic interactions between tedisamil and verapamil.

**METHODS:** In a double-blind, placebo-controlled, four-period cross-over study, 12 healthy volunteers received a 3-day treatment of tedisamil (100 mg BID), verapamil (180 mg BID), a combination of these drugs or placebo. On each study day blood pressure and electrocardiograms (ECG) were assessed. On the third study day cardiac output was measured and blood samples for tedisamil, verapamil and its metabolite norverapamil were drawn.

**RESULTS:** Two subjects were withdrawn from the study because of excessive QT-prolongation. Thus the data of 10 subjects are given. Combined administration of tedisamil and verapamil significantly increased plasma concentration of tedisamil (AUC0-12h: +77%, Cmax: +78%) compared to tedisamil monotherapy, while it decreased plasma concentrations of verapamil (AUC0-12h: -21%, Cmax: -28%) and norverapamil (AUC0-12h: -17%, Cmax: -20%) compared to verapamil monotherapy. These differences were all statistically significant at a p-level <0.05. Compared to placebo, verapamil monotherapy and the combination treatment increased the PR interval by 23.5 (95%CI: 17.9–29.2) msec and 12.2 (5.7–17.0) msec, respectively. Whereas, compared to placebo, QTc was increased by tedisamil monotherapy 27.8 (15.8–39.8) msec and by the combination treatment: 45.7 (33.7–57.7) msec.

**CONCLUSIONS:** This study shows a clinically significant pharmacokinetic interaction between verapamil and tedisamil that is most likely mediated by P-glycoprotein inhibition by verapamil resulting in increased plasma concentration of tedisamil. In line with these pharmacokinetic interactions, an increased effect of the combined treatment was observed for QTc and PR interval. These data imply that combined treatment of oral tedisamil and verapamil should only be undertaken with caution and that frequent ECG monitoring should be performed upon initiation of therapy or during changes in dose regimen.

### PIII-19

EFFECTS OF VERAPAMIL PRE-TREATMENTS ON THE DISTRIBUTION OF <sup>3</sup>H-DOMPERIDONE, A P-GLYCOPROTEIN SUBSTRATE, TO THE HEART AND OTHER TISSUES OF MALE AND FEMALE HARTLEY GUINEA PIGS. L. Couture, J. A. Nash, L. Nguyen, J. Turgeon, Université de Montreal, Charles River Laboratories Preclinical Services Montreal, Montreal, PQ, Canada.

**BACKGROUND/AIMS:** ABC transporters such as the P-glycoprotein (P-gp) were reported to be expressed in normal tissues such as the heart (Couture L, Nash JA, Turgeon J. The ATP-binding cassette transporters and their implication in drug disposition: a special look at the heart. *Pharmacol. Rev.* 58:244–258, 2006). Domperidone, a P-gp substrate, was recognized to be associated with a block of voltage-gated cardiac K<sup>+</sup> channels and drug-induced Long QT syndrome. The aim of our study was firstly to determine the effect of verapamil (also a P-gp substrate) pre-treatments on the distribution of <sup>3</sup>H-domperidone to the heart and other tissues, secondly to compare results from verapamil (11.6 mg/kg) pre-treatments; a pre-treatment of 5 consecutive days (modulation) vs a pre-treatment 2 h prior (competitive inhibition) <sup>3</sup>H-domperidone administration, and thirdly to compare gender.

**METHODS:** Following an IP injection of <sup>3</sup>H-domperidone (2.5 mg/kg) to male and female Hartley guinea pigs, 1 animal from each group was sacrificed at 9 different timepoints up to 7 h post-dose. Tissues were excised and processed by liquid scintillation spectroscopy to determine radioactivity levels.

**RESULTS:** Higher tissue-to-plasma ratios were observed in heart structures (up to 36%) and other tissues of animals pre-treated with verapamil 2 h before the administration of <sup>3</sup>H-domperidone compared to animals pre-treated with verapamil for 5 consecutive days or non pre-treated, without important differences between these two latter groups. Male guinea pigs showed slightly higher levels of <sup>3</sup>H-domperidone in heart structures compared to females. Measurement of QT intervals from ECG showed a tendency for a prolongation of the QT interval for verapamil pre-treated animals.

**CONCLUSION:** The higher levels of <sup>3</sup>H-domperidone in heart of guinea pigs pre-treated with verapamil 2 h before the administration of <sup>3</sup>H-domperidone lead to a possible higher incidence of cardiotoxicities such as the drug-induced Long QT syndrome when domperidone is co-administered with another P-gp substrate. Accordingly, the pharmacodynamic response showed a tendency to a prolongation of the QT interval for those verapamil pre-treated animals. The impairment of P-gp activities by verapamil pre-treatments appeared to be greater by competitive inhibition than by modulation.

### PIII-20

QUANTIFYING THE EFFECTS OF DG-041 ON PLATELET AGGREGATION IN HEALTHY VOLUNTEERS. D. Hermann, PharmD, W. Wang, PhD, P. Van Ess, PharmD PhD, B. Anders, PharmD, D. Hartman, MD, deCODE genetics, deCODE genetics, Brighton, MI.

**BACKGROUND/AIMS:** DG041 is an antiplatelet agent currently under development for the treatment of peripheral arterial occlusive disease (PAOD), MI, and stroke. The DG-041 discovery program was based on an initial genome-wide linkage analysis of PAOD patients implicating chromosome region 1p31. Subsequently, a variant in the gene encoding for the prostaglandin EP3 receptor subtype (PTGER3) was identified as a significant risk factor for the development of severe PAOD. Given the above, DG-041 was developed as a potent and specific inhibitor of the platelet EP3 receptor. To quantify the exposure-response relationship of DG-041 on platelet aggregation to aid dose selection.

**METHODS:** Exposure-response data from single and multiple dose Phase I trials were pooled and described using a nonlinear mixed effects model. The model was then used to compare dose regimens via simulation. Platelet aggregation, induced via the EP3 receptor agonist sulprostone, was measured using an optical aggregometer. Due to the “all or none” nature of the platelet aggregation data the probability of having ≤20% platelet aggregation (Pr20) was modeled using a logistic function. The logistic function included a sigmoid Emax model, a mixture model on IC50, and an inter-subject random effect on IC50. Model parameters were estimated using the Laplacian method as implemented in NONMEM. The predictive distribution for platelet aggregation response at specific time points and DG-041 dosage regimens was determined by repeatedly sampling from the covariance matrix and simulating platelet aggregation effects. The simulation results were uploaded into the Drug Model Explorer<sup>®</sup> software to explore differences between regimens.

**RESULTS:** A total of 1056 data observations from 86 patients were included in the analysis. DG041 inhibited EP3 mediated platelet aggregation in a direct, concentration dependent manner. The estimated Pr20 approached maximal effect with a 400 mg twice daily regimen. Increasing the dose to 800 mg increased the Pr20 at trough by only 2.8 to 6.3% (90% prediction interval). No safety concerns, including bleeding related events were observed across the dose range studied.

**CONCLUSIONS:** Based on modeling and simulation of the biomarker response and safety data, subsequent DG041 trials will evaluate doses up to 400 mg given twice daily.

### PIII-21

SINGLE AND MULTIPLE DOSE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF PRX-08066, A NOVEL 5-HT<sub>2B</sub> ANTAGONIST. G. R. Iyer, PhD, J. Milovanovic, S. Donahue, MD, Epix Pharmaceuticals, Lexington, MA.

**AIM:** To evaluate single and multiple dose safety, tolerability, pharmacokinetics and pharmacodynamics of PRX-08066, a novel 5HT<sub>2B</sub> antagonist, in healthy subjects.

**METHODS:** Three randomized placebo-controlled clinical trials were conducted in separate populations of healthy adult volunteers. In the first 2 trials, oral doses of PRX-08066 or placebo were administered as a single dose (25 mg to 800 mg; N = 40), once daily (25 mg or 100 mg) for 14 days (N = 16), or twice daily (100 mg to 400 mg) for 14 days (N = 24) to evaluate the safety, tolerability and pharmacokinetics (PK) of PRX-08066. In a third study, athletic subjects (N = 15), conditioned to exercise at altitudes >750 m above sea-level, received PRX-08066 (50 mg & 200 mg) or placebo, each twice daily for 3 days, in a cross-over design applying an approximate 2 week washout between dosing periods. During each dosing day, subjects were challenged with hypoxic conditions for 90 minutes to induce increases in systolic pulmonary artery pressure (SPAP). The pharmacodynamics of PRX-08066 was characterized by noninvasive measurement of pulmonary artery pressure using echocardiography. PK and safety were evaluated throughout all three studies.

**RESULTS:** Serum exposures (AUC<sub>0-∞</sub>, AUC<sub>0-24</sub> and C<sub>max</sub>) increased in a dose proportional manner. The terminal half-life ranged from 14 to 19 hours suggesting the feasibility of a once daily dosing regimen. The 200 mg bid dose level demonstrated a statistically significant, 40% reduction in the hypoxia-induced increase in SPAP compared to placebo during hypoxia exercise. Adverse events related to study drug were mild or moderate in severity. A single dose of 800 mg PRX-08066 was markedly associated with dizziness and nausea while a twice daily dose of 400 mg PRX-08066 was not. No apparent trends or clinically important differences between dose groups or dose groups compared to placebo were observed in clinical laboratory data, vital signs, or ECGs.

**CONCLUSIONS:** PRX-08066 was well tolerated at single doses up to 500 mg or twice daily doses up to 400 mg. The PK profile of PRX-08066 is consistent with once daily dosing. In addition, the reduction in SPAP suggests PRX-08066 has the potential to treat patients with pulmonary hypertension associated with hypoxic lung disease or hypoxemia.

### PIII-22

REMODELING OF CAV1.2 CALCIUM CHANNELS IN HUMAN ATHEROSCLEROSIS. D. R. Abernethy, MD, PhD, S. Tiwari, PhD, Y. Zhang, PhD, J. Heller, MD, N. M. Soldatov, PhD, Gerontology Research Center, Johns Hopkins Bayview Medical Center, Baltimore, MD.

**BACKGROUND/AIMS:** Atherosclerosis (AT) is an inflammatory process characterized by proliferation and dedifferentiation of vascular smooth muscle cells (VSMC). Cav1.2 calcium channels are essential for Ca<sup>2+</sup>-signal transduction in VSMC. The pore-forming Cav1.2 $\alpha$ 1 subunit of the channel is subject to alternative splicing. Here we investigated whether the Cav1.2 $\alpha$ 1 splice variants are affected by AT.

**METHODS:** Human VSMC obtained from surgical specimens obtained during vascular reconstruction procedures were isolated by laser-capture microdissection and adjacent regions of arteries affected and not affected by AT were compared.

**RESULTS:** In VSMC from non-atherosclerotic regions, RT-PCR analysis revealed an extended repertoire of Cav1.2 $\alpha$ 1 transcripts characterized by the presence of exons 21 and 41A. AT reduced expression of the Cav1.2 $\alpha$ 1 transcript and caused replacement of the Cav1.2 $\alpha$ 1 splice variants with the unique exon-22 isoform that lacks exon 41A and exhibits higher sensitivity to dihydropyridine Ca<sup>2+</sup> channel blockers at negative membrane potentials. The Cav1.2 remodeling in AT caused changes in electrophysiological properties of the identified Cav1.2 $\alpha$ 1 isoforms including the kinetics and voltage-dependence of inactivation, and recovery from inactivation. Consistent with the pathophysiological state of VSMC in clinical AT, cell culture data pointed to a potentially important association of the exon-22 isoform of Cav1.2 $\alpha$ 1 with proliferation of VSMC.

**CONCLUSIONS:** Thus, localized changes in cytokine expression generated by inflammation in AT affect alternative splicing of the Cav1.2 $\alpha$ 1 gene in the human artery that causes molecular and electrophysiological remodeling of Cav1.2 calcium channels and possibly affects VSMC proliferation. Our findings also raise an intriguing possibility that pharmacological properties of VSMC are locally altered in AT.

### PIII-23

CYP2C9 PHENOTYPE AND CYP2C9 AND VKORC1 GENOTYPES AS MODULATORY FACTORS OF WARFARIN EFFECTS IN CAUCASIAN PATIENTS WITH MULTIPLE DRUG REGIMEN. V. Michaud, PhD student, M. Harvey, K. Goodman, N. Morin, MSc, D. Brouillette, MSc, D. Roy, MD, L. Verret, MSc, N. Noel, MSc, I. Taillon, MSc, G. O'Hara, MD, D. Gossard, MSc, M. Champagne, A. Rosati, S. Simard, M. Vanier, M. Phillips, PhD, A. Ajami, PhD, J. Turgeon, PhD, Universite de Montreal, Xanthus Life Sciences, Montreal Heart Institute, Quebec Heart Institute, Hôpital Maisonneuve-Rosemont, Montreal, PQ, Canada.

**BACKGROUND/AIMS:** Factors such as CYP2C9 and VKORC1 polymorphisms have been used to explain intersubject variability in warfarin pharmacokinetics and effects. Studies have been performed mostly in healthy volunteers or in patients with multiple exclusion criteria. The objective of our study was to assess the role of phenotypic variables and CYP2C9 and VKORC1 polymorphisms in a true clinical setting; in hospitalized patients with multiple drug regimens.

**METHOD:** Patients recruited (n = 144) were taking on average 11.1 ± 4.2 drugs per day. Plasma samples were obtained at 3, 14 and 24 hours after the first dose of warfarin and R- and S-warfarin concentrations determined by a stereoselective LC-MS-MS assay. The three most common CYP2C9 allelic variants were analysed by PCR-RFLP using genomic DNA. Allelic variants of VKOR were determined by sequencing of selected samples. A multiple linear regression model was developed using as covariables phenotype, age, body surface area (BSA), R and S-warfarin levels, CYP2C9 and VKOR genotypes.

**RESULTS:** A major determinant of warfarin dose requirement was S/(S+R) warfarin ratio determined at 14 hours after the first dose. Mean warfarin daily dose was significantly higher among patients with VKORC1 6484CC genotype (2.01, 1.41 and 0.96mg/day/INR for 6484CC, CT and TT, respectively). Patients with VKORC1 9041GG genotype had lower warfarin dose requirement compared to carriers of at least one polymorphic allele (p = 0.02). When patients were stratified according to their CYP2C9 genotype, presence of one polymorphic allele (6484\*T) significantly influenced warfarin dose only in CYP2C9\*2 or CYP2C9\*3 subgroups.

**CONCLUSION:** Results obtained in our study confirm previous data suggesting that age, BSA, CYP2C9 and VKOR genotypes are major determinants of warfarin dose requirements. However, we observed that CYP2C9 activity is the main determinant in hospitalized Caucasian patients taking multiple drugs.

### PIII-24

THE INFLUENCE OF CYCLOOXYGENASE-1 AND GLYCOPROTEIN IIIA GENOTYPES ON ASPIRIN RESPONSE. K. M. Momary, PharmD, N. L. Shapiro, PharmD, L. Brace, PhD, S. S. Shord, PharmD, M. A. Viana, PhD, C. M. Helgason, MD, E. Nutescu, PharmD, L. H. Cavallari, PharmD, University of Illinois at Chicago, College of Pharmacy, Chicago, IL.

**BACKGROUND/AIMS:** Large meta-analyses have demonstrated the efficacy of aspirin in preventing death, MI, and stroke. However, there is substantial inter-patient variability in the inhibitory effects of aspirin on platelet aggregation. Incomplete platelet inhibition with aspirin has been associated with an increased risk of serious vascular events. We sought to determine whether the cyclooxygenase-1 (COX-1) C50T or glycoprotein (GP) IIIA P1<sup>A1/A2</sup> gene polymorphisms are associated with the inhibitory effects of aspirin on platelet aggregation.

**METHODS:** Sixty subjects on aspirin for primary or secondary prevention were enrolled. Blood was obtained for genotyping and determination of *ex-vivo* platelet aggregation (by the method of Born) and salicylate levels (by HPLC). Urine was obtained for assessment of 11-dehydrothromboxane B<sub>2</sub> (dTxB<sub>2</sub>) levels as an additional marker of aspirin response. Genotypes were determined by PCR/RFLP and capillary sequencing. Urine dTxB<sub>2</sub> levels were determined by ELISA via a commercially available assay. Aspirin response and characteristics were compared by genotype.

**RESULTS:** The genotype distributions were in Hardy Weinberg equilibrium. The frequencies of the variant COX-1 50T and GPIIIA P1<sup>A2</sup> alleles were 8% and 12%, respectively. Thirty (51%) subjects were classified as partial responders to aspirin by *ex-vivo* platelet aggregation. The median (range) dTxB<sub>2</sub> and salicylate levels for all subjects were 1156 (385–4424) pg/mg creatinine and 3.0 (0.055–17.5) µg/mL respectively. GPIIIA genotype was not associated with aspirin's effects on platelet aggregation as determined by either the method of Born or dTxB<sub>2</sub> levels. However, the frequency of partial responders to aspirin based on *ex-vivo* platelet aggregation analysis was greater among COX1 50C allele homozygotes compared to T allele carriers (57% vs. 20%;  $p = 0.04$ ). There were no significant differences in dTxB<sub>2</sub> levels, salicylate levels, GPIIIA genotype distribution, or demographic characteristics between COX-1 genotype groups.

**CONCLUSIONS:** Our data suggest that COX-1 genotype is associated with aspirin response as measured by *ex-vivo* platelet aggregation.

### PIII-25 and PIII-26 rescheduled to PI-102 and PI-103

### PIII-27

PROTHROMBIN GENE MUTATION 20209 IS NOT ASSOCIATED WITH STROKE IN PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE. K. A. Neville, MD, MS, K. A. Stegenga, RN, MSN, J. M. Rachel, MT(ASCP), MA, G. M. Woods, MD, S. D. Simon, PhD, M. L. Zucker, MD, Children's Mercy Hospital and Clinics, Saint Luke's Hospital, Kansas City, MO.

**BACKGROUND/AIMS:** Children with sickle cell disease have a greater risk of stroke than the general population. The role of genetic risk factors for thrombosis with regard to this complication remains unclear. The assay used to test for prothrombin mutations in previous studies of sickle cell patients with stroke detected the prothrombin 20210 mutation but not the 20209 mutation, a mutation that may have increased prevalence in African Americans and is likely related to thrombosis. The aim of this retrospective pilot study was to determine the prevalence of prothrombin 20209 and 20210 mutations in a population of pediatric patients with sickle cell disease and a history of overt ischemic stroke.

**METHODS:** A sample size of 20 patients was selected for this pilot study and would have reasonable precision with a prevalence of 25%, since the 95% confidence interval has a width of plus/minus 19%. Seventeen subjects followed in the Comprehensive Sickle Cell

Clinic at Children's Mercy Hospital (CMH) in Kansas City, Missouri with a past history of ischemic stroke as documented by MRI were enrolled in an IRB approved protocol and genotyped for prothrombin mutations 20209 and 20210 utilizing real time PCR amplification on a LightCycler instrument. Mutations present in PCR products were detected by melting curve analysis, which reliably detects both the 20210 G→A and 20209 C→T mutations.

**RESULTS:** Seventeen subjects were enrolled in the study. There were no variant alleles detected for the prothrombin gene at positions 20209 or 20210 (0%, CI = 0%–16%).

**CONCLUSION:** A mutation in the 20209 position of the prothrombin gene appears to be associated with an increased risk of thrombosis. However, this variant allele does not appear to be associated with ischemic stroke in children with sickle cell disease. Further studies are needed to determine what other genetic factors may be associated with stroke in these patients.

### PIII-28

IONTOPHORESIS WITH LASER DOPPLER FLOWIMETRY AS A PHARMACODYNAMIC TOOL FOR HISTAMINE RESPONSE. K. Neville, MD, MS, B. L. Jones, MD, S. M. Abdel-Rahman, PharmD, G. L. Kearns, PharmD, PhD, The Children's Mercy Hospital, Childrens Mercy Hospital, Kansas City, MO.

**BACKGROUND/AIMS:** Epicutaneous histamine (EH) is a well-established test used in clinical medicine. Despite its prior use to assess antihistamine effect, EH has significant limitations which limit its utility as a pharmacodynamic (PD) tool. Histamine iontophoresis with measurement of microvascular blood flow by laser Doppler flowimetry (LD) represents a potential alternative. The purpose of this study was to simultaneously compare EH and LD.

**METHODS:** 20 healthy adults (13 female, 24–45 yr) participated. Histamine iontophoresis (1% histamine at 50 µA for 10 s) was performed on the volar surface of the forearm and microvascular blood flow measured continuously by laser Doppler flowimetry (LD). EH was simultaneously placed on the contralateral extremity with manual assessment of wheal and flare response at standard intervals ( $n = 6$  over 6 hr). PD parameters ( $E_{max}$ ,  $tE_{max}$ ,  $tE_{50}$ ,  $tE_{max-tE_0}$  and AUEC where  $E =$  effect measured in mm for EH and flux for LD,  $t =$  time,  $max =$  maximal effect and  $tE_{50} =$  half maximal effect) were calculated for both LD and EH. Standard regression analysis methods were used to explore associations between LD and EH.

**RESULTS:** Evaluable data were available in 17 subjects. A significant linear association was observed between  $E_{max}$  and AUEC for both EH ( $r = 0.66$ ) and LD ( $r = 0.67$ ); reflective of internal validity. AUEC was also significantly correlated between EH and LD ( $r = 0.55$ ). When the other PD parameters ( $E_{max}$ ,  $tE_{max}$ ,  $tE_{50}$ ,  $tE_{max-tE_0}$ ) were examined between EH and LD, no statistically significant linear or nonlinear associations were found.

**CONCLUSION:** LD appears to be suitable for describing both magnitude and rate of response to histamine challenge. Absence of association for effect parameters between LD (continuous, dynamic measurement) and EH (static, manual measurement) was expected given the nature of each test. While additional validation will be required, LD may well provide a less invasive, more precise and dynamic tool for assessing antihistamine PD as compared to EH.

### PIII-29

CASOPITANT FOR THE PREVENTION OF POST-OPERATIVE NAUSEA AND VOMITING (PONV): POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS (PK/PD). B. M. Johnson, PhD, J. F. Hoke, PhD, R. Bandekar, PhD, L. M. Blackburn, RN, MS, J. Levin, MD, PhD, M. W. Russo, MD, PhD, GlaxoSmithKline, GlaxoSmithKline, Research Triangle Park, NC.

**BACKGROUND/AIMS:** NK-1 receptor antagonists have demonstrated efficacy in the treatment of PONV. The current study evaluates the PK and PK/PD of an NK-1 receptor antagonist, casopitant, after oral administration to female subjects undergoing surgery who are at high risk for PONV.

**METHODS:** Sparse PK data from a dose-ranging Phase II study were analyzed using a nonlinear mixed-effect model, including evaluation of significant covariates (1637 PK samples from 562 female subjects). Subjects received (in addition to ondansetron) placebo; 50, 100 or 150 mg single oral doses of casopitant prior to surgery. A graphical analysis was used to correlate post-hoc estimates of casopitant exposure with complete response rate (no emesis, no rescue therapy, and no premature withdrawal), time- to first emesis, and time- to rescue therapy.

**RESULTS:** A 2-compartment first-order model provided a good description of the PK of casopitant. The oral absorption of casopitant was rapid (lag-time of 13 min and absorption half-time of 16 min). The population estimate of apparent oral clearance was 24.4 L/h/70 kg and exhibited moderate intersubject variability (48%). Covariate analysis identified body-weight as a significant covariate of casopitant central volume of distribution, but not clearance. Age, race, and ethnicity did not correlate with casopitant PK parameters. Complete Response rate did not differ with casopitant exposure (AUC or concentration 24 h post-dose) over the dose range of 50–150 mg. Time-to-event analysis showed that the casopitant exposures produced by 50–150 mg doses provided adequate protection against emesis; however, subjects in the lowest quartile of casopitant exposure were at higher risk for requiring rescue medications.

**CONCLUSION:** The casopitant population PK model provided a good description of the observed data. While casopitant exposure did not correlate with complete response rate, time-to-event analysis suggested exposures produced by doses lower than 50 mg may not provide adequate protection against the need for rescue therapy, and suggests 50 mg may be a minimally effective dose of casopitant for the treatment of PONV in high-risk subjects.

### PIII-30

PHARMACOKINETICS AND PHARMACODYNAMICS (PK/PD) OF CASOPITANT, AN NK-1 RECEPTOR ANTAGONIST, IN PATIENTS UNDERGOING TREATMENT WITH MODERATELY AND HIGHLY-EMETOGENIC CHEMOTHERAPY. B. M. Johnson, PhD, J. F. Hoke, PhD, R. Bandekar, PhD, J. Levin, MD, PhD, M. W. Russo, MD, PhD, GlaxoSmithKline, GlaxoSmithKline, GlaxoSmithKline, Research Triangle Park, NC.

**BACKGROUND:** NK-1 receptor antagonists have demonstrated efficacy in the prevention of chemotherapy-induced nausea and vomiting (CINV) after administration of moderately- and highly-emetogenic chemotherapy (MEC and HEC, respectively). The current study evaluates the PK and PK/PD of casopitant after oral administration to patients undergoing treatment with MEC and HEC.

**METHODS:** Sparse PK data from two dose-ranging Phase II studies were combined and analyzed using a nonlinear mixed-effect model, including evaluation of significant covariates (2802 PK samples from 765 male and female subjects). Subjects received (in addition to ondansetron and dexamethasone) placebo; 50, 100 or 150 mg daily of oral casopitant for 3 days; or a single oral dose of 150 mg casopitant; starting prior to chemotherapy on Day 1. A graphical analysis was used to correlate post-hoc estimates of casopitant exposure with complete response (CR; no emesis, no rescue therapy, and no premature withdrawal) and time-to-emesis.

**RESULTS:** A 2-compartment first-order model adequately described the PK of casopitant; however a mixture model was required to adequately describe oral absorption of casopitant, which in general, was rapid; however 30% of subjects exhibited delayed and slow oral absorption. The population estimate of apparent

oral clearance was 17.4 L/h/70kg and exhibited large intersubject variability (72%) in this patient population. Covariate analysis identified body-weight as a significant covariate of casopitant clearance and central volumes of distribution. There was no conclusive evidence that age (up to 88 yrs), sex, race, and ethnicity correlate with casopitant PK. PK/PD analyses suggested casopitant exposure did not correlate with CR rate in subjects receiving MEC or HEC; however, subjects with lower casopitant AUC (1<sup>st</sup> and 2<sup>nd</sup> quartiles) receiving HEC were at increased risk of emesis, particularly female subjects and during the first 24 h.

**CONCLUSION:** These data suggest high concentrations (and NK-1 receptor occupancy) during the first 24 h may be important for adequate pharmacological response. Simulations suggest oral casopitant administered as a single dose of 150 mg, or followed by 50 mg doses on Days 2 and 3, should provide adequate receptor occupancy and efficacy for the prevention of CINV associated with MEC and HEC.

### PIII-31

POPULATION PHARMACOKINETICS OF ROSIGLITAZONE IN ADULT AND ADOLESCENT PATIENTS WITH TYPE 2 DIABETES MELLITUS. J. Chiu, PhD, B. M. Johnson, PhD, B. R. Patel, PhD, A. K. Miller, PhD, P. N. Mudd, PharmD, MBA, J. F. Hoke, PhD, GlaxoSmithKline, Research Triangle Park, NC.

**BACKGROUND:** Rosiglitazone (ROSI, Avandia<sup>®</sup>) is indicated for use in type 2 diabetes mellitus (T2DM) where it primarily increases sensitivity to insulin. A ROSI population pharmacokinetic (PK) model was developed using the combined PK data from 4 large Phase III studies conducted in adult and adolescent patients with T2DM.

**METHODS:** Studies 1, 2, and 3 were 3–5 arm randomized, placebo controlled, efficacy trials in adult patients (35–80 yrs). Study 4 was a 2 arm randomized, active controlled, study in adolescent patients (10–18 yrs). Sparse steady-state PK samples from multiple visits over a 26 week period were collected. A total of 1309 adult and 93 adolescent patients were included in the analysis (5476 PK samples). Dosing regimens consisted of 1, 2, or 4 mg ROSI administered twice a day or 4 or 8 mg once a day. Demographic data were collected including concomitant medications and use of alcohol and tobacco. Nonlinear mixed-effects modeling was performed using NONMEM. Inter- and intra-subject variability and study specific residual errors were estimated. A step-wise approach was used to evaluate significant covariates of ROSI PK. Visual predictive check and bootstrap procedures were implemented as part of the model evaluation process.

**RESULTS:** A 1-compartment first-order absorption model provided the best description of the observed data. A 2-subpopulation mixture model was included to characterize the oral absorption of ROSI, allowing better description of a small subpopulation of subjects (4.6%) that exhibited delayed (lag-time of 38 min) and slow absorption of ROSI (absorption half-time of 1.1 h). The population estimates of apparent oral clearance (CL/F) and volume (V/F) were 2.73 L/h/70 kg and 16.9 L/70kg, respectively, and exhibited low inter-subject variability (29 and 15%, respectively). Covariate analysis revealed that body surface area (BSA) was the best predictor of CL/F and V/F. After accounting for the effect of BSA, females had 9.1% lower CL/F than males and Caucasians had 10% larger V/F than other races. Age did not correlate with ROSI PK.

**CONCLUSION:** The ROSI population PK model was robust, and while several covariates were deemed statistically significant, none were considered clinically relevant. There was no significant difference in the PK of ROSI between adult and adolescent subjects with T2DM.

### PIII-32

A PHASE 1, SINGLE ASCENDING DOSE STUDY EVALUATING THE SAFETY, PHARMACOKINETICS AND PHARMACODYNAMICS OF SUBCUTANEOUS ADMINISTRATION OF AN ERYTHROPOIETIC MIMETIC ANTIBODY FUSION PROTEIN (CNTO 528) IN HEALTHY MALE SUBJECTS. E. Bouman-Thio, MD, K. Franson, PharmD, J. Burggraaf, MD, A. Cohen, MD, B. Miller, PhD, S. Bai, PhD, S. Marciniak, MBA, J. Yohrling, PhD, T. Jiao, MS, G. Shankar, PhD, K. Ramani, PhD, J. C. Marini, PhD, C. Pendley, PhD, J. Ford, PhD, J. Kowalchick, PhD, E. Bald, PhD, M. Frigo, PhD, B. Frederick, MS, H. Walker, PhD, A. Schantz, PhD, J. Getsy, MD, H. Davis, PhD, Centocor Research and Development, Inc., CHDR, Malvern, PA.

**AIMS:** To assess safety, pharmacokinetics (PK) and pharmacodynamics (PD) of a single SC dose of CNTO 528 in healthy male subj.

**METHODS:** In this randomized, single-site, single-blind, and pbo-controlled study, 16 subj. received a single SC injection of 20 mg (n = 6) or 40 mg CNTO 528 (n = 6) or pbo (n = 4).

**RESULTS: PD:** In subj. treated with CNTO 528, a dose dependent increase in reticulocyte counts and hemoglobin (Hgb) was observed. The Hgb and reticulocyte counts of the SC cohorts were compared to subj. receiving CNTO 528 via a single IV infusion. A near equivalent erythropoietic response was observed when similar doses of IV (mg/kg) or SC (mg) were given. In CNTO 528 treated subj. endogenous EPO concentration increased and then decreased below baseline values. A dose dependent increase in soluble transferrin receptor concentration was observed. Analysis of BFU-E and CD34+ showed no meaningful trend in CNTO 528 treated subj. compared to pbo. **PK:** The C<sub>max</sub> and AUC increased in a dose proportional manner. The mean terminal half-life was about the same for both cohorts (8.7 vs. 6.5 days). The apparent bioavailability, calculated from the mean IV clearance values in another study of three dose levels in different subj., was 110% for the 20 mg and 84% for the 40 mg dose. **Safety:** Administration of CNTO 528 was generally well tolerated. There were no serious adverse events and few CNTO 528-related adverse events (AEs). One subject (administered a single SC dose of 40 mg CNTO 528) had Hgb concentrations >17.8 g/dL, and was phlebotomized on Day 31 of the study. The most common AE across all groups was headache, occurring in 58.3% (7/12) of CNTO 528 treated vs. 50.0% (2/4) of subj. who received pbo. There was no indication that any patterns of AEs or significant laboratory, vital sign, or ECG abnormalities were associated with the administration of CNTO 528. **Immunogenicity:** All 12 subj. who received CNTO 528 were classified as negative for an immune response to CNTO 528.

**CONCLUSIONS:** Single SC administration of CNTO 528 was well tolerated and resulted in dose dependent erythropoietic response. No immunogenicity was observed. PK of SC CNTO 528 was linear and dose proportional. CNTO 528 proved to be safe and effective in elevating and maintaining serum levels of Hgb following a single SC injection to healthy male subj., with excellent bioavailability observed.

### PIII-33

A POPULATION PHARMACOKINETIC ANALYSIS OF ORAL CP-868,596, A HIGHLY SELECTIVE PLATELET-DERIVED GROWTH FACTOR RECEPTOR (PDGFR) INHIBITOR, IN PATIENTS WITH ADVANCED CANCERS. F. Guo, PhD, A. J. Olszanski, MD, A. Sharma, PhD, Pfizer Global R&D, New London, CT.

**AIMS:** To develop a population pharmacokinetic (PopPK) model for CP-868,596, an oral selective inhibitor of PDGFR, under development for the treatment of cancer.

**METHODS:** Concentration data (n = 590) from 35 cancer patients receiving daily oral CP-868,596 in 28-day cycles in an open label First-in-Human dose-escalation study were used. Cohorts of 60 mg BID, and 100, 200, and 280 mg QD administered on an empty stomach, and 60 mg and 100 mg BID administered with food were evaluated. Serum concentrations of CP-868,596 were obtained after

the 1<sup>st</sup> dose and multiple dose administration. Mixed effect analysis (FOCE) in NONMEM and bootstrapping were performed using the log-transformed concentration data. The effect of covariates was assessed. Diagnostic plots, decrease of objective function value ( $\geq 10.83$ ) and predictive check were used as model selection criteria.

**RESULTS:** A 2-compartment 1st-order absorption PK model with intersubject variability (ISV) on CL/F and relative bioavailability (F<sub>1</sub>), interoccasion variability (IOV) on F<sub>1</sub>, the co-administration with food as a categorical covariate on F<sub>1</sub>, and additive residual error was developed. Results revealed linear PK across the dose range evaluated. The ISV of both CL/F and F<sub>1</sub> was decreased by ~20% after adding IOV to F<sub>1</sub>. Inclusion of food as a covariate of F<sub>1</sub> reduced the ISV of F<sub>1</sub> by ~60%; the mean decrease of F<sub>1</sub> due to food was 65% (RSE: 27%) and 30% (RSE: 29%) at 60 and 100 mg BID, respectively. There was no evidence that weight, age, gender, ethnicity, and baseline liver function influenced CP-868,596 disposition.

**CONCLUSION:** The proposed PopPK model adequately described CP-868,596 PK profiles. Inclusion of food as a covariate of F<sub>1</sub> and adding IOV to F<sub>1</sub> improved the fit and reduced the variability of PK parameters. Future studies will further assess the apparent dose-dependent food effect on PK, and the clinical importance of the between-occasion PK variability. The proposed PopPK model could be used to design optimal sampling strategies for future studies.

### PIII-34

SUPERIOR *IN VIVO* BIOLOGICAL ACTIVITY OF ERYTHROPOIETIN FUSION PROTEIN PT-401. J. Jeong, PhD, C. Chen, PhD, K. L. Davis, A. Breidbach, D. H. Catlin, MD, H. J. Gomez, MD, PhD, A. J. Sytkowski, MD, Laboratory for Cell and Molecular Biology, Division of Hematology and Oncology, Beth Israel Deaconess Medical Center, Department of Medicine, Harvard Medical School, Olympic Analytical Laboratory, Department of Molecular and Medical Pharmacology, UC, UCLA Olympic Analytical Laboratory, Department of Molecular and Medical Pharmacology, UC, DNAprint Pharmaceuticals, Inc., Boston, MA.

**BACKGROUND/AIMS:** Chronic anemia due to renal failure, cancer, and other causes is frequently treated by administration of recombinant human erythropoietin (EPO, epoetin). Because it has a relatively short *in vivo* half-life in humans of 4–13 hours, frequent doses of EPO are required to achieve the desired therapeutic result. Modified versions of EPO are being developed to address this problem. We now report that the EPO fusion protein PT-401 has significantly enhanced *in vivo* activity in mice over that exhibited by conventional epoetin, suggesting a therapeutic advantage in anemia treatment.

**METHODS:** Recombinant PT-401 was expressed in Chinese hamster ovary (CHO) cells under a CMV promoter with a signal peptide present at the amino terminus. The two EPO domains in PT-401 were connected in tandem with either 3 or 4 repeats of the amino acid sequence GGGGS as a 15 or 20-amino acid linker sequence, respectively. The protein was purified to near homogeneity by sequential column chromatography.

**RESULTS:** The expression levels of PT-401 in supernatant protein-free medium from cloned CHO cells ranged from 4 to 40 mg/L determined by EPO-ELISA, and from  $2.0 \times 10^5$  to  $4.5 \times 10^6$  IU/L determined by *in vitro* bioassay. CHO cell clones producing PT-401 with the greatest extent of glycosylation, as indicated by SDS-PAGE and isoelectric focusing, were selected. Subcutaneous injection of mice with three doses (days 1, 3 and 5) of PT-401 resulted in percent increases in mean hematocrit of 32.6% (300 IU/kg) or 18.2% (100 IU/kg), while equivalent IU doses of conventional epoetin increased mean hematocrit by 12.5% (300 IU/kg) or 6.4% (100 IU/kg). Importantly, a single dose of PT-401 (100 IU/kg) increased their mean hematocrit by 4.3% within 7 days, while an equivalent unit dose of epoetin had no effect. Additionally, three doses of a different EPO fusion protein under development increased the mean hematocrit by

8.83% per IU injected, which was much greater than that observed with conventional epoetin (0.69%).

**CONCLUSION:** The results show that PT-401 and other EPO-fusion proteins exhibit biological activities superior to those of epoetin, implying significant clinical advantages.

### PIII-35

AN IN-SILICO MODEL FOR PREDICTING THE ERYTHROPOIETIC RESPONSE TO THE NOVEL EPO DIMER PT-401. N. Kabrun, PhD, B. L. Handelin, PhD, H. J. Gomez, MD, PhD, L. T. Herren, PhD, DNAPrint Genomics, Inc, Sarasota, FL.

**BACKGROUND:** Erythropoietin (EPO) and EPO-derived drugs are useful to treat various types of anemia caused by decreased endogenous erythropoietin production and response. However, frequent dosing regimens and patients refractory to treatment has led to the development of novel therapeutics with increased half-life and potency. PT-401, a novel EPO dimer, represents this new class of drug. In order to predict the PK and PD behavior of PT-401 prior to clinical trials, we constructed a computer simulation, EpoFusion, which models the physiological behavior induced by EPO-like drugs.

**METHODS:** EpoFusion was developed with our patented BioFusion<sup>®</sup> methodology, which couples structured knowledge and data synthesis with modeling and simulation. This flexible approach allows representation and simulation of the physiological processes underlying the drug's mechanism-of-action. The central components of EpoFusion are a mechanistic simulation of erythropoietin clearance by receptor binding, internalization, and degradation and a simulation of erythroid maturation and differentiation from BFU-E to RBCs in response to EPO-derived drugs and other biochemical mediators. All parameters in the model are modifiable, supporting prediction of PT-401 clinical effects based on preclinical in-vitro study-derived parameters.

**RESULTS:** EpoFusion was validated against published data for Epoetin alfa treatment of chronic kidney disease patients. The simulation recapitulated PK parameters, e.g. 100 U/Kg IV dose (AUC Reference: 131.9 +/- 8.3 ng h/ml, AUC Model: 134.0 ng h/ml;  $t_{1/2}$  Reference: 8.5 +/- 2.4 hr,  $t_{1/2}$  Model: 8.3 hr).<sup>1</sup> EpoFusion also recapitulated the change in hematocrit (HCT) with 3 times weekly doses of 15, 50, 150, and 500 U/kg IV for 6–12 weeks.<sup>2</sup> The rate of change in HCT obtained by a preliminary model for the four regimens was an average of 94.5% +/- 6.8% of the rate of change observed in the reference data for the corresponding dosing regimens.

**CONCLUSIONS:** EpoFusion accurately reproduces effects of Epoetin alfa therapy in anemic renal disease patients. Additionally, it provides an extensible computational platform that will be used to predict the erythropoietic effects of PT-401 prior to patient administration.

<sup>1</sup> Macdougall *et al.*, J Am Soc Nephrol 1999 10:2392

<sup>2</sup> Eschbach *et al.*, NEJM 1987 316:73

### PIII-36

ERYTHROPOIETIN STIMULATES HUMAN CANCER CELL MIGRATION AND ACTIVATES RHO A THROUGH A MAPK/ERK-DEPENDENT MECHANISM. S. N. Hamadmad, PhD, R. J. Hohl, MD, PhD, University of Iowa, Iowa City, IA.

**BACKGROUND:** Erythropoietin (Epo) receptor (EpoR) is expressed in several human cancers and the functional consequence of this expression is under extensive study. Recent studies have shown that Epo signaling might contribute to the survival, invasion, and drug resistance of cancer cells. Epo is routinely given to cancer patients to counteract the anemia associated with the cancer and/or chemotherapy. Here we investigate the role that Epo plays in cervical cancer and the functional consequences of EpoR expression in these cells.

**METHODS:** We use a human cervical cancer cell line in which EpoR was shown to be expressed. Cell migration was studied using a transwell migration system. Activation of several signaling pathways

was studied by Western blotting. Rho activation assay was performed by pull-down experiments with GST-Rhotekin-RBD fusion protein. Several pharmacological and genetic inhibitors were used to perturb signaling pathways and characterize those that are required for mediating Epo effects.

**RESULTS:** We demonstrate that Epo acts as a chemoattractant for HeLa cancer cells enhancing their migration under serum starved conditions. We show that the effects of Epo are dose- and time-dependent and that they require the activity of two signaling pathways: the MAP kinase and the RhoA GTPase pathways. We demonstrate that Epo activates both pathways in a Jak2-dependent manner and that this activation is required for Epo effects on cell migration. Furthermore, using both pharmacological and genetic inhibitors we demonstrate that the activation of RhoA GTPase is dependent on the activity of the MAP kinase pathway providing the first evidence for interaction between these two signaling cascades.

**CONCLUSION:** This study further extends the role of EpoR in cancer cells and shows that Epo, through activation of several signaling pathways, enhances cancer cell migration. These results also suggest that targeting the Epo pathway or more specifically the MAPK pathway can be a mechanism for inhibition of cancer cell migration in vivo, which is an essential step for the process of cancer metastasis. Both pharmacological inhibitors and antisense techniques might prove efficient for this in the future.

### PIII-37

THE MUCOSALLY-RESTRICTED ANTIGEN GUANYLYL CYCLASE C (GCC) IS A TARGET FOR METASTATIC COLORECTAL CANCER IMMUNOTHERAPY. A. E. Snook, P. Li, J. P. McGettigan, PhD, G. S. Tan, L. Huang, PhD, R. Birbe, MD, S. Schulz, PhD, M. J. Schnell, PhD, J. L. Rothstein, PhD, L. C. Eisenlohr, VMD, PhD, S. A. Waldman, MD, PhD, Thomas Jefferson University, Philadelphia, PA.

**BACKGROUND:** Asymmetry in cross-talk between central and mucosal immune compartments suggests that proteins confined to mucosae engender local, but not central, tolerance. Thus, central vaccination to mucosal antigens should yield immunity against derivative metastatic tumors without autoimmunity. GCC is a receptor for bacterial diarrheagenic enterotoxins whose expression is restricted to intestinal mucosae, but which is over-expressed by metastatic colorectal tumors. Here, GCC immunogenicity in the central compartment was explored to define its utility as a vaccine target for metastatic colorectal cancer.

**METHODS:** The ability of vaccines incorporating the extracellular domain of murine GCC (GCC<sub>ECD</sub>) to induce GCC<sub>ECD</sub>-specific B and T cell responses was quantified in mice. Further, the anti-tumor efficacy of GCC<sub>ECD</sub> vaccination was defined in murine models of subcutaneous and parenchymal colorectal cancer. Moreover, intestinal autoimmunity in the context of anti-tumor immunity was evaluated in GCC<sub>ECD</sub>-vaccinated mice.

**RESULTS:** GCC<sub>ECD</sub>-based vaccines induced robust antigen-specific B and T cell responses in mice. Indeed, T<sub>CD4+</sub> and T<sub>CD8+</sub> cell responses to GCC<sub>ECD</sub> were quantitatively similar to those to bacterial antigens, suggesting that GCC<sub>ECD</sub> is equi-immunogenic to foreign antigens. GCC-protein based vaccines produced a Th2 response characterized by antigen-dependent T<sub>CD4+</sub>-specific cell proliferation and IL-4 production. Similarly, GCC-virus based vaccines produced a Th1 response characterized by antigen-dependent T<sub>CD4+</sub>-specific IFN $\gamma$  production. GCC-virus based vaccines also produced a T<sub>CD8+</sub> response characterized by antigen-dependent T<sub>CD8+</sub>-specific IFN $\gamma$  production. Importantly, GCC-virus based vaccines induced potent anti-tumor immunity in the absence of autoimmunity, inhibiting tumor growth and prolonging survival in a subcutaneous colorectal cancer model and reducing tumor burden in liver and lung models of metastatic colorectal cancer.

**CONCLUSION:** This study reveals for the first time the potent central immunogenicity of mucosally-restricted antigens in the

absence of autoimmunity, suggesting their general utility as novel immune targets for metastatic tumors. Moreover, they underscore the utility of GCC as a specific vaccine target for metastatic colorectal cancer.

### PIII-38

PHASE 1 INVESTIGATION OF INTRAVENOUS ARTESUNATE IN HEALTHY VOLUNTEER SUBJECTS. L. R. Cantilena, Jr., MD, PhD, G. A. Saviolakis, MD, PhD, K. J. Leary, MD, R. S. Miller, MD, A. S. Haeberle, PhD, P. J. Weina, PhD, MD, Uniformed Services University of the Health Science, Walter Reed Army Institute of Research, Bethesda, MD.

**BACKGROUND/AIM:** The currently available parenteral agents for the treatment of severe malaria worldwide and in the United States are quinine or quinidine. Quinine is a quinolinemethanol that is a component of the bark of cinchona trees, and quinidine is the d-isomer of quinine. Both of these agents are effective but the efficacy of quinine is declining and the cardiac risk of IV quinidine is well known. Artemisinins are antimalarial sesquiterpenes from the plant *Artemisia annua* and have documented efficacy against malaria infection. Despite extensive use in Southeast Asia, no FDA approved, GMP formulation for intravenous artesunate (ART) has been available for systematic study in the US until recently. The present study was undertaken to establish the safety of a new GMP ART formulation.

**METHODS:** An ascending, inpatient, single IV dose, double blinded, placebo-controlled phase 1 investigation with ART was conducted in healthy male (32) and female (8) volunteers between 18 and 55 years of age grouped into 5 cohorts of 8 subjects. In each cohort, 6 subjects received ART and 2 received matching placebo IV infusion over 2 minutes. The ART dosage levels for each successive cohort were: 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg. Subjects were intensively monitored immediately before and for 24 hours after treatment. Baseline and serial ECG, hemodynamic and clinical observations were performed during the inpatient and 3 subsequent outpatient follow up visits.

**RESULTS:** No dose limiting toxicity was found for ART at the doses studied. There were no subject drop outs for adverse events or treatment related issues. A dose related decrease in reticulocyte count was noted that peaked 4 days after dosing and returned to normal by study day 7 in most cases. There were no other clinically significant laboratory abnormalities detected. No deleterious hemodynamic or electrocardiographic effects were seen. A transient, reversible sensation of altered or unusual taste, which lasted less than 30 minutes in all cases, was associated with higher doses of ART. All remaining side effects were generally mild and all were reversible.

**CONCLUSION:** Single dose treatment with this formulation of ART was well tolerated in healthy volunteers at the doses studied. Further study with multiple dose regimens and therapeutic use in malaria patients is needed.

### PIII-39

UTILITY OF QUANTITATIVE REAL-TIME PCR TO EVALUATE ANTIMICROBIAL ACTIVITY AND PHARMACODYNAMICS IN *STAPHYLOCOCCUS AUREUS*. J. C. Yang, PharmD, Y. Harigaya, PharmD, D. Brazeau, PhD, A. Forrest, PharmD, P. A. Kelchlin, D. Letina, C. E. Haas, PharmD, P. F. Smith, PharmD, B. T. Tsuji, PharmD, University at Buffalo, Buffalo, NY.

**BACKGROUND:** The use of PCR has increased the speed an accuracy for the identification of *S. aureus*. However, limited investigations have evaluated the use of real time quantitative PCR (qPCR) to evaluate antimicrobial pharmacodynamics (PD) in *S. aureus*. We evaluated the utility of RT-PCR as a tool to evaluate

killing activity and study PD by profiling the *femA* gene in *S. aureus* in response to exposure to vancomycin.

**METHODS:** Methicillin Resistant *Staphylococcus aureus* COL was studied. Time kill experiments were performed for MRSA in log phase growth at  $10^6$  cfu/ml to evaluate bacterial killing in relation to increasing concentrations of vancomycin (0, 1, 2, 4, 8, 16, 32, 64 times the MIC). Serial samples were obtained over 24h to quantify bacterial burden by CFU/ml compared to cycle threshold (CT) by using qPCR. Oligonucleotide primers were designed to amplify a unique conserved region of the *femA* gene in *S. aureus*. Detection was done using SYBR Green. PD analysis was performed by fitting change from baseline in  $\text{Log}_{10}$  CFU/mL at 24 h or  $C_T$  vs. vancomycin concentration: MIC were to a Hill-type model.

**RESULTS:** A strong inverse linear relationship between  $C_T$  and  $\text{Log}_{10}$  CFU/ml was evident, pearson's correlation coefficient = -0.9. *femA* gene expression decreased with increasing concentrations of vancomycin exposure. Vancomycin concentrations  $\geq 8 \times \text{MIC}$  against MRSA demonstrated mean reductions in 24 h CFU  $\log_{10}/\text{ml}$  of 4.9. At concentrations  $\geq 8 \times \text{MIC}$  and achieved killing at undetectable limits; however, relative changes in *femA* expression were present. The EC50 as predicted by CFU/ml = 2.3 was in good agreement with that predicted by qPCR = 2.5.

**CONCLUSIONS:** This study demonstrates qPCR provides rapid and reproducible results that are consistent with those that are obtained by conventional methods. The use of qPCR may have utility where quantification of bacteria are necessary below the limit of detection of traditional counts or in the clinical microbiology laboratory. These data are promising and warrant the basis for further investigations in human bacterial infection.

### PIII-40

PHARMACOKINETICS (PK) OF SILYMARIN (SM) IN HEALTHY VOLUNTEERS AND PATIENTS WITH VIRAL HEPATITIS C (HCV). S. J. Schrieber, PharmD, Z. Wen, PhD, T. E. Dumas, PharmD, M. V. Vourvahis, PharmD, P. C. Smith, PhD, A. D. Kashuba, PharmD, M. W. Fried, MD, R. L. Hawke, PhD, PharmD, University of North Carolina at Chapel Hill, Chapel Hill, NC.

**BACKGROUND/AIMS:** SM, an extract of milk thistle utilized by patients to self-treat liver disease, is comprised primarily of 6 flavonolignans: silybin A and B (SBA and SBB); isosilybin A and B (ISBA and ISBB); silychristin (SC); and silydianin (SD). Previous investigations have only reported the PK for the silybins in healthy subjects. To evaluate the effect of liver disease on SM disposition, we determined the PK for these flavonolignans in patients with chronic HCV and non-cirrhotic (NC) or cirrhotic (C) disease, and in healthy (H) subjects.

**METHODS:** Subjects (H, n = 5; NC, n = 4; C, n = 3) received a single oral 600 mg dose of milk thistle (standardized to 480 mg SM) in a fasted state and 15 blood samples were obtained over 24 h. Plasma concentrations of each flavonolignan were measured by LC-MS before (Free) and after enzymatic hydrolysis with  $\beta$ -glucuronidase + sulfatase (Total). PK parameters were determined for Free, Total, and flavonolignan conjugates (calculated as Total - Free) using a noncompartmental approach with WinNonlin<sup>®</sup>4.1. Relative exposures ( $\text{AUC}_{\text{Rel}}$ ) for each flavonolignan were expressed as a percent of the sum of exposure to all flavonolignans (Sum AUC).

**RESULTS:** Concentrations for Total flavonolignans (2–852 ng/ml) were ~5-fold greater than for Free (2–159 ng/ml). Therefore data are presented in Table 1 for flavonolignan conjugates.  $C_{\text{max}}$  and AUC 0–24hr for conjugates were ~1.7 and 5, and ~2.4 and 5.8-fold higher for NC and C respectively, compared to H. The  $\text{AUC}_{\text{Rel}}$  for SBB and ISBA conjugates accounted for  $\geq 45\%$  exposure to all flavonolignans for all groups. The  $t_{1/2}$  for flavonolignan conjugates in H was ~5–6 h, except for SC and SD which  $t_{1/2}$  was ~9 h, while  $t_{1/2}$  for HCV patients was more variable (3.5–14 h).

**Table 1.** Mean  $\pm$  SD AUC 0–24 hr (ng\**h*/ml) and (% AUC<sub>Rel</sub>) for SM Conjugates

	SBA	SBB	ISBA	ISBB	SC	SD	Sum AUC
H n = 5	187 $\pm$ 83 (8.5)	621 $\pm$ 245 (28.3)	537 $\pm$ 350 (24.5)	262 $\pm$ 160 (11.9)	449 $\pm$ 212 (20.4)	141 $\pm$ 60 (6.4)	2197 $\pm$ 774 (100)
NC n = 4	147 $\pm$ 114 (2.8)	1200 $\pm$ 1046 (22.7)	1187 $\pm$ 1035 (22.5)	652 $\pm$ 541 (12.3)	1833 $\pm$ 1629 (34.7)	262 $\pm$ 176 (5.0)	5281 $\pm$ 4362 (100)
C n = 3	789 $\pm$ 490 (6.2)	3836 $\pm$ 2778* (29.9)	3790 $\pm$ 2414 (29.6)	1755 $\pm$ 1078 (13.7)	1937 $\pm$ 878 (15.1)	714 $\pm$ 612 (5.6)	12821 $\pm$ 7119 (100)

\*H vs C p < 0.05, ANOVA.

**CONCLUSION:** This is the first study to report PK for the major SM flavonolignans in healthy subjects and patients with chronic HCV. Higher SM exposures were observed in patients with liver disease due to increased levels of SM conjugates. These results suggest that SBB, ISBA, and SC may be the primary contributors to the antioxidant effects of SM and should be monitored in clinical trials evaluating the safety and efficacy of SM in liver disease. Supported by NIH grants K24 DK066144 and GCRC RR00046.

### PIII-41

**SAFETY, TOLERABILITY AND PHARMACOKINETICS OF R1626, A NOVEL NUCLEOSIDE ANALOG TARGETING HCV POLYMERASE: RESULTS FROM A PHASE 1 SINGLE DOSE ESCALATION TRIAL IN HEALTHY SUBJECTS.** R. Robson, H. Berns, M. Brandl, S. Fettner, G. Hill, D. Ipe, J. Love, M. Mannino, E. O'Mara, Y. Tu, C. Washington, PhD, Christchurch Clinical Studies Trust, Roche Pharmaceuticals, Roche Pharmaceuticals, Christchurch, New Zealand.

**BACKGROUND/AIMS:** R1479 is a cytidine nucleoside analog, which has been identified as a selective and potent HCV replication inhibitor in vitro. R1626 is a prodrug of R1479 and was designed to significantly improve the oral bioavailability of R1479. The objectives of this study were to evaluate the safety, tolerability and pharmacokinetics of R1479 following single dose administration of R1626 in up to 5 dose levels in healthy subjects.

**METHODS:** A total of 40 healthy male subjects (8 per dose cohort, age range: 18–34 years) were randomized 3:1 to treatment with R1626 or placebo. Subjects received a single oral dose of placebo or 500, 1500, 3000, 6000, or 12000 mg R1626 and were followed up for safety, tolerability and pharmacokinetic assessments for 7 days.

**RESULTS:** R1626 was extensively converted to R1479. R1479 exposure (C<sub>max</sub> and AUC) was linear and near dose-proportional for all doses studied. Mean C<sub>max</sub> of R1479 ranged from 2.96–46.4  $\mu$ g/mL and mean AUC<sub>0– $\infty$</sub>  ranged from 29.5–422.2  $\mu$ g-h/mL. The mean T<sub>max</sub> ranged from 2.7–5.5 hours and mean terminal half-life ranged from 19–28 hours.

R1479 was the major component excreted in urine. Approximately 58–73% (molecular equivalent) of R1626 was recovered in urine as R1479. The renal clearance of R1479 was 7.3–9.9 L/h, similar to glomerular filtration rate.

R1626 was well tolerated at all doses up to 12000 mg. There were no serious adverse events (SAEs) and most AEs were of mild intensity. The most frequently reported AEs were headache and irritation at the site of ECG electrode application. There were no clinically significant changes in laboratory parameters and no clinically relevant changes in vital signs at any dose level.

**CONCLUSIONS:** Orally administered R1626 was efficiently absorbed and converted to R1479, the target moiety, with linear and a near dose proportional pharmacokinetic profile. R1626 was well tolerated up to the highest single dose of 12000 mg administered in healthy subjects.

### PIII-42

**COMPARISON OF THE PHARMACOKINETICS OF ENTECAVIR IN HBV MONO-INFECTED AND HIV/HBV CO-INFECTED SUBJECTS USING POPULATION PHARMACOKINETIC MODELING.** X. Xu, PhD, M. Zhu, PhD, J. Yan, PhD, M. Bifano, Y. Wang, O. Setiadi, F. LaCreta, D. Grasela, M. Pfister, Bristol Myers Squibb, Princeton, NJ.

**BACKGROUND/AIMS:** Entecavir, a nucleoside analogue, was recently approved for the treatment of chronic hepatitis B (HBV). Pharmacokinetics (PK) of entecavir in HIV/HBV co-infected patients was characterized with modeling approach and entecavir exposures were compared in HIV/HBV co-infected and HBV mono-infected subjects in this analysis.

**METHODS:** Study AI463038 was a randomized, double-blind, placebo-controlled Phase II trial designed to evaluate the safety and efficacy of entecavir at 1-mg daily dose in HIV/HBV co-infected patients who had hepatitis B viremia while on lamivudine-containing highly active antiretroviral therapy. The study consisted of a 24-week double-blind, placebo-controlled phase followed by a 24-week open-label phase. Sparse PK samples were collected on Day 1 and during Weeks 2, 12, 24, 36, and 48. This PK dataset (256 data points, 58 subjects) was pooled with data collected from 222 HBV mono-infected subjects (1244 data points), and analyzed with nonlinear mixed effects modeling using NONMEM<sup>®</sup>. “Subject population” was tested as a covariate to evaluate the PK of entecavir in HIV/HBV co-infected and HBV mono-infected subjects. Entecavir exposures in the two patient groups were compared and evaluated by a general linear model.

**RESULTS:** A two-compartment structural model adequately described entecavir PK. The estimated clearance (CL/F), inter-compartment clearance, volume of distribution in central and peripheral compartments were 12.5 L/h, 330 L/h, 115 L and 1830 L, respectively; and inter-individual variability were 46.8, 30.2, 39.2 and 74%, respectively. “Subject population” was found to be an insignificant covariate of CL/F. The geometric mean (GM) of steady state entecavir AUC following 1-mg daily doses in HIV/HBV co-infected subjects was 39.3 ng-h/mL, which was similar to the GM of steady state AUC (dose-normalized to 1 mg) of 38.8 ng-h/mL in HBV mono-infected subjects. The GM ratio was 1.01 with 90% confidence interval (0.91, 1.12) that was within the bioequivalence boundary of 80% to 125%.

**CONCLUSIONS:** The proposed model adequately described the PK of entecavir in both HIV/HBV co-infected and HBV mono-infected subjects. Following 1-mg daily doses, entecavir exposure in the HIV/HBV co-infected subjects was comparable to that in HBV mono-infected subjects.

### PIII-43

**RIFAMPIN ENHANCES EFAVIRENZ METABOLISM IN HEALTHY VOLUNTERS: IMPLICATIONS FOR HIV THERAPY.** Z. Desta, PhD, J. A. Morgan, BSc, L. Li, PhD, B. A. Ward, BSc, S. M. Lemler, RN, S. D. Hall, PhD, D. A. Flockhart, MD, PhD, S. Shen, MD, PhD, Indiana University School of Medicine, Indianapolis, IN.

**BACKGROUND:** Efavirenz in combination with two nucleoside reverse transcriptase inhibitors is a preferred initial treatment for HIV infection. However, treatment with efavirenz-based therapy is made difficult by large interindividual variability in efavirenz pharmacokinetics, and a number of drug interactions contribute to this. Efavirenz is predominantly metabolized by CYP2B6, an enzyme system that is highly induced by rifampin. Since rifampin is frequently prescribed with antiretroviral drugs to treat tuberculosis, it is possible that rifampin reduces effective efavirenz concentration and probably its antiretroviral efficacy.

**METHOD:** In a randomized, double-blinded, placebo controlled crossover design, we determined the effect of rifampin on efavirenz metabolism and pharmacokinetics. Ten female and 10 male subjects (age: 18 to 49 years) were treated with placebo or 600 mg/day rifampin for 10 days. On day 11, a single 600 mg oral dose of

efavirenz was administered with ( $n = 13$ ) and without ( $n = 7$ ) rifampin. Efavirenz, 8-hydroxyefavirenz and rifampin concentrations in plasma (0.5 to 72 h) and urine (12 h) were determined by HPLC. CNS symptoms were assessed using standard questionnaire.

**RESULTS:** Rifampin treatment increased efavirenz oral clearance (2.6-fold) and decreased efavirenz  $AUC_{0-\infty}$  (3.0-fold) and  $C_{max}$  (1.8-fold) compared to placebo pretreatment ( $p < 0.001$ , paired  $t$ -test, two-tailed). Accordingly, there was a marked increase in the plasma concentrations of 8-hydroxyefavirenz ( $AUC_{0-12h}$ ,  $p = 0.0008$ ) and in the amount excreted in urine within the first 12 h of post dosing (amount excreted within the first 12 h post dosing,  $p = 0.007$ ). We observed a significant correlation between baseline efavirenz  $AUC_{0-\infty}$  and rifampin induced efavirenz exposure changes ( $r = 0.65$ ,  $p < 0.002$ ). Rifampin  $AUC_{0-24h}$  was negatively correlated with changes in efavirenz exposure ( $r = 0.76$ ,  $p = 0.007$ ). The incidence of efavirenz-induced CNS side effects appear to be greater in placebo than rifampin treated group.

**CONCLUSIONS:** Our data suggest that rifampin enhance the metabolism of efavirenz in vivo, probably through induction of CYP2B6, and may reduce the antiretroviral efficacy of efavirenz. Efavirenz is a sensitive in vivo CYP2B6 probe and can be used to study the role CYP2B6 plays in human drug metabolism.

### PIII-44

POTENT INHIBITION OF CYTOCHROME P450 (CYP) 2B6-CATALYZED REACTIONS BY VORICONAZOLE IN VITRO. Z. Desta, PhD, B. A. Ward, BSc, P. D. Nguyen, BSc, Indiana University School of Medicine, Indianapolis, IN.

**BACKGROUND:** Voriconazole is a second-generation triazole with potent activity against a broad spectrum of clinically significant fungal pathogens, including *Aspergillus*, *Cryptococcus*, and *Candida* species. Voriconazole is metabolized predominantly by CYP2C19 (and to a small extent by CYP2C9 and CYP3A) and has been shown to inhibit CYP3A, CYP2C19 and CYP2C9 in vitro (IC<sub>50</sub>s, ~8–10  $\mu$ M) and in vivo. As a result, multiple drug interactions with substrates of these enzymes are of major concern. Voriconazole does not appear to inhibit other P450s such as CYP1A2, CYP2D6 and CYP2E1, but little information is available regarding its interaction with CYP2B6. Using human liver microsomes (HLMs) and expressed CYP2B6, we characterize the inhibitory effect of voriconazole on CYP2B6 activity.

**METHODS:** CYP2B6 probe substrates (bupropion, efavirenz and 8-hydroxyefavirenz) were incubated with HLMs (or CYP2B6) in the presence and absence of voriconazole to estimate inhibition constants (IC<sub>50</sub>s, Ki values). Preincubation of the test inhibitor for different times with HLMs (or CYP2B6) and NADPH was performed to gain insight in to the mechanisms of inhibition.

**RESULTS:** Voriconazole potently inhibited CYP2B6 activity as measured by bupropion 4-hydroxylation, 8-OHEFV 14-hydroxylation and efavirenz 8-hydroxylation (IC<sub>50</sub> values respectively were: 1.19  $\mu$ M, 0.76  $\mu$ M and 3.68  $\mu$ M in HLMs; and 3.74  $\mu$ M, 1.86  $\mu$ M and 17.5  $\mu$ M in expressed CYP2B6). The extent of inhibition of CYP2B6 did not differ when voriconazole was preincubated for 0–30 min with HLMs and NADPH before the addition of the substrates. We found no evidence that voriconazole N-oxide, a major metabolite of voriconazole, is involved in CYP2B6 inhibition. Detailed inhibition experiments in HLMs show that voriconazole is a highly potent competitive inhibitor of CYP2B6-catalyzed reactions, with Ki values (<1  $\mu$ M) much lower than the average plasma concentration of voriconazole at steady state (~10  $\mu$ M).

**CONCLUSIONS:** Voriconazole would be anticipated to markedly slow the clearance of drugs predominantly metabolized by CYP2B6; approximately 10-fold change in CYP2B6-catalyzed pathway is predicted. Since voriconazole is relatively more potent inhibitor of CYP2B6 than any of the drug metabolizing CYPs, it could serve as a valuable tool to study CYP2B6 function in vivo.

### PIII-45

A NOVEL METHOD DEMONSTRATING PROTEIN UNBOUND INDINAVIR (IDV) CONCENTRATION IN SEMINAL PLASMA. Y. Cao, MB, T. Parsons, PhD, R. P. Bakshi, PhD, E. J. Fuchs, PA-C, MBA, A. M. Guidos, RN, E. Martinez, BSN, A. D. Kashuba, PharmD, C. W. Hendrix, MD, School of Medicine, Johns Hopkins University, School of Pharmacy, University of North Carolina, Baltimore, MD.

**BACKGROUND/AIMS:** Seminal transmission of HIV is responsible for the majority of HIV incident cases. Moreover, resistance to antiretroviral (ARV) drugs may develop in the male genital tract. Although efforts have been made to characterize the pharmacokinetics of ARVs in semen, the extent of the binding of ARVs to seminal proteins has not been established and the free drug exposure in semen is unknown. Using indinavir (IDV) as a tool, we aimed to 1) establish a method for estimating the fraction of protein-free drug in volume-limited, viscous seminal plasma and 2) determine the extent of ARV binding in semen from volunteers.

**METHODS:** An assay to quantify protein-free drug was developed using a 96-well plate ultrafiltration method (for the collection of free drug) and LC/MS/MS (for drug detection). A single-sequence trial was conducted in an outpatient research clinic using 6 HIV-infected subjects and 6 healthy subjects. Paired semen and blood samples were collected 2 hours after IDV dosing (steady-state with 200 mg q2h for healthy subjects and a single 800 mg dose for HIV-infected subjects). Data are presented as mean (95% confidence interval).

**RESULTS:** The change of drug concentration in ultrafiltrate with centrifugation time was modeled, allowing collection of >40  $\mu$ L of ultrafiltrate from a 100  $\mu$ L sample for drug analysis while minimizing the bias inherent with other methods. In the clinical study, total IDV concentration in seminal plasma was half of that in blood plasma ( $p = 0.04$  for HIV-infected subjects and  $p = 0.05$  for healthy subjects by paired  $t$ -test; Table). The fraction of free IDV in semen from HIV-infected subjects averaged 2.0 (1.5–2.7) fold that in blood plasma. The fraction of free indinavir in semen from healthy subjects averaged 2.8 (2.3–3.3) fold that in blood plasma. Thus, the free IDV concentration in blood and semen was not statistically different ( $P > 0.05$ ).

**CONCLUSIONS:** Samples with limited volume and challenging physical properties such as semen can be assayed for free drug concentrations by ultrafiltration using 96-well plates followed by LC/MS/MS analysis. A greater fraction of IDV in semen is protein-free compared to blood plasma: free IDV concentration can be similar in blood and semen.

**Table:** IDV concentration ( $\mu$ g/mL) in blood and seminal plasma

	Semen (HIV positive)	Blood (HIV positive)	Semen (HIV negative)	Blood (HIV negative)
Total IDV (g/ml)	2.5 (1.4–4.7)	4.7 (3.8–5.7)	0.89 (0.34–2.3)	1.6 (0.91–2.9)
Adjusted free IDV (g/ml)	1.9 (0.86–4.4)	1.9 (1.7–2.2)	0.67 (0.26–1.7)	0.49 (0.29–0.86)
Fraction of free IDV	0.73 (0.58–0.93)	0.37 (0.33–0.41)	0.72 (0.63–0.82)	0.26 (0.23–0.29)

### PIII-46

FOOD DECREASES THE EXPOSURE OF ZIDOVUDINE (ZDV) ADMINISTERED VIA CONTINUOUS INTRAVENOUS INFUSION. Y. Cao, MB, T. Ndovi, MD PhD, C. V. Fletcher, PharmD, A. M. Guidos, RN, C. W. Hendrix, MD, Department of Medicine, the Johns Hopkins University, School of Pharmacy, University of Colorado Health Sciences Center, Baltimore, MD.

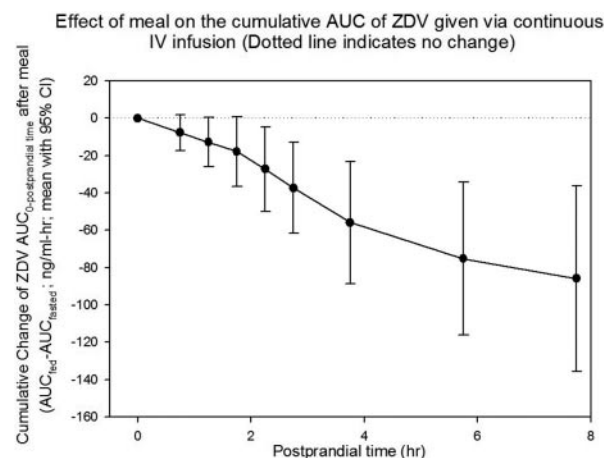
**BACKGROUND/AIMS:** Understanding factors affecting antiretroviral drug concentration helps optimize therapeutic regimens in order to maximize effectiveness and prevent development of

resistance. Zidovudine (ZDV), a widely used antiretroviral drug, is cleared predominantly by hepatic glucuronidation into zidovudine glucuronide (G-ZDV) and has a high hepatic extraction ratio. We tested the hypothesis that a meal decreases ZDV exposure independent of an effect on drug absorption.

**METHODS:** As part of a larger open-label inpatient trial with 7 healthy male volunteers, ZDV was given by continuous infusion at 10 mg/hr starting on Day 1. For this substudy, subjects received only a standardized breakfast meal on the morning of Day 2 and were fasted on the morning of Day 3 until the 8-hour sampling period each day was finished. Blood samples for ZDV and G-ZDV were collected through an indwelling cannula in the arm contralateral to the ZDV infusion at the same time of day on both days (to minimize the potential effect of circadian rhythm on hepatic blood flow and glucuronidation). Data are presented as mean with 95% confidence interval (95% CI).

**RESULTS:** Food decreased the blood ZDV concentration at 1.75 hours postprandially by 13% (1%–24%). The statistically significant decrease lasted 2.25 hours (from 1.75–4 hours postprandially), resulting the corresponding change in AUC (Figure). There was a decreasing trend in the mean fed/fasted concentration ratios of the ZDV metabolite, ZDV glucuronide (G-ZDV) ( $P < 0.01$  by runs test), although the 95% CI at most postprandial time indicates no statistically significant change in G-ZDV concentration.

**CONCLUSIONS:** Food decreases blood ZDV concentrations when ZDV is administered intravenously, thus the decrease is independent of any absorption effects. Since feeding increases hepatic and renal blood flow and ZDV has a high hepatic extraction ratio and some renal elimination, we reasoned that a food-induced increase in hepatic and renal blood flow may explain the food-induced decrease of ZDV concentration. Although this magnitude of ZDV change is unlikely to have clinical significance given the much longer half-life of the active intracellular ZDV triphosphate, this study demonstrates the potential for food to impact drug exposure independent of effects on drug absorption.



**PIII-47**

EFFECTS OF HIV PROTEASE INHIBITOR REGIMEN ON PLATELET FUNCTION AND ENDOGENOUS THROMBIN POTENTIAL (ETP). J. Graff, MD, N. Hentig, MD, A. Foerster, S. Staszewski, MD, S. Klauke, MD, S. Harder, MD, Institute of Clinical Pharmacology, Medical HIV treatment and Research Unit, Frankfurt, Germany.

**BACKGROUND:** More recently, thromboembolic complications in HIV patients have been described. Especially the influence of protease inhibitors on platelet activation and coagulation are currently under discussion.

**METHODS:** HIV positive, therapy naive patients (n = 25) were investigated before and 4–8 weeks after the start of a protease inhibitor (PI) including combination therapy. Therapy consisted of a boosted HIV PI regimen plus reverse transcriptase inhibitors (n = 18) and double PI regimen (n = 7). CD 62P, PAC1, platelet-monocyte interaction [CD41, CD11b (all mean fluorescence intensity)] and CD40L (%+ platelets) were assessed by flow cytometry. To investigate the influence of platelets on coagulation the endogenous thrombin potential (ETP) was determined.

**RESULTS:** CD62P, PAC1 and CD11b expression remained unchanged. In contrast, CD41 (from  $446 \pm 214$  to  $605 \pm 184$ , mean  $\pm$  sd,  $p < 0.001$ ) and CD40L (from  $18 \pm 9$  to  $26 \pm 10$ ,  $p < 0.05$ ) increased significantly. ETP showed no evidence for increased procoagulatory capacity (from  $759 \pm 172$  to  $817 \pm 229$ ).

**CONCLUSIONS:** Effects of HIV therapy und platelet function assessed under field conditions seems to be minor and do not affect all investigated parameters. We found no evidence of generally increased platelet activation in HIV patients under PI regimen.

**PIII-48**

AMERICAN GINSENG (AG) REDUCES OXIDATIVE STRESS MARKERS WITHOUT ALTERING THE PHARMACOKINETICS OF ZIDOVUDINE. L. S. Lee, MD, S. D. Wise, MBChB, FRCP, C. Chan, BSc(Hons), C. Flexner, MD, P. S. Lietman, MD, PhD, Johns Hopkins University, Lilly-National University of Singapore Centre for Clinical Pharmacology, Baltimore, MD.

**BACKGROUND:** Many HIV patients on antiretroviral therapy also take herbal medicines and there is potential for harmful or beneficial pharmacokinetic (PK) and pharmacodynamic interactions. In an in vitro model, we found that American ginseng (AG) induces a phase 2 metabolic enzyme and could increase the clearance of zidovudine (ZDV), reducing its concentrations and compromising anti-retroviral efficacy. Conversely, AG is used for its antioxidant activity and may reverse the oxidative effects of ZDV.

**METHODS:** Open-label two-period fixed-sequence study. Ten healthy volunteers completed the study. Subjects followed a diet designed to exclude phase 2 enzyme inducers for 2 weeks and were then given a single 300mg oral dose of ZDV. Blood and urine were taken from 0 to 8 hours for measurement of ZDV and ZDV-glucuronide concentrations using a validated HPLC/MS method. Markers of oxidative stress, plasma F2-isoprostanes (F2Is) were measured at 0, 2, and 8 hours post-dose, and urine 8-hydroxy-deoxyguanosine (8OHdG) was measured at 0, 0–4 and 4–8 hours post-dose. Subjects then took a ginsenoside-enriched AG extract HT1001 (REMEMBER-FX®) from a single lot, at 200 mg BID, twice the labeled dose, for 2 weeks. They were then given another single 300 mg dose of ZDV and the studies repeated. ZDV PK parameters were compared between pre- and post-AG periods using t-tests. Markers of oxidative stress were compared between pre- and post-AG periods and pre- and post-ZDV dose using mixed effects models.

**RESULTS:** The total ginsenoside content of the AG extract as measured by HPLC was  $8.5 \pm 0.5\%$ . There was no significant effect of AG on formation clearance of ZDV to glucuronide (ratio post- to pre-AG 1.17, 90% confidence interval 0.95–1.45,  $P 0.21$ ), total clearance (ratio 0.97, 0.82–1.14,  $P 0.70$ ),  $AUC_{0-8}$  (ratio 1.03, 0.87–1.21,  $P 0.77$ ) or  $C_{max}$  (ratio 1.13, 0.81–1.58,  $P 0.53$ ). F2I or 8OHdG levels did not increase after a single dose of ZDV. Oxidative stress markers were lower post-AG (F2I ratio post- to pre-AG 0.79, 90% confidence interval 0.73–0.86,  $P < 0.001$ ; 8OHdG ratio 0.74, 0.59–0.92,  $P 0.02$ ).

**CONCLUSION:** Two weeks of AG extract HT1001 did not alter the PK of ZDV and is unlikely to interfere with antiretroviral activity. However, AG reduced oxidative markers and may be beneficial for reducing oxidative effects of long-term ZDV.

### PIII-49

PK/PD EVALUATION OF THE ANTIMICROBIAL ACTIVITY AND PRODUCTION OF BETA LACTAMASE WHEN SULBACTAM IS ADDED TO CEFEPIME (CFP) AND CEFTAZIDIME (TAZ) AGAINST *PSEUDOMONAS AERUGINOSA* (*PsA*). S. M. McCabe, E. Hudzinski, B. T. Tsuji, P. A. Kelchlin, P. F. Smith, State University of New York at Buffalo, Buffalo, NY.

**BACKGROUND:** The development of multi-drug resistant *PsA* is a common problem in the clinical setting and is becoming increasingly difficult to treat. The production of  $\beta$ -lactamase is an important mechanism of resistance for *PsA*. As current treatment options are limited, we evaluated the impact of adding sulbactam, a  $\beta$ -lactamase inhibitor, to TAZ & CFP treatment regimens on *PsA* kill &  $\beta$ -lactamase production, in an *in vitro* PK/PD model.

**METHODS:** *PsA* ATCC 27853 was studied. A one compartment *in vitro* PK/PD model was used to simulate the PK of each drug alone or in combination (1:1 peak concentration ratio) with sulbactam against *PsA* in log-phase growth, at  $10^{6-7}$  CFU/mL. Simulated dosing regimens included 0.5, 1, & 2gm Q8h, Q12h, & by continuous infusion (CI). All regimens were completed in at least duplicate. Bacterial counts (CFU/mL) &  $\beta$ -lactamase activity (spectrophotometric protein corrected nitrocefin assay) were quantified frequently over 24 h.

**RESULTS:** Neither TAZ nor CFP administered alone were effective: both demonstrated initial kill in the model, followed by significant re-growth with induction of  $\beta$ -lactamase. The addition of sulbactam significantly enhanced the activity of both cephalosporins, & resulted in early & sustained suppression of  $\beta$ -lactamase activity. Consistent with %T > MIC, more frequent dosing provided the greatest antipseudomonal activity. The mean log change for TAZ/sulbactam at 0.5gm was -3.78 (CI), -2.64 (q8h), & -1.59 (q12h) CFU/mL; & for 1 gm the mean log change was -4.87 (CI), -3.32 (q8h), & -1.55 (q12h). Results for CFP did not differ from TAZ.

All TAZ/sulbactam regimens demonstrated early and sustained suppression of  $\beta$ -lactamase, as enzyme activity was  $\leq 2$  at all time points and approached undetectable limits at 24h vs CF alone. Increasing the dose of TAZ alone also increased enzyme activity as high as 32 (1g CI).

**CONCLUSIONS:** Monotherapy with TAZ & CFP resulted in minimal antimicrobial activity against this strain of *PsA*, and was correlated with the development of  $\beta$ -lactamase mediated resistance. The addition of sulbactam inhibited  $\beta$ -lactamase activity, and significantly enhanced the antipseudomonal activity of both antibiotics. These data suggest a potential role for the addition of sulbactam to TAZ and/or CFP in the treatment of pseudomonal infections.

### PIII-50

A POPULATION PHARMACOKINETIC MODEL TO CHARACTERIZE THE DISPOSITION OF OLANZAPINE ADMINISTERED ORALLY IN ADOLESCENT PATIENTS. E. Lobo, PhD, T. Quinlan, BS, J. T. Johnson, MS, Q. Hong, PhD, C. Robertson-Plouch, VMD, Eli Lilly & Company, Indianapolis, IN.

**AIMS:** Population pharmacokinetic (PK) modeling was performed to characterize the pharmacokinetics of olanzapine in adolescents, to estimate sources of variability and to identify significant covariates. Comparison of olanzapine PK parameters in adolescents with adults was conducted to guide appropriate dosing recommendations for olanzapine in adolescent patients.

**METHODS:** This was a multicenter, open-label study with 4.5 weeks of olanzapine treatment. A total of 107 patients diagnosed with schizophrenia or bipolar I disorder ages 13 to 17 years were enrolled. Patients received olanzapine orally once daily in the dose range of 2.5 to 20 mg. Four blood samples at steady state were obtained from each patient. Olanzapine concentrations in plasma were determined using a validated HPLC method with electrochemical detection. Similar data in 11 adolescents from 3 previous studies

were also included. A PK model was developed using a nonlinear mixed effect modeling program. The distributions of PK parameters for olanzapine in adolescents were compared to those previously reported in adults (N = 910, 5 to 20 mg) using Kolmogorov-Smirnov two sample test.

**RESULTS:** The pharmacokinetics of oral olanzapine in adolescent patients were described by one-compartment PK model. The typical model estimates for oral clearance (CL/F) was 13.6 L/hr for female patients and 17.5 L/hr for male patients and oral volume of distribution (V/F) was 899 L. The inter-patient variability (40.5% for CL/F, 65.4% for V/F) and the residual error (27%) were moderate. Body weight and gender had a significant influence on CL/F. Olanzapine CL/F increased 1.9 fold in a 3.6 fold body weight range (41 to 148 kg). Males had 30% higher CL/F than females. Approximately 77% of adolescents and adults had comparable CL/F and 69% had comparable V/F values. The median (5<sup>th</sup>–95<sup>th</sup>) average steady state olanzapine concentrations in adolescents (2.5 to 15 mg) is 11.1 (4.5–33.1) ng/mL and in adults (5 to 20 mg) is 10.1 (5.5–20.9) ng/mL.

**CONCLUSIONS:** Pharmacokinetics of oral olanzapine are linear in the dose range of 2.5 to 20 mg in adolescent patients. Given the small magnitude of covariate effects and the inter-patient variability, dose adjustments based on body weight or gender are not necessary. Doses up to 15 mg in adolescents have olanzapine exposure similar to those in adults.

### PIII-51

AGE-ASSOCIATED PROTEIN EXPRESSION OF P-GLYCOPROTEIN (MDR1/P-GP) AND MRP2 IN HUMAN PEDIATRIC LIVER. L. Tang, PharmD, R. N. Hines, PhD, E. G. Schuetz, PhD, B. Meibohm, PhD, University of Tennessee Health Science Center, Medical College of Wisconsin, St. Jude Children's Research Hospital, Memphis, TN.

**BACKGROUND/AIMS:** Human P-gp and MRP2 are membrane associated proteins belonging to the superfamily of ATP-binding cassette (ABC) transporters that mediate the unidirectional transport of various organic anions as well as lipophilic substances conjugated to glutathions, glucuronates, or sulfates. Both P-gp and MRP2 are localized on the canalicular membrane in hepatocytes and are responsible for the transport of various substrates into the bile. This study investigates the developmental changes in protein expression for P-gp and MRP2 in human pediatric liver during the early childhood phase. It is hypothesized that expression of these ABC transporters undergoes developmental changes during the early years of life.

**METHODS:** Human pediatric liver samples from living (n = 43) as well as deceased donors (n = 22) aged 0.3 to 12 years were collected through liver biopsy. Protein extracts enriched in membrane proteins were prepared from liver samples through differential centrifugation. Membrane proteins were then separated by SDS-PAGE and detected by immunoblotting. P-gp and MRP2 protein expression were semi-quantified by comparing their densitometric value relative to that of GAPDH. ANOVA with post hoc pairwise comparisons was performed to assess statistical differences in expression amongst different age groups (A: 0.3–0.7, B: 0.7–2, C: 2–5, D: 5–12 yrs).

**RESULTS:** Mean normalized relative protein expression to GAPDH is presented in the table (statistical significance (p < 0.05) is indicated by italicized numbers).

**CONCLUSION:** Within the investigated age range, there are significant age-associated differences in protein expression for MRP2, but not P-gp, in human pediatric livers.

	A (n = 6)	B (n = 13)	C (n = 13)	D (n = 33)
P-gp	1.24 ± 1.17	1.25 ± 0.84	1.09 ± 0.71	1.03 ± 0.84
MRP2	<i>1.04 ± 1.15*</i>	<i>2.23 ± 1.53</i>	1.38 ± 1.21	<i>1.12 ± 0.91*</i>

\*significant compared to B.

### PIII-52

RECTAL AND ORAL OMEPRAZOLE TREATMENT OF GASTROESOPHAGEAL REFLUX IN INFANTS WITH ESOPHAGEAL ATRESIA OR CONGENITAL DIAPHRAGMATIC HERNIA; A PILOT-STUDY. P. Bestebreurtje, A. A. van Sorge, C. A. Knibbe, M. Duran, S. N. de Wildt, D. Tibboel, Rijnstate Hospital, St. Antonius Hospital, Erasmus MC-Sophia's Childrens Hospital, Arnhem, The Netherlands.

**BACKGROUND:** Omeprazole is frequently used to treat gastroesophageal reflux disease in young infants. Due to their small size, infants require a non-standard dose of omeprazole often administered as extemporaneous formulations. The oral bioavailability of these omeprazole formulations may be unpredictable and may result in variable degrees of drug exposure. An omeprazole suppository appears an effective dosage form in healthy adults, suggesting this to be an interesting alternative to oral administration in children. The aim of this study is to compare the efficacy of oral and rectal omeprazole treatment in infants with gastroesophageal reflux due to esophageal atresia or congenital diaphragmatic hernia.

**METHOD:** First, we developed an omeprazole suppository for pediatric use. Next, in an open-label pilot study, infants with symptomatic GERD, randomly received 1 mg/kg omeprazole orally or rectally. Intraesophageal and intragastric pH was monitored for 48 hrs in 8 patients at 6 and 12 weeks of age, according to existing clinical protocols. The first 24 hrs without medication, to determine if pathological acid reflux was present (Vandenplas classification), the second 24 hrs, with omeprazole treatment, to determine acid suppression efficacy. Primary outcome was efficacy defined as normalizing the 24-hour intraesophageal pH with omeprazole. Secondary outcome was percentage of time that intragastric pH > 4.

**RESULTS:** The 24 hour intraesophageal pH measurements before treatment showed pathological acid reflux in 6 out of 8 patient cases. 2 infants were randomized to omeprazole suppositories and 4 to omeprazole granules. In both patients who received omeprazole rectally, intraesophageal pH normalized. In 2 out of 4 patients who received omeprazole orally, intraesophageal pH normalized. In all patients, both oral and rectal groups, omeprazole significantly increased the percentage of time intragastric pH was above 4.

**CONCLUSION:** This pilot study suggests that rectal omeprazole is an interesting alternative to oral omeprazole in small infants. Oral omeprazole is not effective to treat gastroesophageal reflux effectively in all infants at the dose prescribed. Further studies are needed to determine disposition and efficacy of rectal treatment.

### PIII-53

DRUG PRESCRIPTION MISTAKES AT THE PICU; THE DOCTOR OR THE FORMULARY TO BLAME? I. Ceelie, MD, S. N. de Wildt, MD, PhD, K. Stol, MD, Y. Van der Tuijn, MA ANP, C. Van der Starre, MD, D. Tibboel, MD PhD, ErasmusMC/Sophia, Rotterdam, The Netherlands.

**BACKGROUND/AIMS:** Within the context of the Safety First project, which encourages voluntary reporting of incidents and near incidents in patient care in our pediatric ICU, medication errors are integrated in a patient safety management system. In 2005, 697 of 1,600 (43.6%) reports were related to medication, of which 66 (9.5%) were prescription errors, of which 18 had potentially serious side effects. The top 5 of reported errors was 1) wrong dosage 2) wrong additions to iv solutions 3) wrong concentration 4) wrong route of administration 5) wrong dispersion form. Our aim was to study if the dosage errors could be related to unclear or lacking pediatric dosage guidelines in well-established drug formularies.

**METHODS:** The drugs for which dose errors were reported were: morphine, midazolam, clonidine, amoxicillin/clavulanate and nystatin. For these drugs we compared dosage guidelines in four drug formularies, which are frequently used on our ICU by the residents: 1) Up to Date; Lexi-Comp®, UK 2) MicroMedex®, USA 3) Drug Doses, 2003, F. Shann, Australia 4) "Geneesmiddelenformulierum voor kinderen", WKZ, the Netherlands.

**RESULTS:** For midazolam the maximum dosage recommendation differed up to two fold in the different sources (midazolam; 1,2: 0,12 mg/kg/hour; 3: 0,24 mg/kg/hour) and morphine up to six fold (morphine: 4: 0.01 mg/kg/h, 3: 0.06 mg/kg/h). For nystatin (3: 3 dd 100,000 IU, 2: 4 dd 200,000 IU) and amoxicillin/clavulanate (amoxicilline component: 1,2: 30mg/kg/day, 4: 100 mg/kg/day) this was up to a three fold difference. Two sources (1,2) had no pediatric dosage advice for clonidine. Different notation systems for drug dose (i.e. mg/kg/d in x doses or mg/kg, x times daily) were used for four drugs (midazolam, morphine, amoxicillin/clavulanate and nystatin).

In two sources (1,2) a different dispersion form was noted, which is not available in the Netherlands for amoxicillin/clavulanate.

**CONCLUSION:** Drug prescription errors are made in a small number of frequently prescribed drugs in our ICU. Dosage guidelines for these drugs vary largely between or are completely absent from leading drug formularies. Lack of uniform dosage guidelines for frequently prescribed drugs in the ICU may contribute to drug prescription errors, exposing this vulnerable patient population to an increased risk of serious harm.

### PIII-54

<sup>13</sup>C ERYTHROMYCIN BREATH TEST AS A NON-INVASIVE MEASURE OF CYP3A ACTIVITY IN PRETERM INFANTS: A PILOT STUDY. S. N. de Wildt, M. J. Berns, J. McIntyre, D. Watimena, I. Choonara, J. N. van den Anker, ErasmusMC-Sophia Children's Hospital, Derbyshire Children's Hospital, Children's National Medical Center, Rotterdam, The Netherlands.

**AIM:** The intravenous (IV) <sup>14</sup>Cerythromycin breath test (EBMT) has been successfully used in adults to measure hepatic CYP3A activity, while the oral test appears to reflect both CYP3A and PGP activity. However, to use the same test in newborns is not feasible for obvious ethical reasons (radioactive label). Hence, the aim of this pilot study was to determine if stable isotope labeled <sup>13</sup>Cerythromycin could be used alternatively.

**METHODS:** First, we validated our sampling method, taking exhaled air from the side-port of a tube in ventilated newborns or from a gastric tube positioned 1 cm into the nasopharynx in non-ventilated newborns. Next, newborns who needed treatment with erythromycin for Ureaplasma infection, were given a 10-15 mg/kg <sup>13</sup>C(N-dimethyl) erythromycin dose via nasogastric tube (PO). Pharmacy regulations did not permit IV administration. Exhaled air samples were collected pre-dose and up to 24 hrs post-dose and analyzed for <sup>13</sup>C and <sup>12</sup>CO<sub>2</sub> using GC-MS.

**RESULTS:** We were able to measure <sup>13</sup>CO<sub>2</sub> enrichment in exhaled breath in mechanically ventilated and non-ventilated patients. Three patients (GA 24 6/7-27 4/7 wks, PNA 10-34 days) received oral <sup>13</sup>C-erythromycin. <sup>13</sup>CO<sub>2</sub> did not change significantly from baseline, showed a Tmax of 20 hrs (+12%) and reached a Cmin at 24hrs (-16%) in these patients, respectively. **Discussion:** In patient 1, the erythromycin dose (10 mg/kg) may have been too low to detect a change in exhaled <sup>13</sup>CO<sub>2</sub> after which we increased it to 15 mg/kg. In patient 2, the <sup>13</sup>CO<sub>2</sub> peak at 20 hrs seemed too late to be explained by erythromycin administration, as erythromycin Tmax is 1-3 hrs in neonates < 3 months of age. In patient 3 a decrease in <sup>13</sup>CO<sub>2</sub> from a highly <sup>13</sup>CO<sub>2</sub> enriched baseline, possibly caused by a decrease in glucose infusion rate (which is high in <sup>15</sup>C), may have obscured a <sup>13</sup>CO<sub>2</sub> peak related to erythromycin. After 3 patients this pilot trial was discontinued as at least two out of three patients did not show any increase in <sup>13</sup>CO<sub>2</sub> enrichment related to <sup>13</sup>C-erythromycin administration.

**CONCLUSION:** The lack of a consistent change in exhaled <sup>13</sup>CO<sub>2</sub> response after oral <sup>13</sup>Cerythromycin in this pilot study does not support the routine use of this test in newborns. However, this finding may reflect more advanced ontogeny of PGP than CYP3A activity in newborns and further studies are needed to support this hypothesis.



### PIII-58

SELF REPORTED SAFETY, EFFICACY AND REGULATORY BELIEFS CONCERNING DIETARY SUPPLEMENTS BY RANDOMLY SELECTED CONSUMERS. R. E. Krasnow, MS, J. Knudsen, MD, PhD, L. R. Cantilena, MD, PhD, Uniformed Services University of the Health Science, Bethesda, MD.

**BACKGROUND/AIM:** Currently, dietary supplements (DS) can be sold to the public without premarket testing for efficacy, safety, and purity. Retail sales of DS and herbal medicines currently exceed that of FDA regulated OTC medications. This study examined consumer beliefs about the efficacy, safety and regulation of DS.

**METHODS:** Ninety-four consumers of DS were randomly surveyed outside of stores specializing in the sale of health supplements. Subjects completed an anonymous 27 question survey with both quantitative and qualitative questions regarding: demographic information; DS usage; common sources of DS information; evaluation of DS safety; and knowledge and opinions about DS product regulation. In addition, at the conclusion of the survey, consumers who reported their current DS usage were evaluated for potential drug interactions with self reported concurrent medications.

**RESULTS:** Most surveyed consumers used a DS at least once a day (80%) and the vast majority (85%) believed that all the DS they take are safe. The most common response regarding supplement safety was that subjects were reassured by their own research (38%). Top resources for consumer information regarding DS safety and efficacy were the internet (41%), magazines (31%), and their physicians (27%). 26% of subjects reported that they were not aware that DS were largely untested for safety or efficacy before sale. 77% of consumers agreed that there should be a regulatory agency that ensures products are safe and effective before being sold to the public. The most common belief regarding supplement regulation was that increased regulation would improve public safety (31%). 51% of consumers indicated that their physician is not aware of some or all of the DS they take. No serious drug interactions were detected among the subjects taking DS by self reported history.

**CONCLUSIONS:** Results from this limited survey indicated that consumers of DS believe that the supplements they take are safe. They generally obtain user information independently and approximately half do not fully inform their health care provider of their use of these products. The vast majority of surveyed consumers favored more regulatory oversight for DS products.

### PIII-59

LOW-LEVEL ALANINE AMINOTRANSFERASE (ALT) ELEVATIONS IN OSTEOARTHRITIS (OA) PATIENTS AFTER THE FIRST 2 WEEKS OF TREATMENT WITH DAILY ACETAMINOPHEN (APAP): A RETROSPECTIVE ANALYSIS EVALUATING SUBSEQUENT ALT VALUES WITH CONTINUED APAP THERAPY. E. K. Kuffner, MD, A. R. Temple, MD, K. M. Cooper, MS, J. S. Baggish, MD, D. L. Parenti, MD, McNeil Consumer Healthcare, Fort Washington, PA.

**BACKGROUND/AIMS:** In some OA studies where APAP was given over multiple days, low-level ALT elevations have been occasionally reported. We retrospectively analyzed ALT data after the first 2 weeks of treatment with daily APAP in McNeil-sponsored OA studies to assess the frequency and magnitude of ALT elevations and the rate of resolution while continuing APAP treatment. We analyzed data after the first 2 weeks of APAP for its relevance to ALT elevation data presented in a recent study.

**METHODS:** Transaminase values taken at baseline and after 2 weeks of APAP 3900 mg or 4000 mg/d (n = 513) were evaluated from three 12- to 13-week OA clinical trials.

**RESULTS:** In 3 studies, 449 patients had baseline transaminase activity  $\leq$  the upper limit of the reference range (ULRR) and had a 2-week on-treatment ALT measurement. Of these patients, 368 (82.0%) never had an ALT > ULRR, 81 (18.0%) had an ALT > ULRR, 18 (4.0%) had an elevation > 1.5X ULRR, and 4 (0.9%) had an elevation >

3X ULRR over 2 weeks. The highest elevation observed at 2 weeks was 3.66X ULRR. An ALT value subsequent to the elevation was available for 76 of these 81 patients. On-treatment resolution was documented for 55 patients (72.4%), and a decreasing ALT was documented for an additional 18 patients (23.7%). Three patients did not have documented resolution or decrease in ALT: 2 completed with an elevated ALT (range 1.03-2.21X ULRR), while 1 discontinued due to headache/flu symptoms.

After 12 to 13 weeks, ALT elevations were not associated with a higher frequency of symptoms potentially related to a hepatic origin. No patient had an on-treatment ALT > 3X ULRR in conjunction with hyperbilirubinemia. None of the 513 APAP-treated patients developed hepatotoxicity or hepatic failure at any time during the studies.

**CONCLUSION:** Following 2 weeks of treatment with APAP 3900-4000 mg/d in OA trials, ALT elevations were observed in 18.0% of patients. The elevations were infrequent, low (ALT < 1.5X ULRR in 77.8% of affected patients), and either completely resolved or were resolving in 96.1% of affected patients with continued APAP treatment. These ALT elevations were unaccompanied by symptoms potentially related to hepatic origin, and no patient experienced hepatic injury, dysfunction, or failure after 12 to 13 weeks of therapy.

### PIII-60

ESTIMATING THE ECONOMIC COSTS OF ANTIDEPRESSANT DISCONTINUATION DURING PREGNANCY. L. O'Brien, MSc, G. Koren, MD, The Hospital for Sick Children, Toronto, ON, Canada.

**BACKGROUND:** Mental illnesses such as depression are a public health concern as they carry with them large economic and societal costs. It has been shown that depressed individuals utilize the health care system more than their non-depressed counterparts. In Canada the majority of the direct costs of health care are incurred by the government as they fund the national health care system known as Medicare. This study sought to determine the direct medical costs incurred by the Ontario government resulting from the cessation of antidepressant pharmacotherapy during pregnancy.

**METHODS:** An economic evaluation was conducted using assumptions that were based on data obtained from Statistics Canada, federal and provincial government reports and relevant depression literature.

**RESULTS:** The number of depressed and pregnant women in Ontario that discontinued antidepressant therapy and subsequently had a depressive relapse was estimated to be 1444. The cost of physician services provided to these women was estimated to be \$714,347 (CAD). It was also estimated that \$11,136 (CAD) would be spent on hospitalizations due to untreated depression during pregnancy. Preterm birth and low birth weight (LBW) are two adverse outcomes associated with untreated depression during pregnancy. The total cost of caring for preterm infants born to depressed mothers in the first year of life was determined to be \$4,552,796 while the cost of caring for their LBW infants for the first two years of life was estimated at \$24,393,360. In total the annual cost to the Ontario government due to untreated depression during pregnancy was found to be \$29,671,639, with the majority of costs resulting from the care of preterm and low birth weight infants.

**CONCLUSION:** Depression should be viewed as a major public health issue given its prevalence in North American society and the ensuing health care costs: an estimated \$29,671,639 is spent annually in Ontario on untreated maternal depression. Depression is a treatable disease and as such should be actively managed, particularly in vulnerable populations such as pregnant women.

### PIII-61

SAFETY AND TOLERABILITY OF RANOLAZINE AND HMG COA REDUCTASE INHIBITORS (STATINS) IN PATIENTS WITH CHRONIC ANGINA. J. O. Parker, MD, M. R. Crager, PhD, M. Jerling, A. T. Koren, MD, Kingston General Hospital, CV Therapeutics, Independent Consultant, Kingston, ON, Canada.

**BACKGROUND:** Ranolazine (Ran) was recently approved for the treatment of chronic angina. Ran is a substrate and mild inhibitor of CYP 3A4 and P-glycoprotein (P-gp) and has been shown to increase serum levels of simvastatin by as much as 2-fold due to CYP 3A4 and P-gp interactions. Although this interaction may be unique to simvastatin, because statins are widely used in angina, and other statins are metabolized by CYP 3A4, we sought to evaluate the safety and tolerability of Ran when used with statins in the pivotal Ran angina trials CARISA (Combination Assessment of Ranolazine in Stable Angina) and ERICA (Efficacy of Ranolazine in Chronic Angina).

**METHODS:** The incidence of overall adverse events (AEs), AEs of particular relevance to statins (hepatobiliary, musculoskeletal), and discontinuations due to AEs were evaluated in patients taking placebo + statin vs Ran + statin in CARISA and ERICA. Mean changes from baseline in liver function tests (LFTs) and creatine phosphokinase (CPK) and the incidence of LFTs  $\geq 3x$  upper limits of normal (ULN) and CPK  $\geq 10x$  ULN were also assessed.

**RESULTS:** 44% (369/823) of CARISA and 36% (202/564) of ERICA patients received a statin. The most commonly used statins were simvastatin (20% of patients), atorvastatin (13%), pravastatin (6%) and lovastatin (6%). Overall, hepatobiliary, and musculoskeletal AEs were similar in placebo and Ran treated patients taking statins (TABLE). Discontinuations due to AEs were higher in Ran patients. The combination of Ran + statin did not increase mean values of LFTs or CPK. Small, clinically non-significant reductions in LFTs and CPK were observed in Ran + statin compared to placebo + statin patients. No cases of CPK  $\geq 10x$  ULN or LFTs  $\geq 3x$  ULN were observed in Ran + statin patients. One patient in CARISA and 2 patients in ERICA on placebo + statin experienced LFTs  $\geq 3x$  ULN.

**CONCLUSION:** Statin use was common in the Ran pivotal trials. Simvastatin and other statins metabolized by CYP 3A4 (atorvastatin, lovastatin) represented the majority of statin use. No evidence of statin-related toxicity was observed based on AE and laboratory data. More discontinuations due to AEs were observed in Ran compared to placebo patients on statins.

Event	CARISA (N = 823)			ERICA (N = 564)	
	Placebo + Statin (N = 120)	Ranolazine 750 mg bid + Statin (N = 119)	Ranolazine 1000 mg bid + Statin (N = 130)	Placebo + Statin (N = 93)	Ranolazine 1000 mg bid + Statin (N = 109)
	Overall AEs	40 (33%)	44 (37%)	52 (40%)	39 (42%)
Hepatobiliary AEs	1 (1%)	0 (0)	1 (1%)	0 (0)	1 (1%)
Musculoskeletal AEs	6 (5%)	2 (2%)	5 (4%)	6 (6%)	3 (3%)
Discontinuation for AE	5 (4%)	9 (8%)	12 (9%)	1 (1%)	1 (1%)

### PIII-62

SAFETY OF TRANSDERMAL OXYBUTYNIN IN THE TREATMENT OF OVERACTIVE BLADDER AMONG PATIENTS USING OTHER TRANSDERMAL MEDICATIONS: RESULTS OF THE MATRIX STUDY. L. T. Pizzi, PharmD, MPH, M. McIlwain, BS, N. V. Dahl, PharmD, Jefferson Medical College, Watson Laboratories, Philadelphia, PA.

**BACKGROUND/AIMS:** Safety and health-related quality of life outcomes were evaluated in the Multicenter Assessment of Transdermal Therapy in Overactive Bladder with Oxybutynin (MATRIX) study. An analysis of safety was conducted for the overall study population and for participants who used other patch medications concurrently with OXY-TDS.

**METHODS:** Participants in this open-label, community-based, randomized, prospective study were  $\geq 18$  years of age and had  $\geq 1$

symptom of overactive bladder (OAB). Most concomitant medications were allowed, except for investigational products or other OAB treatments. Participants were treated for as long as 6 months with the oxybutynin transdermal system (OXY-TDS) at the FDA-approved dosage of 3.9 mg/day. Participants changed the patch twice weekly, and were instructed to rotate the application site. All participants who received a dose of OXY-TDS were included in the safety analysis, which was based on adverse event reporting during clinic visits at months 1, 3, and 6. Safety was assessed with descriptive statistics.

**RESULTS:** Members of the study population (N = 2878) were mostly female (2508; 87.2%) and Caucasian (2406; 83.6%); participants ranged in age from 18 to 100 years, with a mean of 62.5 years. During the 6-month study, 1326 participants (46.1%) reported an adverse event of some kind, regardless of relation to study treatment. Most adverse events (2483/2830; 87.7%) were of mild or moderate intensity. Adverse events possibly related to OXY-TDS treatment occurred in 863 participants (30.0%). Most common of these were application site reactions, which were experienced by 402 participants (14.0%). Sixty-six participants (2.3%) used another patch concurrently with OXY-TDS; these individuals had a mean age of 62.6 years and were mostly women (61; 92.4%). Participants most commonly used patches with estrogens (28 participants), nitroglycerin (14 participants), and anesthetics (8 participants). Among participants concurrently using a patch in addition to OXY-TDS, 11 (16.7%) had an application site reaction, regardless of relation to study treatment.

**CONCLUSION:** Open-label OXY-TDS treatment for OAB was well tolerated by community-dwelling adults, including those concurrently using other patch medications.

### PIII-63

DRUG PRESCRIBING-RELATED PHARMACODYNAMIC FACTORS THAT INCREASE THE RISK OF TORSADES DE POINTES IN PATIENTS RECEIVING ANTIPSYCHOTIC THERAPY: CROSS-SECTIONAL PREVALENCE STUDY IN A TEACHING PSYCHIATRIC HOSPITAL. J. Westphal, MD, PhD, D. Gregoire, PharmD, C. Nonnenmacher, PharmD, Etap. Public Sante Alsace Nord, Brumath (Strasbourg), France.

**BACKGROUND/AIMS:** Some of the acknowledged pharmacodynamic factors that increase the risk of torsades de pointes (TdP) related to antipsychotics prolonging the QT interval (QT/APs) are high dose antipsychotic (AP) therapy and drug combinations (DCs) that result in either additive effects on AP-related QT prolongation or potentiating effects (through bradycardia or hypokalemia) on the risk of TdP. The aim of the study was to evaluate the prevalence of these drug prescribing factors in the AP-receiving inpatient population of a 415-bed teaching psychiatric hospital.

**METHODS:** A 1-day cross-sectional study of all the ongoing drug regimens in the inpatient population of the hospital was performed by the pharmacy department. The screening/rating tools used for reviewing AP daily doses and AP-containing DCs were the drug product labels and the knowledge base of the French Agency for Health Products. Hazardous/contraindicated DCs or DCs requiring precaution for use were included. Only drugs prescribed on a regular schedule were considered.

**RESULTS:** The 306 adult patients receiving AP therapy present on the study day were included: 167 (54.6%) were treated with  $\geq 1$  QT/AP. In this 167-patient group: 1) prevalence of QT/AP-containing DCs at increased risk of TdP was 40.7% (95%CI 33.3%–48.1%, n = 68), and occurrence of these DCs correlated ( $p < 0.01$ ) with the number of drugs concomitantly prescribed; 2) polypharmacy with QT/APs and combinations of QT/APs with bradycardia- or hypokalemia-inducing drugs accounted for, respectively, 66.2% (45/68) and 33.8% (23/68) of these DCs. Prevalence of orders for nonAP drugs potentiating the risk of TdP was similar in the 167 patients treated with QT/APs and in the 139 patients treated with other antipsychotics (i.e., nonQT/APs), respectively, 13.8% and 17.3% ( $p = 0.39$ ). There were 41 orders for maximal daily doses or overriden maximal daily doses of QT/APs (prevalence 24.5%, 95%CI

18%-31%), of which 19 (46.3%) were included in DCs at increased risk of TdP. Prevalence of such orders was lower (15.1%, 95%CI 9.1%-21%) in the patients with nonQT/APs ( $p = 0.04$ ).

**CONCLUSION:** These data show that high dose AP therapy and QT/AP-containing DCs at increased risk of TdP are frequently prescribed in our hospital, irrespective of the differential cardiac safety across the individual antipsychotics.

### PIII-64

ASSESSMENT OF PHARMACOKINETIC INTERACTION BETWEEN VILDAGLIPTIN AND SIMVASTATIN. S. P. Ayalasonmayajula, PhD, K. Dole, Pharm D, Y. L. He, PhD, M. Ligueros-Saylan, MD, Y. Wang, PhD, J. Campestrini, PhD, H. Humbert, PhD, W. P. Dole, MD, G. Sunkara, PhD, Novartis Pharmaceuticals Co., Novartis Pharmaceuticals Co., East Hanover, NJ.

**BACKGROUND:** Vildagliptin is a potent and selective DPP-4 inhibitor, intended for the treatment of type 2 diabetes. Co-administration of HMG-CoA-reductase inhibitors (statins) and oral hypoglycemic agents is frequently required to treat patients with diabetes and dyslipidemia. Therefore, this study was conducted to determine the steady state pharmacokinetic interaction between vildagliptin and simvastatin.

**METHODS:** An open label, single center, multiple dose, three period, cross-over study was conducted in 24 healthy subjects. All subjects received once daily doses of either vildagliptin 100 mg or simvastatin 80 mg or the combination vildagliptin 100 mg + simvastatin 80 mg for seven days with an inter-period washout of 7 days. Plasma levels of vildagliptin, simvastatin and its active metabolite, simvastatin  $\beta$ -hydroxyacid, were determined using a validated LC/MS/MS method. Pharmacokinetic and statistical analyses were performed using WinNonlin and SAS, respectively.

**RESULTS:** The 90% confidence intervals of  $C_{max,ss}$  and  $AUC_{0-\tau}$  of vildagliptin, simvastatin and simvastatin  $\beta$ -hydroxyacid during co-administration were within the 80–125% range as compared between mono and combination treatments. Values for  $T_{max}$ ,  $t_{1/2}$ , and  $CL/F$  were also not affected by co-administration. Thus, the rate and extent of absorption of vildagliptin and simvastatin were not affected when co-administered, nor was the metabolic conversion of simvastatin to its active hydroxyl acid. Each drug and the combination appeared to be safe and well tolerated.

**CONCLUSIONS:** The pharmacokinetics of vildagliptin, simvastatin and simvastatin  $\beta$ -hydroxyacid were not altered when vildagliptin and simvastatin were co-administered. Both vildagliptin and simvastatin were well tolerated when administered alone or in combination.

### PIII-65

PHARMACOKINETIC INTERACTION STUDY BETWEEN METFORMIN AND A NOVEL DIPEPTIDYL PEPTIDASE-IV (DPP-IV) INHIBITOR PSN9301 IN HEALTHY SUBJECTS. R. R. Boinpally, PhD, J. Wolf, M.S., A. Savell, M.S., G. Clark, PhD, M. Hamilton, PhD, J. Rachman, MD, OSI Pharmaceuticals, OSI-Prosiding, Boulder, CO.

**BACKGROUND/AIMS:** DPP-IV inhibitors reduce the breakdown of the active form of the insulinotropic hormone GLP-1, stimulating insulin secretion and thus acting as antidiabetic agents. PSN9301 is a novel DPP-IV inhibitor that is likely to be given in combination with metformin in clinic. We have studied the potential PK interaction of this combination as both agents are excreted via renal tubular secretion.

**METHODS:** 24 healthy subjects were treated with single (day 1) as well as multiple (tid on day 2) oral doses of PSN9301 (360 mg HCl salt), metformin (850 mg) and their combination in a three-way cross over design with a washout period of one week between treatments. Plasma and urine samples were collected at predetermined time intervals and PSN9301, its metabolites and/or metformin were quantified

using validated HPLC-MS/MS methods. PK analyses were conducted using WinNonlin®.

**RESULTS:** Median (range)  $C_{max}$  of PSN9301 following a single dose either alone or in combination with metformin was 4.70 (2.23–8.30) vs 4.58 (2.29–8.66)  $\mu\text{g/mL}$ , and the corresponding AUC values were 6.51 (4.23–9.18) vs 6.91 (3.28–9.26)  $\mu\text{g}\cdot\text{hr/mL}$ . Median  $T_{max}$  was 0.5 hr for both treatments. Median  $C_{max}$  of PSN9301 following tid dosing either alone or in combination with metformin was 6.97(3.81–9.08) vs 5.85 (2.90–9.03)  $\mu\text{g/mL}$ , and the corresponding AUC values were 19.9 (13.9–28.0) vs 20.5 (11.9–24.7)  $\mu\text{g}\cdot\text{hr/mL}$ . For metformin single dose, given alone or in combination with PSN9301,  $C_{max}$  was 1.17 (0.71–2.06) vs 1.23 (0.71–1.76)  $\mu\text{g/mL}$  and AUC was 6.92 (4.05–12.9) vs 8.48 (4.80–12.1)  $\mu\text{g}\cdot\text{hr/mL}$ . Median  $T_{max}$  was 1.5 hr for both treatments. For metformin tid dosing, either alone or in combination with PSN9301,  $C_{max}$  was 1.40 (0.81–2.71) vs 1.55 (0.90–2.32)  $\mu\text{g/mL}$  and AUC was 18.6 (10.0–30.9) vs 21.3 (13.6–33.6)  $\mu\text{g}\cdot\text{hr/mL}$ .

**CONCLUSION:** The results of this study indicate that no clinically relevant PK interaction exists between PSN9301 and metformin in humans and thus the combination may be safely given without dose adjustment to type 2 diabetic patients with adequate renal function.

### PIII-66

DOSE PROPORTIONALITY AND MULTIPLE-DOSE PHARMACOKINETICS OF ONDANSETRON HCL ORAL SPRAY (OOS) IN HEALTHY ADULT MEN AND WOMEN. R. Chavira, MA, N. S. Teuscher, PhD, D. Stypinski, PhD, G. Berk, MD, MDS Pharma Services, Hana Biosciences, Inc, Lincoln, NE.

**BACKGROUND:** Ondansetron HCl Oral Spray (OOS) is the first oral spray 5-HT<sub>3</sub> antagonist to deliver ondansetron, the most commonly prescribed anti-emetic for the treatment of chemotherapy induced nausea and vomiting. OOS achieves therapeutic drug levels by delivering a micromist of concentrated ondansetron HCl over the oral mucosa. OOS offers a desirable alternative to patients requiring anti-emetic therapy who have difficulty swallowing or tolerating solid dosage forms. OOS 8 mg has been shown in controlled human studies to be bioequivalent to the 8 mg ondansetron tablet.

**METHODS:** Two studies were conducted to investigate the dose proportionality and multiple-dose PK of OOS in healthy adult men and women: Study 1 was a randomized, crossover study, where 32 subjects received single doses of 4, 8, and 12 mg OOS separated by 72 hr. Plasma samples were collected through 24 hr after dosing in each period.

Study 2 was an open-label, multiple-dose, non-randomized study, where 15 subjects received 8 mg OOS every 12 hr for a total of 7 consecutive doses. Plasma samples were collected predose, through 12 hr after the first dose, and through 24 hr after the last dose. PK parameters in both studies were calculated using noncompartmental methods.

**RESULTS:** Study 1: 31 subjects received all treatments and were included in the PK analysis.  $C_{max}$  and  $AUC_{0-\text{inf}}$  after single 4, 8, and 12 mg doses of OOS were 13.9, 29.6, and 49.5 ng/mL and 109.5, 229.0, and 368.9 ng<sup>h</sup>/mL, respectively. This represents a 2.1- and 3.6-fold increase for  $C_{max}$  and 2.1- and 3.4-fold increase for  $AUC_{0-\text{inf}}$  for a 2- and 3-fold increase in dose of OOS.  $T_{1/2}$ ,  $CL/F$ , and  $T_{max}$  were similar for the 3 doses, ranging from 5 to 6 hr, 39 to 43 L/hr, and 2 to 2.5 hr, respectively.

Study 2: 11 subjects received full doses and were included in the PK analysis.  $C_{max}$  and  $AUC_{0-12}$  were 36.5 vs 32.7 ng/mL, and 240.2 vs 197.2 hr<sup>h</sup>ng/mL, and  $T_{max}$  and  $CL/F$  were 2 vs 2.5 hr and 36.43 vs 35.12 L/hr, after the last dose compared to the first dose.

**CONCLUSION:** Ondansetron oral spray administered in doses of 4, 8 and 12 mg to healthy adults exhibits  $C_{max}$  and AUC that increase proportionally with increasing dose.  $T_{1/2}$ ,  $CL/F$ , and  $T_{max}$  are dose-independent.

Ondansetron oral spray 8 mg administered twice daily demonstrates linear, time-independent PK with no significant accumulation following 4 days of dosing.

### PIII-67

EFFECT OF MULTIPLE DOSE OMEPRAZOLE ON THE SINGLE DOSE PHARMACOKINETICS OF TORCETRAPIB AND ATORVASTATIN. D. Chen, PhD, R. LaBadie, MPH, T. T. Thuren, MD, PhD, C. L. Shear, DrPH, M. A. Gibbs, PhD, Pfizer Global Research and Development, Groton/New London, CT.

**BACKGROUND:** Torcetrapib (T) is a potent and selective cholesteryl ester transfer protein inhibitor in Phase 3 testing in combination with atorvastatin (A) for treating dyslipidemias and altering the progression of atherosclerosis.

**METHODS:** This was an open-label, fixed-sequence, 2-period study including repeat dosing with omeprazole (40 mg QD) and a single dose of fixed dose combination T/A (60 mg/10 mg) in healthy adult subjects. All subjects (N = 50) received a single dose of T/A on Day 1. Following a washout period of 21 days, another dose of T/A was administered on Day 5 of the 8-days of omeprazole dosing.

**RESULTS:** There were no serious adverse events or deaths reported from this study. The ratios of geometric means for  $T C_{max}$  and  $AUC_{last}$  were 136% and 126%, respectively, indicating that both peak T concentration and total exposure were increased in the presence of omeprazole. The ratios of geometric means for  $A C_{max}$  and  $AUC_{last}$  were 89% and 100%, respectively. The bounds of the 90% confidence interval (CI) for both the  $C_{max}$  and  $AUC_{last}$  ratio of test/reference were completely contained within the 80%–125% range.

**CONCLUSIONS:** Treatments with T/A administered alone, as well as co-administered with omeprazole, were generally very well tolerated. In the presence of omeprazole, T exposure increased by approximately 30%, while for A, the 90% CI for ratios of geometric mean ( $C_{max}$  and  $AUC_{last}$ ) values were contained within the range of 80 to 125%, indicating the absence of a clinically relevant effect of omeprazole on the pharmacokinetics of A.

### PIII-68

A PHARMACOKINETIC STUDY OF THE COMBINED ADMINISTRATION OF AGI-1067, A NOVEL ANTI-ATHEROSCLEROTIC AGENT, AND THEOPHYLLINE AND WARFARIN IN HEALTHY SUBJECTS. A. Chilton, PhD, R. Scott, MD, S. Lam-Wong Liang, PhD, R. Teng, PhD, AtheroGenics Inc, AstraZeneca LP, Alpharetta, GA.

**BACKGROUND/AIMS:** AGI-1067 is a novel anti-atherosclerotic agent with antioxidant properties in phase III evaluation for the prevention of clinical events in patients with coronary artery disease. This open-label, non-randomized, sequential, two-way interaction study was designed to assess the effect of multiple doses of AGI-1067 dosed to steady state on the single-dose pharmacokinetics (PK) of theophylline, a CYP1A2 substrate, and warfarin, a typical CYP2C9 substrate, and the effect of single doses of theophylline and warfarin on the steady-state PK of AGI-1067.

**METHODS:** Healthy male and female subjects (n = 30), in the fed state, received a single dose of theophylline 200 mg on Day 1, a single dose of warfarin 25 mg on Day 8, AGI-1067 300 mg once daily on Days 12–40, a single dose of theophylline 200 mg on Day 33 and a single dose of warfarin 25 mg on Day 37. Serial venous blood samples for the determination of AGI-1067, theophylline, and R- and S-warfarin were collected following administration of AGI-1067 and/or theophylline and warfarin. In addition, INR values were determined after warfarin dosing on Days 8 and 37.

**RESULTS:** No pharmacokinetic interaction was detected between theophylline and AGI-1067 in pharmacokinetic parameters ( $C_{max}$  and  $AUC_{0-tau}$ ) using 90% confidence intervals of geometric mean ratios. Co-administration of warfarin did not alter AGI-1067  $C_{max}$  or  $AUC_{0-\infty}$ . R- and S-warfarin  $C_{max}$  was unchanged by AGI-1067, but R- and S-warfarin  $AUC_{0-\infty}$  was significantly decreased by approximately 40%. The mean  $t_{1/2}$  of both R- and S-warfarin was also decreased by about 50–80%. INR values were significantly decreased (p < 0.0001) by 15–32% with the combination of AGI-1067 and warfarin, compared to warfarin alone.

**CONCLUSION:** No PK interaction between AGI-1067 and theophylline was observed in this study, suggesting that AGI-1067 does not inhibit or induce CYP1A2 and would not alter the metabolism of drugs metabolized by CYP1A2. Warfarin administration had no effect on AGI-1067 PK, while AGI-1067 dosing increased the elimination of both R- and S-warfarin, reducing the systemic exposure to R- and S-warfarin by about 40% and the mean INR values by 15–32%.

### PIII-69

A PHARMACOKINETIC INTERACTION STUDY BETWEEN AGI-1067, A NOVEL ANTI-ATHEROSCLEROTIC AGENT, AND DESIPRAMINE AND DIGOXIN IN HEALTHY SUBJECTS. A. Chilton, PhD, R. Scott, MD, S. Lam-Wong Liang, PhD, R. Teng, PhD, AtheroGenics Inc, AstraZeneca LP, Alpharetta, GA.

**BACKGROUND/AIMS:** AGI-1067 is a novel anti-atherosclerotic agent with antioxidant properties in phase III evaluation for the prevention of clinical events in patients with coronary artery disease. This open-label, non-randomized, sequential, two-way interaction study was designed to assess the effect of multiple doses of AGI-1067 dosed to steady state on the single-dose pharmacokinetics (PK) of desipramine, a CYP2D6 substrate, and digoxin, a P-glycoprotein (P-gp) substrate. The effect of single doses of desipramine and digoxin on the steady-state PK of AGI-1067 was also assessed.

**METHODS:** Healthy male and female subjects (n = 30), in the fed state, received a single dose of desipramine 100 mg on Day 1, digoxin 0.25 mg on Day 8, AGI-1067 300 mg once daily on Days 11–40, a single dose of desipramine 100 mg on Day 31 and a single dose of digoxin 0.25 mg on Day 36. Serial venous blood samples were collected for the determination of the study drugs. Additionally, urine samples for digoxin measurements were collected on Days 8–13 and 36–41.

**RESULTS:** AGI-1067 steady-state exposure parameters ( $C_{max}$ ,  $AUC_{0-tau}$ ) were both unchanged by the presence of desipramine and digoxin, using 90% confidence intervals of geometric mean ratios. Co-administration of AGI-1067 did not alter digoxin pharmacokinetic parameters ( $T_{max}$ ,  $C_{max}$ ,  $AUC_{0-\infty}$  or  $t_{1/2}$ ) or the mean  $t_{1/2}$  of desipramine. Urinary excretion of digoxin was also unchanged when co-administered with AGI-1067. However, desipramine  $C_{max}$  and  $AUC_{0-\infty}$  were significantly decreased by co-administration with AGI-1067 (21% and 29%, respectively).

**CONCLUSION:** No PK interaction between AGI-1067 and digoxin was observed in this study, indicating that AGI-1067 does not inhibit or induce P-gp and would not alter the disposition of drugs transported by P-gp. Co-administration with desipramine had no significant effect on the steady-state PK of AGI-1067. AGI-1067 administration seemed to decrease the absorption of desipramine, resulting in a 30% decrease in the systemic exposure to desipramine.

### PIII-70

PHASE I TRIAL FOR PHARMACOKINETIC CHARACTERISTICS OF FACTIVE (GEMIFLOXACIN MESYLATE) INTRAVENOUS FORMULATION WITH FACTIVE TABLET FORMULATION. S. H. Cho, MD, J. L. Ghim, MD, S. M. Choe, MD, Y. H. Kim, MD, D. K. Kim, PhD, K. S. Bae, MD, PhD, Asan Medical Center, University of Ulsan, LG Life Sciences, Ltd., Seoul, Republic of Korea.

**BACKGROUND/AIMS:** Factive® (gemifloxacin mesylate) is administered orally, but alternative routes of drug delivery may be required when oral administration is not feasible. The purpose of the present study was to compare the pharmacokinetics of an IV formulation of Factive® to oral tablet in Korean subjects.

**METHODS:** A single-dose, randomized, open-label, 2-way crossover pharmacokinetics and bioequivalence comparison study of an 1-hour IV infusion of Factive® 200 mg and a 320 mg oral tablet was conducted in 16 healthy subjects. Plasma Factive® concentrations were determined by HPLC/MS/MS. The comparison of bioequivalence was based on the 90% CIs around the geometric mean ratios for AUC (IV/oral).

**RESULTS:** Sixteen subjects participated in the study. All subjects were male Koreans. Their mean (SD) age was 27.2 (5.3) years, mean weight 67.3 (7.4) kg, and mean height 173.5 (4.4) cm. The IV infusion and oral tablet were similar in terms of AUC (9.12 and 9.44 hr x ug/mL, respectively) and the geometric mean IV/oral ratio for AUC was 0.94 (90% CI, 0.82–1.07).  $C_{max}$  of the IV infusion was higher than that of oral tablet (2.90 and 2.03 ug/mL, respectively). Bioavailability of the oral tablet formulation was 68.99 % as compared to the IV formulation of gemifloxacin mesylate.

**CONCLUSIONS:** The AUC of an 1-hour IV infusion of Factive® 200 mg and a oral tablet of Factive® 320 mg was bioequivalent.

### PIII-71

PHARMACOKINETIC AND PHARMACODYNAMIC PROFILES OF BR-A657, A SELECTIVE AT1 RECEPTOR ANTAGONIST, IN PATIENTS WITH ESSENTIAL HYPERTENSION. Y. J. Chung, MD, J. R. Kim, MD, K. S. Lim, MD, J. W. Kim, MD, B. H. Kim, MD, M. G. Kim, MD, MS, T. E. Kim, MD, J. Y. Cho, PhD, K. S. Yu, MD, PhD, S. G. Shin, MD, PhD, I. J. Jang, MD, PhD, Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea.

**BACKGROUND:** BR-A657, an angiotensin receptor blocker under clinical development, acts by selectively blocking the binding of angiotensin II to the angiotensin type 1 receptor. The purpose of this study was to investigate pharmacokinetic and pharmacodynamic profiles of BR-A657 in patients with essential hypertension.

**METHODS:** A randomized, double-blind, placebo-controlled, parallel-group, multiple-dose study was conducted at the Clinical Trial Center, Seoul National University Hospital. In this study, 38 patients with essential hypertension received BR-A657 or placebo once daily for 4 weeks (10 subjects in 20 mg, 10 subjects in 60 mg, 10 subjects in 180 mg and 8 subjects in the placebo group). Serial blood samples were collected via an indwelling venous cannula on Day 1 and Day 28. Steady-state  $t_{max}$ ,  $t_{1/2}$ ,  $C_{max}$  and  $AUC_{0-24}$  were estimated. Levels of plasma renin activity and plasma aldosterone were obtained to evaluate pharmacodynamic profiles in multiple-dose.

**RESULTS:** At steady-state, mean  $t_{max}$  and  $t_{1/2}$  ranged from 1.7–2.5 hours and 7.5–9.9 hours, respectively, across the 20 mg, 60 mg, and 180 mg dosage groups. Mean  $C_{max}$  values were  $34.2 \pm 49.9$  ug/L in 20 mg,  $83.8 \pm 37.1$  ug/L in 60 mg and  $365.4 \pm 243.3$  ug/L in 180 mg group. Average  $AUC_{0-24}$  values were  $174.0 \pm 147.2$  ug-h/L,  $426.9 \pm 134.4$  ug-h/L, and  $1418.5 \pm 575.7$  ug-h/L at each dose level. All doses resulted in increases in plasma renin activity around 6 hours post-dose. Plasma aldosterone levels decreased until 6 hours and increased afterwards. Neither plasma renin nor aldosterone showed any distinct pattern over time in the placebo group. The maximal changes of plasma renin activity and aldosterone were observed at 6 hours after dosing. The mean increases in renin activity ranged 1.3–4.0 fold at 6 hours and the mean aldosterone levels decreased to 42–49 % at 6 hours among dosage groups.

**CONCLUSION:** At steady state, mean  $C_{max}$  and  $AUC_{0-24}$  of BR-A657 showed dose-proportional increases over a dosage range from 20 mg to 180 mg. The fact that plasma aldosterone level declined despite increased plasma renin activity implies BR-A657 inhibited the increase in aldosterone by blocking the action of angiotensin II in Renin-Angiotensin-Aldosterone system effectively.

### PIII-72

POPULATION PHARMOKINETIC ANALYSIS OF INDIPLON. B. Frame, MSc, B. Lalovic, R. Miller, DSc, B. W. Corrigan, PhD, M. Hutmacher, PhD, J. Grundy, PhD, Pfizer Global Research and Development, Neurocrine Biosciences, Ann Arbor, MI.

**OBJECTIVES:** To develop a population pharmacokinetic model to describe the indiplon plasma concentration time relationship in healthy volunteers and insomnia patients.

**METHODS:** Plasma concentration-time data collected in 15 clinical pharmacology (Phase 1) studies and 4 Phase 2 studies in insomnia patients were pooled for NONMEM analysis (741 subjects; 8275 observations; dose range: 5–40 mg Age range: 42–81 years) creatinine clearance, hepatic impairment, age, gender, weight, race, subject status, (healthy volunteer, subject with transient insomnia, or subject with chronic insomnia), concomitant medications, daytime/nighttime indiplon administration, fed/fasted status, and drug formulation were tested as potential explanatory covariates. Posterior predictions were used to conduct model goodness of fit and suitability for simulation.

**RESULTS:** A compartmental model parameterized with random interindividual effects on CL, Vd, F1, and F2 adequately described the data. The posterior check demonstrated that the final model adequately described the data for simulation purposes. Renal function was not a significant covariate in the model. Gender, hepatic impairment, weight, CYP3A inhibition, and time of administration (day/night) were included in the model as covariates.

**CONCLUSIONS:** The model describes the indiplon plasma concentration-time relationship, and can be used to adequately predict concentrations at selected times after oral dosing at various indiplon dose level, age and gender groups of interest.

### PIII-73

INTERACTION STUDY OF ORAL ALBENDAZOLE AND MEBENDAZOLE WITH SHORT AND LONG TERM ORAL RITONAVIR IN HEALTHY VOLUNTEERS. N. Corti, MD, A. Heck, MD, K. Rentsch, PhD, W. Zingg, MD, C. Pauli-Magnus, MD, Division of Clinical Pharmacology and Toxicology, University Hospital Zurich, Division of Clinical Pharmacology and Toxicology, University Hospital Zurich, Institute for Clinical Chemistry University Hospital Zurich, University Children's Hospital Zurich, Zurich, Switzerland.

**BACKGROUND/AIMS:** Concomitant therapy with protease inhibitors and mebendazole (MEB) or albendazole (ALB) is indicated in HIV-infected patients with concurrent helminthic disease, which is commonly observed in developing countries. Low bioavailability and extensive hepatic metabolism makes MEB and ALB prone to metabolic drug interaction. Recent publication of a potentially life threatening pharmacokinetic interaction between ritonavir and MEB and ALB supports an involvement of CYP3A4 inhibition as underlying mechanism. The purpose of this study was therefore to evaluate the effect of short term and long term treatment with ritonavir on MEB and ALB metabolism.

**METHODS:** 16 healthy volunteers were divided in two groups ( $2 \times n = 8$ ) and administered a single oral dose of 1000 mg MEB or 400 mg ALB. Serum pharmacokinetic parameters of MEB and ALB were studied in the absence (day 0) and the presence of co-treatment with ritonavir 400 mg bid (day 1 and day 8) to evaluate the inhibiting and inducing effect of ritonavir.

**RESULTS:** After short term ritonavir no significant changes in  $AUC_{0-24}$ ,  $C_{max}$ , total clearance and  $t_{1/2}$  were observed for MEB, ALB and its major metabolite ALB sulfoxide. In contrast long term ritonavir caused a significant reduction of MEB  $AUC_{0-24}$  and  $C_{max}$  ( $207 \pm 158$  ug\*h/l versus  $85.9 \pm 53$  ug\*h/l;  $p = 0.008$  and  $31 \pm 26$  ug/l versus  $11 \pm 6$  ug/l;  $p = 0.042$ ). Similarly  $AUC_{0-24}$  and  $C_{max}$  of ALB and ALB sulfoxide decreased significantly ( $100 \pm 111$ ug\*h/l versus  $24 \pm 21$  ug\*h/l;  $p = 0.018$  and  $15 \pm 11$ ug/l versus  $4.9 \pm 5.2$  ug/l;  $p = 0.044$  and  $5443 \pm 4726$  ug\*h/l versus  $2352 \pm 1896$  ug\*h/l;  $p = 0.008$  and  $453 \pm 399$  ug/l versus  $230 \pm 151$  ug/l;  $p = 0.042$ , respectively). No significant changes were observed for overall clearance and  $t_{1/2}$ .

**CONCLUSIONS:** Short term ritonavir has no significant inhibitory effect on MEB and ALB kinetics, while significant induction of metabolism is observed under long-term treatment. The absence of an inhibitory effect makes a CYP3A4 or MDR1 mediated mechanism of drug interaction unlikely. Alternatively, an induction of UDP-glucuronosyl-transferase involved in MEB and ALB Phase II metabolism might offer an explanation for the effect of ritonavir on MEB and ALB disposition. Dose adjustment of MEB and ALB may be necessary

after starting treatment with ritonavir to assure effective plasma concentration levels.

### PIII-74

ALISKIREN, A NOVEL ORAL DIRECT RENIN INHIBITOR FOR THE TREATMENT OF HYPERTENSION, DOES NOT AFFECT CARDIAC CONDUCTION OR REPOLARIZATION. S. Ayalasomayajula, C. Yeh, S. Vaidyanathan, B. Flannery, H. A. Dieterich, D. Howard, M. P. Bedigian, W. P. Dole, Novartis Pharmaceuticals Corporation, East Hanover, NJ, Novartis Pharma AG, Basel, Switzerland, Novartis Institutes for Biomedical Research, Cambridge, MA.

**BACKGROUND/AIMS:** In recent years, there has been an increased awareness of the potential for drugs to have unwanted effects on cardiac conduction and repolarization, including an increased risk for the development of cardiac arrhythmias. Aliskiren will be the first in a new class of oral direct renin inhibitors developed for the treatment of hypertension. This study assessed the effects of aliskiren on cardiac safety, as assessed by changes in ECG PR, QRS and QT intervals, in healthy subjects.

**METHODS:** This was a multicenter, double-blind, parallel-group study in 315 healthy subjects (18–65 years). Subjects were randomized to receive aliskiren (ALI) 300 mg, ALI 1200 mg, moxifloxacin 400 mg (MOX, positive control), or placebo (PBO) once daily for 7 days (steady-state drug levels). Digital ECGs obtained at baseline and day 7 were compared at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 23 hours post-dose. At each timepoint, three ECGs were obtained within 5 minutes and values for QRS, PR, QT and QTcF (Fridericia's formula) intervals averaged to obtain the intervals for each time point. The sample size was sufficient to determine if the upper 90% confidence interval for the difference in change in QTcF at any time point was less than 10 msec for an increase in QTcF of 3 msec or less.

**RESULTS:** Comparison of ALI to PBO showed that changes in QTcF were all within the predefined range for point estimates (<5 msec) and confidence intervals (< 10 msec) with only one exception. In the ALI 1200 mg group, the point estimate at 23 hrs for change in QTcF was 5.2 msec (90% confidence interval 2.2–8.1 msec). In contrast, significant QTcF prolongation occurred with MOX at each time point up to 4 hours after dosing. The incidence of QTcF > 450 msec was 0% with ALI 300 and 1200 mg, 4% with PBO and 6% with MOX. QTcF increase >30 msec was more frequent with MOX (14%) than ALI 300 mg (0%), ALI 1200 mg (1%) or PBO (1%). There was no correlation between QTcF and  $C_{max}$  or AUC of aliskiren. Aliskiren had no effect on PR or QRS duration.

**CONCLUSIONS:** Aliskiren at the highest anticipated therapeutic dose (300 mg) and a 4-fold higher dose (1200 mg) had no effect on cardiac conduction (PR and QRS intervals) or on repolarization (QTcF interval) following daily dosing for 7 days (steady state) in healthy volunteers.

### PIII-75

THE PHARMACOKINETICS OF THE ORAL DIRECT RENIN INHIBITOR ALISKIREN ALONE AND IN COMBINATION WITH THE P-GLYCOPROTEIN MODULATORS KETOCONAZOLE, DIGOXIN AND ATORVASTATIN IN HEALTHY SUBJECTS. S. Vaidyanathan, C. Reynolds, C. Yeh, M. Bizot, H. A. Dieterich, D. Howard, W. P. Dole, Novartis Pharmaceuticals Corporation, East Hanover, NJ, Novartis Pharma SAS, Rueil-Malmaison, France, Novartis Pharma AG, Basel, Switzerland, Novartis Institutes for Biomedical Research, Cambridge, MA.

**BACKGROUND:** The P-glycoprotein (P-gp) transporter has been shown to play an important role in the absorption, distribution, metabolism and excretion of certain drugs. Aliskiren, a novel orally active direct renin inhibitor, has been shown in *in vitro* assays to be a substrate but not an inhibitor of P-gp. We investigated the pharmacokinetic interaction between ALI and several drugs

known to be modulators of P-gp: ketoconazole (KETO; inhibitor), digoxin (DIG; substrate) and atorvastatin (ATOR; substrate and inhibitor).

**METHODS:** Three open-label, multiple-dose studies investigated the steady-state pharmacokinetic interactions between ALI 300 mg od and DIG 0.25 mg od (n = 22), ATOR 80 mg od (n = 21), or KETO 200 mg bid (n = 21) in healthy subjects. Subjects received ALI and DIG or ATOR alone or in combination in a 2-period crossover design; the KETO study had a single-sequence design. Plasma drug concentrations were determined by LC/MS/MS.

**RESULTS:** Co-administration with ALI resulted in small changes in DIG  $AUC_{\tau}$  (–15%; geometric mean ratio [GMR] 0.85 [90% CI, 0.75, 0.97]) and DIG  $C_{max}$  (–9%; GMR 0.91 [0.84, 0.99]), and ATOR  $AUC_{\tau}$  (–9%; GMR 0.91 [0.84, 1.00]) and ATOR  $C_{max}$  (–23%; GMR 0.77 [0.67, 0.88]). Co-administration with ATOR or KETO significantly increased ALI  $AUC_{\tau}$  by 47% (GMR 1.47 [1.29, 1.67]) and 76% (GMR 1.76 [1.64, 1.89]), respectively, and ALI  $C_{max}$  by 50% (GMR 1.50 [1.22, 1.85]) and 81% (GMR 1.81 [1.57, 2.09]), respectively. Co-administration with DIG had no significant effect on ALI exposure. ALI was safe and well tolerated alone and in combination with each drug. The most common adverse events were headache and dizziness (mild or moderate in severity); rates were similar for all ALI treatments.

**CONCLUSIONS:** ALI is not an inhibitor of P-gp-mediated transport *in vivo* as ALI had no clinically relevant effect on exposure of DIG or ATOR (P-gp substrates). ALI is a substrate for P-gp *in vivo* as ATOR and KETO (P-gp inhibitors) increased ALI exposure by 50% and 81%, respectively, indicating that P-gp-mediated intestinal efflux has a role in reducing the total absorption of orally administered ALI. Interactions of ALI with P-gp substrates are unlikely to occur. Based on the overall safety profile of ALI and <2-fold increase in exposure with P-gp inhibitors, initial dose adjustment will not likely be required during co-administration.

### PIII-76

LACK OF PHARMACOKINETIC INTERACTION BETWEEN ALISKIREN, A NOVEL DIRECT RENIN INHIBITOR, AND CELECOXIB IN HEALTHY VOLUNTEERS. A. Agarwal, S. Vaidyanathan, H. A. Dieterich, C. Yeh, D. Howard, W. P. Dole, Novartis Pharma AG, Basel, Switzerland, Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA, Novartis Institutes for Biomedical Research, Cambridge, MA, USA.

**BACKGROUND:** Many elderly patients with hypertension receive concomitant medication for chronic pain. This study assessed the pharmacokinetics of aliskiren, a novel orally active direct renin inhibitor efficacious for the treatment of hypertension, and celecoxib alone and in combination in healthy volunteers.

**METHODS:** In this open-label, multiple-dose study, 22 healthy volunteers (ages 18–45 yrs) received celecoxib 200 mg twice daily for 4.5 days, followed by a 5-day washout period. Subjects then received aliskiren 300 mg once daily for 7 days followed by co-administration of aliskiren once-daily and celecoxib twice daily for 4.5 days. Blood samples were taken at frequent intervals after dosing on the last day of each treatment period for the measurement of plasma drug concentrations and determination of pharmacokinetic parameters.

**RESULTS:** At steady state, co-administration of aliskiren and celecoxib had no significant effect on aliskiren AUC (geometric mean ratio: 0.88, [90% CI 0.77, 1.00]) or  $C_{max}$  (geometric mean ratio: 1.00, [0.78, 1.27]). Co-administration had no significant effect on celecoxib AUC (geometric mean ratio: AUC 0.95 [0.88, 1.03]) or  $C_{max}$  (geometric mean ratio: 1.02 [0.88, 1.18]). The most frequently reported adverse events were headache (celecoxib = 0, aliskiren = 4, aliskiren + celecoxib = 0) and dizziness (celecoxib = 1, aliskiren = 1, aliskiren + celecoxib = 4). All adverse events were mild or moderate in severity and none required drug discontinuation.

**CONCLUSIONS:** Aliskiren and celecoxib have no significant pharmacokinetic interactions at steady state in healthy volunteers.

**Table 1.** Aliskiren PK Parameters (n = 22; values are mean ± SD)

	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (ng.h/mL)
Aliskiren	241.6 ± 167.7	2.7 ± 1.8	1480 ± 888
Aliskiren + celecoxib	235.5 ± 183.3	2.0 ± 1.3	1266 ± 727

**Table 2.** Celecoxib PK Parameters (n = 22; values are mean ± SD)

	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (ng.h/mL)
Celecoxib	902 ± 326	2.6 ± 1.1	6324 ± 2559
Celecoxib + aliskiren	921 ± 299	2.9 ± 1.2	5972 ± 2146

### PIII-77

ASSESSMENT OF PHARMACOKINETIC INTERACTION BETWEEN ALISKIREN, A NOVEL DIRECT RENIN INHIBITOR, AND METFORMIN IN HEALTHY VOLUNTEERS. C. Reynolds, S. Vaidyanathan, H. A. Dieterich, C. Yeh, D. Howard, W. P. Dole, Novartis Pharmaceuticals Corporation, East Hanover, NJ, Novartis Pharma AG, Basel, Switzerland, Novartis Institutes for Biomedical Research, Cambridge, MA.

**BACKGROUND:** Hypertension and type 2 diabetes are common comorbidities. This study assessed the pharmacokinetics of aliskiren, a novel direct renin inhibitor for the treatment of hypertension, and the oral hypoglycemic agent metformin in healthy volunteers.

**METHODS:** In this open-label, multiple-dose study, 22 healthy volunteers (ages 18–45 yrs) received metformin 1000 mg orally once a day for 4 days, followed by a 4-day washout period. Subjects then received aliskiren 300 mg once a day for 7 days followed by co-administration of aliskiren and metformin for 4 days. Blood samples were taken to determine plasma drug concentrations at frequent intervals after dosing on the last day of each treatment period.

**RESULTS:** At steady state, AUC and C<sub>max</sub> for aliskiren were lower when co-administered with metformin compared with aliskiren alone (geometric mean ratios: AUC 0.73 [90% CI 0.64, 0.84]; C<sub>max</sub> 0.71 [0.56, 0.89]), but these changes were not considered clinically relevant. AUC and C<sub>max</sub> for metformin were similar when co-administered with aliskiren compared with metformin alone as the geometric mean ratios (AUC 0.88 [0.80, 0.96]; C<sub>max</sub> 0.89 [0.80, 0.99]) fell within the equivalence range (0.80, 1.25). The most frequently reported adverse events were gastrointestinal (abdominal pain/discomfort, diarrhea) occurring in 4 subjects during administration of metformin, 2 subjects during administration of aliskiren and 6 subjects during co-administration of aliskiren and metformin. All adverse events were mild or moderate in severity and none required drug discontinuation.

**CONCLUSIONS:** Co-administration of aliskiren with metformin resulted in a modest decrease in aliskiren exposure (<30%), with no notable effect on the pharmacokinetics of metformin in healthy volunteers.

**Table 1.** Aliskiren PK Parameters (n = 19; values are mean ± SD)

	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (ng.h/mL)
Aliskiren	329.4 ± 228.1	1.5 ± 1.7	2278 ± 1404
Aliskiren + metformin	209.7 ± 94.5	1.5 ± 1.5	1547 ± 705

**Table 2.** Metformin PK Parameters (n = 21; values are mean ± SD)

	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (ng.h/mL)
Metformin	1953 ± 532	2.3 ± 0.6	12528 ± 3222
Metformin + aliskiren	1715 ± 378	1.6 ± 0.8	10966 ± 2616

### PIII-78

ASSESSMENT OF THE PHARMACOKINETIC INTERACTION BETWEEN THE ORAL DIRECT RENIN INHIBITOR ALISKIREN AND FUROSEMIDE: A STUDY IN HEALTHY VOLUNTEERS. C. Zhao, S. Vaidyanathan, H. A. Dieterich, C. Yeh, D. Howard, W. P. Dole, Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA, Novartis Pharma AG, Basel, Switzerland, Novartis Institutes for Biomedical Research, Cambridge, MA.

**BACKGROUND:** Aliskiren is a novel orally active direct renin inhibitor developed for the treatment of hypertension. Furosemide, a loop diuretic, is used for the treatment of heart failure and chronic renal failure with hypertension. Aliskiren is primarily eliminated unchanged in the feces and shows moderate protein binding (approximately 50%); furosemide is excreted mainly unchanged in the urine and shows high plasma protein binding (up to 99%). This study assessed the pharmacokinetics of aliskiren and furosemide administered alone and in combination.

**METHODS:** In this open-label, multiple-dose study, 21 healthy volunteers (ages 18–45 years) received oral furosemide 20 mg once daily for 3 days followed by a 3-day washout. Subjects then received oral aliskiren 300 mg once daily for 7 days, followed by co-administration with furosemide for an additional 3 days. Plasma drug concentrations were determined at frequent intervals for up to 24 h on the last day of each treatment period.

**RESULTS:** At steady state, co-administration of furosemide with aliskiren reduced aliskiren C<sub>max</sub> by 20% (geometric mean ratio 0.80 [90% CI: 0.65, 0.97]) with no change in aliskiren AUC (mean ratio 0.93 [0.84, 1.04]). Co-administration with aliskiren resulted in a 28% decrease in furosemide AUC (mean ratio 0.72 [0.64, 0.81]) and a 49% decrease in furosemide C<sub>max</sub> (mean ratio 0.51 [0.39, 0.66]). Aliskiren was well tolerated alone and in combination with furosemide. The most common adverse event was headache, which was reported by 7 subjects during the study; the majority of adverse events were mild in intensity.

**CONCLUSIONS:** Co-administration of aliskiren and furosemide reduced exposure to furosemide. Since inhibition of the renin-angiotensin system may enhance diuretic sensitivity (Bindschedler et al, Eur J Clin Pharmacol [1997] 52: 371–378), the clinical significance of the reduced furosemide exposure is uncertain. Furosemide had no major effect on the pharmacokinetics of aliskiren.

### PIII-79

LACK OF AN EFFECT OF EZETIMIBE ON THE PHARMACOKINETICS OF TORCETRAPIB/ATORVASTATIN IN HEALTHY SUBJECTS. K. Diringier, BS, M. A. Gibbs, PhD, N. Amin, PharmD, R. LaBadie, MS, T. T. Thuren, MD PhD, C. L. Shear, DrPH, Pfizer Global Research and Development, Groton/New London, CT.

**BACKGROUND:** Torcetrapib (T), a potent and selective inhibitor of cholesteryl ester transfer protein, can substantially elevate HDL-C. Atorvastatin (A), an inhibitor of HMG-CoA reductase, is approved for clinical use as an agent for lowering LDL-C. The fixed combination of T/A is currently in Phase 3 testing for treating dyslipidemias and slowing the progression of atherosclerosis. It is likely that some patients will require Ezetimibe in addition to T/A.

**METHODS:** The current study was a fixed-sequence, open-label, 2-period study to assess the effect of repeat daily doses of EZE 10 mg on the pharmacokinetics of a single dose of fixed combination T/A 60 mg/10 mg in healthy adult subjects. Pharmacokinetic (PK) parameters for T and A were calculated from the individual plasma concentration-time profiles in the absence (N = 53) and presence (N = 49) of steady-state EZE.

**RESULTS:** All agents were well tolerated. Visual inspection of mean and individual plasma EZE trough concentration-time profiles revealed that the majority of subjects achieved steady-state conditions within 6 days of daily dosing. The mean plasma T and A PK profiles and PK parameter estimates for EZE+T/A and T/A alone were

similar. The point estimates (90% confidence intervals [CIs]) of the ratios (with/without EZE) for torcetrapib AUC and  $C_{max}$  were 99.4% (95.7%, 103%) and 96.1% (87.1%, 106%), respectively. The point estimates (90% CIs) of the ratios (with/without EZE) for atorvastatin AUC and  $C_{max}$  were 103% (93.4%, 113%) and 95.0% (87.2%, 104%), respectively. Ninety percent CIs for ratios of mean AUC and  $C_{max}$  values were contained within the range of 80% to 125%, indicating that there was no clinically relevant effect of EZE on the pharmacokinetics of T or A.

**CONCLUSION:** These results indicate that T/A and EZE can be safely co-administered.

### PIII-80

EFFECT OF STEADY-STATE DILTIAZEM OR RIFAMPIN ON THE SINGLE DOSE PHARMACOKINETICS OF TORCETRAPIB/ATORVASTATIN. K. Diringer, BS, N. Amin, PhD, R. Labadie, PhD, T. Thuren, MD, PhD, C. Shear, PhD, M. Gibbs, PhD, Pfizer, Inc., Pfizer, Inc, Groton, CT.

**AIM:** The objectives of this study were to evaluate the effect of steady-state Diltiazem 240 mg QD (DILT) or Rifampin 600 mg QD (RIF) on the single dose pharmacokinetics (PK) of fixed combination torcetrapib/atorvastatin 60/40 mg/mg (T/A).

**METHODS:** This was a randomized, open-label, single period study in healthy adult subjects. Each subject was randomized to one of the following treatments: T/A alone [Day 1]; DILT [Days -6 through 21] + T/A [Day 1]; or RIF [Days -6 through 21] + T/A [Day 1]. Following T/A dosing on Day 1, blood samples were collected from all subjects up to 72 h post-dose and 504 h post-dose for the analysis of A and T, respectively.

**RESULTS:** When T/A was administered in the presence of DILT, exposures to both A and T increased approximately 51% and 63%, respectively, though the terminal phase did not appear markedly different for T, and the half-life of A was similar when administered alone (8 h) and in the presence of DILT (12 h). When T/A was administered in the presence of RIF, mean A AUClast and  $C_{max}$  increased ~12% and ~190%, respectively. The mean elimination half-life of A was markedly shorter following co-administration of T/A with RIF. T exposure was ~72% lower and the elimination phase appeared shorter when T/A was administered in the presence RIF compared to T/A administered alone.

**CONCLUSIONS:** The increases in total exposure to T and A observed following co-administration of T/A + DILT are consistent with inhibition by DILT of CYP3A-mediated metabolism. The decrease in exposure to T observed following co-administration of T/A + RIF is consistent with induction of CYP3A-mediated metabolism by rifampin. The effect of RIF on the PK of A suggests inhibition by RIF of organic anion transporting polypeptide-C (OATP-C) mediated A transport, in addition to the CYP3A inductive effects of RIF.

### PIII-81

PHARMACOKINETICS OF VILDAGLIPTIN AND VALSARTAN DURING CO-ADMINISTRATION. K. Dole, PharmD, Y. He, PhD, M. Ligueros-Saylan, MD, Y. Wang, J. Campestrini, PhD, F. Pommier, PhD, W. Dole, MD, G. Sunkara, PhD, Novartis Pharmaceuticals, Novartis Pharmaceuticals, East Hanover, NJ.

**BACKGROUND:** Vildagliptin, a selective DPP-4 inhibitor, is being developed for the treatment of type 2 diabetes. Hypertension and heart failure occur commonly in patients with diabetes. Valsartan, an ATI receptor blocker, is frequently used to treat hypertension and heart failure. Vildagliptin is primarily metabolized by hydrolysis, whereas valsartan is mainly eliminated by biliary excretion as unchanged drug. In this study we investigated the potential for pharmacokinetic drug interactions between vildagliptin and valsartan.

**METHODS:** The study was an open label, single center, multiple dose, three period, cross-over study in 28 healthy subjects. All subjects received once daily doses of vildagliptin 100 mg, valsartan

320 mg, or the combination vildagliptin 100 mg + valsartan 320 mg for seven days with an inter-period washout of 7 days. Plasma vildagliptin and valsartan levels were determined using a validated LCMS/MS method. PK and statistical analyses were performed using WinNonlin and SAS.

**RESULTS:** During co-administration with valsartan, the 90% confidence intervals for  $C_{max,ss}$  and  $AUC_{0-\tau}$  of vildagliptin were within the 80–125% equivalence range compared to exposures for vildagliptin given alone.  $C_{max,ss}$  and  $AUC_{0-\tau}$  for valsartan were increased by 14% and 24%, respectively, during drug co-administration (90% CI for valsartan  $C_{max,ss}$  and  $AUC_{0-\tau}$  were 98–134% and 109–141%, respectively). The oral clearance (Cl/F) and terminal elimination half life of valsartan were comparable between treatments. The most common reported adverse events were headache and gastrointestinal complaints (most mild in severity).

**CONCLUSIONS:** The steady state pharmacokinetics of vildagliptin were not affected when co-administered with valsartan. Exposure to valsartan was slightly increased during co-administration with vildagliptin which was not considered clinically relevant.

### PIII-82

MODEL-BASED EVALUATIONS TO SELECT AND CONFIRM DOSES IN THE CLINICAL DEVELOPMENT OF EXENATIDE. M. Fineman, L. Phillips, D. J. Jaworowicz, B. Cirincione, E. Ludwig, K. Taylor, P. A. Kothare, A. D. Baron, T. Grasela, Amylin Pharmaceuticals, Inc., Cognigen Corporation, Eli Lilly and Company, San Diego, CA.

**BACKGROUND:** Population pharmacokinetic (PK) and pharmacodynamic (PK/PD) models based on early Phase 1 and 2 data for the new antidiabetic medication exenatide (EX) supported transition from weight-based to fixed 5 and 10  $\mu$ g subcutaneous BID dosing for Phase 3 trials (CPT 2002;71:P29). The model-based dose selection process was designed to target exenatide exposure that would maximize glucose reduction and mitigate gastric AEs. The models were iteratively refined with the inclusion of additional Phase 2 and 3 data to further describe the PK and exposure-response (ER) relationships. These models confirmed the appropriateness of the selected doses and allowed for the quantitative assessment of the influence of intrinsic variables such as age, weight, and gender.

**METHODS:** 4870 EX concentrations from 229 patients with type 2 diabetes (subj) across 10 clinical trials were applied to the existing PK model (1-CMT with nonlinear plus linear absorption and linear elimination). The relationship between EX  $AUC_{0-3h}$  and glucose (gluc)  $AUC_{0-3h}$  (540 pairs from 183 subj) was assessed in an inhibitory sigmoid- $E_{max}$  model. Covariate evaluation included the influence of selected subject descriptors, clinical chemistries, concomitant oral antidiabetic agent use, and anti-ex antibody (Ab) status on PK and PK/PD. Model validation was performed by bootstrapping.

**RESULTS:** For the PK model, weight and anti-EX Ab status were significant covariates for CL/F and V/F, and gender for absorption rate. In the PK/PD model, significant factors for  $E_{max}$  were baseline gluc AUC and anti-EX Ab status. The magnitude of these effects did not support dose adjustment. The population mean estimated decrease in gluc was ~27% and 33% for the 5 and 10  $\mu$ g bid doses, respectively. Both doses approach the asymptotic portion of the exposure-response curve. With EX doses of 5  $\mu$ g, 55% of subj achieved 75% of their predicted maximum glucose reduction. With 10  $\mu$ g doses, 90% achieved that same target.

**CONCLUSION:** This model-based drug development approach demonstrated the value of the learn-and-confirm paradigm. Dose selection was supported by prelim PK and PK/PD models in a small number of subj, and confirmed with larger numbers of subj from Phase 2 and 3 trials. These results verified that EX doses of 10  $\mu$ g BID optimize clinical benefit in terms of glycemic control.

**PIII-83**

THE EFFECTS OF GRADED CONCENTRATIONS OF ORAL ETHANOL ON THE *IN-VIVO* BIOAVAILABILITY OF OXYMORPHONE HCL EXTENDED RELEASE (ER) TABLETS. W. D. Fiske, PhD, G. Photivihok, I. H. Benedek, PhD, Endo Pharmaceuticals, Chadds Ford, PA.

**BACKGROUND/AIMS:** To evaluate the single-dose bioavailability of oxymorphone HCl extended release (ER) 40 mg tablet when co-administered with graded concentrations of aqueous ethanolic solutions under fasted conditions. *In-vitro* dissolution studies have shown that ethanol does not cause premature release (dose dumping) of oxymorphone from the tablet.

**METHODS:** This was an open-label, single-dose, randomized, 4-period crossover study in healthy volunteers conducted at a single clinical research facility. Thirty subjects were enrolled, and 25 completed all 4 periods. Each subject was randomly assigned to receive a single oral dose of oxymorphone ER 40 mg tablet co-administered with one of three ethanolic solutions or water over 4 periods, with each period separated by 7 days. The 4 aqueous solutions evaluated were: 240 ml of 40% ethanol, 240 ml of 20% ethanol, 240 ml of 4% ethanol and 240 ml water (0% ethanol). Serial blood samples for oxymorphone plasma concentration determination and PK were obtained through 48 hours post dose administration. Subjects were administered naltrexone (50 mg) to minimize the potential for significant opioid-related AEs.

**RESULTS:** Graded concentrations of ethanol showed a dose response for peak oxymorphone plasma concentration (C<sub>max</sub>). Increases of 70%, 31% and 7% were seen with co-administration of 240 ml 40%, 20%, and 4% ethanol solutions, respectively. There was no effect on AUC. There were no serious or unexpected AEs in this study. The most frequent AE was vomiting.

**CONCLUSIONS:** The results of this study indicate that there is an interaction between ethanol and oxymorphone HCl ER 40 mg tablets when the two are co-administered. This interaction is manifested as an ethanol dose-related increase in peak oxymorphone plasma concentration (C<sub>max</sub>). There was no effect on AUC. The nature of the interaction is unknown. *In-vitro* dissolution studies suggest this is not a deterioration of the formulation itself caused by ethanol. Further, *in-vitro* dissolution studies may not be predictive of *in-vivo* alcohol effects.

**PIII-84**

NO CLINICALLY RELEVANT CHANGES IN THE EXPOSURE OF LOVASTATIN OR PRAVASTATIN WITH CONCURRENT ADMINISTRATION OF LY518674. N. A. Farid, PhD, C. J. Harris, PharmD, E. Abu-Raddad, PhD, M. Wang, PhD, D. C. Howey, MD, Eli Lilly and Company, Indianapolis, IN.

**BACKGROUND/AIMS:** LY518674, 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)methyl]-5-oxo-1H-1,2,4-triazol-3-yl]propyl]phenoxy]-2-methylpropionic acid, is a potent agonist at the human peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ). Fibrates, such as gemfibrozil, are ligands of the nuclear receptor PPAR $\alpha$ . Gemfibrozil increased the exposure to lovastatin acid by 280% when given with lovastatin, and that of pravastatin by 202%. The objective of this study was to evaluate the effect of LY518674 coadministration on the exposure of lovastatin or pravastatin. The 2 representative statins were selected because of their differing metabolic pathways: lovastatin is a CYP3A substrate and pravastatin is primarily conjugated with glucuronic acid.

**METHODS:** A single center, open-label, fixed sequence (replicate design for pravastatin), two-period study in 52 healthy subjects. Safety and pharmacokinetic parameters of lovastatin and pravastatin were evaluated in the absence and presence of LY518674. In Part A LY518674, 0.1 mg once daily, was given on Days 1 to 4 and lovastatin 40 mg was given on Days -1 and 4. In Part B, LY518674, 0.1 mg once daily, was given on Days 1 to 6 and pravastatin 40 mg was given on Days -3, -1, 4, and 6.

**RESULTS:** Administration of either statin with LY518674 was well tolerated with no serious adverse events. Lovastatin AUC<sub>0-12</sub> increased 19% with LY518674 coadministration (p-value = 0.003) and C<sub>max</sub> did not significantly change, while these parameters for lovastatin  $\beta$ -hydroxyacid decreased by 43% (p-value < .001). Slight increases in pravastatin AUC<sub>0- $\infty$</sub>  (18%, p-value < .001) and C<sub>max</sub> (26%, p-value < .001) were observed with LY518674 coadministration.

**CONCLUSION:** The statistically significant changes in lovastatin, lovastatin acid, or pravastatin exposure observed following LY518674 administration are not perceived to confer increased risk for adverse effects driven by lovastatin or pravastatin exposure. These results are in contrast to what has previously been shown with gemfibrozil. Thus concurrent use of LY518674 with lovastatin or pravastatin should not produce clinically relevant increases in the exposure to these statins.

**PIII-85**

SINGLE AND MULTIPLE DOSE PHARMACOKINETICS OF PPAR ALPHA AGONIST LY518674. E. Abu-Raddad, PhD, A. Long, BS, C. Ernest, BS, C. J. Harris, PharmD, M. Wang, PhD, N. Farid, PhD, D. C. Howey, MD, Eli Lilly and Company, Indianapolis, IN.

**BACKGROUND/AIMS:** LY518674 is a potent and selective agonist of the human peroxisome proliferator-activated alpha (PPAR $\alpha$ ) receptor. Our objective was to characterize the pharmacokinetics (PK) of LY518674 after single- and multiple-dose administration.

**METHODS:** Serial plasma samples were collected from healthy obese subjects administered LY518674 in single escalating doses of 0.01 to 60 mg, or 0.025 to 10 mg QD for 14 days per os. LY518674 concentrations were determined using a validated HPLC/MS/MS method. PK parameters were calculated using standard noncompartmental methods.

**RESULTS:** LY518674 was rapidly absorbed and had a biphasic decline that was more evident in doses of 0.1 mg and above due to assay limitation. AUC and C<sub>max</sub> increased in proportion to dose upon single- and multiple-dose administration. Steady state conditions were achieved within 2 days after the first dose with mean accumulation ratio less than 1.3, consistent with the half-life of LY518674. Single- and multiple-dose PK were similar. Mean apparent clearance after 14 daily doses (3.73 L/h) was not different from that after the first dose. PK parameters are described below as geometric mean (CV%).

Dose, mg	N	C <sub>max</sub> (ng/mL)	AUC* (ng·h/mL)	t <sub>1/2</sub> (h)
<b>Single dose</b>				
0.01	6	0.257 (23.3)	1.82 (17.9)	4.77 (17.4)
0.1	7	3.35 (44.5)	29.2 (39.5)	7.23 (32.5)
1	7	30.1 (18.5)	226 (17.3)	9.76 (16.9)
10	8	297 (27.9)	2060 (36.7)	8.11 (20.9)
30	6	1070 (40.0)	7640 (40.7)	9.04 (14.5)
60	6	1750 (22.7)	11900 (37.9)	6.96 (23.5)
<b>Multiple dose</b>				
0.025	6	0.903 (20.5)	5.93 (28.3)	4.72 (32.1)
0.1	7	3.92 (31.9)	25.6 (33.0)	5.78 (21.9)
1	6	51.1 (26.0)	340 (21.5)	9.04 (24.7)

\* AUC(0- $\infty$ ) for single-dose and AUC <sub>$\tau$ ,ss</sub> for multiple-dose administration. The dose-limiting toxicity after single dose administration was gastrointestinal effects at the 60mg dose level. The dose-limiting toxicity after multiple dose administration was severe myopathy at the 10mg dose level.

**CONCLUSION:** LY518674 exhibits dose and time independent pharmacokinetics in healthy obese subjects.

### PIII-86

THE INFLUENCE OF RENAL IMPAIRMENT ON THE PHARMACOKINETICS OF VILDAGLIPTIN. Y. L. He, PhD, B. Flannery, BS, Y. Wang, J. Campestrini, PhD, M. Ligueros-Saylan, MD, W. P. Dole, MD, D. Howard, PhD, Novartis, Novartis, Cambridge, MA.

**BACKGROUND:** Vildagliptin is a potent and selective DPP-4 inhibitor in clinical development for the treatment of type 2 diabetes. The major elimination pathway is hydrolysis and 23% of an oral dose is excreted as parent in the urine. Kidney is also demonstrated to be one of the major organs that contributes to the hydrolysis metabolism. The objective of this study was to investigate the influence of renal impairment (RI) on the pharmacokinetics (PK) of Vildagliptin.

**METHODS:** The PK of Vildagliptin was determined in subjects with mild (GFR = 50–80 mL/min), moderate (GFR = 30–50 mL/min), severe (GFR < 30 mL/min) renal impairment (RI), and subjects with end stage renal disease (ESRD), compared to subjects with normal renal function (HV) matched for age, gender and body weight. Each group consisted of 6 subjects. Each subject received a single oral dose of 100 mg Vildagliptin. Blood samples were collected to determine plasma concentrations of Vildagliptin and its major inactive metabolite (LAY151) with LC-MS/MS.

**RESULTS:** Compared to HV, exposure to vildagliptin in subjects with various degrees of RI and ESRD was increased ( $C_{max}$ : 8–66%;  $AUC_{0-\infty}$ : 32–134%). There was considerable variability in the  $C_{max}$  and  $AUC_{0-\infty}$  among groups. Renal clearance ( $CL_R$ ) in HV was 12.4 L/h, and a reduction in  $CL_R$  was observed in subjects with RI, which also correlated with the GFR ( $R^2 = 0.75$ ). In contrast, the increase in exposure ( $AUC_{0-\infty}$ ) or  $CL/F$  of vildagliptin in RI (average 70% for all subjects with RI) did not correlate with the GFR. Exposure to the inactive metabolite (LAY151) increased in subjects with RI and the magnitude of increase in the exposure was correlated with the severity of RI.

**CONCLUSION:** The changes in exposure to vildagliptin does not correlate with GFR, and the average increase was less than 2-fold when pooled all subjects with RI. Dose adjustment for vildagliptin is not considered necessary for subjects with RI, and this is further supported by the clinical safety data in long term trials.

### PIII-87

PHARMACOKINETIC COMPARISON OF EXTENDED-AND IMMEDIATE-RELEASE ORAL FORMULATIONS OF SIMVASTATIN IN HEALTHY KOREANS. S. Jang, BS, J. Choi, MD, PhD, M. Park, MD, K. Kim, MD, PhD, K. Park, PhD, MD, Yonsei University College of Medicine, Seoul, Republic of Korea. Supported by Brain Korea 21 Project for Medical Science, Yonsei University.

**BACKGROUND:** An extended-release (ER) formulation of simvastatin would be expected to have more efficient hepatic uptake by sustained delivery of the drug to the liver. This study compared the pharmacokinetics of ER and immediate-release (IR) formulations of simvastatin after multiple-dose given in healthy subjects.

**METHODS:** This was designed as a randomized, multiple-dose, parallel study. 29 subjects were randomly assigned to the newly-developed test-formulation (ER,  $n = 15$ ) and reference-formulation (IR,  $n = 14$ ) of simvastatin. Each subject received an oral dose of 40 mg every morning for 8 consecutive days. Blood samples were collected at 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 13, 17, 24 hours after dosing on day 1 and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 13, 17, 24, 36 and 48 hours after dosing on day 8. Plasma concentrations were analyzed by the LC/MS/MS method, and  $AUC_{last}$  (AUC from dosing to the last sample time),  $C_{max}$ ,  $t_{max}$ , and  $t_{1/2}$  were determined by a non-compartment method using WinNonlin, for both simvastatin and simvastatin acid.

**RESULTS:** For simvastatin acid, which is the active compound of the drug, for day 1,  $AUC_{last}$ ,  $C_{max}$ ,  $t_{max}$  for ER vs IR formulation were on the average 23.2 vs 31.2 ng·hr/ml, 2.2 vs 4.5 ng/ml, and 8.7 vs 4.0 hr, respectively, and for day 8,  $AUC_{last}$ ,  $C_{max}$ ,  $t_{max}$ ,  $t_{1/2}$  for ER vs IR formulation were on the average 57.6 vs 41.4 ng·hr/ml, 3.4 vs

5.2 ng/ml, 8.4 vs 4.6 hr, 13.1 vs 4.5 hr, respectively. These results show that the ER formulation has smaller  $C_{max}$ , later  $t_{max}$  and longer  $t_{1/2}$  compared with the IR formulation, reflecting the ideal characteristics of slow-release formulation. Although not statistically significant ( $p = 0.2256$ ),  $AUC_{last}$  for the ER formulation for day 8 was larger than IR while it was smaller for day 1, which may be caused by a parallel design of using different subjects for two groups, yielding considerable interindividual variation. The results with simvastatin were similar with simvastatin acid.

**CONCLUSION:** This study shows that the new ER formulation of simvastatin may have ideal characteristics of slow-release formulation in most of the noncompartmental pharmacokinetic measures in Korean populations. To better evaluate the characteristics of the ER formulation, integrated results including more subjects' kinetic data as well as dynamic data may be needed.

### PIII-88

PHARMACOKINETIC INTERACTIONS BETWEEN RANOLAZINE AND HMG-CoA REDUCTASE INHIBITORS IN VITRO AND IN VIVO. M. Jerling, MD, PhD, CV Therapeutics, Palo Alto, CA.

**BACKGROUND/AIMS:** Ranolazine is approved by the FDA for the treatment of chronic angina in combination with amlodipine, beta blockers or nitrates, in patients who have not achieved adequate response with other antianginals. It is a CYP3A and P-glycoprotein (Pgp) substrate. The kinetic interactions with the HMG-CoA reductase inhibitors atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin and simvastatin was evaluated in vitro, and the interaction with simvastatin in healthy volunteers.

**METHODS:** HMG-CoA reductase inhibitors were incubated with human liver microsomes with quantification of parent compound and metabolites. Inhibition constants for ranolazine in these assays were determined. In the clinical study 18 healthy volunteers received a single 80 mg simvastatin dose on Day 1, ranolazine 1750 mg in the morning of Day 3 followed by 1000 mg bid up to Day 9, and simvastatin 80 mg qd Days 6–9.  $AUC_{0-\infty}$  after the first simvastatin dose and  $AUC_{\tau}$  on Day 9 at steady-state were compared for simvastatin lactone, simvastatin acid, 6'-exomethylenesimvastatin, 3'-hydroxysimvastatin, and HMG-CoA reductase inhibitor activity.

**RESULTS:** In the microsomal assays ranolazine weakly inhibited CYP450-dependent metabolism of all statins except pravastatin with  $K_i$  values >20  $\mu$ M and  $IC_{50}$  values >46  $\mu$ M. Simvastatin had the highest intrinsic clearance. All statins except pravastatin were Pgp substrates where the difference between basal-to-apical and apical-to-basal transport was lowest for atorvastatin and similar for the other statins. Ranolazine inhibited Pgp-mediated transport of all statins except pravastatin across MDCK-MDR1 cell monolayers with the lowest  $IC_{50}$  value of 39.5  $\mu$ M for simvastatin. In humans ranolazine increased AUC 1.59-fold for simvastatin lactone (90% CI 1.37–1.84), 1.39-fold for simvastatin acid (1.14–1.71), 1.32-fold for 6'-exomethylenesimvastatin (1.04–1.67), and 1.59-fold for HMG-CoA reductase inhibitor activity (1.45–1.74). AUC decreased for 3'-hydroxysimvastatin.

**CONCLUSION:** In vitro results indicate that simvastatin is the statin most sensitive to interactions with ranolazine through CYP3A and Pgp inhibition. Ranolazine at the maximum labeled dose increased AUC for simvastatin compounds and HMG-CoA reductase inhibitor activity less than 1.6-fold in humans.

### PIII-89

EARLY MORNING SPOT URINE VOID IS AN IDEAL ALTERNATIVE TO 24 HOUR URINE COLLECTION FOR DETERMINATION OF BIOMARKERS OF EXPOSURE IN ADULT SMOKERS. S. Kapur, S. Mohamadi, R. Muhammad, R. Serafin, Q. Liang, S. Feng, H. Roethig, PM USA, Richmond, VA.

**BACKGROUND:** Cigarette smoke exposure in adult smokers (SM) can be determined by measuring urinary excretion of selected smoke constituents or metabolites. Complete 24-hour urine (24H)

collections are difficult and inconvenient in ambulatory studies; therefore spot urine collection (SU) (single collection at a specific time point) might be a useful alternative. We have previously shown that there is a good correlation and statistical agreement between SU and 24H urine collections for most of the biomarkers of tobacco exposure (BOE) (Kapur et al. Poster Presentation, ASCPT 2006).

**METHODS:** In this open labeled, forced switching ambulatory study, 20 adult conventional cigarette smokers were switched to an electrically heated smoking system (Roethig et al. *J Clin Pharmacol*, 45(2):133–45, 2005) as a prototype cigarette for a period of 8 days. Early morning SU void and 24H urine samples were collected at baseline and post-baseline periods. The following biomarkers of exposure were evaluated: nicotine and its five metabolites, Nicotine equivalents (NE), mono-hydroxy butenyl mercapturic acid (MHBMA, a metabolite of butadiene), total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, metabolite of tobacco specific nitrosamines), 3-hydroxy propyl mercapturic acid (3-HPMA metabolite of acrolein), s-phenyl mercapturic acid (S-PMA, metabolite of benzene) and total 1-hydroxypyrene (1-OHP).

**RESULTS:** Mean percent change from baseline in 24H and SU (creatinine adjusted and non-adjusted) were very similar and statistically non-significant ( $p > 0.05$ ) for the selected biomarkers of exposure except for MHBMA. The mean ( $\pm$  SD) percent change from baseline in NE in 24H urine and SU were  $-27.53 \pm 35.37$  mg and  $-23.98 \pm 30.79$  mg respectively ( $p > 0.05$ ). For NNAL, mean percent change from baseline in 24H urine and SU were  $-52.33 \pm 18.04$  ng and  $-52.09 \pm 23.14$  ng respectively ( $p > 0.05$ ). When adjusted for creatinine, mean percent change in NE for 24H and SU were  $-30.87 \pm 35.45$  mg/g and  $-25.94 \pm 27.49$  mg/g respectively ( $p > 0.05$ ). Similarly, when adjusted for creatinine mean percent change from baseline in NNAL for 24H and SU were  $-54.39 \pm 18.69$  ng/g and  $-52.31 \pm 23.61$  ng/g respectively ( $p > 0.05$ ).

**CONCLUSION:** Early morning SU collections would be ideal for ambulatory or surveillance studies to evaluate biomarkers of exposure in SM.

### PIII-90

TAK-475, A SQUALENE SYNTHASE INHIBITOR: MASS BALANCE AND EXCRETION STUDY. A. Karim, PhD, A. Abeyratne, PhD, F. Siebert, BS, MT, L. Hetman, BS, K. Teshima, MSc, T. Kondo, PhD, Takeda Global Research & Development, Takeda Pharmaceutical Company, Deerfield, IL.

**BACKGROUND:** TAK-475, a squalene synthase inhibitor, has shown lipid-lowering effects of potential clinical utility; pharmacokinetic and metabolic studies were conducted during clinical development.

**METHODS:** Disposition kinetics of TAK-475 and its two major active metabolites (M-I and M-II) were evaluated in 8 fasting healthy males. After oral administration of a single 100 mg ( $\sim 100 \mu\text{Ci}$ ) dose of [ $^{14}\text{C}$ ]TAK-475 in suspension, whole blood, plasma, urine, and stool were collected. Radioactivity in each sample was determined using liquid scintillation counting and metabolic profile determined by accelerator mass spectrometry (AMS). Plasma concentrations of TAK-475 and active hydroxylated and carboxylated metabolites (M-I and M-II, respectively) were also determined using liquid chromatography/tandem mass spectroscopy.

**RESULTS:** Plasma radioactivity associated with unchanged [ $^{14}\text{C}$ ] TAK-475 showed rapid absorption, with a  $T_{\text{max}}$  of 3.5 h. Scintillation counting of whole blood, plasma, and red blood cells (RBCs) showed plasma radioactivity at 0.75 h (1.9 ng/g) and reached the maximum drug-derived radioactivity concentration of 53.10 ng/g at 3 h. No radioactivity was detected in RBCs up to 4 h postdose. Using AMS, quantifiable radioactivity was found in whole blood and plasma at 0.25 h postdose. Maximum mean radioactivity concentration was seen at 6 h in pooled plasma (33.2 ng Eq/mL) and pooled whole blood (23.8 ng Eq/g). Mean total radioactivity in pooled RBCs increased from 4.47 ng Eq/g at 1 hr to a maximum of 9.37 ng Eq/g at 4 h postdose. The RBC/plasma ratio indicates insignificant selective uptake of radioactive materials in RBCs.

An average of 98.6% of the administered dose was recovered in feces, due to biliary excretion of absorbed drug and/or its metabolites; only 0.2% of the radioactive dose was recovered in urine; total recovery was 98.8%. Recovered radioactivity in feces was in the form of TAK-475 (2.2%), M-I (72.5%), M-II (0.8%), and unspecified metabolites (24.5%). The metabolic profile in pooled plasma at 1, 2, and 4 hours postdose was comprised of TAK-475 ( $< 2\%$ ), M-I (62–72%), and M-II (5–14%).

**CONCLUSIONS:** TAK-475 is rapidly absorbed and converted to active metabolites after oral administration; the major route of TAK-475 excretion is biliary; the primary active metabolite in plasma and feces is M-I.

### PIII-91

COMPARISON OF THE PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF S-AMLODIPINE AND AMLODIPINE RACEMATE IN HEALTHY MALE SUBJECTS. M. G. Kim, MD, MS, J. R. Kim, MD, K. S. Lim, MD, J. W. Kim, MD, B. H. Kim, MD, Y. J. Chung, MD, T. E. Kim, MD, K. S. Yu, MD, PhD, S. G. Shin, MD, PhD, I. J. Jang, MD, PhD, Department of Pharmacology and Clinical Pharmacology Unit, Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea.

**BACKGROUND/AIMS:** SK310 is an S-form enantiomer of amlodipine, a third-generation dihydropyridine calcium antagonist that is prescribed in the management of angina and hypertension. The S-form of amlodipine is the therapeutically active component, while the "R" form is essentially inactive. This study was performed to compare the pharmacokinetic (PK) and pharmacodynamic (PD) properties of a newly developed S-amlodipine gentisate (SK310), with a racemate (amlodipine besylate, Norvasc<sup>®</sup>) in healthy male subjects.

**METHODS:** A randomized, double-blind, double-dummy, two-period, two-way crossover study was conducted in 24 healthy male Korean subjects. All subjects received a single oral dose of 10 mg amlodipine besylate and a single oral dose of 5 mg S-amlodipine gentisate with a two-week washout period in between. Blood samples for determination of S- and R-form enantiomer concentrations of amlodipine were obtained during a 168-hour period after dosing, and plasma concentrations were determined by LC-MS/MS. Systemic vascular resistance, cardiac index, and stroke volume were measured by impedance cardiography. Safety assessments were also performed.

**RESULTS:** The mean plasma concentration-time profiles of S-form enantiomer of amlodipine were virtually similar after oral administration of the S-amlodipine gentisate or amlodipine racemate. The ratios (90% confidence intervals) of geometric mean values were 1.08 (0.98–1.18) for  $C_{\text{max}}$  and 0.96 (0.88–1.04) for AUC, respectively, indicating that the two drugs were pharmacokinetically equivalent in terms of S-form enantiomer. Significant changes from baseline values in systemic vascular resistance, cardiac index, and stroke volume were observed in two drugs, but the changes for S-amlodipine gentisate were comparable to those for amlodipine besylate. No clinically relevant changes were observed in safety profiles.

**CONCLUSIONS:** The 5 mg of S-amlodipine gentisate and 10 mg of amlodipine besylate were pharmacokinetically equivalent in terms of S-form enantiomer and showed similar PD characteristics in healthy male subjects.

### PIII-92

PHARMACOKINETIC INTERACTIONS BETWEEN UDENAFIL AND KETOCONAZOLE IN HEALTHY VOLUNTEERS. J. W. Kim, MD, J. R. Kim, MD, K. S. Lim, MD, B. H. Kim, MD, M. G. Kim, MD, MS, Y. J. Chung, MD, T. E. Kim, MD, J. Y. Cho, PhD, K. S. Yu, MD, PhD, I. J. Jang, MD, PhD, S. G. Shin, MD, PhD, Department of Pharmacology and Clinical Pharmacology Unit, Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea.

**BACKGROUND/AIMS:** Udenafil (DA-8159), a phosphodiesterase type 5 (PDE5) inhibitor, is effective therapy for erectile dysfunction. The primary aim of the present study was to investigate

the effect of the potent CYP3A4 inhibitor ketoconazole on the pharmacokinetics (PK) of DA-8159 and its major metabolite DA-8164.

**METHODS:** An open-label, one-sequence, two-period, two-treatment study was conducted at the Clinical Trial Center, Seoul National University Hospital. In period 1, 12 healthy male volunteers received a single dose of 100 mg DA-8159 orally. In period 2, they received 400 mg ketoconazole once daily for 3 days and a single dose of 100 mg DA-8159 coadministered on the 3rd day of ketoconazole therapy. Serial blood samples were collected at defined intervals for 72 h in each period. Plasma concentrations of DA-8159 and DA-8164 were determined by liquid chromatography-tandem mass spectrometry. PK parameters were estimated by a non-compartmental analysis.

**RESULTS:** Following ketoconazole coadministration,  $C_{max}$  and AUC of DA-8159 increased from 313 ng/mL to 574 ng/mL (1.85-fold, 90% confidence interval, 1.64–2.20,  $P < 0.001$ ) and from 2143 ng<sup>\*</sup>h/mL to 6682 ng<sup>\*</sup>h/mL (3.12 fold, 2.79–3.46,  $P < 0.001$ ), respectively. On the contrary,  $C_{max}$  and AUC of DA-8164 decreased from 187 ng/mL to 19 ng/mL (0.10-fold, 0.08–0.12,  $P < 0.001$ ) and from 2744 ng<sup>\*</sup>h/mL to 934 ng<sup>\*</sup>h/mL (0.34-fold, 0.27–0.43,  $P < 0.001$ ) during ketoconazole coadministration, respectively. Metabolic ratio of the AUC of DA-8164 to that of DA-8159 after DA-8159 administration alone was  $1.33 \pm 0.42$  and was diminished to  $0.15 \pm 0.07$  after ketoconazole coadministration.

**CONCLUSION:** The effect of ketoconazole on DA-8159 is consistent with inhibition of the CYP3A4-mediated metabolism. Ketoconazole coadministration increases exposure to DA-8159 up to 3.12-fold in healthy volunteers.

### PIII-93

EFFECT OF MOZAVAPTAN (OPC-31260) ON THE DEXTROMETHORPHAN N-DEMETHYLATION AS A PROBE FOR CYP3A4 ACTIVITY IN HEALTHY VOLUNTEERS. T. Koue, Y. Tadayasu, M. Kubo, T. Funaki, PhD, M. Shimomura, N. Matsumoto, MD, M. Kainuma, S. Hasegawa, MD, PhD, A. Ohnishi, MD, PhD, Otsuka Pharmaceutical Co., Ltd., Sekino Clinical Pharmacology Clinic, Daisan Hospital, Jikei University School of Medicine, Osaka, Japan.

**BACKGROUND/AIMS:** Mozavaptan (OPC-31260, MZV) is an oral vasopressin ( $V_2$ ) receptor antagonist having an aquaretic effect that has been approved in Japan for the treatment of SIADH. MZV and some of its metabolites have been shown to have an inhibitory effect on CYP3A4 activity *in vitro*. To evaluate the effect of MZV on CYP3A4 activity using dextromethorphan (DTM) as a probe in healthy volunteers.

**METHODS:** The study was an open, one-sequence crossover design. Subjects were healthy males comprising two groups, CYP2D6 extensive metabolizers (EM group, CYP2D6  $*1/*1$ ,  $*1/*2$ ,  $*1/*10$ ,  $*2/*10$ ,  $n = 10$ ) and CYP2D6 intermediate metabolizers (IM group, CYP2D6  $*5/*10$ ,  $*10/*10$ ,  $*10/*14$ ,  $n = 6$ ), as DTM is also a probe for CYP2D6. Subjects were administered 30 mg of DTM on Day 1, 30 mg of MZV once a day on Days 3 through 11 (9 days), and both 30 mg of MZV and 30 mg of DTM on Day 12. Cumulative 24-hour urine was collected after administration on Days 1 and 12, and the concentrations of DTM and 3-methoxymorphinan (3MM), a metabolite of DTM formed via CYP3A4, were determined by LC-MS/MS. The ratio of 3MM to DTM (3MM/DTM) was calculated as an indicator of CYP3A4 activity.

**RESULTS:** 3MM/DTM ratios for 9 subjects in the EM group could not be calculated because the urinary concentrations of 3MM were below the lower limit of quantification after co-administration of MZV. In all of the remaining subjects ( $n = 7$ ), the 3MM/DTM ratios after co-administration of MZV were lower than that after administration of DTM alone.

**CONCLUSION:** These results suggest that MZV has an inhibitory effect on CYP3A4 activity *in vivo*, a finding consistent with that reported *in vitro*.

### PIII-94

EFFECTS OF ITRACONAZOLE ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF MOZAVAPTAN (OPC-31260) IN HEALTHY VOLUNTEERS.

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**BACKGROUND:** Mozavaptan (OPC-31260, MZV) is an oral vasopressin ( $V_2$ ) receptor antagonist having an aquaretic effect that has been approved in Japan for the treatment of SIADH. It has been suggested that CYP3A4 is the main metabolic enzyme of both MZV and some of MZV metabolites (including active metabolites) from *in vitro* studies.

**AIMS:** To evaluate the effects of a CYP3A4 inhibitor, itraconazole (ITZ), on the pharmacokinetics (PK) and pharmacodynamics (PD) of mozavaptan.

**METHODS:** The study was an open, one-sequence crossover design with a one-week washout period. Subjects were healthy males randomized into two groups, a test group ( $n = 24$ ) and a control group ( $n = 6$ ), as the one of the purposes of the study was to evaluate the PD of MZV. The study consisted of three periods, period 1, inhibitor pretreatment period, and period 2. In period 1, all subjects received 30 mg of MZV once under a fasting condition. In the inhibitor pretreatment period, only subjects in the test group were administered 100 mg of ITZ once a day after a meal. In period 2, subjects in the test group were concomitantly administered 30 mg of MZV and 200 mg of ITZ under a fasting condition and subjects in the control group were administered 30 mg of MZV alone. In both period 1 and period 2, blood and urine samples were collected for determination of the concentrations of MZV and its metabolites and PD markers (free water clearance, urine osmolality, and urine volume).

**RESULTS:** All  $AUC_{72h}$  and most  $C_{max}$  of MZV and its active metabolites were clearly increased by co-administration of ITZ. For MZV,  $C_{max}$  increased by 47% and  $AUC_{72h}$  increased by 80%.  $C_{max}$  and  $AUC_{72h}$  of the total active compounds (MZV + active metabolites) increased by 65% and 196%, respectively. However, the decreasing effect of the active compounds on urine osmolality was only slightly amplified and no notable impact on the active compounds' increasing effects on free water clearance and urine volume were seen with co-administration of ITZ.

**CONCLUSION:** The co-administration of ITZ with MZV increased exposure to MZV and its active metabolites. However, the increased exposure to the active compounds had no clear effect on PD.

### PIII-95

GLUTATHIONE-S-TRANSFERASE THETA 1 (*GSTT1*) AND *CYP2D6* NULL GENOTYPES HAVE A LOWER CLEARANCE FOR CHLOROFORM ABSORBED DURING A SHOWER. H. Lee, MD, PhD, M. Romkes, PhD, R. Branch, MD, L. Backer, PhD, MPD, Q. Lan, PhD, B. Blount, PhD, J. Nuckols, PhD, C. Lyu, PhD, S. Kieszak, PhD, M. Brinkman, PhD, S. Gordon, PhD, K. Cantor, PhD, Center for Drug Development Science, UCSF, Center for Clinical Pharmacology, University of Pittsburgh, National Center for Environmental Health, Centers for Disease Control and Prevention, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Department of Environmental & Radiological Health Sciences, Colorado State University, Battelle/Centers for Public Health Research & Evaluation, National Center for Environmental Health, Centers for Disease Control and Prevention, Washington, DC.

**BACKGROUND:** To test if genetic polymorphism plays a role in affecting the clearance of chloroform (CF), a drinking water disinfection by-product, absorbed during showering with hot tap water.

**METHODS:** Using 297 concentrations from 99 subjects whose blood was drawn before, 20, and 40 minutes after turning on a 10-minute shower, a population pharmacokinetic (PK) model for CF

was developed. A nonlinear mixed effects analysis was performed using NONMEM and the effects of various demographic and pharmacogenomic covariates on the PK parameters were explored.

**RESULTS:** A one-compartment open linear PK model with a hypothetical depot compartment for continuous dosing from the inhalational and transdermal routes adequately described the time-concentration profile of CF. Baseline endogenous fluctuation of CF was also modeled. The apparent clearance of CF for *GSTT1* and *CYP2D6* null genotypes was lower than that of the heterozygous or wild type variant, i.e., 308 [166–450, 95% confidence interval] vs. 497 [394–600] L/min, respectively. Due to the small number of the null genotypes ( $n = 3$ ), the difference did not reach a statistical significance.

**CONCLUSIONS:** These results imply that *GSTT1* and *CYP2D6* null genotypes have a lower clearance for CF than that of the wild or heterozygous type. Further exploration using a defined model is warranted.

### PIII-96

CORRELATION OF TEMSIROLIMUS (CCI-779) PHARMACOKINETIC EXPOSURE TO SAFETY IN HEALTHY ADULTS AND PATIENTS WITH CANCER FOLLOWING ONCE-WEEKLY IV TREATMENTS. C. Leister, MS, B. Hug, J. Burns, K. Smith, K. Matschke, J. Boni, Wyeth Research, Wyeth Research, Collegeville, PA.

**BACKGROUND:** Temsirolimus (CCI-779, TEMSR), a novel inhibitor of the mammalian target of rapamycin (mTOR), is in clinical development as an IV formulation for treatment of solid and hematologic malignancies. Because pharmacokinetic (PK) exposure may influence the adverse event (AE) profile, the effect of temsirolimus active moieties exposure on AE severity in patients was evaluated.

**METHODS:** Pooled analysis included 68 healthy adults receiving a single median (5th, 95th percentile) dose of 12.5 (1, 25) mg, and 174 patients with cancer receiving multiple (median = 7) 75 (15, 320) mg doses of intravenous TEMSR. AE severity was the maximum severity reported within 8 weeks of the first treatment. A rating of 1 was the lowest severity and 3 the highest. Zero (0) severity was used if an AE did not occur. AEs occurring in  $\geq 15\%$  of subjects were evaluated. Using stepwise logistic regression, PK parameters of TEMSR  $C_{max}$ , TEMSR AUC plus sirolimus AUC (AUCsum) and number of doses administered within 8 weeks of first administration were evaluated as predictors of AE occurrence. Significant positive exposure predictors were fixed, and a second stepwise logistic regression was performed to identify additional covariates predictive of AE occurrence. Effects of age, gender, body weight, body surface area, hematocrit, and interferon-alpha comedication were examined. Final models were reassessed for AE severity.

**RESULTS:** For a typical healthy subject (56 year old white male weighing 75.6 kg with hematocrit of 37.8%) receiving a 25-mg dose, the median  $C_{max}$  was 586 ng/mL and was associated ( $p < 0.0001$ ) with model-predicted occurrence and severity of mucositis (moderate 34%, mild or moderate 80%). Probability of acne occurrence (22%) increased with increasing  $C_{max}$  and decreasing hematocrit. Correlations in patients yielded a multiplicity of other exposure/covariate associations, but were considered confounded because  $C_{max}$  observations were missing for 28% of patients, and AE responses were more variable.

**CONCLUSION:** In healthy adults, occurrence and severity of mucositis and rash were predicted by temsirolimus  $C_{max}$  following a single dose. Similar findings in patients with cancer could not be confirmed because  $C_{max}$  was missing for many, which prevented identification of a robust AE association.

### PIII-97

THE EFFECT OF LOW AND HIGH FAT MEALS ON THE PLASMA AND URINE PHARMACOKINETICS OF NIACIN AND ITS METABOLITES. J. H. Leu, PharmD, PhD, R. M. Menon, PhD, D. S. Tolbert, PhD, E. A. Cefali, PharmD, PhD, Kos Pharmaceuticals, Inc, Weston, FL.

**BACKGROUND/AIM:** The objective of this study was to compare the pharmacokinetics of niacin and metabolites following administration of extended-release niacin (ER niacin) under fasting conditions, a low-fat meal, and a high-fat meal.

**METHODS:** Twenty-seven male and female subjects were enrolled in this open-label, single-dose, randomized, three-period crossover food effect study. Each subject received  $2 \times 750$  mg of ER niacin on three separate occasions under fasting (Treatment A), low-fat meal (Treatment B), or high-fat meal (Treatment C) conditions. Blood samples for analysis of niacin and nicotinic acid (NUA) were obtained immediately prior to dosing and for 24 hours following dosing. Urine samples for analysis of niacin, NUA, N-methylnicotinamide (MNA), and N-methyl-2-pyridone-5-carboxamide (2PY) were collected 24 hours prior to dosing and 96 hours after dosing.

**RESULTS:** Twenty-three subjects completed the study (13 males and 10 females). In comparison of the low-fat meal versus the high-fat meal treatments, the  $\log_e$  transformed NUA  $C_{max}$  was comparable between treatments at 3.2 ng/mL and 3.19 ng/mL for Treatments B and C, respectively with a ratio of 99% (90% CI; 81–125). The total urinary excretion (niacin, NUA, MNA and 2PY) were 71% for Treatment B and 72% for Treatment C with 90% CI at 95–106 therefore niacin rate and extent of absorption were comparable under both fed conditions. Regarding comparison of the fed arms versus the fasted arm, the  $\log_e$  transformed NUA  $C_{max}$  ratios were significantly higher at 154% (90% CI; 122–188) and 152% (90% CI; 123–190) for Treatments B and C, respectively. The total urinary recovery for niacin and metabolites under fasting conditions (56%) was significantly different ( $p < 0.001$ ) than that for fed treatments, ratio 127% (90% CI; 121–134) and 128% (90% CI; 122–135) indicating significantly lower bioavailability under fasting conditions. ER niacin was well tolerated in this study with all adverse events consisting of mild vasodilation, pruritus, and rash.

**CONCLUSION:** The rate and extent of absorption for niacin following administration with a low-fat meal or a high-fat meal were comparable. The administration of ER niacin following a 10-hour fast resulted in lower bioavailability in rate and extent relative to the two fed conditions. ER niacin was well tolerated in this study.

### PIII-98

PHARMACOKINETICS AND PHARMACODYNAMICS OF EXENATIDE LONG-ACTING RELEASE AFTER SINGLE-AND MULTIPLE-DOSING. L. MacConell, K. Taylor, D. Zhuang, P. Kothare, K. Mace, W. Li, S. Flanagan, M. Fineman, Amylin Pharmaceuticals, Inc., Eli Lilly and Company, Alkermes Inc., San Diego, CA.

**BACKGROUND/AIMS:** The incretin hormone, glucagon-like peptide-1 (GLP-1) secreted by the gut in response to ingestion of food has several effects, including stimulating glucose-dependent insulin secretion and reducing appetite. The incretin mimetic, exenatide shares several effects with GLP-1, leading to improved glycemic control. Current studies assessed the pharmacokinetics (PK) and pharmacodynamics (PD) of single and repeated weekly subcutaneous injections of exenatide long-acting release (LAR) formulation in patients with type 2 diabetes (T2DM).

**METHODS:** 62 and 45 patients with T2DM were treated in single and repeat dose exenatide LAR trials, respectively. Single dose patients received LAR (2.5 mg, 5.0 mg, 7.0 mg or 10.0 mg) or PBO. Doses were escalated based on safety and tolerability of the preceding lower dose. Weekly dose PK was predicted from non-parametric superposition of single dose data. Weekly dose patients received LAR (0.8 mg or 2.0 mg, targeting the low and high concentrations

achieved by therapeutic doses of exenatide BID) or PBO for 15 weeks. Plasma exenatide, fasting (FPG) and postprandial plasma glucose (PPG) and body weight were assessed. Data were analyzed non-compartmentally.

**RESULTS:** Plasma exenatide concentrations were measurable for approximately 9 weeks after a single injection; exposure increased dose dependently. Reductions in FPG and PPG correlated with increasing exenatide concentrations. Repeat doses of LAR, predicted plasma exenatide concentration profiles consistent with observed data. Weekly dosing yielded mean steady-state exenatide plasma concentrations spanning the targeted therapeutic range, leading to significant reductions in mean PPG and body weight with 2.0 mg LAR. Mean FPG was significantly reduced independent of dose. Generally, adverse events were transient and mild to moderate in intensity.

**CONCLUSIONS:** Single dose PK supported and predicted weekly dose PK. Weekly LAR doses targeting the therapeutic exenatide plasma concentration range were well tolerated. Dose-dependent effects on PPG and body weight, but not FPG, suggested differential exposure-response relationships across the pharmacology of exenatide. These data indicated LAR prolonged the rate of exenatide delivery to the circulation, while retaining the PD action of exenatide.

### PIII-99

ASSESSMENT OF TIME TO STEADY STATE: NONLINEAR MIXED MODELING VERSUS OTHER TRADITIONAL APPROACHES. L. Maganti, D. Panbianco, Ph.D, Merck & Co., Merck & Co., Rahway, NJ.

**BACKGROUND:** An estimate of the time it takes for a drug's plasma concentration to reach steady state needs to be obtained for regulatory labeling, if the drug is intended for chronic use. In this paper, various methods proposed in the literature for estimating the steady state pharmacokinetics, including the aggregate and individual modeling of trough concentrations, are reviewed and compared with the results obtained from nonlinear mixed modeling. Case studies obtained from our phase I studies were used to compare the estimates obtained from different approaches.

**METHODS:** The nonlinear mixed modeling approach invokes the use of nonlinear mixed effects models to directly estimate both population and individual estimate of time to 90% of steady state ( $T_{90}$ ). The population and individual estimates obtained from this approach were compared with those obtained from various other approaches, including aggregate (stepwise tests for linear trend, Helmert transformation, equality of means) and individual (mono-exponential modeling, quadratic plateau regression, individual slope test) modeling for assessment of time to steady state.

**RESULTS:** Using nonlinear mixed modeling approach, estimate of the half-life is obtained for each individual and for the entire sample as well. The population estimates were comparable to those obtained from helmert and stepwise linear trend analysis in most case studies. However, in some cases, because of the huge variability in a subject's concentrations, the software used was unable to estimate the parameters, or the estimates returned were unrealistic.

**CONCLUSION:** Nonlinear mixed modeling approach, which provides both individual and population estimates, may be routinely used for estimating steady state pharmacokinetics in multiple dose studies.

### PIII-100

POPULATION PHARMACOKINETIC ANALYSIS OF VERNAKALANT HYDROCHLORIDE INJECTION (RSD1235) FOR ATRIAL FIBRILLATION OR ATRIAL FLUTTER. Z. L. Mao, Ph.D, H. Kastrissios, Ph.D, Y. Gao, MD, J. Keirns, Ph.D, Astellas Pharma US, Inc., Pharsight Corporation, Deerfield, IL.

**BACKGROUND/AIMS:** Vernakalant hydrochloride injection (RSD1235) is a mixed frequency-dependent  $\text{Na}^+$  and atrial-preferential  $\text{K}^+$  channel blocker that effectively and rapidly terminates atrial fibrillation (AF). This analysis was designed to develop a population

pharmacokinetics model for vernakalant in patients with AF or atrial flutter (AFL) and to determine the influence of patient factors on pharmacokinetics.

**METHODS:** Data from a randomized, double-blind, placebo-controlled, multicenter phase 3 study were used. Patients with AF or AFL (lasting 3 h to  $\leq 45$  d) received a 10-min infusion of vernakalant 3 mg/kg or placebo and then, if AF or AFL persisted after 15 min, a second 10-min infusion of vernakalant 2 mg/kg or placebo. Blood samples for pharmacokinetic evaluation were collected before and after the first and second infusions, at 1 min after conversion to sinus rhythm, and at 1 additional time point between 15 min and 24 h.

**RESULTS:** The pharmacokinetic model was generated from data from 128 patients given vernakalant (including 89 men, 118 whites, 26 with congestive heart failure [CHF], 35 receiving a concomitant CYP 2D6 inhibitor, 79 receiving a concomitant beta-blocker; median age, 62 years). Plasma concentration vs time data were fit best by an open 2-compartment mammillary pharmacokinetic disposition model with first-order elimination. Both body weight and sex were correlated with the volume of the central compartment ( $V_c$ ), with sex being the stronger predictor. The final parameter estimates for  $V_c$  were 46.2 L in men and 23.9 L in women; for the volume of the peripheral compartment ( $V_p$ ), 93.2 L; for intercompartment clearance, 208 L/h; and for systemic clearance, 31.2 L/h. Age, CHF, renal function, and concomitant CYP 2D6 inhibitor or beta-blocker use did not correlate with vernakalant clearance. The pharmacokinetics of vernakalant were not influenced by race, although there were few nonwhite participants.

**CONCLUSION:** The pharmacokinetic data for vernakalant were best described by a 2-compartment linear model with rapid first-order elimination from the central compartment.  $V_c$  differed by sex. The pharmacokinetics of vernakalant were not influenced by age, CHF, renal function, or concomitant CYP 2D6 inhibitor or beta-blocker use in this study population.

### PIII-101

THE INFLUENCE OF ORAL DELIVERY RATE ON NIACIN PHARMACOKINETICS. R. Menon, Ph.D, J. Leu, Pharm D, Ph.D, D. Tolbert, Ph.D, E. Cefali, Pharm D, Ph.D, Kos Pharmaceuticals, Weston, FL.

**BACKGROUND/AIMS:** Immediate-release niacin and extended-release niacin are used in the treatment of hypercholesterolemia. Niacin is metabolized by two major pathways. The pharmacokinetic profile of niacin and its metabolites is influenced by niacin absorption rate due to saturable first-pass metabolism. The objective of the study was to quantitate the effect of niacin absorption rate with plasma and urine pharmacokinetics of niacin and its major metabolites.

**METHODS:** Twelve, healthy, male subjects were enrolled in the open-label, dose-rate escalation study. Each subject received orally, in sequential order, each of the treatments consisting of 2000 mg niacin in solution approximating 3 different niacin absorption rates at 50, 100, and 200 mg/10 min. Blood samples were obtained just prior to dosing and serially for approximately 17 hours thereafter. Urine was collected for 24 hours pre-dose and for 96 hours thereafter. Subjects reported and were queried for adverse events including flushing incidence (FI). Plasma was analyzed for niacin, nicotinic acid (NUA), nicotinamide (NAM) and nicotinamide-N-oxide (NNO) concentrations. Urine was analyzed for the parent compound and its metabolites NUA, NAM, NNO, N-methylnicotinamide (MNA) and N-methyl-2-pyridone-5-carboxamide (2PY) concentrations.  $C_{max}$ ,  $T_{max}$  and  $AUC(0-t)$  were determined from the plasma data and percent of niacin dose excreted in the urine was determined from the urine data. The pharmacokinetic parameters were evaluated statistically, but FI was not.

**RESULTS:** Niacin and NUA in plasma showed a supraproportional increase in  $C_{max}$  and  $AUC(0-t)$  with increased absorption rate, while plasma NAM and NNO parameters were not as different. The total niacin dose recovered in the urine as the molar sum of niacin and metabolites was comparable across dosing rates. However, urine recovery of niacin and NUA combined increased proportionately

with increased absorption rate while 2PY and MNA recovery decreased with increased absorption rate. FI also increased proportionately with absorption rate.

**CONCLUSION:** Increased niacin absorption rate resulted in accumulation of niacin and NUA in plasma and recovery in the urine that was proportional to absorption rate. FI increased with niacin absorption rate and urine recovery of niacin and NUA.

### PIII-102

PHARMACOKINETICS AND PHARMACODYNAMICS OF KS01017 FOLLOWING SINGLE DOSE ADMINISTRATION. R. Menon, PhD, D. Tolbert, PhD, J. Leu, PharmD, PhD, E. Cefali, PharmD, PhD, Kos Pharmaceuticals, Weston, FL.

**BACKGROUND/AIMS:** KS01017 is being developed as an anti-diabetic drug with beneficial lipid effects. The effect of single-dose administration on pharmacokinetic (PK) and pharmacodynamic (PD) parameters of KS01017 was studied in Type II diabetic patients.

**METHODS:** KS01017 and placebo were administered as oral solutions in 12 type II diabetic men and women, 18 to 60 years of age in this single-blind, multiple-dose, 4-way crossover study. Placebo solution, 100 mg, 200 mg and 500 mg KS01017 were administered as 10 divided doses over 4.5 hours to mimic a sustained-release formulation. Following dosing, serial plasma and serum samples were collected for up to 24 hours to quantitate KS01017, glucose, insulin and free fatty acid (FFA). Non-compartmental PK and PD parameters were calculated and compared between treatments.

**RESULTS:** The 90% confidence intervals for dose-normalized Cmax and AUC (0-t) values indicated that KS01017 was dose proportional at the 3 doses given in the study. The mean terminal half-life of 1.6 hour was comparable for all three active treatments. The three active treatments showed a significant decrease in FFA and glucose Cmin as compared to placebo. Glucose and FFA AUC(0-24) were lower for all three active treatments compared to placebo, but were statistically significantly different for only glucose. Insulin Cmin and AUC (0-24) were lower for the active treatments, but not statistically different from placebo. There did not appear to be a dose dependent effect on the PD parameters at the 3 doses used in the study. Adverse events related to drug administrations were mild and observed following the 200 mg and 500 mg dose only.

**CONCLUSION:** KS01017 showed dose-proportional kinetics at the doses administered in the study. KS01017 was well tolerated and showed beneficial effects on glucose, insulin and FFA values following single dose.

### PIII-103

PHARMACOKINETICS OF PROPOFOL IN PATIENTS WITH MAJOR BURN. D. Yim, MD, PhD, H. Chae, MD, E. Hong, DVM, A. T. Han, MD, PhD, The Catholic U. of Korea, Hallym Univ., Seoul, Republic of Korea.

**BACKGROUND/AIMS:** To appraise the influence of major burn on the pharmacokinetics of propofol.

**METHODS:** PK study of propofol was performed in 20 burned patients (burned area >20%) and 20 unburned patients undergoing surgery. After i.v. bolus injection of propofol 2 mg/kg, arterial blood samples were drawn at 0, 15, 30, 45, 60th seconds, 2, 3, 5, 10, 15, 30, 45, 60th minutes, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5th hours. Plasma concentration of propofol was measured by HPLC based fluorescence detection. NONMEM (Ver. 5.1.1) was used to build the population

PK model with contributing covariates. The structural model building and covariate search were carried out using First order (FO) method. To determine the final model, the structural model and covariates found using the FO method were confirmed using first order conditional estimation (FOCE) method.

**RESULTS:** In burned patients, all of the PK parameters were 1.5 to 3 times greater than those of the unburned. Body weight was the only covariate influencing clearance (CL) regardless of burn. The deepest compartment volume of distribution (Vd) in burned patients increased with the burned body surface area. The other covariates such as albumin or days after burn were not included in the model.

**CONCLUSION:** To the best we know of, the current result is the first report on the PK changes of protocol in burned patients. More than doubled Vd and CL in the burn suggests that its dosage regimen may need to be increased accordingly.

### PIII-104

CONCOMITANT ORANGE JUICE DOES NOT AFFECT FUROSEMIDE EXPOSURE ALLOWING US TO SHOW THAT FUROSEMIDE INTRASUBJECT VARIABILITY IS MUCH LESS THAN PREVIOUSLY SUPPOSED. H. Zheng, MD, PhD, L. Frassetto, MD, Y. Huang, PhD, L. Benet, PharmD, PhD, UCSF, San Francisco, CA.

**BACKGROUND/AIMS:** Recently, fruit juices have been reported to reduce the bioavailability of a number of orally administered BCS Class 3 and Class 4 drugs, which are believed to require a gut uptake transporter to achieve consistent drug exposure. Furosemide is a loop diuretic that is incompletely and previously thought to be erratically absorbed from the gastrointestinal tract. We studied the effect of 2 doses of orange juice, the fruit juice most commonly taken with medication, on orally administered furosemide.

**METHODS:** A prospective randomized, open-labeled, 3-way crossover, single dose clinical pharmacokinetic study was done on the General Clinical Research Center at UCSF. Upon initiation of the study, 10 healthy volunteers were recruited and randomized to the order of the 3 treatment regimens. At each visit, a 40mg oral dose of furosemide was given with: a) 20oz non-calcium containing Ocean Spray orange juice, b) 20oz water, or c) 6oz non-calcium containing Ocean Spray orange juice plus 14oz water. Each visit was at least one week apart. Blood samples were drawn at 0, 30, 60, 120, 180, 240, 360, and 480min. Subjects collected urine for the fractions of 0-4, 4-8 and 8-24 h. The furosemide concentration in plasma and urine were determined by LC/MS.

**RESULTS:** There were no significant differences or even trends for any furosemide pharmacokinetic measurement among the 3 treatment regimens: Cmax (p = 0.9); Tmax (p = 0.23); T1/2 (p = 0.16); AUC0-8 (p = 0.71) and AUC0-∞ (p = 0.54); CLr (p = 0.56). This lack of an effect, however, allowed calculation of intrasubject variability of drug exposure (AUC), where %CV ranged from 3.5 to 40.6%, but averaged 14.7%, versus intersubject variability for each treatment where %CV ranged from 52.3 to 68.3% and averaged 58.6%.

**CONCLUSION:** No effect of orange juice on oral furosemide pharmacokinetic profiles was observed. Although this drug exhibits a large degree of intersubject variability, oral furosemide is not a highly variable drug and it unexpectedly exhibits a relatively low intrasubject variability, suggesting that patients receiving oral furosemide would be expected to maintain consistent drug availability at each dose administration.

### PIII-105

QUANTIFICATION OF SEROTONIN 5-HT<sub>1A</sub> RECEPTORS IN HUMANS WITH [<sup>11</sup>C](R)-(-)-RWAY: RADIOMETABOLITE(S) LIKELY CONFOUND BRAIN MEASUREMENTS. X. Zhang, MD, F. Yasuno, MD, PhD, S. Zoghbi, PhD, J. Liow, PhD, J. Hong, MS, J. McCarron, PhD, V. Pike, PhD, R. Innis, MD, PhD, Molecular Imaging Branch, NIMH, NIH, Bethesda, MD.

**BACKGROUND/AIMS:** The 5-HT<sub>1A</sub> receptor is a target for drug therapy in the treatment of anxiety and depress. Each of the two most commonly used PET tracers for the 5-HT<sub>1A</sub> receptor has at least one significant deficiency. [*carbonyl*-<sup>11</sup>C]WAY-100635 is difficult to synthesize and has very low uptake in cerebellum. [<sup>18</sup>F]FCWAY undergoes defluorination and generates significant radioactivity in skull. [<sup>11</sup>C](R)-(-)-RWAY has been recently developed and shown to be a promising radioligand for imaging brain 5-HT<sub>1A</sub> receptors with PET in rodents and nonhuman primates.

**METHODS:** Six healthy volunteers (age, 29 ± 10 y; range, 22–47 y; weight, 88 ± 27 kg) participated in this first in human study. [<sup>11</sup>C](R)-(-)-RWAY was administered intravenously over ~60 s, with injected activity of 664 ± 104 MBq, specific activity of 124 ± 149 GBq/μmol, and mass dose of carrier of 5.3 ± 3.4 μg. Dynamic PET scans were acquired using a GE Advance tomograph.

**RESULTS:** At 80 min after radiotracer injection, activity ratios were about three for brain receptor-rich regions compared to cerebellum. However, the washout from brain was unexpectedly slow relative to plasma clearance of the parent radiotracer. This disparity between brain and plasma activity was quantified with distribution volume calculated from increasingly truncated brain imaging data. In both receptor-rich regions and cerebellum, distribution volumes were unstable and increased continuously from 90 to 150 min by about 30%. This increasing distribution volume was unlikely due to the variations or errors of plasma input at later time points, since a similar truncation of plasma time points from 150 to 90 min did not significantly affect the analysis of the brain data. When the metabolites of [<sup>11</sup>C](R)-(-)-RWAY in human and monkey were compared, a moderate lipophilic radiometabolite was present at a significantly higher percentage of total plasma radioactivity in human than in monkey.

**CONCLUSION:** The relatively slow washout of activity from brain and the temporal instability of distribution volume likely reflect the accumulation of radiometabolite(s) in human brain. Although prior studies in rodents and nonhuman primates showed [<sup>11</sup>C](R)-(-)-RWAY to be a promising radiotracer, we suspect that a species difference in metabolism caused this serious deficiency in humans.

