

### OIII-A-I

POPULATION PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS OF VERNAKALANT HYDROCHLORIDE INJECTION (RSD1235) IN ATRIAL FIBRILLATION OR ATRIAL FLUTTER. Z. L. Mao, PhD, Y. Gao, MD, S. Kshirsagar, PhD, J. Keirns, PhD, Astellas Pharma US, Inc., Pharsight Corporation, Deerfield, IL.

**BACKGROUND/AIMS:** Vernakalant hydrochloride injection (RSD1235) is a novel, intravenous antiarrhythmic agent under investigation for the rapid conversion of atrial fibrillation (AF) to sinus rhythm (SR). In this population pharmacokinetic-pharmacodynamic (PK/PD) analysis, the exposure-response relationships of vernakalant for efficacy and safety were evaluated and the factors influencing its pharmacodynamics were identified.

**METHODS:** Efficacy, safety, and pharmacokinetics data were obtained from a randomized, double-blind, placebo (PBO)-controlled, multicenter phase 3 study. Patients with AF or atrial flutter (AFL) (lasting 3 h to  $\leq 45$  d) received either vernakalant 3 mg/kg or PBO via a 10-min infusion and then, if AF or AFL persisted after 15 min, vernakalant 2 mg/kg or PBO via a second 10-min infusion. In this analysis, efficacy endpoints were the incidence of SR conversion within 25 min of the first infusion in patients with recent onset AF (lasting 3 h to  $\leq 7$  d) and the time to SR conversion in this patient group. Safety endpoints, analyzed for the total population, were the QTc interval prolongation, corrected by Fridericia's formula (QTcF), and systolic blood pressure (SBP) changes.

**RESULTS:** The incidence of AF conversion to SR within 25 min was significantly higher with vernakalant than with PBO (43.2% vs 1.2%;  $P < .001$ ). A simple binary logistic regression model confirmed that vernakalant markedly increased the 25-min SR conversion rate. None of the covariates examined improved model fit, including age, sex, body weight, cardiovascular disease history, AF duration, arrhythmia type, or concomitant medication usage. The safety data for vernakalant were best described by sigmoidal Emax models. The maximum increase in QTcF is expected to be 6.0 ms in patients who demonstrated conversion to SR within 90 min and 20.4 ms in those remaining in AF. The EC<sub>50</sub> was 1720 ng/mL. Age and concomitant antiarrhythmic medication use affected baseline QTcF. The typical Emax for SBP was 3.25 mm Hg, and the EC<sub>50</sub> was 1140 ng/mL. Age affected baseline SBP.

**CONCLUSION:** PK/PD modeling confirmed that vernakalant markedly increased the incidence of AF-to-SR conversion. Maximum changes in QTc interval were smaller in patients with SR conversion than in those without. Maximum changes in SBP were relatively small.

### OIII-A-II

BAYESIAN MONITORING AND BOOTSTRAP TRIAL SIMULATION: A NEW PARADIGM TO IMPLEMENT ADAPTIVE CLINICAL TRIALS DESIGNS IN DEPRESSION. R. Gomeni, PhD, E. Merlo-Pich, MD, GSK, Verona, Italy.

**AIMS:** To develop a methodology for real-time monitoring of accumulating efficacy data in clinical trial testing antidepressant drugs and to implement a Bayesian and trial simulation-driven decision rule to progress/discontinue treatment arms.

**METHODS:** A longitudinal model was developed to analyse time course of HAMD-17 scores using a Bayesian three-stage hierarchical approach and to estimate Posterior Probability of Superiority (PPS) with respect to placebo. Bayesian monitoring strategy was based on the joint assessment of PPS and Predictive Power. Once accumulating data satisfied the futility stopping criteria, a non-parametric bootstrapping trial simulation was used to support the final decision to terminate/progress arms according to a scenario-based risk analysis.

**RESULTS:** A longitudinal model based on the combination of Weibull and linear function accurately described population and individual HAMD-17 time-course. The performance of the Bayesian monitoring and the trial simulation was evaluated on the retrospective analysis of 3 clinical trials (967 subjects in two positive and one negative trial) on Major Depressive Disorders (MDD). The analysis conducted on the whole and on the truncated databases at different calendar date simulating the accrual process demonstrated the good predictability properties of the model. The performances of the decision strategy based on non-parametric bootstrapping trial simulation was illustrated in the analysis of a trial with heterogeneous and delayed recruitment. The application of this strategy reversed the decision recommended by standard application of the futility stopping rule.

**CONCLUSIONS:** Real-time monitoring of clinical trial in MDD can be effectively implemented by applying longitudinal data modelling. The inclusion of trial simulation approach based on the available data to explore alternative trial outcomes improved the overall predictive performance. This methodology could be used as an effective decision tool by an Independent Monitoring Board.

### OIII-A-III

A PENALIZED MIXTURE MODEL APPROACH IN GENOTYPE/PHENOTYPE ASSOCIATION PHARMACOGENETICS STUDIES. L. Li, PhD, R. Jason, MS, Y. Jin, MD, S. Gonzales, MD, A. Nguyen, BS, S. Todd, PhD, Z. Desta, PhD, S. Christopher, D. A. Flockhart, MD, PhD, Indiana University, Indianapolis, IN.

**BACKGROUND:** In translational research, a pharmacogenetic association study has a two-fold aim: test whether genetic variables or their combinations are associated with a pharmacokinetics/pharmacodynamic phenotype; and determine how those combinations are clustered to predict the phenotype. Although multiple comparison procedures can detect genetic effects on phenotypes, they cannot perform clustering.

**METHOD:** Our proposed mixture model approach concurrently detects main and interaction effects of genetic variables through a likelihood ratio test (LRT), and performs phenotype cluster analysis based on genetic variable combinations. Its performance is demonstrated with four examples: ESR2 effects on hot flashes in a Tamoxifen trial; ABCB1 and ABCG2 effects on patient survival in a docetaxel trial; CYP2D6 polymorphism effects on Tamoxifen metabolite; CYP2B6 polymorphism effect on its protein expression. Our mixture model's performance is also compared to other methods, multi-dimension reduction (MDR) and restricted partition method (RPM), in simulation studies.

**RESULTS:** In the tamoxifen trial, the mixture model detects an ESR2-2 effect on hot-flash development ( $p$ -value = 0.045). Patients with ESR2-2 AA genotype have lower risk to develop hot-flashes (3/13=23%) compared to patients with AG or GG genotypes (46/70 = 65%). In the docetaxel trial, the mixture model detects an interaction effect between ABCB1 and ABCG2 polymorphisms ( $p$ -value = 0.022). The genotype subgroup ABCB1 TT and ABCG2 CA has a much higher survival rate (4/4 = 100%) than the other four genotype subgroups (12/46 = 26%). To predict NDM/endoxifen ratio, 35 CYP2D6 genotypes are partitioned into three groups ( $p$ -value = 0.04). To predict CYP2B6 protein expression, seventeen CYP2B6 genotypes are categorized into three clusters ( $p$ -value = 0.002). The biological validities of both partitions are examined using established function of CYP2D6 and CYP2B6 alleles. In addition, simulation studies suggest that the mixture model is more powerful than MDR, and has higher recovery rate than RPM.

**CONCLUSIONS:** The mixture model is a unified approach that can perform genotype clustering and hypothesis testing simultaneously in investigating genotype/phenotype associations in pharmacogenetic studies.

### 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 P450D6\*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