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Application of Pharmacometric Methods to Improve Pediatric Drug Development

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APPLICATION OF PHARMACOMETRIC METHODS TO IMPROVE PEDIATRIC DRUG DEVELOPMENT

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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List of Abbreviations

Abbreviation	Interpretation
ADAS-COG	Alzheimer's disease assessment scale – cognitive score
BOV	between-occasion variability
BSV	between-subject variability
BLA	Biologic License Application
BPCA	Best Pharmaceuticals for Children Act
BSA	body surface area
CDC	Center for Disease Control and prevention
CHLA	Children's Hospital Los Angeles
CL	clearance
Cmax	maximum plasma drug concentration
CROWN	Creating an Optimal Warfarin dosing Nomogram
CTS	clinical trial simulation
CV	co-efficient of variation
CYP2C9	cytochrome P450 2c9
DDI	drug-drug interactions
EOP2A	End-of-Phase-IIA
ER	exposure-response
FDA	Food and Drug Administration
FDAMA	Food and Drug Administration Modernization Act
FOCEI	first-order conditional estimation with interaction
FPG	fasting plasma glucose
HAMD-17	Hamilton depression rating scale
HbA1c	hemoglobin A1c
HVOD	hepatic veno-occlusive disease
EC_{50}	concentration of drug to elicit half maximal response
IND	Investigational New Drug
INR	international normalized ratio
IV	intravenous

KA	first-order absorption rate constant
LOQ	limit of quantitation
NCA	non-compartmental analysis
NDA	New Drug Application
NM	non-linear mixed-effects
OFV	objective function value
PCA	pro-coagulant complex activity
PD	pharmacodynamics
PK	pharmacokinetics
PREA	Pediatric Research Equity Act
RCCT	randomized concentration-controlled trial
RDCT	randomized dose-controlled trial
RECT	randomized effect-controlled trial
RSE	relative standard error
SD	standard deviation
SE	standard error
$t_{1/2}$	half-life
TDM	therapeutic drug monitoring
Tmax	time for maximum plasma concentration
TS	two-stage
UCI	upper limit of 95% confidence interval
UPDRS	unified Parkinson disease rating scale
V	volume of distribution
VKORC1	vitamin K epoxide reductase sub-unit c1
WSV	within-subject variability

Abstract

APPLICATION OF PHARMACOMETRIC METHODS TO IMPROVE PEDIATRIC DRUG DEVELOPMENT

By Mallika A. Lala, Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Major Co-advisors: Jogarao V.S. Gobburu and F. Douglas Boudinot

Pharmacometrics is a quantitative science that is rapidly changing the landscape of drug development, and particularly so for the pediatric population. The motivation behind the research underlying this dissertation is to contribute towards the improvement of pediatric drug development by the astute application of pharmacometric methods. Two distinct research areas have been focused upon: 1- improving pediatric pharmacokinetic (PK) trial design and 2- improving pediatric dosing of warfarin by using a genetics-based dosing regimen.

The first project examined in detail the feasibility of and simulation-based methodology for implementing a recent regulatory PK quality standard. The focus was on designing pediatric PK trials that employ sparse sampling and population analysis methods, using a simulation-estimation platform. The research provided clarity on the impact of various trial design elements, such as PK sampling, adult data inclusion, PK variability and analysis method on sample size adequacy to honor the standard.

The PK quality standard was found to be practically feasible in terms of sample size adequacy. Informative sampling schedule for a given number of PK samples per subject is assumed during trial design. Recommendations are made to: 1- use prior adult or pediatric data for trial design and analysis, wherever possible and 2 - use one-stage population analysis methods and biologically feasible covariate models for designing pediatric PK studies.

The second project involved derivation of the first ever pediatric warfarin dosing regimen, including starting dose and titration scheme, based on pharmacogenetics (Cyp2c9 *1/*2/*3 and VKORc1 -1629 G>A polymorphisms). While extensive research and several dosing models for warfarin use in adults exist, there is paucity of data in pediatrics. A validated adult warfarin population PKPD model was bridged using physiological principles and limited pediatric data to arrive at a pediatric PKPD model and dosing regimen. Pediatric data (n=26) from an observational study conducted at the Children's Hospital Los Angeles (CHLA) was used to qualify the pediatric model.

A 2-step pediatric starting dose based on body weight (<20 kg and ≥20 kg) for each of 18 (6 Cyp2c9 x 3 VKORC1) genotype categories is proposed. The titration

scheme involves percentage changes relative to previous dose, based on latest patient INR. The dosing regimen targets a major (≥ 60%) proportion of INRs within therapeutic range of 2.0-3.0, by the second week into warfarin therapy. Simulataneously, bleeding and thromboembolic risks are minimized via minimal proportions (≤ 10% and ≤ 20%) of INRs > 3.5 and INRs < 2.0, respectively. In simulations, the proposed dosing regimen performed better on target INR outcomes than the standard-of-care dosing used in the CHLA patients. Given the challeneges in and low likelihood of conducting pediatric warfarin clinical studies, the proposed dosing regimen is believed to be an important advance in pediatric warfarin therapy. Prospective warfarin studies in pediatrics using the proposed dosing regimen are recommended to refine and validate the suggested dosing strategy.

CHAPTER 1

Pharmacometrics: Concepts and Applications to Drug Development

ABSTRACT

Pharmacometrics is the science of quantitaive clinical pharmacology that impacts decision-making throughouht the drug development and regulatory review process. It is based primarily on pharmacokinetic and pharmacodynamic modeling and simulation with applications including among others, clinical trial design and dose optimization. Through the channel of quantitiative drug, disease and trial models, pharmacometric methods have the unqiue ability to leverage all prior and current information from diverse sources including clinical pharmacology, pathophysiology and statistics.

This chapter provides an introduction to the genral applications of pharmacometrics as well as concepts and methods employed, including non-linear mixed effects modeling and population analysis. Further, several case studies are cited, where pharmacometric analyses played a role in drug development and/or regulatory decision making. Finally, a future perspective on the field is provided with considerations for wider adoption of pharmacometrics to improve the efficiency of drug development programs.

WHAT IS PHARMACOMETRICS?

Introduction

Pharmacometrics is the science of quantitaive clinical pharmacology that influences decision-making throughouht the drug development and regulatory review process. It is an amalgamation of several research areas, including among others, pharmacokinetics (PK), pharmacodynamics (PD), pathophysiology and statistics. Pharmacometrics comprises of an array of techniques that are primarily based on modeling and simulation of data, which include but are not limited to population pharmacokinetic (PPK) analysis, exposure-response (E-R, or PK-PD) determination for drug efficacy and safety, clinical trial simulations and disease progression modeling.

Several organizations have discussed the increasing importance of modeling and simulation for enhancing drug development [1-4]. The pharmaceutical industry has conducted surveys to evaluate the role of pharmacometric analysis in their drug development process. A study at Parke-Davis [5] found that in almost half (5 of 12) of the cases reviewed, the population analysis provided information that influenced the direction of individual development programs and may have facilitated review and approval. A similar study at Hoffmann La Roche [6] found that a modeling and simulation guided approach contributed toward making clinical drug development more rational and efficient, by better dose selection for clinical trials and time savings up to several months.

The following sections of this chapter describe the general applications of pharmacometrics during drug development and regulatory review, as well as different concepts and methods employed. Case studies, which bring out the role that pharmacometric analyses have played in various aspects of drug development, and a future perspective on the field, are also provided.

Quantitative disease-drug-trial models

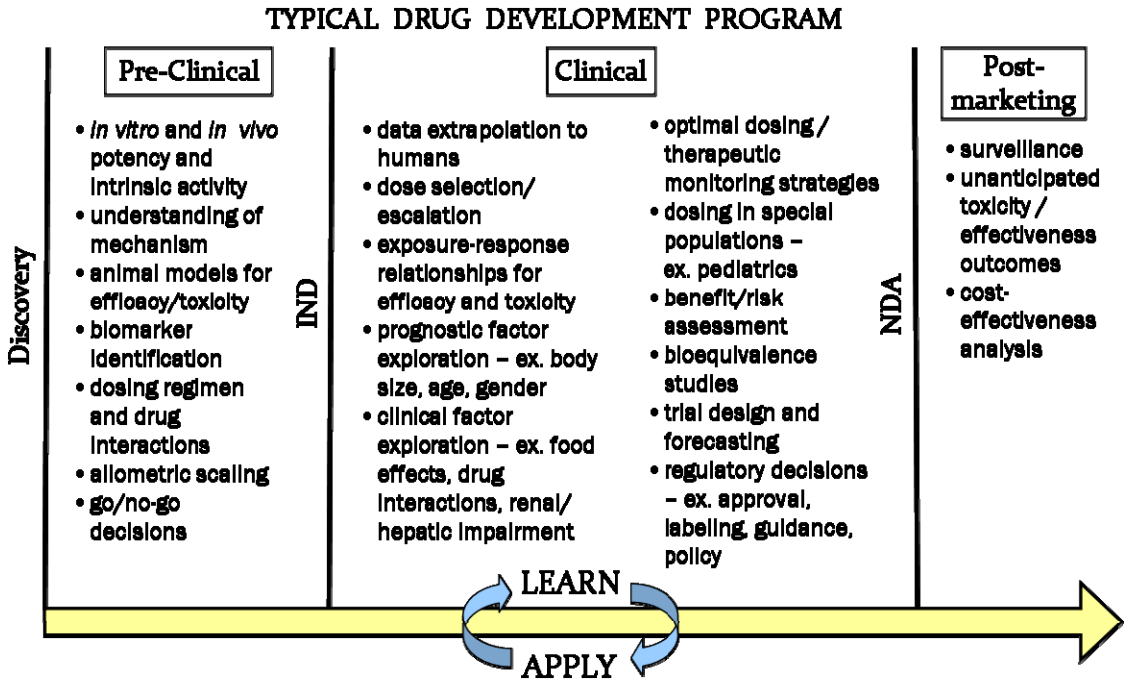
Disease-drug-trial models may be considered mathematical expressions of the time course of biomarkers, clinical outcomes, placebo effects, drug effects, and trial execution characteristics [7]. Accrual of information from across drug development programs enables efficient future planning, for which quantified disease, drug, and trial information can serve as a helpful guide.

Disease models quantify the relevant biological system in the absence of drug (detailed discussion in *Section 2*). Drug models characterize the exposure-response relationship for both efficacy and safety of drugs. Among other decisions, such models drive the determination of optimal dosing regimens. Using drug models early on can reduce unexpected safety/efficacy outcomes during the late clinical phase [8;9]. Trial models attempt to account for patient characteristics and behaviors such as eligibility criteria, baseline variables and their correlation, protocol adherence [10] and dropout rates, which may significantly influence outcomes in clinical trials. Trial models have great potential contribution towards more efficient and successful future clinical trials.

Applications

Pharmacometrics can be applied at all stages of the drug lifecycle, right from the pre-clinical phase through clinical development and regulatory review, as well as post-marketing. Potential applications range from molecule screening and identification of biomarkers and surrogates, to dosing regimen and trial design selection and optimization, to prognostic factor and benefit/risk evaluation. These methods have the unique ability to leverage all prior and current information, providing a rational, scientifically sound framework to maximize knowledge and efficiency of drug development programs. The many and varied applications of pharmacometrics are illustrated in Figure 1.

Figure 1: Potential applications of pharmacometrics throughout drug development



Clinical trial design

It has been observed over time that registration trials fail to demonstrate effectiveness or safety, often due to ignorance of prior knowledge, both drug-specific and non-specific (placebo-effect or natural disease progression) and/or employment of one-size-fits-all dosing strategies [1;11]. Disease-drug-trial models and clinical trial simulations are useful tools that can help reduce such trial failures. Potential benefits include upfront comparison of candidate study designs, dose and safety outcomes selection, sample size and power determination, and evaluation of drug interactions and co-morbidities [12]. The resources needed to perform the pharmacometric analyses are negligible compared with the costs of unsuccessful trials.

For instance, nesiritide, developed for the treatment of acute congestive heart failure, was initially not approved because the dosing regimen used in the first registration trial was sub-optimal. Modeling led to suggestion of a new, optimal dosing regimen, and results of simulated trials based on this regimen matched well with those of the second registration trial that led to eventual approval of the drug [13]. In retrospect it appears as though an early dose optimization could have saved three years of drug development time and one failed clinical trial.

Another instance, is for a drug to treat type 2 diabetes mellitus [14], a semi-mechanistic model to describe the time course of FPG and HbA1c was developed and extensive simulations were performed to evaluate two different trial designs: genotype-stratified and biomarker enrichment designs. The biomarker-enrichment design with a bid dosing

regimen was proposed for future trials with an understanding that the trial results would be used to derive an optimal dosing strategy such as genotype-based dosing. An important resulting drug development decision was the need to develop a sustained release formulation of the drug.

Dose optimization

Exploring several dosing strategies in clinical trials is often impractical, costly, and in some cases, unethical. Under such circumstances, simulations can be used to explore all competing dosing schemes and select an optimal strategy. If no single dosing scheme is able to achieve target drug exposures in majority of patients, there may be need for dose individualization and therapeutic drug monitoring (TDM). Modeling and simulation can help forecast this need and provide a TDM strategy [1]. This was observed in case of an oral suspension product for prophylaxis of invasive fungal infections in high-risk patients [15]. E-R analysis revealed very high variability in exposures across patients and the need for TDM to maximize effectiveness for all patients, and supported conducting a post-marketing study to evaluate benefit of proposed TDM. The analysis also supported inclusion of administration conditions to optimize drug absorption, emphasizing the importance of adequate plasma concentrations, in the drug label.

Usually, only dosing regimens 'directly' studied in clinical trials are proposed in drug labels. However, a drug model may effectively be used to explore the suitability of intermediate doses that are not directly studied but could potentially offer similar effectiveness as studied dosing regimens [16;17]. But extrapolating outside the studied

dose range may not be feasible. The ability of a well-developed exposure-response relationship to support approval of a dosing regimen not directly studied in clinical trials is in fact one of the strongest merits of modeling and simulation. Unfortunately, this tool is not being fully exploited currently.

Covariate / Prognostic factor determination

Apart from dose-ranging studies, the clinical pharmacology characterization of a new drug involves a number of bridging studies to identify influential covariates or prognostic factors such as body size, age, gender, food intake, co-morbidities, co-medications, and others. While effectiveness and safety data may not be collected in bridging studies, they could be simulated from a previously developed drug model.

For instance, Sular is a once-a-day controlled release formulation of the drug Nisoldipine, which is approved in the United States for the treatment of hypertension. Food was found to increase the bioavailability (Cmax increases up to 245%) of the controlled release product. The influence of these higher drug concentrations on lowering of blood pressure was evaluated using simulation of the drug effect under fed condition from a previously developed exposure-response model [18]. Even though the Sular label recommends administration on an empty stomach for optimal bioavailability, these simulations alleviated the safety concern of a large drop in blood pressure, should the drug be administered with food. Hence, there is no safety warning in the label for the drug to not be administered in a fed condition.

Special populations

Pharmacometric analyses enable the understanding of unique clinical pharmacology features in special populations such as pediatrics, geriatrics, renal/hepatic impairment, and others. A case in point is docetaxel, where the exposure-response relationship in patients with cancer was successful in identifying a sub-population, patients with liver impairment, to be more prone to grade 4 neutropenia [19]. This important finding improved the safety profile of the drug and was the basis of the dosing recommendation for patients with hepatic insufficiency in the label. The drug development program of docetaxel exemplifies the value added by prospective modeling and simulation while planning clinical trials.

The FDA offers a six month extension on the marketing exclusivity for a new drug, should the sponsor fulfill the requirement of a written request to characterize the exposure-response relationship of the drug in pediatrics. Hence, one of the most sought out special populations to study for labeling changes is pediatrics. A well-defined exposure-response relationship of a drug in adults, be it for a biomarker, surrogate or clinical endpoint, can facilitate development of the same drug for use in pediatrics. Modeling and simulation is a powerful tool that can be used to provide plausible trial design, rational dosing recommendations and useful labeling information in pediatrics when sufficient understanding of adult and pediatric pharmacology is available [20].

For instance, a pediatric population analysis [21], and further modeling and simulation [22], provided the labeled dosing recommendations for the anti-arrhythmic agent sotalol

in pediatrics aged 1 month to 12 years. The E-R analysis found drug effects in pediatrics to be consistent with adults. In this case, dosing for patients < 2 years of age was selected specifically based on modeling, and not studied directly in trials.

Regulatory considerations

The United States Food and Drug Administration (FDA) routinely utilizes pharmacometric methods as an aid in making regulatory decisions during the investigational new drug (IND), biologics license application (BLA) and new drug application (NDA) review processes. The role of pharmacometric analyses in various regulatory decisions are summarized in Table 1.

A survey of 42 NDAs submitted between 2000 and 2004, which included a pharmacometric component, revealed that pharmacometric analyses were pivotal in regulatory decision making in more than half of the cases. Of the 14 reviews where such analyses were key to approval related decisions, 5 identified the need for additional trials, while 6 identified reduction in the burden of conducting additional trials [1].

The proceedings of an advisory committee meeting for cardio-renal (CR) drug products are noteworthy [23]. The meeting devoted 50% of the total time to discuss the role of exposure-response in CR drug development. The advisory committee concluded that model-dependent analysis to learn about the shape of the exposure-response curve and more innovative designs to potentially allow both, frequentist and Bayesian types of data analysis were needed.

Table 1: Summary of the types of regulatory decisions influenced by pharmacometric analyses

Regulatory Decision	Role of Pharmacometric Analyses
Trial design guidance	• Selection of dose or exposure range for registration trials • Derivation of optimal sampling schemes (PK and PD)
Approval	• Development of approval criteria • Evaluation of: o evidence of effectiveness o benefit-risk o targeted safety studies (ex: QT evaluation) o clinical implications of failed bioequivalence studies
Labeling	• Recommendation of dosing strategy: o dose and regimen o individualized doses, where required o therapeutic drug monitoring, where required o dosing in special populations (ex: pediatrics) o drug interactions • Evidence for warnings and precautions
Policy	• Evaluation of: o alternative primary analysis methods o competing recommendations for guidances o bioequivalence criteria

The FDA issues guidance to industry to facilitate a smoother drug development and approval process. The guidance to industry on population pharmacokinetics [16] emphasizes the role of modeling and simulation in designing and analyzing trials. The FDA Modernization Act (FDAMA) [17] has a section for "extrapolation from existing studies" which emphasizes the ability to use knowledge from previous clinical trials for approval of the same drug product for pediatric use, or for establishing equivalence of alternative formulations, provided the original trial yielded well-defined exposure-response relationships. The FDA has also implemented End-of-Phase-IIA (EOP2A) meetings with sponsors [24] and published the Critical Path Initiative [25], which again emphasize the usefulness of pharmacometrics in enhancing drug development. The premise for all these regulatory initiatives is that with efficient planning, sponsors can economize valuable drug development time and resources, which is in public health interest, as well as reap full advantage of the resulting incentives.

DISEASE MODELS

A disease model is a mathematical representation of a given biological (or pathological) system in the absence of drug that attempts to quantify the time course of the disease [7]. There are three major sub-models that capture the relevant aspects of disease modeling, namely, the relationship between biomarkers and clinical outcomes, the natural disease progression, and the placebo effect. In addition, there are three general approaches to building any disease model: systems biology, semi-mechanistic, and empirical modeling. The main features of the three approaches are summarized in Table 2.

Biomarkers and clinical outcomes

In several cases, particularly when clinical endpoints occur after prolonged periods of time, biomarkers are used as outcomes in clinical trials rather than the actual clinical endpoints. Characterization of the relationship between biomarkers and clinical outcomes for both efficacy and safety for a particular disease condition, is thus a very important aspect of disease modeling, and can help develop surrogate endpoints. Such models can then aid in trial design optimization and risk projection based on biomarker data. Systems biology models, although complex, are very useful for this purpose [26]. They are based on an understanding of the underlying biological system, much like physiologically-based models. They represent the system at the molecular level, with an ability to account for pathological disturbances. The model parameters are estimated from multiple, detailed *in-vitro* and *ex-vivo* experiments [7].

On the other hand, semi-mechanistic and empirical models are predominantly data driven and tend to disregard details of related diseases [27]. Semi-mechanistic models sufficiently simplify the biological system to be able to describe the available data well, and could be the first step toward a systems biology model. Empirical disease models are essentially mathematical expressions used to interpolate between observed data, and seldom relate to the underlying biology. Even so, such models are useful, depending on the problem at hand. Empirical models are simple and frequently all that is available, and are often invaluable in making go/no-go decisions and designing pivotal trials. The empirical parametric hazard model [28] that describes the relationship between the change in tumor size and survival is one such example.

It may be correct to say that every model will include some empirical component. For instance, in the case of diabetes, a detailed systems biology model with more than 50 parameters [29], as well as a semi-mechanistic model [30] have been proposed. While the systems biology model takes into account glucose and HbA1c data, as well as other related information such as blood pressure, cardiac output, family history, cholesterol, and smoking status, the semi-mechanistic model focuses on just the glucose and HbA1c information. Similarly, the outputs of the systems biology model include risks of retinopathy, nephropathy, and neuropathy, while the semi-mechanistic model is restricted to prediction of changes in glucose and HbA1c. Having said that, the systems biology model will still need to establish a relationship between change in blood pressure and/or glucose and a binary event such as myocardial infarction, thus incorporating an empirical component [7].

Natural disease progression

The natural disease progression aspect of disease modeling aims at describing the time course of changes observed in the clinical outcome. Drug therapy may alter natural progression of the disease, and such models can then provide insights into the management of several diseases [31]. For this purpose, empirical models have been used most commonly. The natural progression of Alzheimer's disease as measured by the Alzheimer's Disease Assessment Scale – Cognitive score (ADAS-COG) and that of Parkinson's disease using total Unified Parkinson Disease Rating Scale (UPDRS) have been described using empirical models [32-34]. However mechanistic models, which are

more generalizable, are also being studied. A mechanistic disease progression model for arthritis in rats has been proposed [35].

Placebo effect

The effect in a placebo group refers to the psycho-socially induced biochemical changes in a patient's brain and body that in turn may affect both, the natural course of a disease, and response to therapy [36]. Thus, even though the placebo-effect is not directly related to the disease, it can significantly impact outcomes. This is particularly true for disease conditions that are measured symptomatically, such as pain and depression. Therefore, modeling the magnitude and time course of placebo effect has value in discerning true drug effects and also aids in estimating sample size during trial design. Recently, a Bayesian model that describes the time course of the Hamilton Depression Rating scale (HAMD-17) clinical score in the placebo arms of antidepressant trials, combined with a dropout mechanism, has been developed [37]. This model provides new insights on the validity of the results of several longitudinal registration trials currently used for new drug products. A placebo model for Crohn's disease trials [38] is also available.

Table 2: Comparison of systems biology, semi-mechanistic and empirical approaches to disease models

Feature / *Approach*	*Data source*	*Validation*	*Complexity & Resources*	*Application*
Systems biology models	Wide range – underlying biology, inter-relationships with related systems, multiple detailed experiments etc.	Extremely challenging	High – Diverse expertise involved.	• target identification • dose selection • trial design optimization • risk projection based on biomarker data
Semi-mechanistic models	Limited range – one or more experiments; related systems not considered	Relatively simple	Low – Lesser expertise involved.	• go/no-go decisions • dose selection • trial design optimization
Empirical models	Limited range – one or more experiments; may not accommodate design variations and related systems not considered			

POPULATION ANALYSIS

Conceptual framework

A population model typically comprises structural and statistical model components. Structural models are deterministic in nature, and account for population or 'fixed effects' (primary model parameters), but do not account for variability. The typical value of systemic clearance (CL) for a 70 kg individual and the mean potency (EC50) of a drug are examples of fixed effects. A population model suite would include four structural models: PK model, PD model, covariate (or prognostic factor) model and disease progression model.

Statistical models are stochastic in nature, and account for the variability or 'random effects' seen at both, the individual and the observational levels. A population model suite would include three statistical models: between-subject variability (BSV) model, between-occasion variability (BOV) model, and within-subject variability (WSV) model. Random effects models usually assume that the between-subject and between-occasion errors (η) are normally distributed with mean zero and variance Ω^2, and that the within-subject or residual errors (ε) are normally distributed with mean zero and variance σ^2. BSV signifies deviations among different subjects and BOV signifies deviations among different occasions. WSV signifies deviation between predicted and observed values for each subject, and may be the result of measurement error or even model-misspecification.

Nonlinear mixed effects models are called so because they attempt to account for both, fixed and random effects together. The "mixed effects" concept is depicted in Figure 2.

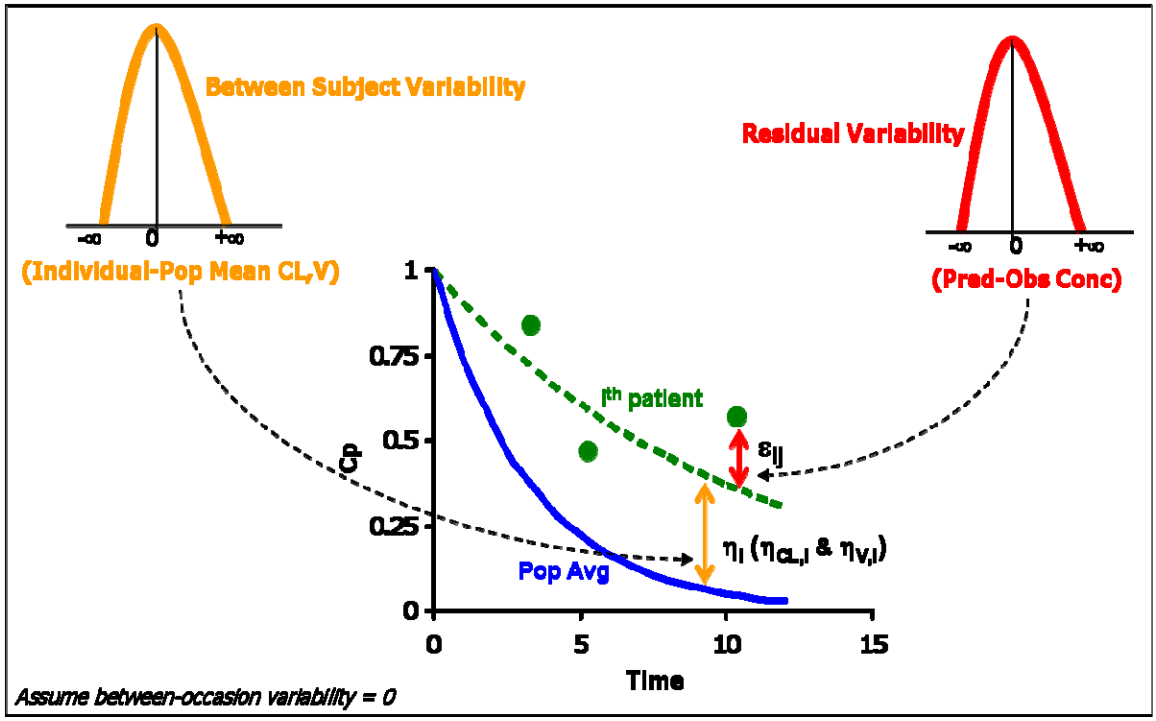
Consider a one-compartment PK model where the drug is given as an intravenous bolus and the volume of distribution (V) is identical in every individual (no BSV for V). Then, the concentration in the 'ith' subject at the 'jth' time point (C_{ij}) can be described using the following equations:

$$C_{ij} = \frac{Dose}{V} \cdot e^{-\frac{CL_i}{V} \cdot t} + \varepsilon_{ij} \qquad \text{Eqn. (i)}$$

$$CL_i = CL_{POP} + \eta_{CL,i} \qquad \text{Eqn. (ii)}$$

where; CL_i is the estimated clearance of the 'ith' subject, CL_{POP} is the estimated population mean clearance, $\eta_{CL,i}$ is the difference between the population mean and individual clearances and ε_{ij} is the residual error of the 'jth' sample of the 'ith' subject.

Figure 2: Conceptual framework for nonlinear mixed effects modeling



Analysis methods

A primary goal of population analysis is to estimate the mean value of relevant parameters (such as CL, V and EC50) in the population of interest, the variances in these parameters as well as residual variability of observations. Another goal is to explain the observed BSV using patient covariates such as body size, age, genotype etc. In addition, estimating individual PK parameters (such as CL_i and V_i) is required to impute concentrations for performing E-R analysis and any other simulations at a later stage.

The known methods for performing a population analysis are: naïve pooled, naïve averaged, two-stage (TS), and nonlinear mixed effects (NM) or one-stage analysis. The main features of these analysis methods are summarized in Table 3.

In naïve pooled analysis, individual observations from all subjects are pooled (as though all data came from a single, giant subject) to obtain average PK parameters. A minor variation of this method is the naïve averaged analysis which involves determination of the mean of the data at each time point. Both these methods provide only the central tendency of the model parameters and no random effects are estimated. These methods are used more often for pre-clinical data and are appealing because of their simplicity. However, since between-subject variability is not estimated and cannot be accounted for using covariates, the potential applications of naïve pooled or naïve averaged analyses are very limited.

In two-stage analysis, the first stage involves estimation of the average parameters for each subject from their individual observations, while the second stage involves the estimation of the population mean and variance of the parameters, after adjusting for covariates, if necessary. Estimates of both, the central tendency and the inter-individual variability can be obtained reasonably well. The TS method requires collection of rich data to have sufficient samples per subject (greater than the number of model parameters to be estimated), which is the usual requirement with experimental data. One concern is this method assumes that the individual parameters, estimated in stage one, are known without any uncertainty. More serious drawbacks include the inability to model sparse data and concentration (or dose) dependent nonlinear processes. The conventional PK non-compartmental analysis (NCA) is a type of two-stage population analysis approach.

In non-linear mixed effects analysis, data from all subjects are simultaneously modeled to yield estimates of both, population mean parameters as well as variance. Since both stages of the TS method are performed in one step, the NM technique is also known as the 'one-stage' method. Individual parameters are calculated post-hoc, subsequent to this one-stage optimization. Nonlinear mixed effects modeling is perhaps the most powerful technique for analyzing both rich and sparse data, and does not share the drawbacks of the other methods discussed earlier. One of the main advantages of the NM method is its ability to conduct meta-analyses which enables incorporating all data across a drug development program. The primary disadvantage of this method is that sophisticated software are required for the analysis, which mandates special training for its use, while learning resources are limited. In addition, these analyses can be highly time-consuming.

Table 3: Main features of the common population analysis methods

Feature / *Method*	*Covariate exploration*	*Uncertainty at observational level*	*Uncertainty at subject level*	*Relative Complexity & Time involved*
Naïve Pooled	Indirect – A model with known relevant covariates can be imposed.	Ignored – Mean estimates will be unduly closer to outliers (extreme observations).	Ignored – All subjects are weighted equally, regardless of number of observations per subject.	Low
Naïve Averaged	Indirect – Subjects can be divided into groups based on relevant covariates.			
Two-Stage	Convenient – A covariate model can be estimated in stage 2.	Accounted – Models will not be unduly influenced by extreme observations.		
One-stage	Convenient – A covariate model can be included in the optimization step.		Accounted – Subjects with more data are also weighted more.	High - Special training is required.

Model qualification

All models are required to be qualified and credible for their wider adoption. Validation implies a procedure of utmost robustness. However, the fact that the true model and its parameters are not known discourages the use of the term 'validation' for population PK-PD models. Hence, qualification may be a better suited term.

The purpose for which the model is being developed should be clearly specified as a pre-requisite before undertaking any model building. Based on the purpose of the model, qualification methods can test either the descriptive capacity or the extrapolation capacity of a given model. Developing an acceptable descriptive model is critical for making labeling recommendations. However, drug labels, usually, do not extrapolate results beyond the range of data observed.

Adequate description of the data at hand will ensure that the proposed model and its parameters are qualified to make reliable inferences, within the range of the data studied. This can be assessed using the routine diagnostic tests such as goodness-of-fit plots (independent variable versus observed and individual/population model predictions), summary statistics, and precision of the parameter estimates. A model and its parameters may be deemed 'qualified' to perform the particular task(s) if they satisfy certain pre-specified criteria. Application of a predictive check to a model and its parameters along with Monte-Carlo simulations [39;40] is an effective method used for qualification of population models.

Physiological interpretation of model parameters is one of the most important aspects of model qualification. A model and its parameters may be deemed 'credible' to perform a particular task(s) if the conceptual foundation on which the model was proposed is satisfactory to a panel of experts. It is important to note that there is no formal means to assess whether a model can be used for extrapolation. Hence the credibility of the model i.e. whether the model was derived from sound mechanistic principles, which appear reasonable to subject matter experts, is important. Thus, a model (and its parameters) may be considered qualified to predict beyond the range of the data used for building the model if the descriptive capacity of the model is acceptable and the model is credible.

TYPES OF DATA AND TRIAL DESIGNS

Data

Pharmacometrics data (referring to PK/PD measurements) that may be collected during clinical trials, in general, are of two types – rich data and sparse data. Typically, rich data, which refers to several (10-20) samples from each subject, is collected under controlled conditions in trials conducted in a small number of patients over a short duration of time. Data from each subject can be analyzed independent of the others, in most cases, and then summarized. Such kind of data is the best for building structural models. Dose-escalation studies, bioequivalence studies, and bridging (for prognostic factor effects) studies are examples of trials where rich data are collected.

On the other hand, late phase clinical trials that are conducted in a large number of patients and for relatively longer durations, typically collect sparse data. Few (1-5) samples are taken from each individual due to practical limitations, which makes it challenging to analyze the data from each subject separately. Sparse data are most suited to build statistical models. Pivotal or registration safety-efficacy trials are examples of studies that tend to collect sparse data.

Trial Designs

Broadly, three of the most commonly used trial designs that employ population analyses are: parallel, cross-over, and titration. In a parallel study design, subjects are randomized to one of several treatment options, for instance, control, dose1, dose2 or dose3. Such a design supports the estimation of population exposure-response characteristics well, but not that of individual characteristics. In a cross-over design, each subject receives all the treatment options. This is the most powerful study design for estimating the individual exposure-response relationships. However, such trials are longer in duration and may experience carry-over effects from previous treatments. The titration design is one where patients are usually initiated at a low dose, which is then gradually increased either until no additional benefit is observed, or until dose-limiting toxicity occurs. This design resembles clinical practice most closely and individual exposure-response determination is possible. However, it may so happen that patients who are less sensitive to the drug need higher doses, making it (falsely) appear as though the response decreases after a certain dose. In several cases, particularly for the cross-over and titration designs, sophisticated data analysis such as mixed-effects modeling is required.

Further, based on the assignment of randomized groups in the trial, there are different designs possible. Subjects may be randomized to receive a particular dose or concentration of the test drug or to a particular effect elicited by the drug. Accordingly, such trials are referred to as Randomized Dose Controlled (RDCT), Randomized Concentration Controlled (RCCT), or Randomized Effect Controlled (RECT) trials. An active control group is used where a placebo control is considered unethical.

In an RDCT, the different doses of the drug to be tested are randomly administered to the subjects. Data are then collected throughout the trial and analyzed using an appropriate method. Such trials are the most commonly seen design due to the relatively simple execution and analysis involved.

In an RCCT, a set of target drug concentration levels are selected based on the exposure-response relationship established from previous studies. Subjects are then randomized to one of these pre-specified target concentrations [41]. Such a design obviates a dose-titration period during which the dose that ensures achieving concentrations within the selected target range (ex.: 5 ± 0.5 μg/L) is identified. A variation of the RCCT design is when doses are pre-specified based on a certain demographic variable. For instance, body weight adjusted doses are routinely administered in pediatric studies. Similarly, in an RECT, subjects are randomly assigned to a pre-specified target effect level. Again, the target effects are chosen based on prior knowledge of the drug's exposure-response, and the dose is titrated accordingly.

RCCT and RECT designs have similar requirements such as prior exposure-response relationship to select the appropriate target concentration or effect ranges, an efficient and sensitive analytical assay method with a short turn-around time, and sufficient strengths of the formulation to allow for any required dose adjustments. Candidate drugs for such trial designs are those where the PK has a large unexplained variability (RCCT) and those where the PD has a large unexplained variability (RECT). In addition, when the measured effect (desired/undesired) is symptomatic, for instance, effects such as pain or nausea that are 'felt' by patients, the RECT could be applicable. When the symptoms are not obvious, the RCCT may be a better choice. Unfortunately, very few drug development programs utilize RCCT or RECT designs, perhaps due to their complicated execution and data analysis, relative to the RDCT design, as well as the cost of implementing TDM if the drug is approved [42;43]. Notably, trials for immunosuppressant drugs used in transplantation generally employ the RCCT design.

CASE STUDIES

Pharmacometric analyses have been employed at various stages of the drug development process. Several case studies where such analyses have had pragmatic value in decision making are discussed. Table 4 summarizes all presented cases while a few selected cases have been discussed in detail.

Tacrolimus – liver, kidney, heart transplantation

Background:

Tacrolimus is an immunosuppressive agent indicated for the prophylaxis of organ rejection in allogeneic liver, kidney, or heart transplants. A large amount of variability has been observed in the PK and PD of this drug. Pharmacometric methods have been employed throughout the drug development stages of tacrolimus, to select rational dosing regimens and optimize therapy [44].

Key questions:

1. What is a safe and effective dosing regimen for first-time-in-man clinical studies?
2. What is a rational target therapeutic concentration range for tacrolimus?
3. What is an optimal initial dose of tacrolimus for late phase clinical trials?
4. What is an optimal TDM strategy for managing patients on tacrolimus therapy?

Role of Pharmacometrics:

The starting dose of tacrolimus (0.15 mg/kg/day IV) used in early phase clinical trials was extrapolated from a synthesis of safe doses in two animal models (rat and dog). The target concentration range for monitoring the drug therapy during these trials was also based on the same animal models, augmented with *in vitro* PD modeling using the IC_{50} values from mixed lymphocyte reactions. Collectively, all the animal models studied were also highly predictive of the systemic toxicities observed with tacrolimus in humans. A pilot compassionate-use early clinical study in patients with refractory liver rejection suggested that the 0.15 mg/kg starting dose was clinically effective, but toxic in some patients, and doses had to be individualized to the patient. A reduced starting dose

(0.05 mg/kg/day IV) was predicted by simulations before onset of the pivotal trial, and the need for this dose reduction was dramatically confirmed during the U.S. and European multicenter registration trials. In addition, an Artificial Intelligent Modeling System (AIMS) was developed to efficiently guide dosing and monitoring of patients on tacrolimus. The AIMS-based TDM led to clinical and pharmacoeconomic benefits in a subsequent prospective pilot clinical study.

Impact:

Pre-clinical models proved to be a reliable guide for identifying a safe and effective dose and a therapeutic concentration range for tacrolimus. Implementation of the AIMS improved the TDM strategy by 3-4 fold reduction in number of blood samples drawn and a reduction in length of hospitalization after liver transplantation. Thus, modeling and simulation enabled more efficient trial design and data analysis of the RCCTs conducted during development of tacrolimus and improved the cost-effectiveness of therapy.

Degarelix – prostate cancer

Background:

Degarelix is indicated for the treatment of advanced prostate cancer patients. During its clinical development, the primary end-point used in trials was suppression of testosterone levels (< 0.5 ng/ml) from day 28 of treatment initiation through 1 year of therapy in 90% patients. The dosing goals were to achieve this challenging end-point. The sponsor conducted five early and late phase dose-finding clinical studies but was unable to derive

an optimal dosing regimen. An end-of-phase 2a meeting was arranged between the FDA and the sponsor to discuss a better drug development plan for degarelix.

Key question:

What is a rational dosing regimen that would maximize the effectiveness of degarelix in advanced prostate cancer patients?

Role of Pharmacometrics:

Population analysis was conducted to develop an exposure-response model for degarelix based on the five dose finding studies conducted by the sponsor [45;46]. The FDA suggested alternative dosing strategies and clarified the regulatory expectations of the NDA. For initial suppression of testosterone levels by day 28, a higher loading dose requirement was explored. A lower maintenance dose was derived to sustain the testosterone suppression through 1 year of drug therapy. Using a mechanistic E-R model and extensive clinical trial simulations an optimal dosing regimen was derived. All pharmacometric analyses were conducted by the sponsor, under the guidance of the FDA. The model-based regimen was used in a registration trial that resulted in positive outcomes and led to approval of degarelix for this indication.

Impact:

Degarelix was approved for use in advanced prostate cancer based on a registration trial that employed a modeling and simulation derived dosing regimen, which several prior clinical studies failed to derive. Trials in prostate cancer patients are challenging and costly and early interaction between the sponsor and the FDA enabled more cost-efficient drug development and a smoother review process.

Busulfan – bone marrow transplantation

Background:

Busulfex, an intravenous formulation of the drug busulfan, is used in combination with cyclophosphamide as an immunosuppressive conditioning regimen for bone marrow ablation prior to hematopoietic stem cell transplantation. The drug was initially approved for use in adults with chronic myelogenous leukemia. The dose-limiting toxicity associated with busulfan is potentially fatal hepatic venoocclusive disease (HVOD). Clinical studies suggested that a therapeutic window of 900-1500 umol/L/min in adults was appropriate to balance safety (occurrence of HVOD and leukemic relapse) and efficacy (successful engraftment). The FDA issued a written request (WR) to the sponsor to determine the PK of busulfan in pediatrics (aged 4-17 years) and the optimal dosing regimen for this population that would achieve target exposures.

Key question:

What is the appropriate dosing strategy for busulfex in pediatric patients?

Role of Pharmacometrics:

A population PK study was conducted to characterize the PK of intravenous busulfan in pediatrics and provide dosing recommendations [47]. Clinical studies indicated that the therapeutic window was similar for pediatric and adult patients. However, this was confounded by the increased variability in the PK of oral busulfan seen in pediatric patients compared with adults. Hence a target therapeutic window with a lower, more conservative threshold for toxicity, than in adults, was used for pediatric patients (900-1350 umol/L/min). Body weight, body surface area, age and gender were explored for

their impact on pediatric dosing. Simulations suggested that the mg/kg and mg/m^2 based dosing regimens were similar in their efficiency. Exposures obtained by different dosing regimens, with 1 to 7 dosing steps including various combinations of weights and doses, were evaluated. All the dosing regimens explored had, at best, 60% patients achieving target exposures after the first dose. Notably, the model revealed that the unexplained between-subject variability (25%) was larger than the within-subject variability (6%), indicating that BSV is the key determinant of therapeutic success. This finding coupled with the narrow therapeutic window for busulfan, supported implementation of therapeutic drug monitoring for optimizing drug therapy.

Impact:

Based on the modeling and simulation, and practical considerations, a 2-step dosing regimen was proposed from this study: 1.1 mg/kg for patients weighing ≤ 12 kg and 0.8 mg/kg (adult dose) for patients weighing > 12 kg. In addition, considering that about 40% patients may not achieve target exposures after the first dose, even with the optimized regimen, a TDM strategy was proposed to enhance therapeutic targeting. These dosing recommendations, which had not been directly tested in clinical trials, were incorporated into the drug label.

Table 4: Summary of case studies, where pharmacometric analysis had an impact on decision making, during different stages of drug development.

Drug	Stage	Key Questions	Decision Impacted	Comments
5c8, mAb [48]	☒ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☐EOP2A ☐ Post-marketing	☒ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☒ Go/no-go ☒ Dose optimization ☐ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	*Perceived impact of model developed:* • optimize sample collection in experiments • anticipate exposure-response in humans • quantify other antigen-provoked responses • project utility of 5c8 in treatment of antibody-mediated autoimmune disease
rPSGL-Ig [49]	☒ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☐EOP2A ☐ Post-marketing	☒ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☒ Go/no-go ☒ Dose optimization ☐ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• developed allometric models across animal species to predict PK and dose range for first-time-in-man clinical trial
Tacrolimus [44]	☒ Pre-clinical ☒ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☒ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☒ Improved trial design ☒ Approval ☐ Labeling ☐ Special population – dose selection	• derived early phase trials starting dose using two animal models • derived target conc. range for RCCT trials and TDM using animal and in-vitro PD models • derived final starting dose for pivotal trial using simulations • improved TDM strategy and cost-efficiency

Rivoglitazone [50]	☐ Pre-clinical ☒ Early Clinical ☐ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☒ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• developed a 'best-in-class' compound using modeling and simulation • selected biomarker/end-point, dose, sampling, washout, eligibility & discontinuation criteria, and forecasted trials for late clinical phase • built disease model from related drug information
Mycophenolate mofetil [51;52]	☐ Pre-clinical ☒ Early Clinical ☐ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• derived dosing regimen for a late phase clinical trial (RCCT) using E-R model based on a pilot study
Degarelix [45;46]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☒ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☒ Approval ☐ Labeling ☐ Special population – dose selection	• explored alternative dosing strategies based on five phase 1/ 2 studies • selected final dosing regimen for registration trial that eventually led to drug approval
Piperacillin/ Tazobactam [53]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☒ Special population – dose selection	• recommended 2-step weight-based PIP/TAZ pediatric dosing regimen in drug label for patients aged ≥ 2 months • verified no new safety concerns than those in adults

		☐ Benefit/ risk evaluation		
Busulfan [1;47]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☒ Special population – dose selection	• recommended 2-step weight-based pediatric dosing regimen in drug label • proposed TDM strategy in label to enhance therapeutic targeting
Everolimus/ Cyclosporine [15]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☒ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☒ Approval ☐ Labeling ☐ Special population – dose selection	• projected likely outcomes of altered dosing schemes • proposed new dosing regimen that reduced renal toxicity while maintaining efficacy thus improving benefit/risk profile than seen in registration trial • cardio-renal advisory committee recommended new regimen to be evaluated in future trial
Apomorphine [1]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☒ Special population – dose selection	• demonstrated a 50% increase in exposure in renal impairment • derived maximum recommended dose and titration strategy and dose adjustment in renal impairment in drug label

Zoledronic acid [1]	☐ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☐EOP2A ☒ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☒ Special population – dose selection	• suggested a correlation between risk of renal deterioration and drug exposure • recommended dose adjustments in mild and moderate renal impairment patients in drug label
Oxcarbeazepine [1;54]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☒ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☒ Approval ☒ Labeling ☒ Special population – dose selection	• found no important differences in placebo and drug effects between adults and pediatrics • supported evidence for approving drug as monotherpay in pediatric patients with partial seizures • derived dosing instructions in drug label • saved additional controlled trials
Micafungin [15]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☒ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☒ Approval ☒ Labeling ☐ Special population – dose selection	• derived dosing recommendation and supported approval of drug for esophageal candidiasis • provided evidence for label to indicate greater potential for liver toxicity at approved dose

Varenicline [15]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☐ Special population – dose selection	• showed much higher drug exposures in renal impairment • found baseline smoking status and age to be prognostic of abstinence from smoking • found marginal dose increase to increase effectiveness but also significantly increase toxicity (nausea) • recommended lowering dose in case of intolerance to adverse effects in drug label
Docetaxel [19]	☐ Pre-clinical ☒ Early Clinical ☐ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☒ Special population – dose selection	• identified a sub-population (liver impairment patients) more prone to grade 4 and febrile neutropenia • recommended reduced dose in label for patients with liver insufficiency to improve safety profile of drug
Nesiritide [1;13]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☒ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☒ Approval ☐ Labeling ☐ Special population – dose selection	• explored alternative dosing regimens for reasonable benefit-risk profile • proposed dosing regimen for use in subsequent registration VMAC (Vasodilation in the Management of Acute CHF) trial that led to drug approval

Sotalol [1;21;22]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☒ Special population – dose selection	• found drug effects in pediatrics to be consistent with adults • found sponsor's dosing recommendations to be acceptable for patients aged ≥ 2 years • derived more specific dosing for neonates and infants aged < 2 years in drug label
Nisoldipine [18]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☐ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☒ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☐ Special population – dose selection	• alleviated safety concern of large drop in blood pressure upon administration of drug in fed condition, given significant food effect on increasing bioavailability of controlled-release product
Oral suspension product for prophylaxis of invasive fungal infections in high-risk patients [15]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☐ Improved trial design ☐ Approval ☒ Labeling ☐ Special population – dose selection	• revealed need for TDM to maximize effect for all patients • supported inclusion of conditions to optimize drug absorption and importance of ensuring adequate plasma concentrations in label • supported need for post-marketing study to evaluate benefit of proposed TDM

Drug to treat type 2 diabetes mellitus [14]	☐ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☒ EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☒ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• evaluated 2 trial designs: genotype-stratified and biomarker enrichment designs, using semi-mechanistic model for FPG and HbA1c • proposed biomarker-enrichment design for future trials that would help derive optimal genotype-based dosing • revealed need to develop sustained release drug formulation
Drug to treat a life-threatening rheumatologic disorder [1]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐ EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☒ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• showed that biomarker was predictive of clinical outcome but a 65% reduction would achieve significance, after two failed registration trials • recommended exploring doses that achieve greater reduction in the biomarker or maximal tolerated dose for future trials
Drug to treat a debilitating neurological disorder [15]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐ EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☐ Dose selection ☐ Covariate determination ☒ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☐ Improved trial design ☒ Approval ☐ Labeling ☐ Special population – dose selection	• showed that reduction in symptoms was related with drug dose while withdrawal effects were significant and consistent, after 1 failed and 1 successful registration trial • supported evidence of effectiveness for drug approval • saved additional clinical trial

Drug to treat a mild, moderate, or severe life-threatening disease [15]	☐ Pre-clinical ☐ Early Clinical ☒ Late Clinical ☐EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☐ Dose selection ☐ Covariate determination ☒ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• identified non-responder sub-group: patients with mild disease • showed consistent effectiveness in patients with moderate and severe disease • elucidated inconsistent results from previous trials • recommended future study in only moderate and severe disease patients
New class of antivirals [14]	☐ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☒EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• distinguished QD and BID dosing regimens using a mechanistic viral-dynamic model that previous models could not achieve • allowed assessment of impact of variability, dosing regimen, patient compliance and dropout on trial outcomes • proposed a lower dose BID regimen for future trials
Drug to treat insomnia [14]	☐ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☒EOP2A ☐ Post-marketing	☐ Molecule screening ☒ Trial / experimental design ☒ Dose selection ☐ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☐ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• recommended healthy subject studies for selecting doses for sleep onset but not for sleep maintenance evaluation • recommended patient trial durations of more than 30 days for reliable identification of doses and persistent sleep maintenance

Pro-drug to treat a life-threatening disease [14]	☐ Pre-clinical ☐ Early Clinical ☐ Late Clinical ☒EOP2A ☐ Post-marketing	☐ Molecule screening ☐ Trial / experimental design ☒ Dose selection ☒ Covariate determination ☐ Evidence of effectiveness ☐ Benefit/ risk evaluation	☐ Go/no-go ☒ Dose optimization ☒ Improved trial design ☐ Approval ☐ Labeling ☐ Special population – dose selection	• revealed body weight to be prognostic for toxicity and effectiveness and that per kg dosing of both test and reference drugs would allow more appropriate investigation of non-inferiority • indirectly, also derived optimal dosing of reference drug for wider application across other development programs

PERSPECTIVE

Learn-Apply paradigm

The strongest merit of model-based drug development lies in its ability to incorporate the entire base of relevant prior knowledge into decision-focused recommendations for the future. A Learn-Apply paradigm is being proposed as an effective means to leverage pharmacometric methods and enhance drug development [55]. Accordingly, learning refers to transforming information (such as clinical trial data) into knowledge while applying refers to utilizing this knowledge to make informed decisions (such as confirmation of effectiveness, dose selection etc). This is an extension to the learn-and-confirm philosophy in modeling that has been promoted by Lewis Sheiner [56].

Currently, pharmacometric models are typically developed at the end of phase 3. A more prudent way to economize time and costs to develop models is by maintaining a progressive model building philosophy. The essence of progressive model building is to continuously update the current model as new knowledge is accrued. The advantages are

at least two-fold: the ability to ‘carry-forward’ knowledge all along the development of a given drug product, and the ability to divide a big problem into several small components that are easier to solve. For instance, in the case of developing a "best-in-class" compound, model-based drug development can use the wealth of knowledge from predecessor drugs with a similar mechanism of action [50]. Right from the phase 1 stage, efficacy and safety drug models can be developed based on preclinical data of the new drug, as well as clinical experience with predecessors. As the clinical development advances, the models can be continually updated, and thus the characteristics of the new drug would become increasingly well defined. However, implementation of such a paradigm calls for more open collaboration of scientists from all disciplines and an institutional commitment to use the ‘current’ model while designing the next trial.

Future considerations

The late-phase attrition rates in drug development are alarmingly high at both, the registration trial and the regulatory review stages, and it is believed that timely application of pharmacometric methods can enhance future development plans and reduce these attrition rates [1-7;11;57].

Quantitative disease-drug-trial model suites can serve as a valuable tool for improving future drug development and should be increasingly employed to design trials using clinical trial simulations. The FDA has set a target to design 50% of all pediatric trials using simulations by 2015 and 100% by 2020. Upon development of and experience with a particular disease-drug-trial model suite, a standardized template can be created for the

trial design, data analysis and review for all drugs under that indication. Consortia on specific topics are perhaps effective means for developing such model suites.

Early-on interaction between the FDA and drug sponsors may help in more efficient planning. The End-of-Phase 2A (EOP2A) meetings are a good platform to facilitate this goal via more rational dose selection and trial design and reduction in number of cycles involved in the NDA review [24].

However, modeling and simulation must not be viewed as a substitute for clinical trials altogether, nor seen as a tool to salvage failed trials, which were poorly-designed, for regulatory approval. The aim is simply to employ these techniques into a continuous learn-apply paradigm, capitalize on prior knowledge, improve trial design, and support evidence for approval and labeling of drugs.

Increased collaboration between the industry, academia and the FDA is essential for the growth and wider application of pharmacometrics. In addition, increased interaction across the board between experts, such as clinicians, pharmacometricians and statisticians is a must for better appreciation of this field. Finally, training in this area is currently not offered by many academic institutions, and this may be an important step forward in the future.

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CHAPTER 2

Pharmacometric Analyses Impact Pediatric Drug Approval and Dosing

ABSTRACT

The purpose of this article is to review case studies where pharmacometric analyses, also known as PK/PD modeling and simulation, have contributed to decision making in pediatric drug development and regulatory reviews. Most prominently, pharmacometric analyses support dose selection for clinical trials, evidence of effectiveness for regulatory approval and dosing recommendations for pediatric labeling. In addition, the article provides a future perspective on adopting pharmacometric analyses to improve pediatric pharmacotherapy and drug development.

INTRODUCTION

The need to improve pediatric pharmacotherapy and drug development has a long history with regulators and public health professionals. As the potential harm of extensive off-label drug use in pediatrics began to surface, several legislative initiatives were undertaken to generate pediatric-specific data. The aim was to ensure that the pediatric patient population no longer remained a therapeutic orphan [1]. In the U.S., the Food and Drug Administration Modernization Act (FDAMA) in 1997, the Best Pharmaceuticals for Children Act (BPCA) in 2002 and the Pediatric Research Equity Act (PREA) in 2003, have jointly provided an impetus to pediatric clinical studies and useful pediatric prescribing information in drug labels [2;3]. The European Council (EC) and the European Parliament have also promoted major regulatory changes in the way pediatric studies are planned and conducted in Europe. The regulation provides financial incentives, and requires a Pediatric Investigation Plan (PIP) for all new products and some existing products (new indication, new formulation, new dosage form, etc.), similar to the pediatric Written Request (WR) in the U.S. [4].

These initiatives have been largely successful in stimulating pediatric investigations. The desire to generate prospectively planned data for pediatrics is now being realized. As of a recent update, FDA has issued 386 pediatric written requests for several important diseases. Thus far, 173 approved drugs have obtained pediatric exclusivity by fulfilling the elements of written requests, as agreed upon by the FDA and sponsors. FDA has made labeling changes for many drug products (n>160) and the majority of these changes resulted in new pediatric safety and effectiveness information [5].

In the early 1990s, it was accepted that pediatric-specific data were either impossible or difficult to generate. In recent times, while financial incentives have led to an increase in the amount of information available to treat the pediatric population, there still remains a concern regarding the generation of good quality data to guide pediatric pharmacotherapy.

- Off-label use of medication continues to be a major concern in pediatrics [6;7]. According to a 2005 study (677 patients), prescribing information in all age categories was available for less than 35% of commonly prescribed medications [7]. There are data from a 2004 survey (7901 patients) to indicate that 96% of cardiovascular-renal, 86% of pain, 80% of gastrointestinal, and 67% of pulmonary and dermatologic medication prescriptions either did not follow the prescribing recommendations or such information was not available [6]. Additionally, younger children were more likely to be treated with off-label strategies. For example, 92% (out of 238 patients) received one or more courses of an unapproved drug [8].

- Many pediatric investigations fail to generate useful data due to challenges unique to pediatric drug development. A recent study found that about half of the pediatric antihypertensive pivotal dose-response trials failed [9]. A retrospective analysis of such trials revealed that poor dose selection, lack of acknowledgement of differences between adult and pediatric populations and lack of pediatric formulations were associated with trial failures [9].

These concerns point to the unique nature of pediatric drug development. Such drug development programs are typically short (one or two clinical trials) and generally do not involve mortality/morbidity end-points. Drug approval is often based on matching systemic exposures or effect on pharmacodynamic biomarkers, to those in adults. Another major challenge is our understanding of and ability to account for the impact of growth and maturation on clinical pharmacology. It is expected that different drug exposures and/or altered response to the drug would be achieved in pediatric patients as compared with adults [10]. Altered clinical pharmacology along with ethical and logistical constraints together pose challenges to the design and analysis of pediatric trials as well as to pediatric therapeutics.

Towards that end, there has been a growing interest in exploring means to enhance pediatric drug development [4;11]. Pharmacometric analysis methods are an important tool to improve the success of pediatric clinical trials and, therefore, pediatric pharmacotherapy. As summarized by Manolis and Pons, pharmacometric analyses consist of characterization and prediction of pharmacokinetics/pharmacodynamics (PK/PD), extrapolation from adults to children, interpolation between pediatric age subsets and optimal use of scientific literature and in vitro/preclinical data. Pharmacometric analyses can be employed to design informative studies using knowledge about disease pathophysiology, drug pharmacology (from adults and/or pediatrics), and organ maturation in pediatrics.

The focus of the present article is to review case studies where pharmacometric analyses have influenced three major areas: pediatric trial design and therapeutics guidelines, evidence of effectiveness, and dosing recommendations for pediatric labeling. For each of these areas, case studies are grouped according to a best fit scenario and one of the case studies is discussed in detail. In addition, the article also provides future perspectives for the application of modeling and simulation to improve pediatric trial design and therapeutics.

APPLICATIONS OF PHARMACOMETRIC ANALYSES

Trial Design and Therapeutics Guidelines

A prospective clinical trial is one of the best ways to generate information to derive useful prescribing guidelines for pediatric drug use. For several drugs, however, there are either no data from prospectively planned clinical trials or pediatric trials have failed to achieve their primary objective. While it is possible that some drugs approved for adults may not be effective in the pediatric population, it is important to derive evidence-based support. Table provides examples where pharmacometric analyses were used to design future pediatric studies based on adult data or studies for drugs where there had been a failed pediatric trial for the same drug.

A case in point is the antihypertensive drug esmolol. The pediatric registration trial failed to demonstrate effectiveness of the drug [12]. However, an external clinical study of the same drug and indication in pediatrics, that was conducted even before the registration

trial commenced, found the drug to be effective and reported a distinct exposure-response (E-R) relationship for reduction in blood pressure [13]. The registration trial investigated 125-500 ug/kg/min esmolol doses in spite of the external clinical study showing 700 ug/kg/min of esmolol to be effective in reducing blood pressure in pediatric patients with acute hypertension after cardiac operations. The results of these studies clearly demonstrate that the choice of doses studied in the registration trial was a key determinant of trial failure. It is unlikely that a pediatric trial of esmolol will be repeated. Thus, inefficient trial design led to a potentially effective treatment not being approved for use in pediatrics, a common theme among antihypertensive trials conducted in the early 2000s.

Pharmacometric analyses can provide a rational basis for making important choices while designing pediatric trials using available information. Important trial design aspects such as dose range to be studied, sample size and PK sampling, trial duration, and analysis methods should be carefully selected [9;14]. A case study to systematically design a pediatric clinical trial based on adult data (without pediatric data) for anti-hypertensive drugs is available [14]. Presumably, such systematic use of available information may have helped appropriate dose selection for a registration trial of esmolol (discussed above).

On the other hand, there are several instances of drugs that have been in clinical use for years in pediatrics, but optimal dosing strategies were unknown. In many such cases, pharmacometric analyses have provided insights to retrospectively derive dosing

information or to design subsequent trials to further optimize dosing strategies and perhaps guide therapeutic decisions. Such examples are also cited in Table 1.

Table 1: Pharmacometric analyses to design pediatric trials using existing data in pediatrics and/or adults

Drug	Problem Statement	Pharmacometric Analyses Contributions
Topotecan*[15]*, ***Furosemide****[16]*, ***Vancomycin****[17]*, ***Ondansetron*** *[18]*	Lack of clear guidelines for pediatric use despite years of clinical experience	• Explored competing dosing regimens • Recommended optimal dosing strategy for use in future trials and/or clinical practice
Fluconazole[11;19], ***Actinomycin-D*** *(AMD) [20]*		• Developed dosing guidelines that were successfully employed in subsequent trials • Examined the success (in terms of efficacy and safety) of dosing strategies and designed a prospective efficacy trial
Carvedilol*[21-23]*, ***Esmolol*** *[12]*	Failure of pediatric trial for drug approved in adults	• Provided insights into failed trial • Recommended optimal dosing strategy for use in future trials • Recommended appropriate end-point for future trials
Famciclovir*[24]*, ***Teduglutide****[25]*	Prospective clinical trial design	• Recommended prospective study design including elements such as dose selection, sample size and PK sampling

Evidence of effectiveness

The primary analysis methods for drug approval (treatment vs placebo comparison using standard hypothesis testing) and some endpoints (mortality/morbidity) used in adult trials may not be feasible or practical in pediatrics. Occasionally, a pivotal trial may fail to meet its primary endpoint due to avoidable reasons. Model-based endpoints may then be used in select instances, along with prior knowledge from adults and related pediatric data, to provide primary or supportive evidence of effectiveness for approval in pediatrics. Model-based endpoints are expected to be more powerful than standard hypothesis testing and hold unique value in pediatric trials due to challenges identified above. Table 2 provides a summary of case studies where pharmacometric analyses were considered suitable to support evidence of effectiveness for pediatric drug approval.

Under certain circumstances, regulations allow the use of well established exposure-response knowledge from one population for the approval in another [26]. Pharmacometric analysis was useful in bridging consistent drug effect of *d,l* sotalol hydrochloride on a surrogate (heart rate) in pediatrics and adults. Sotalol was originally approved in adults to treat life-threatening ventricular fibrillation and tachycardia, and for maintenance of sinus rhythm in patients with symptomatic atrial fibrillation and flutter. A clinical study assessing the antiarrhythmic and beta blocking effects of sotalol on QTc and heart rate in pediatrics ranging from neonates to 12-year-old children formed the basis of approval for sotalol's use in pediatric patients. A biomarker study and ensuing pharmacometric analyses led to the judicious dosing recommendation in pediatrics [27].

Table 2: Pharmacometric analyses to provide primary or supportive evidence of effectiveness

Drug	Problem Statement	Pharmacometric Analyses Contributions
Oxcarbazepine (Trileptal) [28]	• Unethical to conduct a monotherapy trial in pediatrics	• Provided evidence for approving the drug (first ever) as monotherpay in pediatric patients with partial seizures • Drug approved without additional controlled trials and model-derived dosing instructions included in label
Candesartan Cilexitil (Atacand) [29]	• Trial in patients aged 6 to <17 yrs failed potentially due to poor dose selection and primary analysis method • No other approved ARB for patients aged <6 yrs and no new pediatric trial expected	• Supported evidence of effectiveness in patients aged 6 to <17 yrs • Provided rational dosing recommendations in drug label
Sotalol (Betapace) [27]	• Impractical to conduct a mortality trial in pediatrics	• Demonstrated consistent drug effect on surrogate (heart rate) in pediatrics and adults • Proposed dosing for patients aged ≥ 2 years • Derived dosing recommendations for neonates and infants in drug label.

Dosing Recommendation

The most successful application of pharmacometric analyses to pediatric drug development has been deriving dosing recommendations [30]. In several instances these recommendations have been successfully incorporated into the drug label (Table 3). In others, such as voriconazole [31] and leflunomide [32], dosing strategies have been proposed to guide therapeutic decisions. Occasionally, pediatric doses not directly studied in trials have been approved and included in the drug labels (see Table 3 for specific examples). In fact, in some therapeutic areas such as anti-virals and anti-infectives, drugs are frequently approved for pediatric use by extrapolating effectiveness from adult data. Drug exposures that are shown to be safe and effective in adults are typically considered target exposures for pediatrics. Suitable pediatric dosing regimens are then derived based on matching exposures between pediatrics and adults. In addition, as described previously (Table 1) there are several cases of drugs that have already been in clinical use for pediatrics where pharmacometric analyses have been used after-the-fact to recommend dosing strategies in order to improve therapeutics.

Pharmacometric analyses have been used to optimize dosing recommendations after trial results are obtained based on the therapeutic goal. According to the FDA's pediatric study decision tree [33] there are three broad approaches to conduct pediatric studies to seek drug approval and dosing recommendations: the PK-Only approach, the PK-Biomarker approach, and the PK-Efficacy approach. Depending upon the disease, expected response to intervention, and prior information available (from adult or related pediatric data), one of the three approaches is selected for the pediatric drug development

program. Table 3 below provides a summary of case studies, categorized by approach used, where pharmacometric analyses were employed to derive pediatric dosing recommendations for labeling.

Table 3: Pharmacometric analyses to derive pediatric dosing recommendations for labeling

Drug	Pharmacometric Analyses Contributions
PK-Only Approach	
Piperacillin/ Tazobactam (Zosyn) [34]	• Derived a 2-step body weight based dosing regimen to include in the label
Busulfan (Busulfex) [35]	• Derived a 2-step, body weight based dosing regimen to include in the label • Derived therapeutic drug monitoring strategy to enhance therapeutic efficiency
Levofloxacin (Levaquin) [36]	• Recommended dosing regimen to balance efficacy and safety that was not directly studied in a pediatric trial
PK-Biomarker Approach	
Argatroban [37]	• Provided dosing strategy for pediatrics that matched therapeutic response and risk with adults
Levetiracetam (Keppra) [38]	• Demonstrated that a higher dose (3 mg/kg) that was not directly studied may offer better effectiveness than the lower dose (2 mg/kg) that was studied in the pivotal trial; Both doses incorporated into the label
Tipranavir (Aptivus) [39]	• Recommended higher dose (of two doses) studied in the trial based on benefit/risk evaluation • Explored different dosing strategies and recommended a body weight-based dosing regimen along with original BSA-based dosing
PK-Efficacy Approach	
Fenoldopam (Corlopam) [40]	• Recommended capping the pediatric dose (at 0.8 mcg/kg/min) based on benefit/risk evaluation

FUTURE PERSPECTIVE

There are several areas within the realm of pediatric drug development that could highly benefit from future research [41]. Pharmacometric analyses have the potential to contribute towards many of these areas.

- Pediatric dosing decisions will gain much higher success if developmental ontogeny is well understood and routinely incorporated. This is particularly true for disposition pathways such as non-renal elimination pathways and transport systems where effect of maturation is not well established.
- Pediatric clinical trials will be much more informative with new biomarkers (surrogates) that are well suited to the pediatric population and powerful analysis methods (such as model based endpoints).
- Pediatric clinical trials also need a sound rationale for sample size selection. Established methods to derive sample size are missing for typical PK and PKPD studies because these studies are not designed with a goal to derive statistical significance. We are exploring methods such as defining an acceptable precision standard to derive an objective basis for sample size selection.
- Pediatric pharmacotherapy also needs powerful quantitative techniques to identify safety signals to optimize treatment strategies. This may involve identification of useful biomarkers that are predictive of adverse drug events. It is important to enable detection of safety signals even with data from a small number of subjects.

CONCLUSION

In summary, the case studies presented in this article exemplify that pharmacometric analyses have had a significant impact on improving pediatric pharmacotherapy. Wider adoption of these methods will bring objectivity to decision making during pediatric drug development, improve trial success rates, and provide a more rational basis for decisions in pediatric therapeutics.

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CHAPTER 3

Simulation-based Methodology for using PK Quality Standard to Design Pediatric Trials in the Population Analysis setting

ABSTRACT

The objective of this research is to evaluate the feasibility and methodological challenges while implementing a pharmacokinetic (PK) quality standard, in the population analysis setting. It is important for pediatric trials to yield good quality PK data to enable making reliable dosing decisions. The quality standard aims to ensure both, rational pediatric PK trial design and consistency in regulatory review. A simulation-based method for designing pediatric trials to be prospectively powered to meet the quality standard is proposed.

A simulation-estimation platform, aiming to optimize the pediatric sample size that met the PK quality standard under different scenarios, was used to explore the impact of several trial design elements. In general, reasonable sample sizes (range: 16 - 64 pediatric subjects) were required to meet the quality standard even with sparse sampling schedules (2-3 samples per subject). Increasing sample size and PK samples per subject

increased the precision of parameter estimates. Sample size requirements to achieve target precision progressively increase with increasing between-subject PK variability (low-30%, medium-50% and high-70%). Inclusion of rich adult data, in general, reduced required pediatric sample sizes (eg. from N=64 to N=48 for 70% variability). However, a ceiling effect is observed in the extent adult data can inform the model and reduce pediatric sample size adequacy (no additional benefit of 24 vs. 12 adults). A comparison of population mean analysis and individual post-hoc analysis methods found the former to be more powerful and less biased. Finally, all trends were moreover the same for i.v. and oral administration models.

In conclusion, the PK quality standard is practically feasible in terms of sample size adequacy. A simulation-based approach to design pediatric PK trials using the standard is described. Informative sampling schedule for a given number of PK samples per subject is assumed during trial design. The recommendations are: 1- to use prior adult or pediatric data for trial design and analysis, wherever possible and 2- to use one-stage population analysis methods with biologically plausible covariate models for designing pediatric PK studies.

INTRODUCTION

The pediatric population has been described as a therapeutic orphan [1] because historically, pediatric drug development was deprived of the patronage given to drug development programs in adults [2;3]. However, over the past decade, concerns and actions of health care professionals, researchers and regulators have together led to an impetus in pediatric clinical research [4;5]. Recent legislative initiatives in combination have dramatically stimulated changes in pediatric drug labeling [6]. The aim is to provide useful prescribing information for pediatric pharmacotherapy, such as rational dosing and identification of risks of therapies. Eventually, the hope is to amend the long drawn deficit in pediatric drug development.

Under any pediatric drug development program, pharmacokinetic (PK) information is a key driver of decisions including but not limited to dosing, approval or labeling. According to the FDA's pediatric study decision tree [7], a PK trial, where suitable, may serve as the basis for drug approval in pediatrics. In fact in some therapeutic areas, such as anti-virals and anti-infectives, regulatory approval of drugs for use in pediatrics is primarily based on PK studies. In other areas, PK data from pediatric studies may have a crucial role to play in determining the doses to be tested in pivotal safety and effectiveness trials. Occasionally, drug doses not directly studied in pediatric trials are approved and included in the label for pediatric use based on prior exposure-response characterization from adults or pediatrics. PK data is also useful in supporting evidence of effectiveness and safety related labeling decisions. Hence it is important that all pediatric drug development programs, typically comprising only few (1-2) trials, yield

good quality PK data. Trial design elements, particularly sample size and PK sampling schedule, have a significant impact on the quality of the resulting PK data. Making rational choices during the study design stages itself can save time and costs, ensure good quality PK data and safeguard the interpretability of study results as well as pediatric exclusivity granted to sponsors.

Despite the recent progress in pediatric drug development, there have been multiple unexpected and disappointing results, particularly for pediatric PK studies. One case in point is for the drug metoprolol, where data collected in pediatric trials could not be used efficiently. Three pediatric dose levels (0.2, 1 and 2 mg/kg) were studied in the trial with single trough PK sampling. However, 60% of the samples for dose group 0.2 mg/kg were below LOQ. Firstly, this raised a scientific/regulatory concern due to the (avoidable) complexity involved in the exposure-response analysis, which served as the basis for approval of the drug. Secondly, collecting unusable data from pediatrics raises ethical questions in its own standing.

Another, and perhaps the most important, aspect of pediatric trial design that warrants attention is sample size selection. The pediatric PK studies submitted to the FDA have vastly variable sample sizes. Often a clear rationale is not provided, which leads to inconsistency and introduces subjectivity during regulatory review. There was an instance where a pediatric written request stipulated a sample size of 24 pediatric subjects to be studied, based on no scientific rationale. But due to challenging recruitment rate,

that sample size requirement could not be met, adversely affecting the pediatric exclusivity determination.

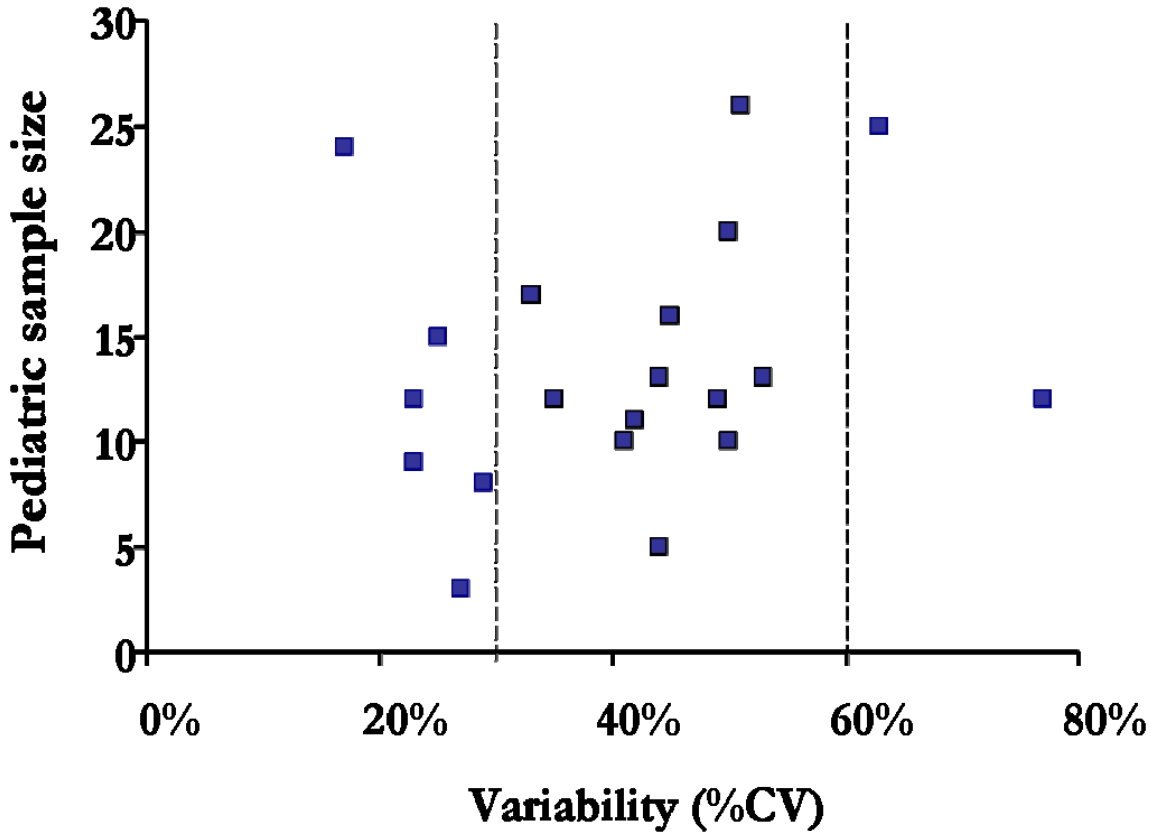
Heretofore, the choice of sample size for pediatric PK studies seems to have been a logistic decision rather than a scientific one. Figure 1 presents the sample sizes used vs. variability (%CV) reported on clearance, in eight randomly selected pediatric PK trials, for illustration. One can observe that there is no correlation between sample size and %CV, which brings out the lack of rationale behind sample size selection. Typically, these studies have included a small number of patients, particularly in the lower age range, which is inadequate for estimating PK parameters with good precision.

These instances draw attention to the need for rational pediatric trial design. In case of pediatric PK studies, there is lack of objective criteria to design a trial, which are available for mainstream efficacy trials. The consequences may be detrimental as is apparent from the cases cited above. In recognition of need for a uniform criterion to define PK data quality, a regulatory requirement has been recently initiated as part of the pediatric written request [8]. The FDA recommends using a pre-defined target on the precision of primary PK parameter estimates, such as mean clearance and volume of distribution, as a quality standard for PK data. The requirement is to prospectively power (at least 80%) a pediatric trial to target a 95% confidence interval within 60% and 140% of the geometric mean estimate of primary PK parameters, for each pediatric age group studied. The aim is to provide guidelines for bringing objectivity into pediatric PK trial design.

Figure 1: Sample size vs. %CV in 8 pediatric PK studies chosen randomly from the literature and submissions to FDA.

[1]Studies cover different age groups ranging from 2 days – 18 years.

[2]Vertical dashed lines represent different variability levels



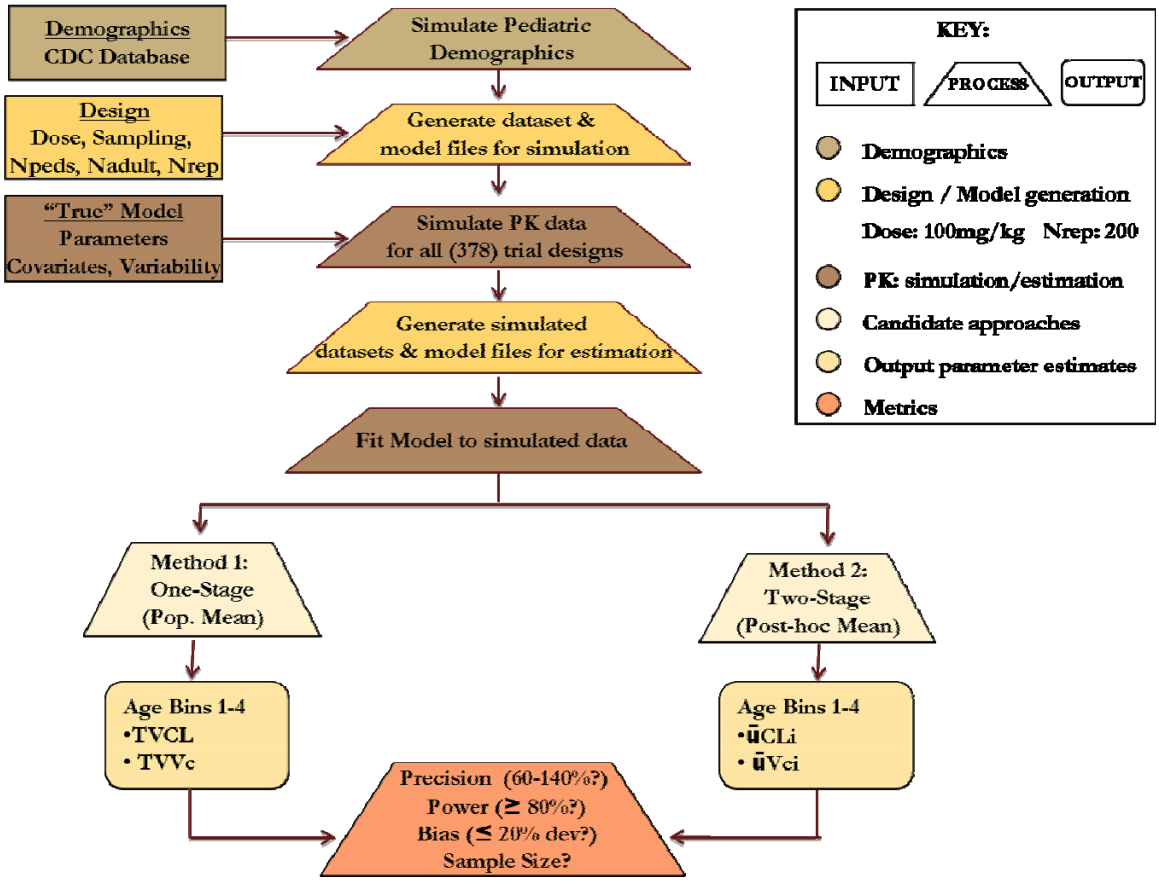
An objective criterion, such as the proposed PK quality standard, is one way of ensuring more rational pediatric trial design. Implementation of the standard will lead to greater consistency and efficiency in analysis of pediatric studies and their regulatory review. However, there remain methodological research questions on how to select the optimal sample size while designing pediatric trials, so as to prospectively power the study to

meet the precision standard. Frequently, pediatric studies collect sparse PK data (1-3 samples per subject) and population analysis techniques are used for the design and data analysis of such trials. Methods on optimizing the PK sampling schedule for population analyses have been published previously [9-11]. The current study focuses on simulation-based methodology to derive sample size, and assess the impact of different elements, while designing pediatric studies to meet the proposed PK quality standard, in the population PK setting.

METHODS

A simulation-estimation method was developed for the current study. A pediatric population was simulated in terms of demographics and PK observations under different trial design scenarios with increasing sample sizes. The parameters of the PK model were then estimated using the simulated data and relevant metrics for the estimates of mean systemic clearance and central volume of distribution were determined. Figure 2 is a flow chart describing all the computations carried out by the tool. The following sections detail the steps involved during the simulation-estimation for a base case trial design scenario with n=16 pediatric subjects, 2 PK samples per subject (1 and 3 hours), 30% between-subject variability in clearance and volume, no adult data included, oral drug administration, and using population mean estimates for analysis. 250 replicate datasets were simulated for the analysis.

Figure 2: Flow chart of computations carried out by the simulation-estimation tool



Answers to the following six research questions were sought through the current study:

1. What are the typical sample sizes for different trial design scenarios that achieve 80% power for target (60-140%) precision and acceptable bias?
2. What is the impact of varying the number of samples per subject?
3. What is the impact of low, medium or high PK between-subject variability?
4. What is the impact of including adult data for estimation?
5. What is the impact of the analysis method used?
6. What is the impact of i.v. vs. oral administration models?

Nominal design

For the present study, the PK model developed previously in pediatrics for Zosyn (piperacillin/tazobactam) [12] was adopted. The model drug used follows one-compartment, dose-proportional PK and has 100% bioavailability with fast absorption (T_{max} about 2 hrs) and a half-life ($t_{1/2}$) of 1.5 hrs. A single 100 mg/kg dose was administered orally to the simulated pediatric subjects.

Demographics

A CDC (Center for Disease Control and prevention) database was used for the simulation of pediatric demographics that included age, gender and weight. The database contained ages from birth-20 yrs, in increments of 1 month, yielding n=240 unique ages. For each unique combination of age and each gender (n=480) there are parameters, including a variability component, to determine the distribution of body weight. Thus, 100 individuals of different body weight for each combination of age and gender were simulated resulting in a virtual bank of n=48000 unique pediatric subjects.

For this study, the pediatric subjects were divided into four age bins as is commonly done during recruitment in pediatric clinical trials. The age bins used were 1 mo to 2 yrs, >2 to 6 yrs, >6 to 12 yrs and >12 to 17 yrs. Subjects were randomly sampled from this bank of pediatrics and evenly distributed into each age bin. All replicate datasets maintained the same set of subjects in terms of covariates.

The bank was also used to determine the "true" mean clearance and volume for each pediatric age bin. Using the covariate model employed for the simulations, the "true" individual clearances and volumes for each virtual subject in the bank were determined and subsequently the geometric mean (non-parametric) of these parameters for each age bin was arrived at. In addition, we also determined the mean (parametric) clearance and volume predicted by the model at the median age and weight for each age bin.

Simulation-estimation models

The one-compartment model used in the present study was parameterized in terms of total systemic clearance (CL) and volume of distribution for central compartment (Vc), and in case of oral administration scenarios, the first-order absorption rate constant (KA). The between-subject variability (BSV) of the model parameters was described using a lognormal distribution.

An allometric scaling model was used for body weight effect on clearance and volume whereas an Emax-type model was used for age effect on clearance. The covariate models were employed for simulation and estimation as well as to determine the true individual clearance and volume for each subject in the pediatrics bank.

$$CL_i = TVCL \bullet \left(\frac{WT_i}{20}\right)^{allo_{CL}} \bullet \frac{Age}{(Age_i + A_{50})} \bullet e^{\eta_{CL_i}} \quad \text{…. Eqn. 1}$$

$$Vc_i = TVVc \bullet \left(\frac{WT_i}{20}\right)^{allo_{Vc}} \bullet e^{\eta_{Vc_i}} \quad \text{…. Eqn. 2}$$

$$KA_i = TVKA \bullet e^{\eta_{Vci}} \quad \text{…. Eqn. 3}$$

where; η_{CLi} is the difference between individual (CL_i) and population mean (TVCL) clearance on log scale, η_{Vci} is the difference between individual (Vc_i) and population mean (TVVc) volume of distribution on log scale, η_{KAi} is the difference between individual (KA_i) and population mean (TVKA) absorption rate constant on log scale, $allo_{CL}$ and $allo_{Vc}$ are the allometirc exponents that account for the effect of body weight (WT) on clearance and volume respectively, A_{50} is the covariate parameter that accounts for the effect of maturation on clearance and reflects the age at which clearance is half of its maximal (or adult) value. η_{CLi}, η_{Vci} and η_{KAi} were all assumed to follow a normal distribution, independent of each other, with mean of zero and variances of Ω^2_{CL}, Ω^2_{Vc} and Ω^2_{KA} respectively.

The residual error or within-subject variability (WSV) was described using a proportional error model as shown below:

$$Cp_i = Cp_{pred} \bullet \left(1 + \varepsilon_{Cp}\right) \quad \text{…. Eqn. 4}$$

where; ε_{Cp} is the difference between the individual observed plasma concentration (Cp_i) and the individual model prediction (Cp_{pred}) and is assumed to follow a normal distribution with mean of zero and variance of σ^2_{Cp}.

Truncated simulations were performed in order to contain the simulated parameter values within reasonable limits. The distributions of clearance, volume, $t_{1/2}$ and KA were truncated using the generic expression shown below, for each of these parameters:

$$LOLIM = \exp(LnMean - 3 \bullet SD) \quad \text{…. Eqn. 5}$$

$$HILIM = \exp(LnMean + 3 \bullet SD) \quad \text{…. Eqn. 6}$$

where; LOLIM and HILIM are the lower and upper limits desired for the simulated parameters, LnMean is the mean parameter on the log scale and SD is the standard deviation of the parameter on the log scale. For clearance, volume and KA the variances used for simulations (Table 1) determined SD and for $t_{1/2}$ the variances of clearance and volume were added to determine SD.

The parameter values for the "true" one-compartment model used for simulations, with associated covariate effects, are listed in Table 1. During estimation the oral absorption parameters, TVKA and Ω^2_{KA}, were fixed, while all remaining model parameters were estimated. For the population mean analysis, model predictions of mean clearance and volume at the median age and weight, for each of the four age bins, were determined as mean parameter estimates.

Table 1: Values of the one-compartment model parameters used for the simulations

Parameter	Value	Units
TVCL	3.2	L/h /20kg
TVVc	6.2	L /20kg
TVKA	2 (fix)	/h
Ω^2_{CL} (CV)	30, 50, 70	%
Ω^2_{Vc} (CV)	30, 50, 70	%
Ω^2_{KA} (CV)	50 (fix)	%
σ^2_{CP} (CV)	10	%
ALLO_CL	0.75	
ALLO_Vc	1	
A_{50}	0.18	years

TVCL, TVVc, TVKA: typical values of systemic clearance, central volume of distribution and first-order absorption rate constant

Ω^2_{CL}, Ω^2_{Vc}, Ω^2_{KA}: variance in CL, Vc and KA respectively; CV: coefficient of variation

σ^2_{CP}: variance in individual plasma concentrations

ALLO_CL, ALLO_Vc: allometric exponent for weight effect on CL and Vc respectively

A_{50}: covariate parameter for effect of maturation on CL, defined as the age at which clearance is half of the adult value

Data analysis

For each replicate, the precision and bias in the mean clearance and volume parameter estimates were computed. The mean bias and power to achieve target precision standard were then determined, based on all replicates.

Precision metrics

In accordance with the recent regulatory requirement, we assessed precision as a %CV-like metric but in terms of upper bound of the 95% confidence interval (UCI) rather than

standard error (SE). Thus, precision was defined as the ratio of the upper limit of the 95% confidence interval to the mean parameter estimate, or relative UCI (RUCI). Values close to 1 for this ratio imply high precision (or small standard errors) while higher values imply more imprecision (or large standard errors). Then as per the PK quality standard defined previously, the target is for this ratio to be ≤ 140%. Given the lognormal distribution of the parameters (CL and Vc), it is assumed that if the UCI is within 140% of the mean, then the LCI will be within 60% of the mean. Hence we focused only on the UCI for assessing precision.

A percentage expression was used to determine precision on clearance and volume parameters for every replicate and the mean of this metric for all replicates was the 'mean imprecision'. Thus a value of 100 for the metric represents no imprecision (or a 0 standard error) and higher the value of the metric, deviant from 100, lower is the parameter precision.

$$RUCI = \left(\frac{UCI}{Mean} \right) \bullet 100 \qquad \text{…. Eqn. 7}$$

The proportion of replicates where RUCI met the target was determined to be the power to achieve the precision standard. A trial design was considered successful if it achieved 80% power for target precision (i.e. RUCI ≤ 140 for ≥80% replicates).

In order to construct the 95%CI, for each age bin, model estimated mean parameters and standard errors were used. Then the 2-sided $t_{df,\alpha}$ statistic corresponding to the total pediatric sample size for estimating eight model parameters (see Table 1) was used (α =0.025, n=16, df=8, t= 2.306).

Bias metrics

For the purpose of computing bias on the parameter estimates, reference values that may be considered "true" estimates were used. The mean parameter estimates for each age bin obtained from the virtual pediatrics bank, as described above were considered as the reference values.

The metric used to compute bias in the parameter estimates was the percent deviation from the reference value, calculated as follows:

$$\%Deviation = \left(\frac{P_{est} - P_{true}}{P_{true}} \right) \bullet 100 \qquad \text{…. Eqn. 8}$$

where; P_{est} is the estimated value of the parameter by fitting the model to the simulated data and P_{true} is the reference value for the model parameter. The mean bias for all replicates was determined. A deviation within 20% of the reference value was regarded as acceptable bias. A trial design was evaluated not only in terms of precision but also acceptable bias, for research purposes.

We also considered the bias in the covariate parameter estimates and variance estimates (both BSV and WSV) as well as shrinkage in post-hoc estimates to assess their reasonable estimation (results not shown). However these metrics were excluded from the power analysis for the trial design.

Scenarios explored

We were interested in exploring the impact of varying different trial design elements on meeting the requirements of the PK quality standard. Table 2 provides a summary of the different scenarios explored in our simulations. There were 756 unique scenarios explored. Different combinations of all the following key design elements were evaluated:

1. Between-subject variability (3 scenarios): The base case trial design was with low (30%) between-subject variability. Scenarios with medium (50%) and high (70%) between-subject variability were also simulated.
2. Samples per subject (3 scenarios): Three sparse sampling schedules, in terms of number of PK samples (1, 2, or 3) per subject, were explored.

   While it is recognized that single trough sampling may be irrelevant for population PK analysis and using such sparse sampling schedules is discouarged, this scenario has been included for research completion purposes. For the single trough sampling scenarios the estimation was carried out differently, based on previous recommendations [13]. Accordingly, only the TVCL, $allo_{CL}$, Ω^2_{CL} and σ^2_{Cp} parameters were estimated, when rich adult data was included. In absence of adult data, even the residual variability parameter (σ^2_{Cp}) was not estimated.
3. Adult data inclusion (3 scenarios): The designs were varied in terms of inclusion of rich adult data (10 samples per subject) in the estimation, exploring three scenarios: pediatric data alone, or with additional rich PK data from 12 or 24 adults.

4. Analysis method (2 scenarios): Two different analysis methods were assessed. Thus all metrics were determined on both, population mean estimates (a one-stage approach) as well as individual post-hoc estimates (similar to a two-stage approach). For the latter analysis method, the geometric means of individual post-hoc estimates of clearance and volume were empirically determined, for each age bin. In order to construct the 95%CI, for each age bin, we used standard errors of age- and weight-normalized post-hoc individual estimates and a 2-sided $t_{df,\alpha}$ statistic corresponding to the pediatric sample size for that particular age bin (α = 0.025, df = n-1) .
5. Drug administration (2 scenarios): Scenarios were simulated using both i.v. as well as oral administration models, for comparison.
6. Sample size (7 scenarios): For each scenario, trial designs with increasing pediatric sample sizes (n = 16, 24, 32, 40, 48, 64 and 80) were simulated, to determine the design that met the quality standard.

Table 2: Trial design scenarios explored during simulations

Design element	ORAL	I.V.
SAMPLING	• 2 per subject (1, 3 h) • 3 per subject (1, 3, 4 h) • Single trough (4 h) Rich sampling used for adult data: 10 per subject (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 h)	• 2 per subject (0.1, 2h) • 3 per subject (0.1, 2, 3 h) • Single trough (3 h) Rich sampling used for adult data: 10 per subject (0.1, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 h)
VARIABILITY	For Cl and Vc: • Low (30%) • Medium (50%) • High (70%) For KA: 50% fixed for all cases	For CL and Vc: • Low (30%) • Medium (50%) • High (70%)
ADULT DATA	• No adult data • Rich data from 12 adults • Rich data from 24 adults	
ANALYSIS METHOD	• Population mean estimates, for each pediatric age bin • Individual post-hoc estimates, for each pediatric age bin	
SAMPLE SIZE	• 16 • 24 • 32 • 40 • 48 • 64 • 80 Distributed evenly across four age bins	

Software

An R environment was employed for the simulation-estimation procedure with system calls to NONMEM and SAS. For the simulations, random numbers were generated using a six digit seed. 250 replicates were simulated for each trial design. NONMEM version VI with Compaq Visual FORTRAN 6 compiler was used to conduct the simulations. SAS version 9.2. was used for the estimations. The estimation method used in SAS was QPOINTS=1 (equivalent to LaPlace in NONMEM). R version 2.9.1. was used to create the automated program script and carry out data manipulation, data analysis and graphics generation.

RESULTS

Figure 3 is a comparative display of the impact of all aspects of trial design explored on meeting the PK quality standard.

What are the typical sample sizes for different trial design scenarios that achieve 80% power for target (60-140%) precision and acceptable bias?

As expected, power increases with increasing sample size. The trends remain similar for both clearance and volume estimates and for all scenarios tested. Figure 4 is a representation of the trend for power to achieve target precision against sample size, by age bin, for a sample scenario (oral, 50% variability, 2 PK samples, no adult data).

In general, the bias in the parameter estimates was acceptable - deviation within 20% of reference values. This result is expected [13]. The mean bias in most scenarios tended to be positive, and was generally higher for age bin 1 (1mo - 2yrs) than other age bins. In general, mean bias w.r.t. simulated data was higher (up to +40% for age bin 1) than that w.r.t. true data i.e. pediatrics bank. Figure 5 presents the mean bias, by age bin, for the same sample scenario (oral, 50% variability, 2 PK samples, no adult data).

Figure 3 displays the smallest sample size that achieves 80% power for target precision and acceptable bias, for all age bins, across all trial scenarios. The sample size selected as a success for a trial design was one for which the criteria were met for all age bins.

Figure 3: **Impact of all trial scenario elements explored on sample size adequacy to meet PK quality standard.**

[1] Results shown are for all oral administration scenarios; i.v. scenario results were similar.

[2] Where dotted lines are not visible on the graph, they overlap with solid lines.

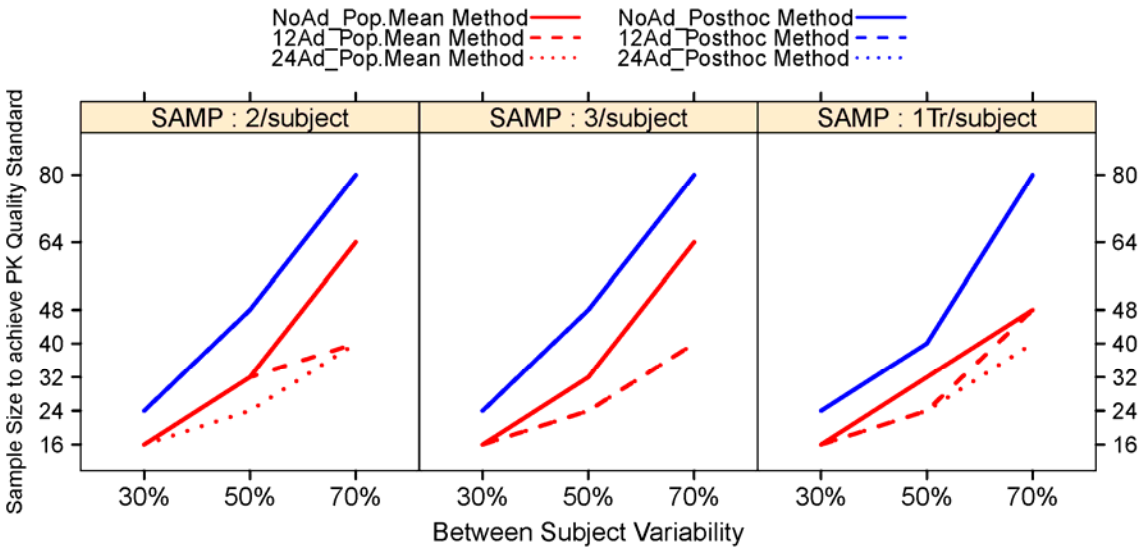


Figure 4: Power to achieve target precision standard (on mean clearance) at all sample sizes explored, by age bin, for a sample scenario (oral, 50% variability, 2 PK samples per subject, no adult data).

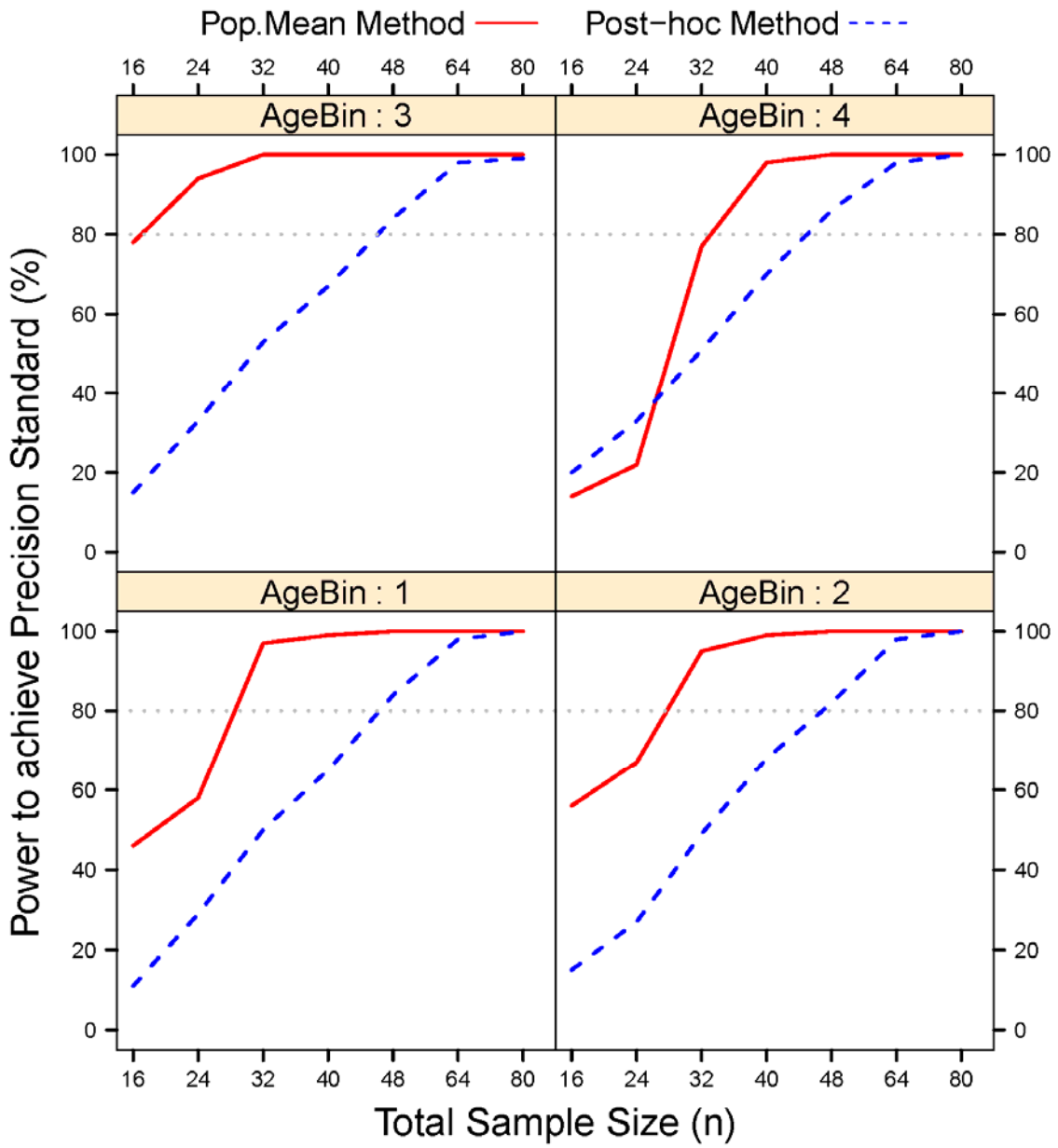
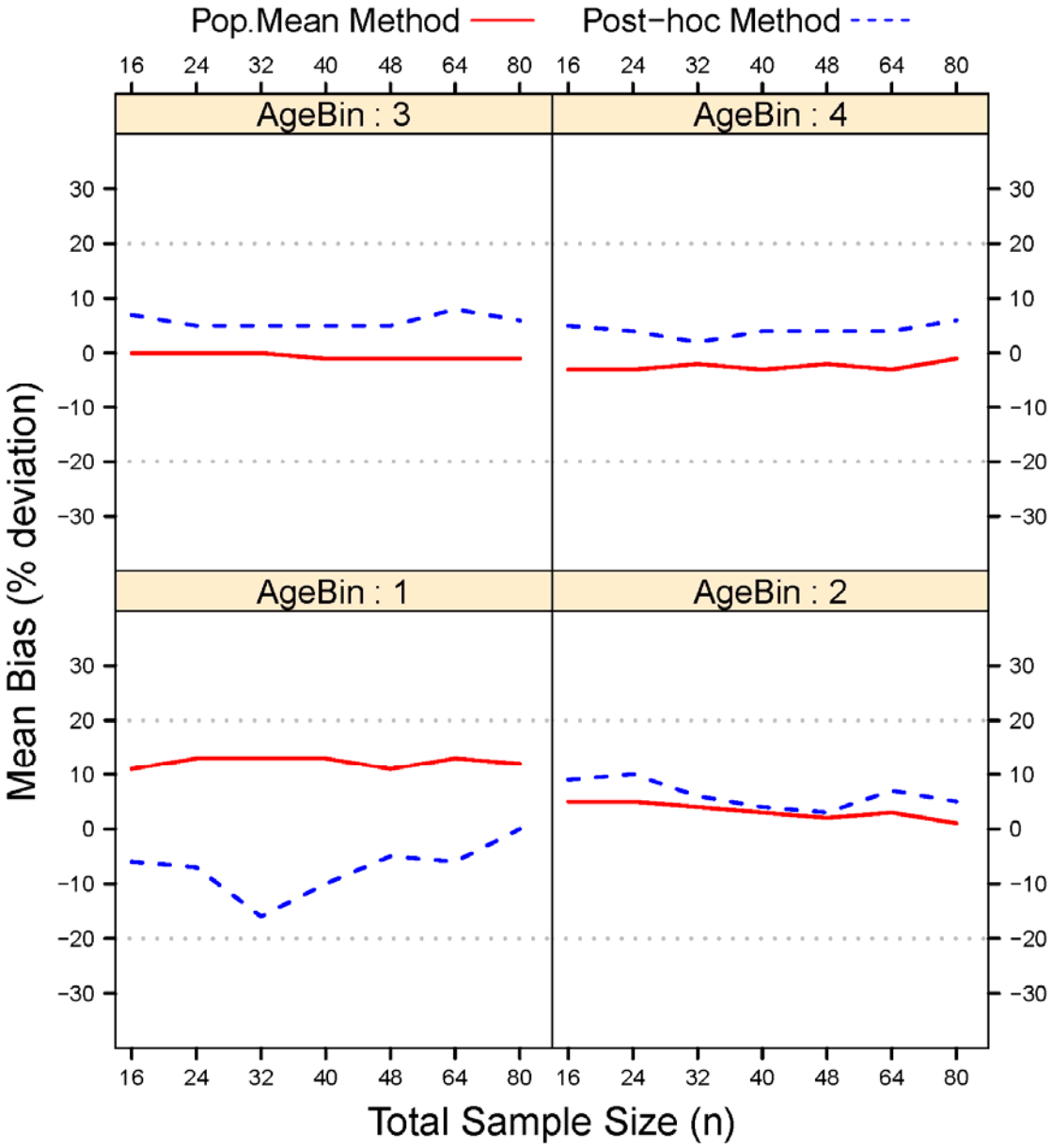


Figure 5: Mean bias (in mean clearance) at all sample sizes explored, by age bin, for a sample scenario (oral, 50% variability, 2 PK samples per subject, no adult data).



What is the impact of varying the number of samples per subject?

Scenarios with 3 PK samples per subject consistently achieved higher power than that achieved with 2 PK samples per subject. However, this translated into lower sample size requirements only in a few cases. This can be seen in Figure 3. Mean bias was not different for 2 vs. 3 samples per subject. Shrinkage was in general ≤ 20% and was consistently greater in cases of 2 samples per subject over 3 samples per subject, as expected.

In this study, the results of single trough sampling scenarios are not directly comparable to those of scenarios with 2 or 3 samples per subject because the estimation was carried out differently for single trough sampling, as described in section 2.5. We assessed precision and bias for only the mean clearance estimates. The power trends however remain similar as for cases with 2-3 samples per subject. The bias was generally acceptable at lower variability scenarios but at high (70%) variability, the mean bias in clearance estimates was up to 30% deviation. However, post-hoc estimates are susceptible to high shrinkage in case of such sparse data, which was observed in our study for single trough sampling scenarios in absence of adult data (30-70% shrinkage).

What is the impact of low, medium or high PK between-subject variability?

Between-subject variability was the trial design element with maximum bearing on power and sample size requirements to meet the precision standard. As would be the case in conventional power analyses, higher PK variability resulted in lower power and higher

sample sizes, consistently in all scenarios. Variability did not have implications on bias estimates.

The residual error estimation was dependent on variability. While a 10% proportional residual error was used for simulations, at low and medium variability scenarios (30%, 50%) this estimate was 15-17% whereas at high (70%) variability scenarios the residual error estimates were higher, 20-30%. In addition, the A_{50} parameter estimate was more biased at higher variability.

What is the impact of including adult data for estimation?

In case of the population mean analysis method, inclusion of adult data (rich sampling) from 12 adults significantly improves the power to achieve target precision. This results in a smaller number of required pediatric subjects when adult data is included in the estimation. For instance, n=64 and n=48 met the precision standard for a 70% variability scenario without and with adult data, respectively. However, a ceiling effect was observed in the inclusion of adult data. In most cases, including rich data from 24 adults did not offer significant increase in power or reduction in sample sizes over including rich data from 12 adults.

The inclusion of adult data does not significantly impact the outcomes of the individual post-hoc analysis method. The power/sample size to meet the precision standard, in general, remains unaffected whether adult data is included or not. Figure 3 presents the

impact of inclusion of adult data on minimum sample size requirements to achieve precision standard for all scenarios.

Adult data inclusion had no significant impact on mean bias in clearance and volume estimates. However, it did significantly reduce the bias in estimation of the A_{50} parameter. Shrinkage in single trough sampling scenarios was significantly lowered (≤20%) in presence of adult data. Adult data also significantly improved the estimation of BSV parameters, in all cases.

What is the impact of the analysis method used?

The population mean analysis method is a more powerful analysis approach than the individual post-hoc method i.e. allows use of smaller sample size to meet precision standard, consistently for all scenarios. This observation is clear in Figure 3. Population mean analysis also resulted in consistently lower mean bias in estimation, compared with individual post-hoc analysis.

For population mean analysis, as far as the covariate parameter estimates, the $allo_{CL}$ and $allo_{V}$ were generally well estimated (≤ ±20% deviation), although the $allo_{CL}$ was generally under estimated. The A_{50} parameter was always significantly over predicted with 50-300% deviation from the reference value (0.18) used for simulations. As far as variance, these parameters were generally reasonably estimated (≤ ± 20% deviation) but the tendency was towards underprediction. This is expected, since we used truncated simulations to restrict the simulated clearance and volume estimates within reasonable

bounds. In case of single trough sampling, the BSV on clearance was considerably under-estimated (-50 to -80% deviation). Adult data inclusion significantly reduced the bias in estimation of variance (-20 to -40% deviation).

For individual post-hoc analysis, as far as shrinkage in parameter estimates, it was generally ≤ 20%. As expected, shrinkage was greater with lesser number of PK samples per subject and was significant (30-70%) for single trough sampling. However, this reduced (to ≤20%) in presence of adult data.

What is the impact of i.v. vs. oral administration models?

All trends in power, sample size, and bias remained moreover similar between i.v. and oral administration scenarios.

DISCUSSION

What are the typical sample sizes for different trial design scenarios that achieve 80% power for target (60-140%) precision and acceptable bias?

The study aimed to address the practical feasibility of implementing the precision standard, in terms of sample size. Hence the inferences are focused on the estimates of sample size adequacy and trends across different scenarios explored, and not on the specific numbers arrived at for sample size. They are intended to serve as guidelines while designing pediatric trials in keeping with the quality standard.

In general, reasonable sample sizes were found to be adequate to meet the proposed quality standard, ranging from 16-64 subjects, even with sparse sampling and high between-subject variability. Hence we consider the proposed quality standard to be practically feasible. Further, the estimation bias with these sample sizes was also found to be acceptable (deviation from true values within 20%). While bias cannot be assessed in real trials, it is re-assuring that the simulations do not suggest major bias in the estimates.

In absence of adult data, the sample size for trial design success was driven by both the extreme age bins, bin 1 (1mo-2yrs) and bin 4 (12-17yrs). In presence of adult data, the sample size was mainly driven by bin 1. Bins 2 (2-6 yrs) and 3 (6-12 yrs) invariably complied with the criteria, for a given sample size, as long as bins 1 and 4 did. This result is expected given the covariate model we have used, where body weight is the main driver for clearance and volume estimates. Estimation precision is always lower at the extreme ends of the data range. Hence precision on parameter estimates was consistently found to be poorest for age bin 1 (also lowest body weight group), and in absence of adult data, even for age bin 4 (also highest weight group). Thus, the total sample size that can be used in a pediatric trial in order to be powered to meet the quality standard may be lowered by recruiting fewer subjects in the middle age bins 2 and 3, and more subjects in age bins 1 and 4. However, this decision is more of a regulatory issue than a research focus of the current study.

What is the impact of varying the number of samples per subject?

Sparse sampling schedules are most common in pediatric trials. While we explored scenarios with either 2-3 or single trough PK sample per subject, the results are generalizable. Increasing the number of PK samples per subject, even within the realm of sparse sampling schedules, adds information to the model to aid better precision. If rich pediatric PK data are available, non-compartmental analysis (NCA) may be considered, in which case the sample size determination is fairly straightforward using variability estimates from adult data or relevant prior pediatric data [8].

Of note is the fact that apart from number of samples, the sampling time schedule is of critical importance during study design and has bearing on parameter precision as well. However, several researchers have proposed methods to optimize PK sampling schedules while designing a population study [9-11], and a thorough account of this aspect of optimal trial design is beyond the scope of the present research. It is assumed that optimal sampling time points for a given number of samples are pre-determined based on a previous method.

As mentioned previously, we do not encourage the use of single trough sampling for population PK studies, in recognition of its limitations. With only single trough data, estimating volume parameters at all, let alone with good precision, is an unreasonable expectation. Previous research has shown that the bias in estimation may be higher with single trough sampling and that both between-subject as well as residual variability parameters together may not be well estimated [13]. However, there still are cases where

pediatric PK studies collect only single trough samples. At times practical restrictions, for instance anemia or other health conditions in pediatric patients would only permit single trough data to be collected. Further, large registration trials frequently collect only single trough PK samples. Hence we incorporated this scenario in our simulations. Only clearance parameters may be estimated within the precision standard with reasonable sample sizes, given the assumption that prior information on the structural model is available.

What is the impact of low, medium or high PK between-subject variability?

PK variability is the predominant determinant of sample size as would be expected in any power analysis. This parameter may differ considerably across drugs. We explored three levels of between-subject-variability in order to generalize the methods to a wide range of drugs. With sophisticated analytical methods and assays available today, we do not anticipate the residual variability to be significantly high. Also structural PK models are seldom severely mis-specified. Hence we did not explore the impact of varying this parameter.

What is the impact of including adult data for estimation?

While designing a pediatric study, it is important to leverage prior information that may be available in the form of either adult data for the same drug in question, or as relevant adult or pediatric data from related drugs or indications. Most pediatric drug development programs occur after the drug is approved in adults. Hence during pediatric trial design, it is likely that a population PK model of the drug based on adult data would be available.

For the analysis, sparse pediatric PK data may then be combined with the rich adult data in order to estimate the relevant PK parameters in pediatrics. Hence inclusion of rich adult data is also an important consideration while designing pediatric studies.

Rich adult data would additionally inform the structural model, leading to improved estimation precision. Accordingly, we did observe an increase in precision and lowering of pediatric sample size requirements, upon inclusion of adult data in the simulations. However, the concern with including rich adult data in design or analysis of pediatric trials would be the undue influence of the adult data on estimation, leading to perhaps falsely high precision, simply by virtue of the large possible adult sample size. This concern was addressed in our simulations. Importantly, we used a $t_{df,\alpha}$ statistic corresponding to only total pediatric sample size while constructing the 95% CI on parameter estimates, avoiding an undue impact of adult sample size on precision. We found a ceiling effect in terms of amount of adult data included. In most cases, rich data from 24 adults did not offer a pediatric sample size advantage over using rich data from 12 adults. In the few cases this did happen, the gradient was reduced, and with more adult data (48 adults) there was no added benefit (results not shown).

Both observations made with regard to adult data inclusion are useful. The first one re-iterates the importance of using prior information where available, while the latter alleviates the concern of adult data driving the parameter precision and leading to unrealistically low pediatric sample size requirements.

What is the impact of the analysis method used?

There could be different ways to assess whether the precision standard is met. For instance, in the case of clearance, one option is to estimate the population mean clearance for each pediatric age group to be studied while the other option could be to use the geometric mean of individual post-hoc clearance estimates, for each pediatric age group. Hence both analysis methods were explored. Given rich PK sampling, conventional NCA, which is a two-stage analysis method, is comparable with the post-hoc analysis approach used in our study. However, the body weight and age effect on post-hoc individual estimates were normalized while determining precision so as to make the precision assessment comparable with the population mean method. NCA and post-hoc methods yield similar sample size outcomes at equivalent variability levels. The population mean analysis was more precise and less biased than post-hoc analysis, which is in fact a merit of mixed-effects modeling. Thus, the population-mean or one-stage approach is the most powerful analysis method for pediatric data.

What is the impact of i.v. vs. oral administration models?

While an i.v. administration model is the simplest simulation template in terms of parameters, most pediatric drugs are oral formulations. Hence both scenarios were evaluated. However, since PK samples during the absorption phase would rarely be available in pediatrics we chose to fix the absorption parameters (KA and BSV_KA) based on adult values. Thus in terms of parameters estimated, the i.v. and oral scenarios were the same. The only difference was additional variability (50%) contributed to the

model by the KA parameter in the oral scenarios. As expected then, the outcomes and trends were similar for both i.v. and oral cases.

Metrics used

The quality standard specifies the 95% confidence interval (CI) rather than standard error (SE) for the precision standard. Using CI takes the combined effects of both SE and $t_{df,\alpha}$ into consideration for calculating precision. In case of small sample sizes (<30), which are commonly used for pediatric studies, this is an important consideration for precision, rather than using SE alone. Under an asymptotic normal distribution assumption, the precision standard specified would be equivalent to achieving a relative standard error (RSE) on the mean parameter estimate within 20%. In our study, we empirically constructed the 95% CI using model-generated SE estimates and $t_{df,\alpha}$ values corresponding to the pediatric sample size used, avoiding undue influence of adult data on precision. A non-parametric bootstrap would be the alternative way to construct the required 95% CI, but this technique would be computationally very intensive and was not considered justified for the scope of this study.

The bank of virtual pediatric subjects generated may be considered the true population of interest for this study. Hence the non-parametric mean clearance and volume parameters, for each age group, derived from the bank were used as reference values or true mean estimates. These estimates matched well with the parametric mean estimates derived using the covariate models for clearance and volume with median demographic values, for each age group. Hence, we elected to use the same approach to determine the

population mean parameter estimates, for each age group, under the population mean analysis scenarios.

Scenarios

The aims of the study were to address the simulation methodology to design a pediatric trial, along with the impact of different trial design elements, while targeting the precision quality standard. Hence a simple one-compartment PK model was used in the simulations. However, the methods used can also be applied for scenarios that differ based on the underlying PK model, the covariate model used, the dose administered or the number of and division into age bins, even though such scenarios were not explored in this study. Scenarios were chosen to assess the impact of what we believe are the key pediatric trial design elements.

Conclusions

The following are the salient findings of this research:

1. Plan well at the design stage to ensure an informative pediatric trial.
2. The PK quality standard of 60-140% precision with 80% power is practically feasible. Reasonable sample sizes are adequate to comply with the standard and it may be implemented using a simulation-based approach.
3. Pre-determined optimal sampling times for a given number of PK samples per subject is important during trial design.

4. Use prior adult data or pediatric data for trial design and analysis, wherever possible. Inclusion of adult data will not unduly drive precision and sample size to achieve the quality standard.
5. Use one-stage population mean analysis methods, with biologically plausible covariate models, for pediatric PK studies.
6. Allometric and Emax-type age-effect covariate models are feasible to use in simulations, while designing pediatric trials to achieve the PK quality standard.

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CHAPTER 4

Covariate models – do not center at values outside the data range

ABSTRACT

The purpose of this paper is to draw attention to a seemingly obvious concept during centering of covariate effects in a population analysis. A simulation-estimation platform with pediatric data was used to assess the impact of the choice of reference body weight value at which the body weight effect on clearance and volume parameters is centered. It was found that the reference value chosen for centering had implications for not only parameter interpretation but also their precision. Absence of centering or in effect using 1 kg as a reference value led to 20-30% lower mean precision than centering at the median of body weight range. In addition, centering at the upper end of the body weight range led to 5-10% lower mean precision than centering at the median. The results can be generally applied to all covariates underlining the recommendation that covariate effects should be centered at an appropriate value of the covariate. Usually, using the median of the covariate data range as the reference value will lead to most relevant interpretation of model parameters and highest possible precision.

INTRODUCTION

While developing covariate models in population analyses, it is good practice to center the effect of a covariate on a model parameter at a particular reference value of the covariate in order to make the parameter interpretable. It is perhaps a known issue, at least among modelers, that when centering is done at a value outside the present data range there could be instability in estimation and poor parameter precision. However, a systematic study that accounts this phenomenon has not been published previously and we believe it is of value to modelers in general. We used covariate models with body weight effect centered at different reference values to demonstrate two key implications of centering on: 1- parameter precision and 2- parameter interpretation.

METHODS

As part of a larger project, we conducted extensive simulation-estimation of pediatric data. A pediatric population was simulated in terms of demographics and pharmacokinetic (PK) observations under scenarios with increasing sample sizes. The parameters of the PK model were then estimated using the simulated data and precision and bias metrics for the estimates of mean systemic clearance and central volume of distribution were determined. For each sample size scenario, 250 replicate simulation-estimations were carried out.

Nominal Design

A single 100 mg/kg dose of a hypothetical drug was administered intravenously (bolus) to the simulated pediatric subjects. The drug follows one-compartment, dose-proportional PK and has a half-life of about 1.5 hrs. A sparse sampling schedule design with 3 samples per subject (0.1, 2 and 4 hrs) was employed. The different sample size scenarios used were 16, 24, 32, 48 and 72 pediatric subjects.

Demographics

We used a CDC database for the simulation of pediatric demographics that included age, gender and a relationship to determine body weight. Using this information we generated a bank of n=48000 unique virtual pediatric subjects. The subjects were divided into four age bins (1 mo to 2 yrs, >2 to 6 yrs, >6 to 12 yrs and >12 to 17 yrs) as is commonly done during recruitment in most pediatric clinical trials. The weight range of simulated subjects was 5 kg - 80 kg (median = 20 kg). For each scenario, the desired total number of subjects was randomly sampled from this bank of pediatrics, with equal number of subjects into each age bin.

Simulation Models

The one-compartment model used in the present study was parameterized in terms of total systemic clearance (CL) and volume of distribution for central compartment (Vc). The between-subject variability (BSV) of the model parameters was described using a

lognormal variance model. The allometric exponential covariate model was used to account for effect of body weight on both clearance and volume.

$$CL_i = TVCL \bullet WT_i^{allo_{CL}} \bullet e^{\eta_{CL_i}} \quad \text{… Eq. 1}$$

$$Vc_i = TVVc \bullet WT_i^{allo_{Vc}} \bullet e^{\eta_{Vc_i}} \quad \text{… Eq. 2}$$

where; η_{CLi} is the difference between individual (CL_i) and typical value or population mean (TVCL) clearance on log scale, η_{Vci} is the difference between individual (Vc_i) and typical value or population mean (TVVc) volume of distribution on log scale, $allo_{CL}$ and $allo_{Vc}$ are the allometric exponents for the effect of individual body weight (WT_i) on clearance and volume respectively. η_{CLi} and η_{Vci} were both assumed to follow a normal distribution independent of each other, with mean of zero and variances of ω^2_{CL} and ω^2_{Vc} respectively. The residual error or within-subject variability (WSV) was described using a proportional error model as shown below:

$$Cp_i = Cp_{pred} \bullet \left(1 + \varepsilon_{Cp}\right) \quad \text{… Eq. 3}$$

where; Cp_i is the individual observed plasma concentration, Cp_{pred} is the individual model prediction and ε_{Cp} is the residual error assumed to follow a normal distribution with mean of zero and variance of σ^2_{Cp}.

The parameter values for the "true" one-compartment model and associated covariate effects used for simulations are listed in Table 1.

Table 1: The one-compartment model parameters used for simulations

Parameter	Value	Units
Mean Clearance	3.2	L/h/20kg
Mean Volume	6.2	L/20kg
BSV_Clearance	30	% CV
BSV_Volume	30	% CV
Residual error	10	% CV
$allo_{CL}$	0.75	
$allo_{V}$	1	

BSV: between-subject variability; CV: coefficient of variation

$allo_{CL}$, $allo_{V}$: allometric exponent for weight effect on clearance and volume respectively

Estimation models

For the current study, for every simulated replicate, three estimation cases were carried out, based on the centering of the body weight effect in the covariate model:

- Case 1: Covariate effect not centered (effective reference value = 1 kg)
- Case 2: Covariate effect centered at upper end of data range (reference value = 70 kg)
- Case 3: Covariate effect centered at median of data range (reference value = 20 kg)

Accordingly, one of the following three covariate models was employed:

$$TVCL = THETA_{CL} \bullet \left(WT_i\right)^{allo_{CL}} \quad \text{… Eq. 4}$$

$$TVCL = THETA_{CL} \bullet \left(\frac{WT_i}{70}\right)^{allo_{CL}} \quad \text{… Eq. 5}$$

$$TVCL = THETA_{CL} \bullet \left(\frac{WT_i}{20}\right)^{allo_{CL}} \quad \text{… Eq. 6}$$

Correspondingly, covariate models were also applied for TVVc, using the $allo_{Vc}$ parameter. All model parameters were estimated.

Metrics

We assessed the resulting precision and bias of mean parameter estimates (clearance and volume) for each replicate. The mean precision, power to achieve a target precision and mean bias, based on all replicates, were then computed for each sample size scenario.

In accordance with a recent regulatory requirement of a precision standard on primary PK parameter estimates [1], we assessed precision as a %CV-like metric but in terms of upper bound of the 95% confidence interval (UCI) rather than standard error (SE). Using CI takes the combined effects of both SE and $t_{df,\alpha}$ into consideration which is important in case of small sample sizes (<30) that are commonly used for pediatric studies rather than considering SE alone. Thus, precision was defined as follows:

$$precision = \left(\frac{UCI}{Mean} \right) \bullet 100 \qquad \text{…. Eq. 7}$$

Values close to 100 for this ratio represent high precision (or small standard errors) while higher values, deviant from 100, represent more imprecision (or large standard errors). We determined precision on clearance and volume parameters for every replicate and the mean of this metric for all replicates was the "Mean Imprecision" for a particular sample size scenario.

In order to assess power, we used a pre-determined target on parameter precision, again based on the regulatory requirement [1], defined as precision ≤ 140%. Under an asymptotic normal distribution assumption, the precision target specified would be equivalent to achieving a relative standard error (RSE) on the mean parameter estimate within 20%. The proportion of replicates where the mean precision met the target was determined to be the power to achieve target precision for that sample size scenario. In order to construct the 95%CI, we used model estimated mean parameters and standard errors and a $t_{df,\alpha}$ statistic corresponding to the total pediatric sample size used at a 2-sided $\alpha = 0.05$.

For the purpose of computing bias on the parameter estimates, the model parameters used for simulation (Table 1) were considered to be the "true" estimates. The percent deviation from the true value was the metric used, calculated as follows:

$$bias = \left(\frac{P_{est} - P_{true}}{P_{true}} \right) \bullet 100 \qquad \text{…. Eq. 8}$$

where; P_{est} is the estimated value of the parameter by fitting the model to the simulated data and P_{true} is the true value for the model parameter.

Software

We employed an R environment for the simulation-estimation platform with system calls to NONMEM. For the simulations, random numbers were generated using a six digit seed. The estimation method used was FOCEI. NONMEM version VI with Compaq Visual FORTRAN 6 compiler was used to conduct the simulations and estimations. R

version 2.9.1. was used to create the automated program script and carry out data manipulation and analysis.

RESULTS

Precision and bias of mean parameter estimates

We found a dramatic improvement in the precision of the estimates of primary model parameters (mean clearance and volume) when the body weight effect in the covariate model was centered at the median (Case 3, reference value = 20 kg) as compared to at an extreme value outside the data range (Case 1, reference value = 1 kg). Absence of centering, or in effect using a 1 kg reference value, led to 20-30% lower mean precision and up to 85% lower power, than centering at the median of body weight range. The outcomes are less dramatic for centering at the upper end of the body weight range (Case 2, reference value = 70 kg), which led to 5-10% lower mean precision and up to 6% lower power than centering at the median (refer Table 2).

As we can see in Table 2, when body weight effect is centered at the median of the simulated data the precision on clearance and volume is high (100% power) at all sample sizes. However, when 1 kg is used for centering the mean precision is poor (<50%) at lower sample sizes and reasonable precision is obtained only at much higher sample sizes (n>50) than would be expected.

The bias in parameter estimates was unaltered by the choice of reference value used for centering body weight effect on clearance and volume.

Table 2: Mean and power for precision on clearance and volume estimates in absence and presence of appropriate centering.

Sample Size	Reference Value = 1 kg		Reference Value = 20 kg		Reference Value = 70 kg	
	Mean Imprecision (%)	*Power (%)*	*Mean Imprecision (%)*	*Power (%)*	*Mean Imprecision (%)*	*Power (%)*
Clearance						
16	156	15	114	100	125	94
24	149	22	112	100	122	98
32	142	46	110	100	120	100
48	136	78	108	100	116	100
72	131	98	107	100	114	100
Volume						
16	157	15	116	100	126	94
24	150	15	115	100	122	96
32	143	42	111	100	120	100
48	137	74	109	100	117	100
72	132	98	107	100	114	100

Age range: 1 month – 16 years; Body weight range: 5 – 80 kg

Mean imprecision = (95% UCI/Mean parameter)•100;

Power=% replicates where mean imprecision ≤ 140

DISCUSSION

Impact of centering on parameter precision

In most population analyses, the precision of model parameters, usually in the form of standard error (SE), is used as a diagnostic tool for the model. A lower SE indicates higher precision on parameter estimates, which is desirable and reflects well on the model itself.

However, caution must be exerted while making this interpretation. The precision on a parameter estimate is sensitive to both, sample size and the available data range, and in turn to the reference value that a covariate effect is centered at. It is expected that any software will run into difficulties while estimating a parameter in a data range where little or no information is available. Hence, for instance, if a reference value of 1kg is used to model the body weight effect on primary parameters then the parameter estimate may be very imprecise because it will require the model to extrapolate to an extreme covariate range relative to the data present. However, if the sample size is sufficiently large, the imprecision may not be significant. Similarly, if centering at 70 kg (commonly used in adult population analyses) is applied while modeling pediatric data [2-4], then the mean parameter estimates could again have misleadingly lower precision because the observed data would be concentrated at a lower weight range than the reference value.

Accordingly, as we found in Case 1, the precision on population mean clearance and volume estimates was unexpectedly poor (refer Table 2) for a population analysis given low between-subject-variability (30%) and residual variability (10%). We can attribute

the poor precision to inappropriate choice of centering at 1 kg, which is an extreme low value outside of the available of body weight range. In Cases 2 and 3 the parameter precision was as expected for a population analysis. However, Case 3 (centering at the median of the body weight range) had higher mean precision relative to Case 2 (centering at the upper end of the weight range).

Thus, if a poor choice of reference value is made for centering a covariate effect, it may adversely affect the parameter precision. In turn, dosing decisions can potentially be affected - either if the parameter precision is subsequently used in simulations to derive dosing regimens, or by erroneously rejecting reasonable model parameter estimates based on precision. Some researchers use both mean parameter point estimate as well as precision to conduct simulations to derive dosing recommendations, and caution must be exerted while selecting reference values for centering covariate effects in such cases. However, the choice of centering is irrelevant when the allometric exponent is fixed to a constant during modeling or when sampling covariance between parameter estimates is ignored during simulation.

We also found in our study that the choice of centering does not affect the overall model significance (OFV) nor the precision or significance of other covariate effects. We simulated a categorical covariate (gender) effect on clearance using a proportional model, along with body weight effect, and then estimated the gender effect using the three different reference values for body weight effect. Reference value had no impact on the precision or statistical significance of the gender effect. This result is expected since in

this case the gender effect parameter is estimated for all levels of body weight. However, in a case where a different mean clearance parameter is estimated for each gender and the determination of a significant gender effect depends on the difference between mean clearance estimates, and their respective precision (95% CI), an erroneous decision about gender effect may be made if inappropriate centering of body weight effect is used.

Interpretation of parameter estimates

The primary reason for centering covariate effects used in a population PK model is to make the model parameters interpretable. In our study, in Case 1, absence of appropriate centering limited the interpretability of resulting mean parameter estimates because according to the models used (Eq. 4) the parameters represented the mean clearance and volume for a non-existing individual weighing 1 kg. On the other hand in Cases 2 and 3, when centering of the body weight effect was done at realistic body weight values (Eq. 5 and 6), the resulting model parameters were interpretable.

Further, if a proportional model is used to incorporate a covariate effect, for instance age [5], according to the model depicted below (Eq. 9) then A_{eff} is a parameter that represents the effect of age on clearance and TVCL would be interpreted as the typical value of clearance for an individual with age = 0 years. Again, this is an unrealistic population mean clearance estimate that is not interpretable. The interpretation of model parameters becomes even more complicated when multiple covariates are involved for a single model parameter.

$$TVCL = THETA_{CL} \bullet (1 + A_{eff} \bullet Age_i) \quad \text{… Eq. 9}$$

Hence it is good practice to center the individual covariate effects at a reference covariate value that would make the model parameter interpretable and plausible. The proportional model for age effect (Eq. 9) used above can be modified as follows to make TVCL meaningful:

$$TVCL = THETA_{CL} \bullet (1 + A_{eff} \bullet (Age_i - 50)) \quad \text{… Eq. 10}$$

TVCL now represents the typical value of clearance for an individual aged 50 years, which is a useful interpretation of the parameter since 50 years is a plausible age for an adult.

For most relevant interpretability of model parameter estimates, the choice of the reference value to be used for centering covariate effects should depend not only on the range of covariates present in the data being analyzed but also the population of interest. For instance, in Case 2 in this study, 70 kg is a reasonable reference value for centering body weight effect in an adult population model but, in terms of parameter interpretation, not so suitable for pediatrics [2-4] or an adult obese population. Therefore, typically the median or mean of the covariate range in the data available serves as a good choice for the reference value [6-9]. In Case 3, the mean parameter estimates were for a 20 kg pediatric subject making their interpretation most relevant for the population of interest to this study. However, we do recognize that the advantage of using a uniform centering reference value for all populations is the convenience of comparing mean parameter estimates across all studies, pediatric and adult.

In some population PK analyses, model parameters may be expressed per kg (e.g. clearance is reported as L/h/kg), even when a centered model is used and the allometric exponent is not 1 [10;11]. Per kg parameter expression is also implied when centering of body weight effect is omitted [5;12]. Both these approaches not only limit the interpretability of the clearance parameter but can also lead to erroneously determining clearance values at higher body weights.

Other covariate effects

The choice of reference value for centering body weight effect in a population model is perhaps most critical since it is the most commonly used covariate. However, the same rationale applies to any continuous covariate used in the model. For categorical covariates too, a similar rationale holds true. For instance, in case of a categorical covariate with multiple levels, such as genotype, a mean clearance parameter for a particular genotype category may be estimated, along with different effect parameters for the remaining genotype categories. In such cases, the choice of genotype category for which the mean clearance parameter (or intercept) is estimated will dictate its precision. This is similar to choice of reference value for a continuous covariate. The genotype category with highest number of subjects in the available data will have a mean clearance estimate with highest precision. However, the precision of the different genotype effect parameters (or slope) remains independent of the choice of reference genotype category. The interpretation of a model parameter is also based on defining the reference covariate category (or typical population) that it represents. In case of genotype, it would perhaps

be most advisable to use the genotype with highest prevalence in the population of interest as the reference category.

Conclusion

Centering covariate effects used in population models is important. While doing so the choice of the reference covariate value at which its effect is centered is critical for both, model parameter interpretation and precision. However, interpretation and presentation may always be altered by re-parameterizing the model in terms of covariate values, as applicable for the circumstance at hand. The model applied for estimation may not always directly produce the parameters desired for interpretation. A final recommendation would be to use model parameterization that yields stable and precise estimation, and also parameters with relevant interpretation, for which the median or mean of continuous covariate data is an ideal choice.

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CHAPTER 5

A Genetics-based Pediatric Warfarin Dosing Regimen derived using Pharmacometric Bridging

ABSTRACT

The objective of this research was to derive a genetics-based pediatric dosing regimen for warfarin, including starting dose and titration scheme, using modeling and simulation.

Whilst several algorithms have been suggested for warfarin dosing in adults, pediatric specific dosing algortims are absent. Even so, warfarin continues to be extensively used as an anticoagulant in the pediatric population. A model-based approach was used to arrive at a proposed pediatric dosing regimen that was based on warfarin dosing in adults and pharmacokinetic/pharmacodynamic (PK/PD) principles. Pediatric data on warfarin dosing and INR came from a study conducted at the Children's Hospital of Los Angeles (CHLA). The dosing regimen targeted a major (≥ 60%) proportion of INRs within therapeutic range of 2.0-3.0, by week two into warfarin therpay. Simulataneously,

the target was to minimize bleeding and thromboembolic risk by minimizing the proportions of INR > 3.5 (to ≤ 10%) as well as those of INR < 2 (to ≤ 20%). The targets were set as used in adults as well as in pediatric studies.

A 2-step pediatric starting dose is proposed based on body weight (<20 kg and ≥20 kg) for each of 18 genotype categories, using differet possible combinations of individual CYP2C9 (*1/*2/*3) and VKORC1 (-1639 G>A) genotypes. The titration scheme involves percentage changes relative to previous dose, based on the latest patient INR. In simulations, the propsed dosing regimen performed better than the empricial dosing used in the CHLA patients, based on consistently maintaining target INR outcomes. To our knowledge, this is the first ever proposed dosing regimen for using warfarin in pediatric patients. However, the research is limited by the small sample size of available pediatric PK/PD data and absence of prospective validation of the dosing regimen. Hence prospective clinical studies with warfarin in pediatrics using the proposed dosing regimen are recommended.

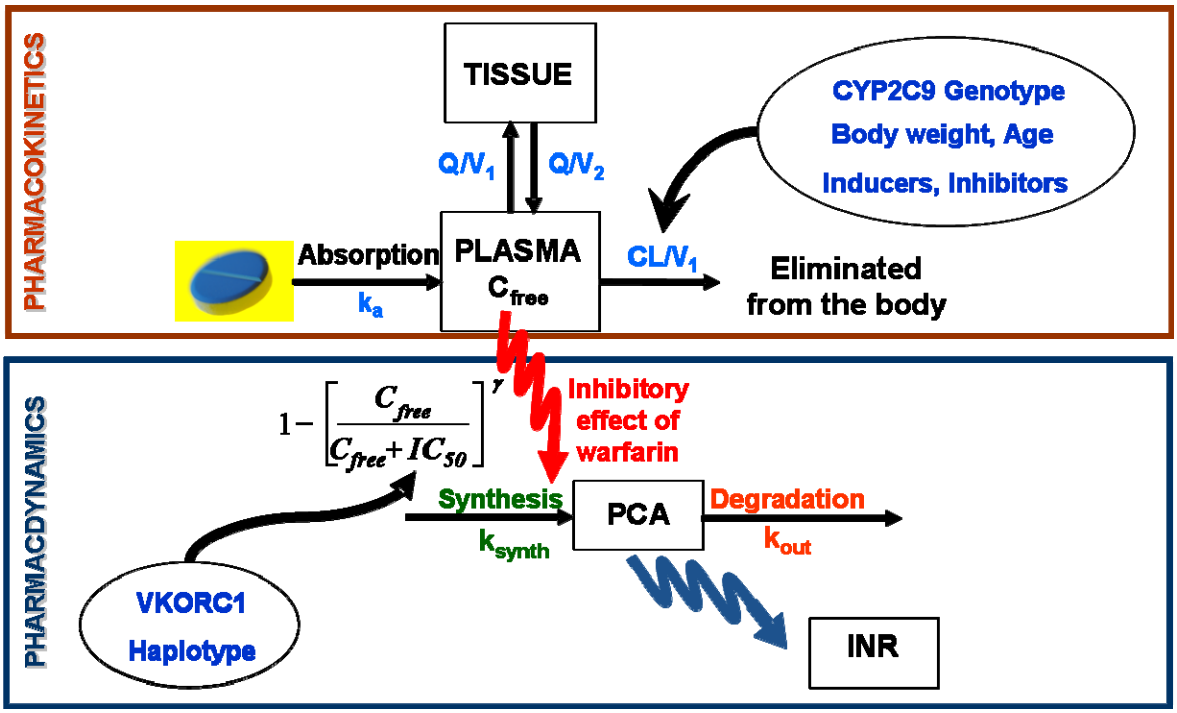
INTRODUCTION

Warfarin is the most widely used oral anticoagulant. It has been used for over 50 years in adults for the treatment and prevention of venous thromboembolism, pulmonary embolism, and thromboembolic events associated with atrial fibrillation, myocardial infarction, cardiac valve replacement and stroke [1;3;4]. The drug is currently not approved for pediatric indications [1]. Management of warfarin therapy is complicated owing primarily to two reasons – its narrow therapeutic index and high inter- and intra- individual variability in drug response. The individualized treatment goal is to maintain patient INR (international normalized ratio) within a therapeutic range, usually 2.0-3.0. Therapy involves potentially fatal thromboembolic risk at lower INRs and hemorrhagic risk at higher INRs [2-5].

Various known warfarin pharmacokinetics (PK) and pharmacodynamics (PD) factors contribute to the observed INR variability. Figure 1 is a schematic of the PK-PD of S-warfarin, the potent enantiomer in the raceimic warfarin product administered. Most importantly, polymorphisms in two genes – cytochrome p450 2c9 (CYP2C9) and vitamin K epoxide reductase c1 (VKORC1), which are involved in the PK and PD of warfarin respectively, have been shown to result in increased INRs and reduced warfarin dose requirements [3-10]. The variant alleles *2 and *3 in the CYP2C9 gene reportedly reduce warfarin clearance to about 30% and 15% respectively [4;8]. Patients with these variant alleles also require a longer time to achieve stable dosing, and are at a significantly

increased bleeding risk when compared with patients with the CYP2C9*1*1 (homozygous wild-type) genotype [6;11-13]. Multiple polymorphisms (such as -1639 G>A and 1173 C>T) in the VKORC1 gene, which occur in linkage disequilibrium, have been shown to increase warfarin sensitivity by about 30-50% [13;14], reducing warfarin dose requirements. Collectively, the CYP2C9 and VKORC1 genotypes have been shown to account for approximately 45% of the variability in warfarin dose requirements [7;9;10;15;16]. At a clinical pharmacology advisory committee meeting of the U.S. Food and Drug Administration (FDA) in 2005, there was consensus on existence of sufficient mechanistic and clinical evidence to support lower doses of warfarin for patients with certain polymorphisms in CYP2C9 and VKORC1 genes [17]. Subsequently, the warfarin label was updated in 2007 to include recommendations to genotype subjects for CYP2C9 and VKORC1 before initiating warfarin therapy and use lower doses accordingly [1]. Notably, the prevalence of the CYP2C9 polymorphisms in Caucasians is about 35% [18] and that of VKORC1 polymorphisms is about 40% in Caucasians and very high, i.e. about 85%, in Asians [5]. Therefore, testing for these polymorphisms is being performed to guide dosing in adults, which may improve the clinical safety and efficacy of warfarin [11]. Additional influential factors for warfarin dosing include but are not limited to body weight, age, co-morbidities, drug-drug interactions (DDI) and diet. Extensive research has been undertaken in order to account for the impact of all these factors on warfarin therapy and several algorithms for dosing warfarin in adults have been proposed [7-10;12;15;16;19].

Figure 1: PK-PD of S-warfarin



PK: two-compartment model dose-proportional PK, metabolic clearance via CYP2C9 only

PD: inhibition of synthesis of components of pro-coagulant complex activity (PCA) characterized using inhibitory Emax model; response measured as INR, a derivative of prothrombin time

CL- clearance; V- volume of distribution; ka- first-order absorption rate constant; Cfree- free/unbound plasma drug concentration; EC50- drug concentration to elicit half maximal inhibitory response; ksyth- zero-order rate constant for PCA production; kout- first-order rate constant for PCA degradation [42].

As far as pediatrics, even though not approved, warfarin remains the mainstay of oral anticoagulant therapy for patients with cardiovascular indications for prevention of thromboembolism [20-23]. Two distinct pediatric patient populations receive warfarin frequently; one are infants and young children with congenital heart defects who have undergone Fontan or other surgery, and second are the adolescent patients who have valve

replacement. Some other common pediatric indications requiring warfarin therapy include presence of central venous lines, congenital antithrombin deficiency, and cerebral thromboembolism. As in adults, the management of warfarin therapy is difficult in pediatrics and adverse effects are common [20;24;25]. The bleeding rate in pediatric patients on warfarin has been reported to be about 0.5% per patient year for major bleeding events and to range from 1.9-2.3% per patient year for minor bleeding events [25;26]. While major bleeding may occur even at warfarin doses considered therapeutic, serious hemorrhage risk has been shown to increase with increasing intensity of anticoagulation [27]. The occurrence of recurrent thromboembolic events in pediatric patients while still on warfarin therapy has been reported to range from 1.3-2.3% per patient year [25;26].

Limited clinical studies of warfarin in the pediatric population have been conducted [26;28;29]. Body size and age have been suggested to have an influence on warfarin dose. Some researchers also propose a maturation effect on the fundamental activity of the human coagulation system [30;31]. In addition, the polymorphisms in CYP2C9 and VKORC1 genes have been shown to be associated with lower pediatric warfarin dose requirements [32-34]. It is intuitive that these genetic effects seen in adults would be similar in pediatrics given that the mechanism of action of warfarin, and the coagulation and drug elimination pathways, are the same in pediatrics and adults.

However, all clinically available computer-based pharmacogenetic dosing algorithms for warfarin ignore considerations for pediatrics. In order to provide pediatric patients on

warfarin therapy with the pharmacogenetic advances that are now being integrated into adult care, it is important to develop and validate a pediatric warfarin dosing algorithm. Such an algorithm should integrate the impact of CYP2C9 and VKORC1 genotype with other factors (such as body size, ontogeny of PK-PD determinants, concomitant drugs) on warfarin disposition and effect in pediatrics. A recent study reported enthusiasm among pediatric hematologists for trials to develop a pediatric warfarin dosing algorithm, incorporating pharmacogenetic effects [35]. However, patient recruitment problems have been a severe limitation to such trials [36]. The need for efficient, novel approaches to enable addressing pediatric warfarin dosing has been highlighted [35].

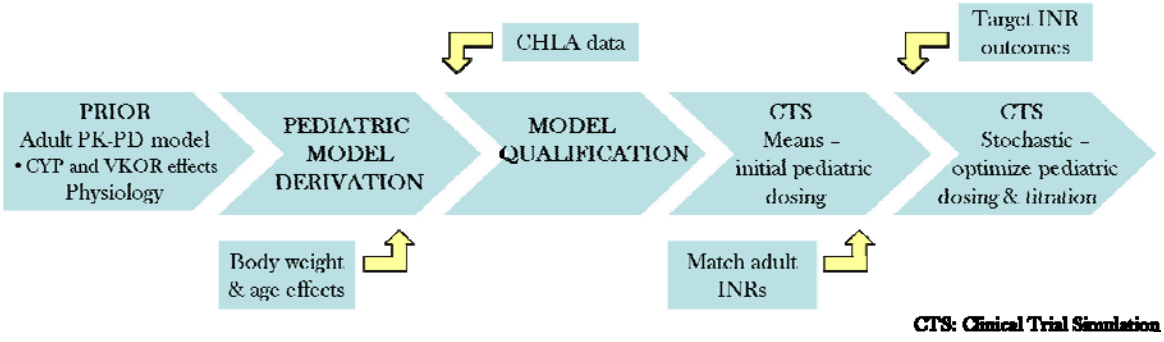
The objective of the current study is to develop a genetics-based pediatric warfarin dosing regimen, including both starting doses and a titration scheme, which can be validated prospectively for pediatric patients. A pharmacometric bridging approach, using modeling and simulation along with limited available pediatric data have been used to assess the potential usefulness of the dosing regimen. The eventual goal is to establish a new standard of care for pediatric patients who require warfarin therapy via an optimal, validated dosing regimen that will guide clinicians for safer and more effective warfarin use in pediatrics.

METHODS

An attempt has been made to leverage all the information previosuly available for the data anlysis in this study, using efficient modeling and simulation methods. Prior information

was available in the form of an accepted adult warfarin PKPD model [14] and warfarin dosing and INR information was available from a limited number of pediatric subjects from Children's Hospital, Los Angeles (CHLA). Our research approach is outlined in Figure 2. Briefly, we derived a pediatric PKPD model using the prior adult PKPD model and knowledge of physiology. By physiology we refer to PKPD principles such as the relation between drug clearance and body size, the maturation pattern of drug metabolizing enzymes and the mechanism of action of warfarin. We then qualified the pediatric model using the CHLA data, which were not used for model derivation. Initial pediatric warfarin doses were estimated by matching target INRs for typical pediatric subjects with adults. The pediatric dosing regimen - starting dose and titration scheme - were then optimized using simulations of several thousand pediatric subjects.

Figure 2: Outline of research approach employed



CHLA data

Patients

Data for the current study came from pediatric patients ≤ 18 years of age who were followed previously (within the last 1 year) and currently (during the study period) in the warfarin clinic of the Division of Cardiology, CHLA. Patients who had received warfarin for less than 7 days were excluded. This was an observational study with patients receiving standard of care warfarin therapy. The clinicians dosed and monitored patients as per their clinical expertise, with each patient treated on a case to case basis, depending on their condition and target INR. Warfarin dosing and INR logs (INR measurements across time) were recorded during regular scheduled visits to monitor warfarin therapy. Informed consent for study participation was obtained at one such routine visit along with a sample of 1.0 ml blood in addition to the routine blood draw. The blood sample was transported to the USC (University of Southern California) pharmacogenetics laboratory where genetic testing was done. A vitamin K dietary intake estimate was performed from a 1-3 day food diary. In addition to genotype and diet, data items collected were age, weight, height, gender, warfarin dose, INR, other medical illness or medications and adverse events. The information was obtained from the existing nurse coordinator's database and documented under the study ID number.

Genetic analysis

Genomic DNA samples were extracted from blood samples using a genomic DNA extraction kit (QIAmp DNA Blood Mini kit, Qiagen, Mississauga, Ontario, Canada). The genotypes for the CYP2C9 *2 (rs1799853) and *3 (rs1057910) and the VKORC1 -1639 G>A (rs9923231) SNPs were determined using real-time quantitative polymerase chain reaction assay based on the 5' nuclease allelic discrimination assay (ABI PRISM 7900 Sequence Detection System, Applied Biosystems, Foster City, California). Genomic DNA (10 ng) was mixed with 2.5 μL of gene specific primers and probes (10X concentrated) and 12.5 μL of polymerase chain reaction universal master mix (Applied Biosystems) to a final volume of 25 μL. Thermal cycler parameters included 10 minutes at 95^{o}C and 50 cycles involving denaturation at 95^{o}C for 15 seconds and annealing/extension at 60^{o}C for 1 minutes. For quality control of genotyping, negative and positive controls were used whenever genotyping was performed. Distributions of the CYP2C9 and the VKORC1 genotypes were compared to the Hardy-Weinberg theoretical distribution using the chi square test. A p-value less than 0.05 was considered statistically significant.

Pediatric warfarin model: derivation & qualification

Prior adult model

Previosuly, a group at the FDA in collaboration with Harvard Partners, Boston MA has developed a warfarin population PKPD model and dosing scheme in adults [14]. The adult model was based on published research on the concentration-effect relationship for

warfarin [8;42] as well as data from an adult warfarin trial, CROWN (creating an optimal warfarin dosing nomogram). The model was built using data from an initial 271 subjects and subsequently validated in the same trial by using model-derived dosing in 117 subjects (*unpublished results*). In the current study, a pediatric warfarin population PKPD model was derived using the adult model and mechanistic reasoning.

Pediatric PK model

The adult PK parameters were first scaled to account for body size effects in pediatrics. A covariate effect for body weight was included in the pediatric model, using previously published and widely accepted allometric scaling principles [37;38]. A body weight effect with allometric exponent of 0.75 was included on systemic and inter-compartmental free clearances while that with an exponent of 1 was included for the central and peripheral free volumes of distribution. Next, the impact of maturation on free warfarin clearance was accounted for, using age as a covariate. The age effect on free clearance in the model is based on a relationship previously developed for the maturation of CYP2C9 using warfarin pediatric data [39]. According to the model used, CYP2C9 enzyme activity increases with age and attains maturity by the age of 2-3 months. Finally, effects of the CYP2C9 genotype (variants *2 and *3) on clearance were included in the model as estimated for adults [8]. These values came from a clinical PKPD study in 150 adult patients on warfarin that included subjects of all relevant polymorphic CYP2C9 genotypes.

Between-subject variability in our pediatric PK model was described for three model parameters - clearance, central volume, and peripheral volume, using a lognormal distribution. The total plasma drug concentrations were corrected for plasma protein binding, which is reported to be 99% [1], in order to achieve free plasma drug levels. The following equations describe the PK model we used:

$$CL_i = TVCL \bullet CYP_{eff} \bullet \left(\frac{WT_i}{20}\right)^{0.75} \bullet \left(\frac{0.821 \bullet Age_i}{0.01 + Age_i} + 0.21\right) \bullet e^{\eta_{CL_i}} \quad \text{… Eqn. 1}$$

$$Vc_i = TVVc \bullet \left(\frac{WT_i}{20}\right)^{1} \bullet e^{\eta_{Vc_i}} \quad \text{… Eqn. 2}$$

$$Q_i = TVQ \bullet \left(\frac{WT_i}{20}\right)^{0.75} \quad \text{... Eqn. 3}$$

$$Vp_i = TVVp \bullet \left(\frac{WT_i}{20}\right)^{1} \bullet e^{\eta_{Vp_i}} \quad \text{... Eqn. 4}$$

$$Cp = \frac{Cp_{free}}{f_u} \quad \text{... Eqn. 5}$$

where; η_{CLi} is the difference between individual (CL_i) and population mean (TVCL) clearance on log scale, η_{Vci} is the difference between individual (Vc_i) and population mean (TVVc) central volume of distribution on log scale, η_{Vpi} is the difference between individual (Vp_i) and population mean (TVVp) peripheral volume of distribution on log scale, Q_i and TVQ are the individual and population mean inter-compartmental clearance,

CYP_{eff} is the covariate parameter that accounts for the effect of CYP2C9 genotype on clearance, WT_i and Age_i are individual body weight and age and Cp, Cp_{free} and f_u are total, free and fraction unbound plasma drug concentrations of S-warfarin. η_{CLi}, η_{Vci} and η_{Vpi} were all assumed to follow a normal distribution, independent of each other, with mean of zero and variances of Ω^2_{CL}, Ω^2_{Vc} and Ω^2_{Vp} respectively.

The pediatric model assumes that immature CYP2C9 clearance is not saturable at therapeutic warfarin concentrations. Plasma protein binding was assumed to be unaffected by maturation. The model does not account for the impact of drug-drug interactions and dietary vitamin K on warfarin PK.

Pediatric PD model

There is no published study that establishes the concentration/dose-response relationship for warfarin in the pediatric population. This relationship is assumed to be similar in pediatrics and adults for pediatric PD model derivation. The assumption is based on two reasons. First, the mechanism of action of warfarin is the same for pediatrics and adults, namely inhibition of synthesis of vitamin K dependent clotting factors in the liver, and drug response in both populations is measured clinically as INR. Second, the concentration-response relationship has been shown to be similar between pediatrics and adults for other anticoagulants, namely argatroban [40] and heparin and low molecular weight heparin [41]. While these drugs have a different mechanism of action than warfarin

and their response is measured in terms of aPTT, the net effect is on clotting factor activity, much as for warfarin. Hence, in absence of pediatric specific warfarin PD data, the adult PD model [14] for warfarin was used for pediatrics as well.

Warfarin exerts anticoagulation by inhibiting synthesis of vitamin K dependent clotting facttos, which results in a decrease in total clotting factor complex activity (PCA). This effect is measured clinically as a subsequent increase in prothrombin time (PT). The INR is a standardized measurement of PT, accounting for variations in lab reagents. The PD model used describes an inhibitory effect of warfarin on INR degradation rather than on synthesis of PCA. Given the inverse relationship between PCA and INR, and the fact that the clinical response to warfarin therapy is measured as INR, such a representation of the effect of warfarin is both logical and intuitive.

The PD effect in patients is driven by free drug levels and hence protein-binding corrected plasma drug concentrations are used in the model. According to the model (Eqn. 6), INR change is dependent on previous INR, free warfarin plasma concentration (Cp_{free}) and drug potency (EC50), which may be defined as the drug concentration required for half maximal inhibitory effect. Patient sensitivity to warfarin, which is dependent on VKORC1 genotype, was captured as differing potency of the drug ($EC50_{VKOR}$) to elicit the same response in subjects with different genotypes.

$$\frac{dINR}{dt} = K_{in} - K_{out} \bullet INR \bullet \left(1 - \left(\frac{Cp_{free}}{Cp_{free} + EC50_{VKOR}}\right)\right) \quad \text{… Eqn. 6}$$

Between-subject variability in our pediatric PD model was described for three parameters - K_{in}, K_{out}, and $EC50_{VKOR}$, using a lognormal distribution. The measurement error on INR was accounted for using an additive error model. The following equations describe the PD stochastic models used:

$$Kin_i = TVKin \bullet e^{\eta_{Kin_i}} \quad \text{… Eqn. 7}$$

$$Kout_i = TVKout \bullet e^{\eta_{Kout_i}} \quad \text{… Eqn. 8}$$

$$EC50_{VKOR_i} = TVEC50_{VKOR} \bullet e^{\eta_{EC50VKOR_i}} \quad \text{… Eqn. 9}$$

$$INR_{obs} = INR_{pred} + \varepsilon_{INR} \quad \text{… Eqn. 10}$$

where; η_{Kini} is the difference between individual (Kin_i) and population mean (TVKin) synthesis rate constant for INR on log scale, η_{Kouti} is the difference between individual ($Kout_i$) and population mean (TVKout) degradation rate constant for INR on log scale, $\eta_{EC50VKORi}$ is the difference between individual ($EC50_{VKORi}$) and population mean ($TVEC50_{VKOR}$) VKORC1 genotype-dependent warfarin potency on log scale and ε_{INR} is the difference between observed (INR_{obs}) and model-predicted (INR_{pred}) INR. η_{Kini}, η_{Kouti}, $\eta_{EC50VKORi}$ and ε_{INR} were all assumed to follow a normal distribution, independent of each other, with mean of zero and variances of Ω^2_{Kout}, Ω^2_{Kin}, $\Omega^2_{EC50VKOR}$ and σ^2_{INR} respectively.

Pediatric model qualification

The prediction capability of the derived pediatric population PKPD model was qualified using CHLA pediatric data. Data were used from only those subjects whose CYP2C9 and VKORC1 genotypes and INR log information were available. We first predicted individual CHLA patient INRs using our model, based on only the demographic and warfarin dosing information from the CHLA data. 200 replicate simulations were performed for each CHLA subject's set of demographic and dosing data, to generate a distribution of predicted INRs across time. For the validation simulations, INR distribution was truncated within values 1.0-6.0. Thus, we generated 5^{th}, 50^{th} and 95^{th} INR prediction percentiles, for each CHLA subject over time. This step was blinded to the INR data from the CHLA study. Next, we overlaid the observed INR-time profiles for each subject from the CHLA trial onto the model INR prediction percentiles. If about 90% of a subject's INR observations fell within the 5^{th} and 95^{th} percentiles, the model predictions were considered reasonable. The proportion of subjects where model predictions matched reasonably well with INR observations were determined to qualify the model's prediction capability.

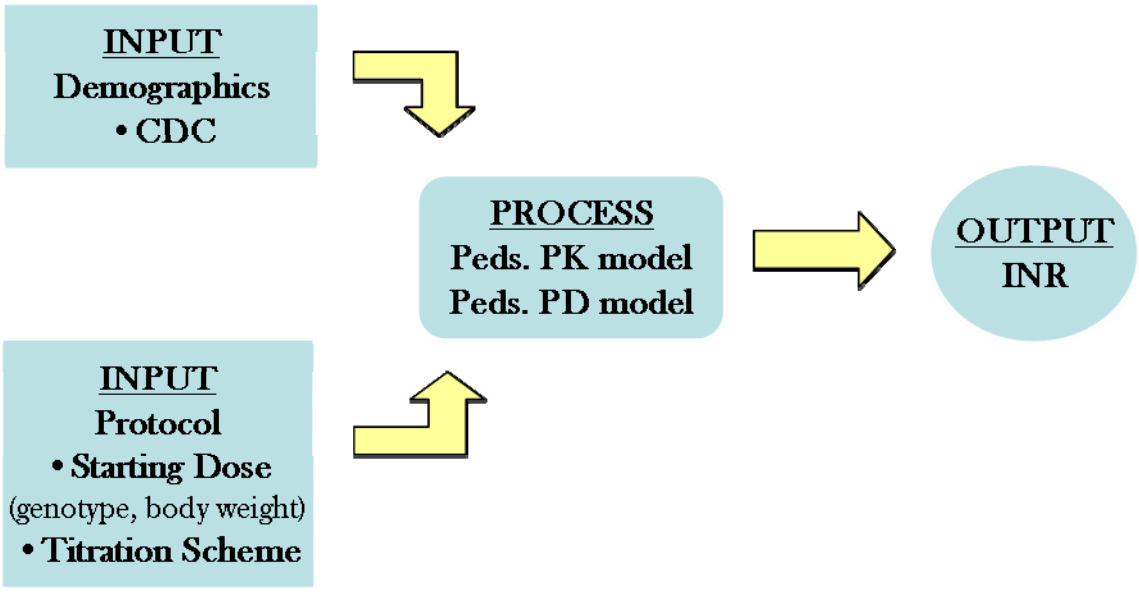
Optimal pediatric dosing: clinical trial simulations

The pediatric PKPD model was employed to investigate optimal warfarin dosing using clinical trial simulations. The aim was to optimize INR outcomes to clinically reasonable targets. Figure 3 is a schematic representation of the simulation process. For purposes of deriving starting dose, each genotype category was treated independently. Given all

possible combinations of genotypes for CYP2C9 (*1*1, *1*2, *1*3, *2*2, *2*3 or *3*3) and VKORC1 (GG, GA or AA), we had 18 unique genotype categories.

Broadly, a two-step approach was used for determining optimal dosing. In the first step we narrowed the starting dosing choices based on deterministic simulations in typical subjects within each genotype. The second step comprised of performing stochastic simulations to derive the best starting and titration dosing.

Figure 3: Clinical trial simulations schematic



Demographics

We used a CDC (center for disease control and prevention) database for the simulation of pediatric demographics that included age, gender and weight. The database contained ages from birth-20 yrs, in increments of 1 month, yielding 240 unique ages. For each unique combination of age and gender there is a parameter set, including a variability component, to determine the distribution of body weight. We simulated 100 individuals of different body weight for each combination of age (1 month - 17 years) and gender resulting in a virtual bank of about 48000 unique virtual pediatric subjects. For preliminary simulations we considered six typical pediatric subjects, covering the entire pediatric demographic range. The six typical subjects represent the mean body weight and age, obtained from the virtual bank, for five different body weight/age groups. The typical demographics were: 5kg/1month; 8kg/6mo; 11kg/1.5yrs; 16kg/4yrs; 28kg/9yrs; 54kg/15yrs. For final simulations, subjects were randomly sampled from the virtual bank of pediatrics. 1000 pediatric subjects for each genotype category were simulated.

Initial pediatric dosing: mean simulations

In the initial step, the target was to match warfarin INRs in typical pediatric subjects with adults. Body weight based dosing (mg/kg/day) was considered suitable for pediatrics. However, given the body-weight clearance relationship is not linear; the mg/kg dose is expected to be different across different body weight groups (lower for subjects with higher body weight). Thus one consideration was to arrive at a reasonable number of

dosing steps by body weight level. We also considered the need to adjust dosing steps based on age group. For the mean simulations all sources of variability in the pediatric model were disregarded. INR outcomes were simulated for the first 30 days of warfarin therapy.

Initial estimates of starting dose (mg/kg/day) for each of the six typical pediatric subjects were derived for each genotype category. Adult starting doses and our pediatric covariate model for clearance [8;14] were used for this purpose. Then using the initial pediatric dose estimates we simulated INR outcomes for the average subject, within each genotype category. The starting dose was adjusted to target, on an average, an INR of 2.5 and/or to match the INR-time profile for typical subjects with that of adults. Doses that rendered mean INR-time profiles closest to the target were considered for the full-fledged stochastic simulations.

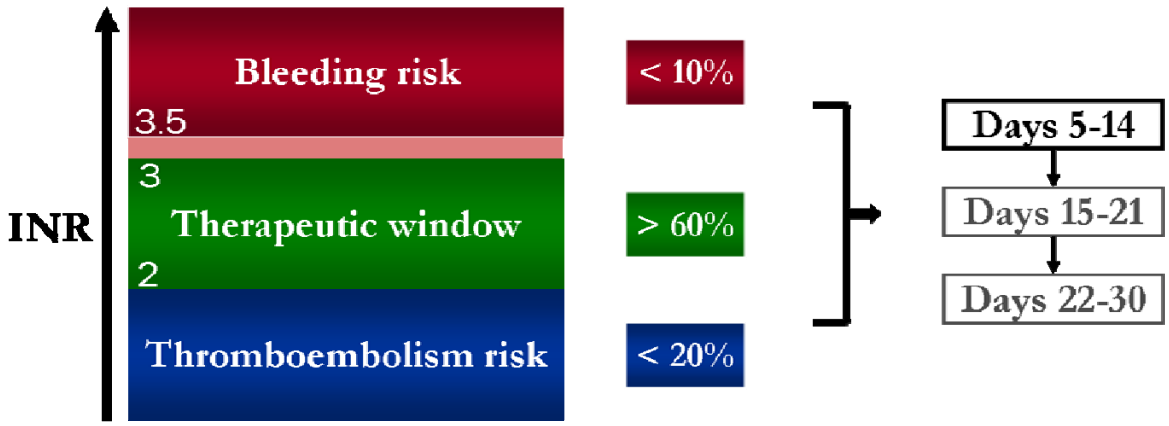
Final pediatric dosing: stochastic simulations

The next step was to perform stochastic simulations in order to optimize the pediatric dosing scheme for the entire population. For these simulations all sources of variability were included in the model, namely, demographic variability, between-subject PK and PD variability and INR measurement error. For each genotype category, the dosing aim was to target a high ($\geq$ 60%) proportion of INRs within therapeutic range of 2.0-3.0, by week two into warfarin therpay. Simulataneously, the target was to minimize bleeding and

thromboembolic risk by minimizing proportions of INR > 3.5 (to ≤ 10%) as well as proportions of INR < 2 (to ≤ 20%). While week 2 (14 days) into therapy was considred the primary time end-point, the INR outcomes through month 1 (30 days) were evaluated for all dosing regimens tested. The target clinical outcomes (depicted in Figure 4) are based on those used commonly in pediatric patients on warfarin [20;24;26;28;29] as well as those desired in adults, as per the expertise of the CROWN trial clinicians [14].

The starting dose as well as titration scheme were optimized as an iterative process, assessing the target INR outcomes for all genotype groups. INR monitoring (and dose titration) in our simulations was performed twice a week, as is done in regular clinical practice. Simulations were performed for first 30 days of warfarin therapy. For the final propsed dosing regimen INR outcomes through 90 days of warfarin therapy were also simulated.

Figure 4: INR target outcomes for pediatric dose optimization



Initially genotype-independent dosing was compared with genotype-based dosing. In the former case, all subjects were given the same body weight adjusted doses as determined for the CYP2C9*1*1 and VKORC1 GA genotype. In the latter case, dosing was as per our proposed regimen – subjects with different genotypes were given different body weight adjusted doses. Comparisons were made with regard to target INR outcomes for all genotype categories.

The proposed dosing regimen was then compared with the empirical dosing as used in subjects in the CHLA study. For the proposed dosing target INR outcomes were as previously defined (Figure 4). However, for the CHLA dosing since the target INR varied across patients, outcomes were in accordance with individual patient target INR range. To maintain consistency with INR outcomes used for the proposed dosing, sub-therapeutic INRs for CHLA dosing were those below the lower limit of the patient's target therapeutic INR range while supra-therapeutic INRs were considered at 0.5 value above the upper limit of the target range.

Finally, we also evaluated the impact of restricting doses proposed by our regimen to available strengths of warfarin formulations, on the target INR outcomes. The lowest dose that is currently feasible to administer to pediatric patients in warfarin clinics is 0.5 mg.

Software

Trial Simulator version 2.2.1 by Pharsight® was used for the mean and stochastic simulations, while determining pediatric doses. NONMEM version VI with Compaq Visual FORTRAN 6 compiler was used for simulations during model validation. For NONMEM simulations, random numbers were generated using a six digit seed. R version 2.9.1 was used for data processing, data analysis and graphics generation. The NONMEM model used is given in Appendix B. The drug model set-up in clinical trial simulator is given in Appendix C.

RESULTS

CHLA Data

A total of 36 pediatric subjects were included in the CHLA study. Of these, 10 subjects were missing genotype and/or INR log data. We were able to use data from 26 pediatric subjects for model qualification. Cohort demographics are provided in Table 1. The mean age of subjects was 4yrs 5mo (range 4 mo-18 yrs) and mean body weight was 23 kg (range 6.9-84 kg). The cohort included a wide range of body weight but only one subject with age < 6 months. While there were a fair number of subjects with the VKORC1 polymorphisms in the study cohort, the CYP2C9*2 polymorphism was rare and the *3 polymorphism was absent. There were three sub-groups in terms of target INR range, which was dependent on indication for warfarin therapy.

Table 1: Description of cohort of pediatric subjects from CHLA

Characteristic	Mean (range) or Number (%)
Age (years)	4.4 (0.33-18)
Body weight (kg)	23 (6.9-84.1)
Height (cm)	107 (65-189)
BSA	0.81 (0.36-2.1)
Warfarin maintenance dose (mg/kg/day)	0.12 (0.04 - 0.3)
Gender:	
Male	16 (61%)
Female	10 (39%)
Race:	
Hispanic	16 (61%)
Caucasian	7 (27%)
African American	2 (8%)
Mixed	1 (4%)
Target INR:	
1.5-2.5	13 (50%)
2.0-2.5	5 (19%)
2.5-3.5	8 (31%)
Indication:	
Valve replacement	8 (31%)
Fontan procedure	12 (46%)
Kawasaki	5 (19%)
Cardiomyopathy	1 (4%)
CYP2C9 genotype:	
*1*1	22 (85%)
*1*2	4 (15%)
*1*3 / *2*2 / *2*3 / *3*3	0
VKORC1 genotype:	
GG	7 (27%)
GA	8 (31%)
AA	11 (42%)

A wide range of doses (0.5-6.5 mg/day) were used in the pediatric subjects by the clinicians in the CHLA study. Starting doses in particular ranged from 0.5-5 mg/day. The dosing and titration choices did not follow a specified algorithm. For instance, two comparable patients weighing 16 kg, with target INR 1.5-2.5, were started on doses of 0.5 mg/day and 1.5 mg/day, respectively. Further, in case of two patients with target INR 2.5-3.5, during monitoring at an INR of 1.4 on day 5, one patient was given a 20% increase in dose while the other didn't receive any dose change.

The patient charts reveal that adherence to dosing assigned was poor in 4 subjects and prolonged times (> 60 days) were needed to arrive at stable dose in 12 (46%) subjects. The median time to achieve stable INR was 137 days. There were 4 major bleeding and 6 minor bleeding events during the study.

Pediatric warfarin model

The parameters of the pediatric model that we derived and used for clinical trial simulation are shown in Table 2.

Table 2: Parameters of the warfarin pediatric population PKPD model used to predict CHLA data and to optimize dosing.

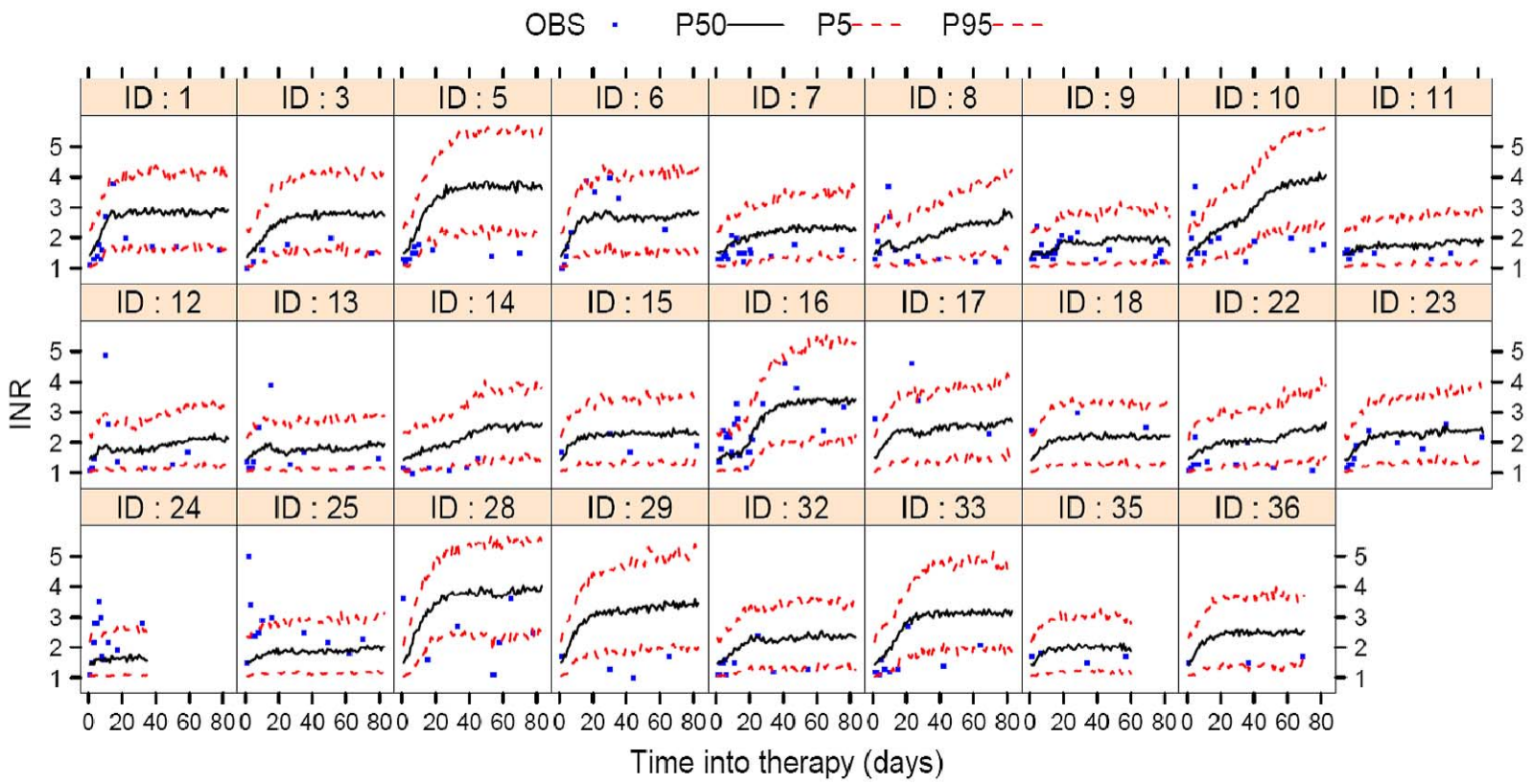
Parameter	*Value (*for a 20 kg subject)*	*Variability*
PK: two-compartment model		
TVCL	0.1207 L/h*	30 %CV
CYP_{eff} *1*1 *1*2 *1*3 *2*2 *2*3 *3*3	 100% 68% 55% 28% 31% 15%	
TVVc	3.45 L*	24 %CV
TVVp	1.65 L*	98 %CV
TVQ	0.05 L/h*	
Allometric exponent for weight effect on: CL and Q Vc and Vp	 0.75 1	
Ka	2 /h	
Bioavailability	50%	
Unbound fraction	1%	
PD: sigmoidal Emax indirect response model		
Kin	0.01953 /h	SD = 0.005
Kout	0.01698 /h	SD = 0.005
$EC50_{VKOR}$ GG GA AA	 3.953 µg/L 3.075 µg/L 2.547 µg/L	SD = 0.783
INR residual error		SD = 0.586

The model qualification outcomes for all 26 subjects are presented in Figure 5. The 5th, 50th and 95th perrcentiles of INR predictions by the model and observed INR values from the CHLA subjects are shown. In about 80% cases (20/26) the observations lay moreover within the 95% prediction intervals. No particular genotype, age or body weight group was associated with cases where the model did not predict the INR time profile well. However, in case of the only two African-American subjects present in the cohort, the model fails to capture the INR profile well. Based on these results, the model was considered reasonable for use in subsequent simulations to determine an optimal warfarin dosing regimen for pediatrics.

Figure 5: Model qualification outcomes: predicted and observed INR over time

P5, P50, P95 – 5th, 50th (median) and 95th percentile model predicted INR.

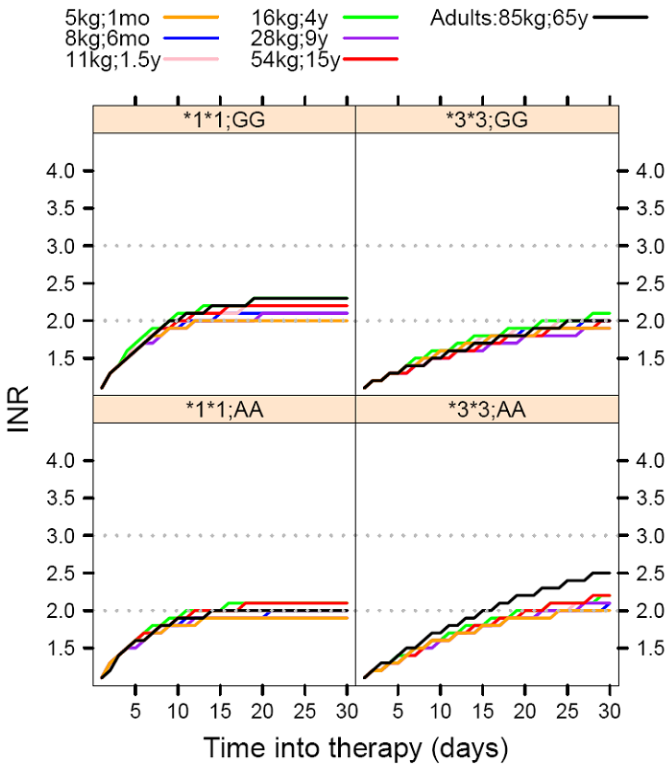
OBS – CHLA subjects observed INR



Initial pediatric dosing – typical subjects

The simulated INR-time profiles for the typical subjects for four representative genotype categories are presented in Figure 6. Based upon these profiles, as well as the clearance-body weight relationship for warafrin as per our model, we found the need to use two different mg/kg doses for higher (≥ 20 kg) and lower (< 20 kg) body weight subjects, within each genotype category. We did not find the need to alter mg/kg dose based on an age cut-off. The selected initial dosing scheme allowed for targeting an INR of 2.0-2.5, on an average, for all genotypes and moreover matched the average adult INR profiles.

Figure 6: INR vs. time profiles by genotype for mean simulations – initial dosing scheme for typical subjects.



Final pediatric dosing – population

For the stochastic simulations, the results were evaluted in terms of the target INR outcomes across time. We refined the starting dose from the initial doses derived for typical subjects, as suitable for each genotype category. We also made minor modifications to the titration scheme from that suggested for adults [14]. Thus, we derived an optimal pediatric warfarin dosing regimen, inclusive of starting dose and titartion scheme to maximize desired INR outcomes. Our final proposed dosing regimen is given in Table 3.

Table 3: Final proposed pediatric warfarin dosing regimen – starting dose and titration scheme

Starting Dose: mg/kg/day < 20 kg ≥ 20 kg

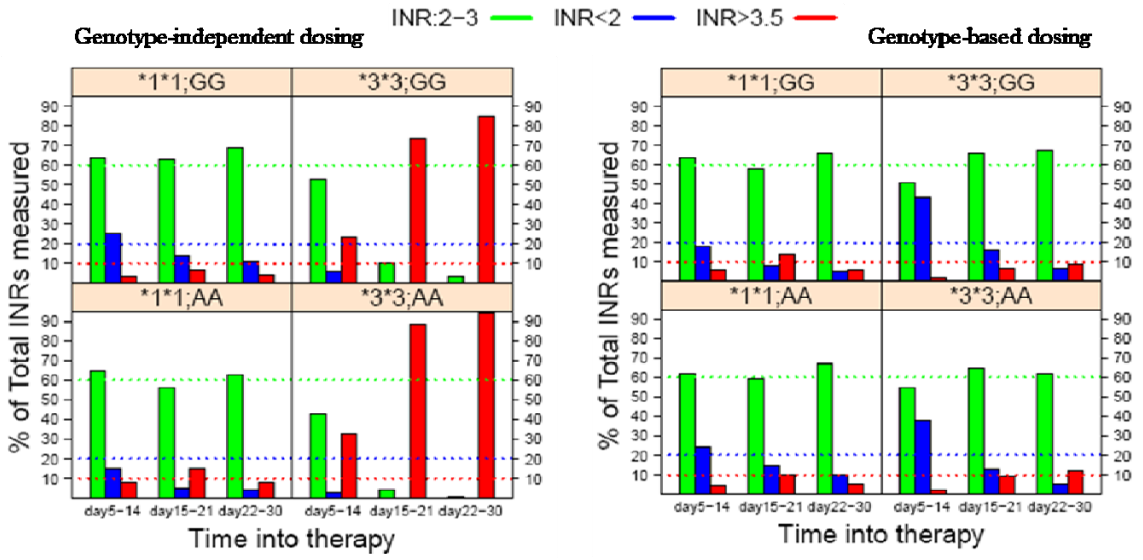
CYP2C9 / VKOR	*1*1	*1*2	*1*3	*2*2	*2*3	*3*3
GG	.13	.08	.065	.035	.035	.017
	.09	.06	.05	.027	.027	.012
GA	.095	.06	.05	.027	.027	.015
	.07	.045	.035	.02	.02	.01
AA	.07	.05	.045	.025	.025	.012
	.05	.04	.03	.017	.017	.01

Titration Scheme

INR	% change from previous dose
< 1.8	+20 %
≥ 1.8 and < 3.2	No change
≥ 3.2 and < 4	-20 %
≥ 4 and < 5	-25 %
≥ 5 and < 6	-30 %
≥ 6	-50 %

The comparison of INR outcomes for genotype-independent and genotype-based dosing are displayed in Figure 7, for four representative genotypes. The genotype-independent dosing results in progressively worse outcomes (dramatic increase in proportions of INR>3.5) as the number of variant CYP2C9 or VKORC1 alleles increases.

Figure 7: Comparison of genotype-based and genotype-independent dosing on patient INR outcomes across time.



The comparison of INR outcomes between CHLA dosing and our proposed genotype-based dosing are displayed in Figure 8 for all (six) genotypes present in the CHLA data. There were vast differences in outcomes between genotype categories under CHLA dosing.

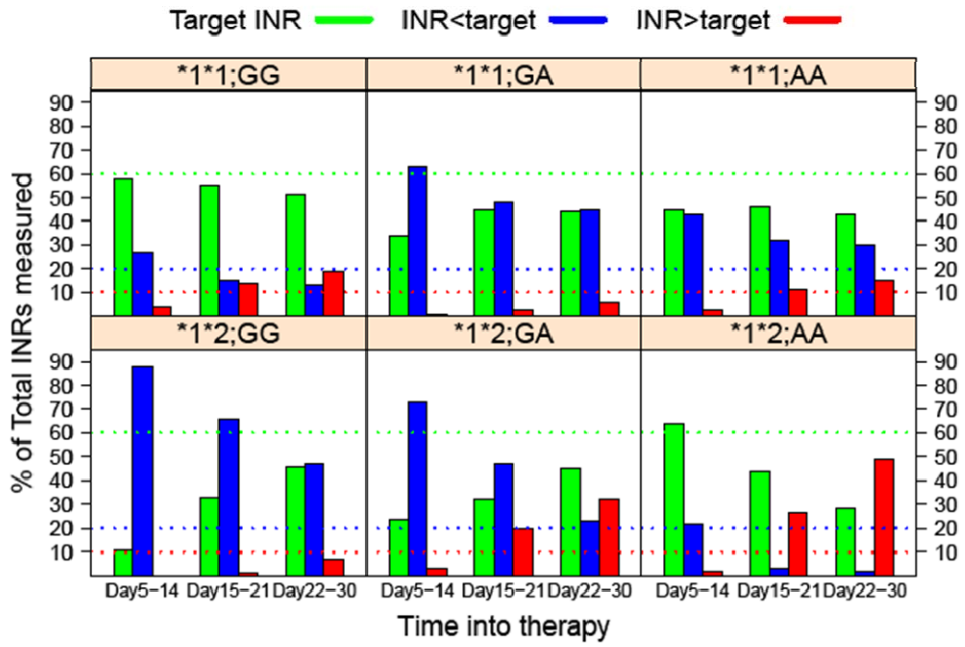
In case of genotype *1*1-GG (homozygous wildtype for both genes) the proportions of INR within target therapeutic range were high (60%) at week 2, with the CHLA dosing. However, there is a decline in this proportion and an increase in proportions of supra-therapeutic INR (to 20%) through month 1. In case of the other extreme end of genotype *1*2-AA (heterozygous variant for CYP2C9 and homozygous variant for VKORC1) while the proportions of INR within target therapeutic range were again high (60%) at

week 2, there is a sharp decline in this proportion (to 30%) and an increase in proportions of supra-therapeutic INR (to 50%) through month 1. In contrast, with the proposed dosing, both therapeutic and supra-therapeutic INRs are consistently around 60% and 10% respectively through month 1.

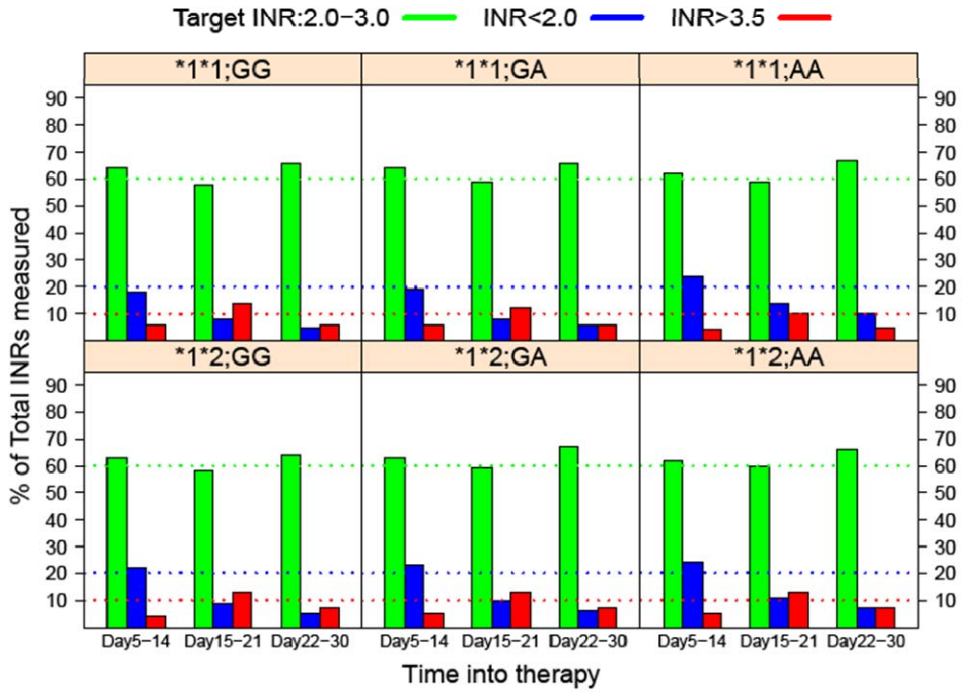
In case of the remaining, intermediate genotype categories, the proportions of INR within target therapeutic range were much lower (10-40%) at week 2 with CHLA dosing and remain lower (upto 45%) through month 1, relative to the proposed dosing (60%). As far as supra-therapeutic INR proportions, there is again an increase observed through month 1 (up to 10- 30%), in all cases. Notably, the proportions of sub-therapuetic INR at week 2 were much higher (40-90%) with CHLA dosing and remain considerably high (20-50%) through month 1, relative to the proposed dosing (< 20%).

Figure 8: Target INR outcomes across time with CHLA standard of care dosing and proposed dosing regimen.

CHLA dosing

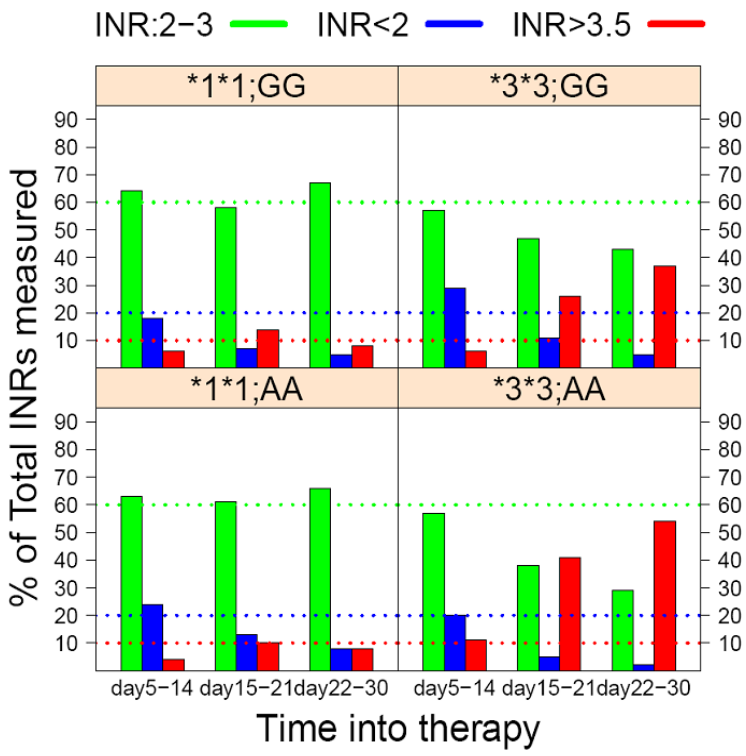


Proposed dosing



Finally, we report the simulated INR outcomes for all genotype categories using our proposed dosing but having imposed restrictions of available warfarin formulation strengths. Here, the lowest dose administered and all dose changes (increase/decrease) during the titration were limited to a minimum of 0.5 mg. The INR outcomes for four representative genotypes are displayed in Figure 9. As expected, proportions of INR >3.5 increase sharply as the number of variant CYP2C9 or VKORC1 alleles increases. This is because the doses administered tend to be higher than those proposed for certain genotypes owing to the formulation strength limitations for low dose requirements.

Figure 9: INR outcomes across time with proposed warfarin dosing and formulation restrictions.



DISCUSSION

There are two primary contributions of the current study:

1- A proposed scientifically based pediatric warfarin dosing regimen that can be reproduced across clinical settings.

2- A tool that can be used by clinicians/researchers to arrive at an optimal pediatric warfarin dosing regimen, should INR outcomes be targeted other than those used in the current study.

The research, in a nutshell, involved leveraging prior information in the form of adult warfarin data, extensive research on warfarin pharmacokinetics and pharmacodynamics and physiology to meet a clinical need. Pharmacometric methods were employed to bridge an adult model and dosing regimen to develop a pediatric warfarin model and propose a dosing regimen. The most relevant aspects of the research are discussed further.

Pediatric model qualification

In perspective, a warfarin population PKPD model built with adult data was appropriately scaled for a pediatric population and used to predict INR outcomes over time for pediatric patients on warfarin. The INR outcomes were predicted well in about 80% of the patients, given limitations in sample size, covaraiate distributions and individual therapeutic INR targets. The mechanisms that the model represents are supported by pharmacological/physiological knowledge. The allometric and maturation models used for scaling PK parameters from adults to pediatrics are those proposed and/or widely

accepted in the literature. Thus, the pediatric model appears useful based on physiology, consistency with adult data, and predictive ability in limited pediatric data.

Having said that, the available CHLA pediatric data represented limited CYP2C9 genotypes. *1*2 was the only genetic polymorphism present in the subjects. In the general Caucasian population the prevelance of *1*2, *1*3, and *2*2/*2*3/*3*3 are 20%, 10% and 5% respectively. In addition, there were no subjects aged < 2 months in order to assess the validity of the CYP2C9 maturation model used for pediatric clearance.

The model makes predictions for the typical subject with a given set of covariates having fixed effects and assumes compliance with dosing regimen. However, an important concern that contributes to INR variability and is difficult to quantify is patient adherence to dosing regimen [2]. In fact, the INR logs of some of the patients where the model appeared unable to predict INR well did reveal poor protocol adherence and exceptional difficulty in achieving stable INR. In most cases, the model predictions follow the dosing patterns (constant or increasing dose) but INR outcomes are counter-intuitive (decreasing or steady). Such observations are classic cases of non-compliance with warfarin therapy.

The model also tended to over predict the INR outcomes for both the African-American subjects present in the cohort. It is known that subjects of the black race have a lower sensitivity to warfarin, requiring higher doses, owing to certain genetic polymorphisms that were not included in the current model. Hence this observation is not surprising but may also be confounded by non-compliance. In addition, particularly in case of

pediatrics, diet can have an influence on INR to a greater extent than captured by the unexplained variability model parameter. These aspects may have led to subjects in the CHLA study straying from their predicted INR profile.

Initial pediatric dosing

The mean simulations led us to conclude that two weight bins with different mg/kg doses, for all genotypes, would result in desired INR profiles on an average. In addition, given that 18 different starting doses are already required based on genotype, we found it to be of practical convenience to have a 2-step dosing regimen based on body weight. There has been speculation about an impact of age in regard to the wide observation that younger children require higher mg/kg warfarin doses than older children and adults, and uncertainty has been expressed about the underlying mechanism [20;26;28;29]. However, we would like to point out that this is neither an unexpected observation nor a consequence of an age or maturation effect. It is an expected outcome based on the nature of the clearance-body weight relationship for the drug. The slope of the relationship is steeper at the lower weight range, which are mostly younger children, and gets shallower at the higher weight range, which are mostly older children. Hence per kg dose is higher for younger, or rather lighter weighing, pediatric subjects. Accordingly, for all genotypes we have proposed a smaller mg/kg dose for subjects weighing ≥ 20 kg and a higher dose for subjects < 20 kg. Notably, the absolute doses (in mg) administered to heavier children would still be higher than absolute doses given to lighter children.

Final pediatric dosing

Based on our pediatric warfarin PKPD model, it is expected that genotype would have a significant effect on warfarin clearance and INR response, and thereby on required doses for target INR. In the simulations, assuming everyone to belong to the same genotype category (CYP2C9*1*1/VKORC1-GA) for dosing purposes resulted in adverse INR outcomes, particularly for the homozygous variant genotypes (*2/*3 and AA). Between the CYP2C9 (PK) and VKORC1 (PD) genetic effects, the polymorphisms altering PK had the most significant impact on dosing. There are two reasons for this; first, the homozygous variant CYP2C9 variant genotypes *2*2, *2*3 and *3*3 had a large magnitude of effect (-70 to -85% on clearance) relative to the VKORC1 homozygous variant genotype AA (-35% on potency). Second, warfarin dose is titrated by monitoring INR, the PD response, and not the drug concentrations which reflect PK. Hence adjusting starting dose based on genotypes relevant for PK is most crucial. Patients with the *2*2/*2*3/*3*3 genotypes have prolonged warfarin half-life (3-6 times longer than *1*1) and the starting dose needs to account for this effect. For patients with genetic polymorphisms, outcomes at week 3 or 4 into therapy are more clinically relevant than week 2. While all results have been presented for only four or six (of eighteen) genotype categories, the conclusions for all scenarios tested remain the same for all genotypes.

In the CHLA study, the target INR range of 2.5-3.5 used for patients with valve replacement matches with that reported in the literature. However, the choice of target INR 1.5-2.5 and 2.0-2.5 for patients undergoing Fontan procedure or diagnosed with Kawasaki are different from that reported widely in the literature [20;24;26;28;29].

Hence for the proposed dosing the commonly accepted and employed target INR range of 2.0-3.0 was selected, to maintain consistency with the literature and to generalize the outomes to most settings. However, the modeling and simulation tool developed can be employed to derive rational pediatric warfarin dosing for optimizing clinical outcomes in terms of any other target INR range or even another INR end-point, if desired.

Comparison of the CHLA warfarin dosing with our proposed genetics-based dosing regimen on individual target INR outcomes reflects the erratic nature of pediatric warfarin dosing practices, and the need for a uniform dosing regimen. The dose and titration choices used in the CHLA study were independent of patient genotype and did not follow a specific, reproducible algorithm. This is common practice for pediatric warfarin dosing. Granted the dosing decisions are based on clinical experience, but this would vary considerably across clinicians and institutions. The first clinical problem with current dosing practices, such as those used in the CHLA study, is that even if good INR control is obtained (which was not common in the study) the dosing employed cannot be reproduced in another setting. The second problem is their moreover empirical nature. Hence a rational, uniform dosing regimen that can be replicated across patients and clinics is required.

While INR control was fairly good initially for the *1*1-GG and *1*2-AA genotype groups with the CHLA dosing, there was an increase in supra-therapeutic outcomes across time, and significantly so for *1*2-AA, which indicates sub-optimal starting dose and/or titration scheme. The INR control was poor for the remaining genotype groups. Their INR outcomes in general reflect clinician attitude to be conservative with warfarin

therapy. In several cases, the INRs tend to remain in the sub-therapeutic range through even a whole month into therapy. This is because there is grave concern, perhaps more so in pediatrics, of overdosing leading to bleeding events. As a result though, the proportions of INRs within the therapeutic window were lower than ought to be targeted. However, while supra-therapeutic INRs remain well below 10% initially, the trend was for these to increase significantly over time, more so in patients with genetic polymorphisms. Thus despite the general conservative dosing, lack of an appropriate starting dose and/or a rational and consistent dose titration scheme can lead to several cases with risky supra-therapeutic INRs after first couple weeks into warfarin therapy.

Based on simulations, we consider our dosing regimen superior to that used in the CHLA study, which may be regarded as the current standard of care. Our regimen succeeded over empirical dosing in maximizing targeted INR outcomes consistently throughout the first month into warfarin therapy. We also expect to see similar results should these dosing regimens be compared in a clinical trial.

Finally, we make a case for the need for a suitable pediatric warfarin formulation. From our simulations it is clear that limiting the lowest dose administered and smallest possible dose change to 0.5 mg is not advisable for pediatric subjects, particularly those with homozygous CYP2C9 and/or VKORC1 polymorphisms. In addition, the oral tablet is not a well-suited dosage form for pediatric patients. In several cases where patients are unable to swallow whole tablets, the tablets are crushed and administered with apple sauce. Such drug administration practices, along with a limitation on lowest dose strength

available, further contribute to the already high variabiltiy in INR outcomes. Hence a more pediatric-friendly warfarin formulation, in terms of both strength and dosage form, is a timely requirement.

Scope of the study

Currently there is no information available on altering warfarin dose in pediatrics based on influential factors. In particular, quantifying the impact of polymorphisms in the CYP2C9 and VKORC1 on pediatric warfarin dosing is of critical therapeutic relevance. To our knowledge, our research brings forth the first ever proposed dosing regimen for using warfarin in pediatric patients as well as a useful tool to derive such dosing. The pediatric PKPD model used complies with what is known of warfarin and general pharmacology, and is consistent with adult warfrain clinical data.

However, we recognize the limitations of the current research. We believe that in general the limitations may be attributed to the paucity of available clinical data on warfarin use in pedtarics. Firstly, we were restricted to a small sample size of pediatric subjects (n=26), which we used to qualify the model. Hence the model was based on adult data and physiological principles rather than pediatric data, and the proposed dosing was based on simulations. Moreover, the available pediatric data was limited in terms of covariate distributions, particualrly CYP2C9 polymorphisms and youngest ages.Another limitation to our study is the absence of prospective validation of the dosing regimen. For any dosing regimen to be widely accepted it must first be shown to be superior on relevant clinical outcomes in a prospective controlled trial. Given the practical limitations

to conducting pediatric warfarin interventional trials, we recommend that more propspective observational studies and experiemntal studies be conducted in pediatrics to update the PKPD model and hence the proposed dosing strategy. However, despite the limitations, we believe we have made efficient use of available information and suggested an important first step towards improving pediatric warfarin dosing.

The research was based on certain assumptions. First, that the concentration-INR response relationship for warfarin is similar between pediatrics and adults. Some researchers have suggested intrinsic developmental differences in the coagulation systems [30;31], precluding extrapolation of dose-response for antithrombotic therapy from adults to the youngest subset of the pediatric population (< 6 months). The second assumption is that the CYP2C9 polymorphisms reduce warfarin clearance to the same extent in pediatrics and adults. Last, we assumed no developmental changes in plasma protein binding and dose-proportional PK throughout the entire pediatric age range. However, again there is dearth of data regarding how ontogeny affects warfarin pharmacokinetics and pharmacodynamics to formally challenge our assumptions. This is particularly true for VKORC1 where the patterns of developmental expression are not yet known.

The eventual goal of studies like ours is to establish a new standard of care for pediatric patients who require warfarin therapy. A genetics-based warfarin dosing nomogram that functions more efficiently than conventional arbitrary dosing at maximizing therapeutic INR outcomes will represent a major advance in pediatric pharmacotherapy. Such a

nomogram could be made widely available to all clinicians, and would enhance the safety and effectiveness of warfarin therapy in pediatric patients. Hence, further research for refining and validating the proposed model and dosing regimen would be useful. Even so, the proposed regimen is based on rational sciene and is recommended for use in pediatric studies and practice.

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CHAPTER 6

Conclusions and Future Research

CONCLUSIONS

Over the past decade there has been significant interest and progress in conducting pediatric clinical research in order to directly generate information for the safe and effective use of drugs in this special population. However, pediatric drug development is challenging and fairly unique is several aspects. Most development programs have just one chance to perform an informative set of trials, generally few in number. In some cases, logistic and ethical constraints, and lack of financial incentives prevent conduct of trials and limit the data available on drug use in pediatrics. Hence novel, efficient approaches such as pharmacometric methods have and can be used to leverage prior and current information and make useful decisions during pediatric drug development. The research underlying this dissertation as well as the several case studies discussed in chapters 1 and 2 highlight the contribution of pharmacometrics in enhancing pediatric drug development.

One aspect of the research undertaken provides evidence of the use of modeling and simulation in improving pediatric trial design. With objective criteria such as the PK quality standard in place, pharmacometric techniques can be successfully applied in better planning of pediatric PK trials, to ensure informative trial outcomes. The other aspect of the research undertaken represents the power of pharmacometric methods in maximizing on limited available information and generating useful dosing guidelines in pediatrics. A warfarin model and genetics-based dosing regimen that was originally built using adult data and validated in a clinical trial was successfully leveraged along with physiological principles to derive a pediatric warfarin model and dosing regimen. The work caters to a heretofore unmet clinical need for a rational, reproducible pediatric warfarin dosing strategy that may be applied across clinical settings.

The broader implications of the current research include, in general, improved pediatric health care and quality of life. Parents of children requiring pharmacotherapy for various conditions as well as the clinicians treating these patients stand to benefit considerably from the wider adoption of such research, as described in this dissertation. The work also represents an advance for pharmacogenetics. For instance, adults are now able to avail of genetics-based warfarin dosing and the research aims at providing similar care for pediatric patients as well. While clinical research historically has been focused on the adult population, the work undertaken represents increased awareness and avenues for bridging the gap between adults and pediatrics. The research is encouargaing for future investments in enhancing pediatric drug development.

FUTURE RESEARCH

The quality standard described currently applies only to pediatric PK trials. However, the likelihood is that future regulation will introduce such standards for exposure-response trials in pediatrics as well. Research on methodology and feasibility for pediatric trial design to achieve good target precision on the slope of the exposure-response relationship is a potential avenue for related future research. In general, all future clinical trials ought to be designed rationally, using modeling and simulation.

Future clnical studies with warfarin in pediatrics would serve as a bonus to our current research. Controlled interventional clinical trials of warfarin in pediatric patients are an unrealistic expectation, given the age of this drug and lack of financial incentive for future trials. However, experimental PKPD studies can help update the model and prospective observational studies may help improve the dosing regimen. Refining and prospectively validating the proposed dosing regimen could lead to wider adoption of rational and standardized dosing for warfarin use in pediatrics, rather than the current empirical standard of care dosing practices. The current research is a step forward in enhancing the safety and effectiveness profile of the most widely used anticoagulant that stands to benefit from future research.

Further, there are several reserach areas within pediatrics that can benefit from modeling and simulation such as quantifying effects of ontogeny and identifying pediatric-specific biomarkers and trial end-points. Moreover, the applications of pharmacometrics go beyond pediatrics to all populations and to all aspects of drug development. Identification

of risk factors for new and existing drug therapies and quantifying pharmacogenetic information is an important research area. Pharmacometric analysis has been used for this purpose for the anti-viral drug nevirapine. The report is presented in Appendix D of this dissertation. In summary, a continuous Learn-Apply paradigm, if adopted, can significantly improve pediatric and overall drug development and therapeutics. Timely application of pharmacometric methods would be an integral part of such a paradigm.

APPENDICES

APPENDIX A

R script, NONMEM model and SAS program files used to run simulation-estimation for pediatric PK quality standard evaluation (Chapter 3)

R script

```
#### EXECUTING SIMULATIONS IN NONMEM AND ESTIMATIONS IN SAS THROUGH R ###
### VARYING BOTH SAMPLE SIZE AND NUMBER OF SAMPLES ###


library(Hmisc)
library(grid)
library(lattice)

setwd("/home/lalam/Peds_Sim")
#setwd("W:/Peds_Sim")

## INPUT SIMULATION SCENARIO: ONE COMP. ORAL FIRST ORDER ABSORPTION ##

## PK MODEL PARAMETERS:

DOSE <- 100   #mg/kg
anchor <- 20  #kg
CL <- 3.2     #L/h/20kg
V <- 6.2      #L/20kg
KA <- 2       #/hr
FBA <- 1      #100%
ALLOCL<- 0.75
A50<- 0.18    #yrs
ALLOV<-1
p<-8

vari<-"MV"
CVCL <- 0.5
CVV <- 0.5
SDCL <-round(sqrt(log(1+CVCL**2)),2)
```

```
SDV <-round(sqrt(log(1+CVV**2)),2)
SDTHF <- round(sqrt(SDCL**2 + SDV**2),2)
CVKA <- 0.5
SDKA <-round(sqrt(log(1+CVKA**2)),2)
sdtimes <-3
OMCL <- CVCL**2
OMV <- CVV**2
OMKA<-CVKA**2
SIG <- 0.01
COV<-c(ALLOCL,ALLOV,A50,OMCL,OMV,SIG)
lim<-20

## TRIAL CHARACTERISTICS:

nBins<-4
nAdult<-24
tAdult<- ifelse(nAdult==12,2.20, ifelse(nAdult==24,2.07, 0))
nABins <- ifelse(nAdult==0,nBins,nBins+1)
nrep1<-250
nrep2<-300
nrep <- ifelse(vari=="LV",nrep1,nrep2)

path<-ifelse(nAdult==0,
paste("/home/lalam/Peds_Sim/ORAL/SPARSE/NoAdult/",vari,"/",sep=""),
paste("/home/lalam/Peds_Sim/ORAL/SPARSE/Adult/",nAdult,"ad/",vari,"/",sep
=""))
#path<-ifelse(nAdult==0,
paste("W:/Peds_Sim/ORAL/SPARSE/NoAdult/",vari,"/",sep=""),
paste("W:/Peds_Sim/ORAL/SPARSE/Adult/",nAdult,"ad/",vari,"/",sep=""))

Nbin1<-c(4,4,4,4,nAdult)
t1<-c(3.18,3.18,3.18,3.18,tAdult)
Nbin2<-c(6,6,6,6,nAdult)
t2<-c(2.57,2.57,2.57,2.57,tAdult)
Nbin3<-c(8,8,8,8,nAdult)
t3<-c(2.36,2.36,2.36,2.36,tAdult)
Nbin4<-c(10,10,10,10,nAdult)
t4<-c(2.26,2.26,2.26,2.26,tAdult)
Nbin5<-c(12,12,12,12,nAdult)
t5<-c(2.20,2.20,2.20,2.20,tAdult)
Nbin6<-c(16,16,16,16,nAdult)
t6<-c(2.13,2.13,2.13,2.13,tAdult)
Nbin7<-c(20,20,20,20,nAdult)
t7<-c(2.09,2.09,2.09,2.09,tAdult)

N1<- Nbin1[1]+Nbin1[2]+Nbin1[3]+Nbin1[4]+Nbin1[5]
N2<- Nbin2[1]+Nbin2[2]+Nbin2[3]+Nbin2[4]+Nbin2[5]
N3<- Nbin3[1]+Nbin3[2]+Nbin3[3]+Nbin3[4]+Nbin3[5]
N4<- Nbin4[1]+Nbin4[2]+Nbin4[3]+Nbin4[4]+Nbin4[5]
N5<- Nbin5[1]+Nbin5[2]+Nbin5[3]+Nbin5[4]+Nbin5[5]
N6<- Nbin6[1]+Nbin6[2]+Nbin6[3]+Nbin6[4]+Nbin6[5]
N7<- Nbin7[1]+Nbin7[2]+Nbin7[3]+Nbin7[4]+Nbin7[5]
```

```
Nsub <- c(N1,N2,N3,N4,N5,N6,N7)    # if other than 7 sizes, also change
                                          in modules 6,7

Tsamp1 <- c(0,4)
Tsamp2 <- c(0,1,3)
Tsamp3 <- c(0,1,3,4)
TsampR <- c(0,0.25,0.5,1,1.5,2,2.5,3,4,6,8)
Nsamp <- c(length(Tsamp2),length(Tsamp3))       # if other than 2
                                     schedules, also change in module 2
RS<-length(TsampR)

Ntrials <- length(Nsub)*length(Nsamp)


## DEMOGRAPHICS:

# ages below entered in months
Bin1lo<-1   #1month
Bin1hi<-24    #2yrs
Bin2hi<-72.5  #6yrs
Bin3hi<-144.5 #12yrs
Bin4hi<-192.5 #16yrs
Bin5lo<- 18 # yrs
Bin5hi<- 65 # yrs

medage1<-1
medage2<-4
medage3<-9
medage4<-14
medage5<-41

medwt1<-9.9
medwt2<-16.1
medwt3<-28.9
medwt4<-50.5
medwt5<-74.7

## PREDEFINED OBJECTS:

simdataitems<-c("ID","TIME","AMT","Y","ABIN","AGE","WT","SEX")

parms<-c("CL","V")
Nparms<-length(parms)
covar<-c("ALLOCL","ALLOV","A50","OMCL","OMV","SIG")
Ncovar<-length(covar)
MEANparms<-paste("MEAN_",parms,sep="")
RSEparms<-paste("RSE_",parms,sep="")
UCIparms<-paste("UCI",parms,sep="")
BIAS1parms<-paste("BIAS1_",parms,sep="")
EQUIV1parms<-paste("EQUIV1_",parms,sep="")
BIAS2parms<-paste("BIAS2_",parms,sep="")
```

```
EQUIV2parms<-paste("EQUIV2_",parms,sep="")
BIASparms<-c(BIAS1parms,BIAS2parms)
EQUIVparms<-c(EQUIV1parms,EQUIV2parms)
PRECparms<-paste("PREC_",parms,sep="")
ESTcovar<-paste("EST_",covar,sep="")
BIAScovar<-paste("BIAS_",covar,sep="")
PRECcovar<-paste("PREC_",covar,sep="")
popestnames<-c(PRECparms,BIASparms,EQUIVparms)
poprawnames<-c(MEANparms,RSEparms)
popest2names<-c(ESTcovar,BIAScovar,PRECcovar)
Npopest<-length(popestnames)+1
Npopraw<-length(poprawnames)+1
Npopest2<-length(popest2names)
pop1names<-
c("ID","AGE","WT","CL","SECL","RSE_CL","UCICL","V","SEV","RSE_V","UCIV")
Npop1<-length(pop1names)
pop2names<-
c("Parameter","Estimate","SE","DF","t","p","alpha","LCI","UCI","gradient"
)
Npop2<-length(pop2names)

indparms<-c("CLi","Vi")
Nindparms<-length(indparms)
MEANposthoc <-paste("MEAN_",indparms,sep="")
RSEposthoc <-paste("RSE_",indparms,sep="")
SHRINKposthoc <- paste("SHRINK_",indparms,sep="")
BIAS1posthoc <- paste("BIAS1_",indparms,sep="")
BIAS2posthoc <- paste("BIAS2_",indparms,sep="")
EQUIV1posthoc <- paste("EQUIV1_",indparms,sep="")
EQUIV2posthoc <- paste("EQUIV2_",indparms,sep="")
PRECposthoc <- paste("PREC_",indparms,sep="")
indrawnames<-c(MEANposthoc, RSEposthoc)
posthocestnames<-
c(PRECposthoc,BIAS1posthoc,BIAS2posthoc,EQUIV1posthoc,EQUIV2posthoc,SHRIN
Kposthoc)
Nindraw<-length(indrawnames)+1
Nposthocest<-length(posthocestnames)+1
posthocnames<-
c("ID","ABIN","AGE","WT","SEX","dose","CLi","SECLi","UCICLi","Vi","SEVi",
"UCIVi","KAi","SEKAi","UCIKAi","TIME","dv","PPRED","IPRED")
Nposthoc<-length(posthocnames)


## MODULE 1: CREATING BANK OF PEDS WITH CDC-BASED AGE-WT RELATIONSHIP ##

data<-read.csv("CDCwtage.csv")
names(data)

nsim<-100
sim<-c()
for (i in 1:nsim){
 data$nrep<-i
```

```
 sim<-rbind(sim, data)
}
set.seed(123)
sim$rn<-rnorm(nrow(sim))
sim$wt<-sim$M*(1+sim$L*sim$S*sim$rn)**(1/sim$L)
sim$CL<-CL*((sim$wt/anchor)**ALLOCL)*(sim$Agemos/((A50*12)+sim$Agemos))
sim$V<-V*((sim$wt/anchor)**ALLOV)

sim1<-sim[sim$Agemos>Bin1lo & sim$Agemos<=Bin1hi,c(1,2,17,18,19)]
sim2<-sim[sim$Agemos>Bin1hi & sim$Agemos<=Bin2hi,c(1,2,17,18,19)]
sim3<-sim[sim$Agemos>Bin2hi & sim$Agemos<=Bin3hi,c(1,2,17,18,19)]
sim4<-sim[sim$Agemos>Bin3hi & sim$Agemos<=Bin4hi,c(1,2,17,18,19)]
sim1$AgeBin<-1
sim2$AgeBin<-2
sim3$AgeBin<-3
sim4$AgeBin<-4

#edit(bank)
bank<-rbind(sim1,sim2,sim3,sim4)
bankmean<-aggregate(log(bank[ ,parms]),by=list(bank$AgeBin),mean,na.rm=T)
bankmean<-exp(bankmean)[ ,parms]
bankvar<-aggregate(log(bank[ ,parms]),by=list(bank$AgeBin),var,na.rm=T)
bankvar<-bankvar[ ,parms]

truemean<-bankmean

meanclad<-CL*((medwt5/anchor)**ALLOCL)*(medage5/(medage5+A50))
meanvad<-V*((medwt5/anchor)**ALLOV)
adultmean<-c(meanclad,meanvad)
if(nAdult>0) bankmean<-rbind(bankmean,adultmean)


## MODULE 1A: SIMULATING COMMON DEMOGRAPHICS FOR ALL CASES ##

simb1a<-sim1a[sample(1:nrow(sim1a),Npbin[1],replace=F), ]
simb1b<-sim1b[sample(1:nrow(sim1b),Npbin[1],replace=F), ]
simb1<-sim1[sample(1:nrow(sim1),Npbin[1],replace=F), ]
simb2<-sim2[sample(1:nrow(sim2),Npbin[1],replace=F), ]
simb3<-sim3[sample(1:nrow(sim3),Npbin[1],replace=F), ]
simb4<-sim4[sample(1:nrow(sim4),Npbin[1],replace=F), ]

simbin5<-sim5[sample(1:nrow(sim5),nAdult,replace=F), ]


simbn1a<-simb1a
simbn1b<-simb1b
simbn1<-simb1
simbn2<-simb2
simbn3<-simb3
simbn4<-simb4

simdemo<-list()
```

```
allsimdemo<-list(list())
k<-7
for (i in Npbin) {

            simbin1a<-simbn1a[sample(1:nrow(simbn1a),i,replace=F), ]
            simbin1b<-simbn1b[sample(1:nrow(simbn1b),i,replace=F), ]
            simbin1<-simbn1[sample(1:nrow(simbn1),i,replace=F), ]
            simbin2<-simbn2[sample(1:nrow(simbn2),i,replace=F), ]
            simbin3<-simbn3[sample(1:nrow(simbn3),i,replace=F), ]
            simbin4<-simbn4[sample(1:nrow(simbn4),i,replace=F), ]
            simbn1a<-simbin1a
            simbn1b<-simbin1b
            simbn1<-simbin1
            simbn2<-simbin2
            simbn3<-simbin3
            simbn4<-simbin4
            simdemo[[6]]<-simbin1a
            simdemo[[7]]<-simbin1b
            simdemo[[1]]<-simbin1
            simdemo[[2]]<-simbin2
            simdemo[[3]]<-simbin3
            simdemo[[4]]<-simbin4
      allsimdemo[[k]]<-simdemo
      k<-k-2
}

## MODULE 1B: CREATING DEMOGRAPHIC DATASETS FOR SIMULATIONS ##

k <- 1
n <- 4

for (i in Ntot) {
      if(n==10) n<-12
            simbin1a<-allsimdemo[[k]][[6]]
            simbin1b<-allsimdemo[[k]][[7]]
            simbin1<-allsimdemo[[k]][[1]]
            simbin2<-allsimdemo[[k]][[2]]
            simbin3<-allsimdemo[[k]][[3]]
            simbin4<-allsimdemo[[k]][[4]]
            simagewt<-rbind(simbin1,simbin2,simbin3,simbin4)
            if(nBins==5) simagewt<-
rbind(simbin1a,simbin1b,simbin2,simbin3,simbin4)
            simagewt<-simagewt[,c(6,2,3,1)]
            names(simagewt)<-c("AgeBin","Age","Weight","Sex")
            simagewt$Age<-simagewt$Age/12
            if(nAdult>0) simagewt<-rbind(simagewt,simbin5)

            demog <- data.frame(1:i)
            names(demog) <- "ID"
            demog<-cbind(demog,simagewt)
            names(demog)<-c("ID","AgeBin","AGE","WT","SEX")
```

```
            demog$AGE<-
ifelse(demog$AGE<1,round(as.numeric(demog$AGE),2),round(as.numeric(demog$
AGE),1))
            demog$WT <- round(as.numeric(demog$WT),1)

            plot<-xyplot(demog$WT~demog$AGE, data=demog,xlab="AGE
(yrs)",ylab="WEIGHT (kg)",

      scales=list(cex=1.5,lwd=2,x=list(log=10,at=c(0,1,2,6,12,16,25,50)))
,
                  panel=function(x,y,...){
                  panel.xyplot(x,y,type="p",...)
                  panel.curve(5.4095+4.6965*10^x-
0.7261*(10^x)^2+0.0721*(10^x)^3-0.002*(10^x)^4, from=log10(1),
to=log10(18), lty=2, col=2)
                  panel.curve(3.9364+4.5254*10^x-
0.7279*(10^x)^2+0.0649*(10^x)^3-0.0017*(10^x)^4, from=log10(1),
to=log10(18), lty=2, col=2)
                  panel.curve(7.5589+4.1842*10^x-
0.4651*(10^x)^2+0.0628*(10^x)^3-0.002*(10^x)^4, from=log10(1),
to=log10(18), lty=2,col=2)
                  panel.curve(3.5802+10.681*10^x-4.037*(10^x)^2,
from=log10(0.0001), to=log10(1), lty=3,col=1)
                  panel.curve(2.5787+9.8283*10^x-3.8735*(10^x)^2,
from=log10(0.0001), to=log10(1), lty=3,col=1)
                  panel.curve(4.4035+12.857*10^x-4.9702*(10^x)^2,
from=log10(0.0001), to=log10(1), lty=3,col=1)
                  }
                  )
            pdf(file=paste("AgeWtPlot",n,".pdf",sep=""),width=9,height=9)
            print(plot)
            dev.off()

            write.table(demog, file=paste("demog_N",n,".csv",sep=""),
            sep=",", quote=F, row.names=F, na=".")

            k <- k+2
            n <- n+2
      }


## MODULE 2: CREATING DATA TEMPLATES & CONTROL STREAMS FOR NM TO USE FOR
SIMULATIONS ##

ctlstrm <- scan(file="runsim_oral_trunc.mod", what="character", sep="\n")

d<-1
k <- 1

for (i in Nsub) {
      for (j in Nsamp) {
            if (j==Nsamp[1]) Tsamp <- Tsamp2
```

```
            if (j==Nsamp[2]) Tsamp <- Tsamp3

            #demog<-
read.csv(file=paste("W:/Peds_Sim/DEMOGS/demog",d,"_",nAdult,"ad.csv",sep=
""))
            demog<-
read.csv(file=paste("/home/lalam/Peds_Sim/DEMOGS/demog",d,"_",nAdult,"ad.
csv",sep=""))
            demog<-demog[!duplicated(demog$ID), ]
            e<-c(rep(j,each=i-nAdult),rep(RS,each=nAdult))
            input<-demog[rep(demog$ID,e), ]
            input$TIME <- c(rep(Tsamp, i-nAdult),rep(TsampR,nAdult))
            input$AMT <- ifelse(input$TIME==0, DOSE*input$WT, 0)
            input$CONC <- rep(".", nrow(input))
            input<-input[ ,c(1,6,7,8,2,3,4,5)]
            write.table(input,
file=paste(path,"run_sim_",nAdult,"/input",k,".csv",sep=""),
            sep=",", quote=F, row.names=F, na=".")

            ctlstrm[3]  <- paste("$DATA input",k,".csv IGNORE=@",sep="")
            ctlstrm[7] <- paste("SUBPROBLEMS = ",nrep,sep="")
            ctlstrm[11] <- paste(" ",CL," FIX ; CL (L/h/20kg)",sep="")
            ctlstrm[12] <- paste(" ",V," FIX ; V (L/20kg)",sep="")
            ctlstrm[13] <- paste(" ",KA," FIX ; TVKA (/h)",sep="")
            ctlstrm[14] <- paste(" ",ALLOCL," FIX ; ALLOCL",sep="")
            ctlstrm[15] <- paste(" ",ALLOV," FIX ; ALLOV",sep="")
            ctlstrm[16] <- paste(" ",A50," FIX ; A50 (YRS)",sep="")
            ctlstrm[18] <- paste(" ",OMCL," FIX ; BSVCL",sep="")
            ctlstrm[19] <- paste(" ",OMV," FIX ; BSVV",sep="")
            ctlstrm[20] <- paste(" ",OMKA," FIX ; BSVKA",sep="")
            ctlstrm[22] <- paste(" ",SIG," FIX ; CVCP",sep="")
            ctlstrm[40] <- paste("      DLTACL =
",sdtimes,"*",SDCL,sep="")
            ctlstrm[41] <- paste("      DLTAV = ",sdtimes,"*",SDV,sep="")
            ctlstrm[42] <- paste("      DLTAKA =
",sdtimes,"*",SDKA,sep="")
            ctlstrm[43] <- paste("      DLTATH =
",sdtimes,"*",SDTHF,sep="")
            ctlstrm[length(ctlstrm)] <- paste("NOPRINT ONEHEADER NOAPPEND
FILE=sdtab",k,sep="")

      write(ctlstrm,file=paste(path,"run_sim_",nAdult,"/runsim",k,".mod",
sep=""))

            k <- k+1
            }
d<-d+1
}


## MODULE 3: EXECUTE NM RUNS FOR SIMULATIONS THROUGH R ON CLUSTER ##
```

```
execute <- character()
execute[1] <- "#!/bin/sh"
execute[2] <- paste("execute -
threads=32",paste("runsim",1:Ntrials,".mod",collapse=" ",sep=""),sep=" ")
setwd(paste(path,"run_sim_",nAdult,sep=""))
write(execute,"sim.pl")
system("perl sim.pl")


## MODULE 4: READ IN THE SIMULATED OUTPUT DATA (sdtab files) FROM NM ##
## SPLIT THE SIMULATED DATA FOR EACH REPLICATE AND CREATE DATASETS TO
INPUT BACK TO SAS ##
## CREATE SAS MODEL FILES FOR FITTING SIMULATED DATA ##

#model <- scan(file="W:/Peds_Sim/fit_oral_sp.sas", what="character",
sep="\n")
model <- scan(file="/home/lalam/Peds_Sim/fit_oral_sp.sas",
what="character", sep="\n")

k <- 1
test <- list()

simparms<- list()
allsim<-list(list())

for (i in Nsub) {
      for (j in Nsamp) {
            for(r in 1:nrep){
                  test[[r]] <- read.table(file=paste("sdtab",k,sep=""),
                        skip=(r-1)*((i*j)+(nAdult*(RS-
j))+1)+(r),header=T,nrows=(i*j)+(nAdult*(RS-j)))

                  simparms[[r]]<-
test[[r]][!duplicated(test[[r]]$ID),c("CL","V","ABIN","WT")]

                  test[[r]] <- test[[r]][ ,simdataitems]
                  names(test[[r]]) <-
c("ID","TIME","AMT","CONC","ABIN","AGE","WT","SEX")

                  dosing <- test[[r]][!duplicated(test[[r]]$ID), ]
                  dosing$TIME<-0
                  dosing$AMT<-DOSE*dosing$WT
                  dosing$CONC <- "."

                  test[[r]]<-test[[r]][test[[r]]$TIME>0 , ]
                  test[[r]] <- rbind(dosing, test[[r]])
                  test[[r]] <- test[[r]][order(test[[r]]$ID), ]
                  test[[r]]$MDV <- ifelse(test[[r]]$AMT==0,0,1)
                  test[[r]]$EVID <- ifelse(test[[r]]$AMT==0,0,1)
                  write.table(test[[r]],
file=paste(path,"fit_model/simdata",k,"rep",r,".csv",sep=""),
                        sep=",", quote=F, row.names=F, na=".")
```

```
                  model[7]<- paste("  DATAFILE=\"",
path,"fit_model/simdata",k,"rep",r,".csv\"",sep="")
                  model[17]<-paste("if id=1001 then do; age=",medage1," ;
wt=",medwt1,"; end;",sep="")
                  model[18]<-paste("if id=1002 then do; age=",medage2," ;
wt=",medwt2,"; end;",sep="")
                  model[19]<-paste("if id=1003 then do; age=",medage3," ;
wt=",medwt3,"; end;",sep="")
                  model[20]<-paste("if id=1004 then do; age=",medage4," ;
wt=",medwt4,"; end;",sep="")
                  model[65]<-paste("   anchor = ",anchor,";",sep="")

                  model[66]<-paste("   TVKA = ",KA,";",sep="")
                  model[67]<-paste("   s2ka = ",OMKA,";",sep="")
                  model[68]<-paste("   F = ",FBA,";",sep="")
                  model[141] <- paste("(pTVCL=",CL,",",sep="")
                  model[142] <- paste("pTVV=",V,",",sep="")
                  model[143] <- paste("pALLOCL=",ALLOCL,",",sep="")
                  model[144] <- paste("pALLOV=",ALLOV,",",sep="")
                  model[145] <- paste("pA50=",A50,",",sep="")
                  model[146] <- paste("ps2cl=",OMCL,",",sep="")
                  model[147] <- paste("ps2v=",OMV,",",sep="")
                  model[148] <- paste("ps2=",SIG,",",sep="")
                  model[149] <- paste("repeats=",lim,")",sep="")
                  model[171] <- paste("proc export data=bins
outfile=\"",path,"model_output/bins",k,"rep",r,".csv\" DBMS=CSV REPLACE;
run;",sep="")
                  model[172] <- paste("proc export data=para
outfile=\"",path,"model_output/parms",k,"rep",r,".csv\" DBMS=CSV REPLACE;
run;",sep="")
                  model[173] <- paste("proc export data=posthoc
outfile=\"",path,"model_output/posthoc",k,"rep",r,".csv\" DBMS=CSV
REPLACE; run;",sep="")

      write(model,file=paste(path,"fit_model/fit",k,"rep",r,".sas",sep=""
))

                  }
            allsim[[k]]<-simparms

            k <- k+1
      }
}


## MODULE 5: FITTING MODEL TO SIMULATED DATA USING SAS THROUGH R ##

setwd(paste(path,"fit_model",sep=""))

runsas <- character()
runsas[1] <- "#!/bin/sh"
```

```
k<-1
for (i in Nsub){
      for (j in Nsamp) {
            for (r in 1:nrep) {
                  runsas[2]<-(paste("/opt/sas92/SASFoundation/9.2/sas92 -
noterminal -log ",path,"fit_model -print ",path,"fit_model "
,path,"fit_model/fit",k,"rep",r,".sas",sep=""))
                  write(runsas,file=paste("fit",k,"rep",r,".bat",sep=""))
                  system(paste("chmod a+x fit",k,"rep",r,".bat",sep=""))
                  system(paste("qsub -o /home/lalam/eofiles -e
/home/lalam/eofiles fit",k,"rep",r,".bat &",sep=""))
            }
      k<-k+1
      }
}

## MODULE 6: READ IN SAS ESTIMATION OUTPUT (lst/bin/parm files) FOR
ANALYSIS : METHOD 1 - POP.MEAN. ##

popest <- list()
popraw<-list()
popest2<-list()
pop1 <- list()
pop2<-list()
allpop1<-list(list())
allpop2<-list(list())
agebin<-1:nBins

k<-1
f<-1
g<-1
finalpop<-data.frame(matrix(NA,ncol=Npopest+2,nrow=Ntrials*nBins*2))
names(finalpop)<-c("METRIC","AgeBin",popestnames,"SCENARIO")
finalpop2<-data.frame(matrix(NA,ncol=Npopest2+2,nrow=Ntrials*2))
names(finalpop2)<-c("METRIC",popest2names,"SCENARIO")

for (i in Nsub) {

      t<-qt(0.975,(i-nAdult-p))

      for (j in Nsamp) {

      popest[[k]]<-data.frame(matrix(NA,ncol=Npopest,nrow=nrep*nBins))
      names(popest[[k]])<-c("AgeBin",popestnames)
      popraw[[k]]<-data.frame(matrix(NA,ncol=Npopraw,nrow=nrep*nBins))
      names(popraw[[k]])<-c("AgeBin",poprawnames)
      popest2[[k]]<- data.frame(matrix(NA,ncol=Npopest2,nrow=nrep))
      names(popest2[[k]]) <- popest2names

      b<-1
      for(r in 1:nrep){
```

```
            output <- scan(file=paste("fit",k,"rep",r,".lst",sep=""),
what="character", sep="\n")
            thetaPos <- grep("Successful", output)
            ifelse(length(thetaPos)==0,
                  pop1[[r]]<-
data.frame(matrix(NA,ncol=Npop1,nrow=nBins)),
                  pop1[[r]]<-
read.csv(paste(path,"model_output/bins",k,"rep",r,".csv",sep="")))
                  names(pop1[[r]])<-pop1names
            ifelse(length(thetaPos)==0,
                  pop2[[r]]<-
data.frame(matrix(NA,ncol=Npop2,nrow=Nparms+Ncovar)),
                  pop2[[r]]<-
read.csv(paste(path,"model_output/parms",k,"rep",r,".csv",sep="")))
                  names(pop2[[r]])<-pop2names

            allsim[[k]][[r]][ ,parms]<-log(allsim[[k]][[r]][ ,parms])
            simmean<-aggregate(allsim[[k]][[r]][
,parms],by=list(allsim[[k]][[r]]$ABIN),mean,na.rm=T)
            simmean<-exp(simmean[1:nBins,parms])

            popmean<- pop1[[r]][ ,parms]
            popRSE<-pop1[[r]][ ,RSEparms]
            popprec<-(100 + t*(popRSE))
            popbias1<-((popmean-truemean)/truemean)*100
            popequiv1<-exp(log(popmean/truemean))
            popbias2<-((popmean-simmean)/simmean)*100
            popequiv2<-exp(log(popmean/truemean))
            popparms <-
cbind(agebin,popprec,popbias1,popbias2,popequiv1,popequiv2)
            popest[[k]][c(b:(nBins*r)),] <- popparms
            poprawpar<-cbind(agebin,popmean,popRSE)
            popraw[[k]][c(b:(nBins*r)),] <- poprawpar

            popcov<-pop2[[r]][(Nparms+1):nrow(pop2[[r]]),pop2names[1:3]]
            popcov$UCI<-popcov$Estimate + t*(popcov$SE)
            popcov$Prec<-(popcov$UCI/popcov$Estimate)*100
            popcov$Bias<-((popcov$Estimate-COV)/COV)*100
            popcovpar <- c(popcov$Estimate,popcov$Bias,popcov$Prec)
            popest2[[k]][r,] <- popcovpar

            b<-b+nBins
      }
      allpop1[[k]]<-pop1
      allpop2[[k]]<-pop2

      pass<-c()
            p.pass<-c()
            for(a in agebin) {
                  for(p in 2:3) {
                  con<-is.na(popest[[k]][popest[[k]]$AgeBin==a,p])
                  ncon<-length(con[con==F])
```

```
                  test1<-
ifelse(is.na(popest[[k]][popest[[k]]$AgeBin==a,p])==T,0,

      ifelse(popest[[k]][popest[[k]]$AgeBin==a,p]<=140,1,0))
                  test2<-(length(test1[test1==1])/ncon)*100
                  pass[p]<-test2
                  }
                  for(p in 4:7) {
                        pass[p]<-NA
                  }
                  for(p in 8:11) {
                        pass[p]<-NA
                  }
                  p.pass<-rbind(p.pass,pass)
            }
            p.pass[,1]<-agebin

            pass2<-c()
            for(p in 1:6) {
                  pass2[p]<-NA
                  }
            for(p in 7:12) {
                  pass2[p]<-NA
                  }
            for(p in 13:18) {
                  con<-is.na(popest2[[k]][,p])
                  ncon<-length(con[con==F])
                  test1<-ifelse(is.na(popest2[[k]][,p])==T, 0,
                              ifelse(popest2[[k]][,p]<=140, 1,0))
                  test2<-(length(test1[test1==1])/ncon)*100
                  pass2[p]<-test2
                  }

      meanpop <- aggregate(popest[[k]][
,popestnames],by=list(popest[[k]]$AgeBin),mean,na.rm=T)
      popest[[k]][c((nrep*nBins+1):(nrep*nBins+nBins)),] <- meanpop
      popest[[k]][c((nrep*nBins+nBins+1):(nrep*nBins+nBins*2)),] <-
p.pass
      popest[[k]][,c(PRECparms,BIASparms)]<-
round(popest[[k]][,c(PRECparms,BIASparms)],0)
      popest[[k]][,c(EQUIVparms)]<-round(popest[[k]][,c(EQUIVparms)],1)
      popest[[k]]$REP <- rep(c(1:nrep,"MEAN","POWER"),each=nBins)
      popest[[k]]<- popest[[k]][
,c(ncol(popest[[k]]),1:ncol(popest[[k]])-1)]
      write.table(popest[[k]],
file=paste(path,"results/method1/",vari,"_Sparse_",nAdult,"ad_M1_output",
k,".csv",sep=""),
            sep=",", quote=F, row.names=F, na=".")

      meanraw <- aggregate(popraw[[k]][
,poprawnames],by=list(popraw[[k]]$AgeBin),mean,na.rm=T)
      popraw[[k]][c((nrep*nBins+1):(nrep*nBins+nBins)),] <- meanraw
```

```
      popraw[[k]]$REP <- rep(c(1:nrep,"MEAN"),each=nBins)
      popraw[[k]]<- popraw[[k]][
,c(ncol(popraw[[k]]),1:ncol(popraw[[k]])-1)]

      popest2[[k]][nrep+1,] <- mean(popest2[[k]][1:nrep,],na.rm=T)
      popest2[[k]][nrep+2,] <- pass2
      popest2[[k]][,c(ESTcovar)]<-round(popest2[[k]][,c(ESTcovar)],3)
      popest2[[k]][,c(PRECcovar,BIAScovar)]<-
round(popest2[[k]][,c(PRECcovar,BIAScovar)],0)
      popest2[[k]]$REP <- c(c(1:nrep),"MEAN","POWER")
      popest2[[k]] <- popest2[[k]][
,c(ncol(popest2[[k]]),1:ncol(popest2[[k]])-1)]
      write.table(popest2[[k]],
file=paste(path,"results/method1/",vari,"_Sparse_",nAdult,"ad_M1_auxout",
k,".csv",sep=""),
      sep=",", quote=F, row.names=F, na=".")


      finalpop[f:(f+nBins*2-1), ]<-
popest[[k]][(nrep*nBins+1):(nrep*nBins+nBins*2), ]
      finalpop$SCENARIO[f:(f+nBins*2-1)]<-k
      f<-f+(nBins*2)

      finalpop2[c(g,g+1), ]<-popest2[[k]][c(nrep+1,nrep+2), ]
      finalpop2$SCENARIO[c(g,g+1)]<-k
      g<-g+2

      k<-k+1
}
}

finalpop<-finalpop[ ,c(ncol(finalpop),1:ncol(finalpop)-1)]
write.table(finalpop,
file=paste(path,"results/method1/",vari,"_Sparse_",nAdult,"ad_M1_summary.
csv",sep=""),sep=",", quote=F, row.names=F, na=".")

finalpop2<-finalpop2[ ,c(ncol(finalpop2),1:ncol(finalpop2)-1)]
write.table(finalpop2,
file=paste(path,"results/method1/",vari,"_Sparse_",nAdult,"ad_M1_auxsum.c
sv",sep=""),sep=",", quote=F, row.names=F, na=".")



## MODULE 7: READ IN SAS ESTIMATION OUTPUT (lst/parm/posthoc files) FOR
ANALYSIS : METHOD 2 - POSTHOC ##

posthocest <- list()
indraw<-list()
allposthoc<-list(list())
posthoc <- list()
posthoc.pkg<-list()
pop2<-list()
```

```
agebin<-1:nABins

finalposthoc<-
data.frame(matrix(NA,ncol=Nposthocest+2,nrow=Ntrials*nABins*2))
names(finalposthoc)<-c("METRIC","AgeBin",posthocestnames,"SCENARIO")

k<-1
f<-1
for (i in Nsub) {
      if (i==Nsub[1]) Nbin <- Nbin1
      if (i==Nsub[2]) Nbin <- Nbin2
      if (i==Nsub[3]) Nbin <- Nbin3
      if (i==Nsub[4]) Nbin <- Nbin4
      if (i==Nsub[5]) Nbin <- Nbin5
      if (i==Nsub[6]) Nbin <- Nbin6
      if (i==Nsub[7]) Nbin <- Nbin7

      Nbin<-Nbin[1:nABins]
      t<-qt(0.975,(((i-nAdult)/4)-1))

for (j in Nsamp) {

      posthocest[[k]]<-
data.frame(matrix(NA,ncol=Nposthocest,nrow=nrep*nABins))
      names(posthocest[[k]]) <- c("AgeBin",posthocestnames)
      indraw[[k]]<- data.frame(matrix(NA,ncol=Nindraw,nrow=nrep*nABins))
      names(indraw[[k]]) <- c("AgeBin",indrawnames)

      b<-1
      for(r in 1:nrep){
            output <- scan(file=paste("fit",k,"rep",r,".lst",sep=""),
what="character", sep="\n")
            thetaPos <- grep("Successful", output)

            ifelse(length(thetaPos)==0,
                  pop2[[r]]<-
data.frame(matrix(NA,ncol=Npop2,nrow=Nparms+Ncovar)),
                  pop2[[r]]<-
read.csv(paste(path,"model_output/parms",k,"rep",r,".csv",sep="")))
                  names(pop2[[r]])<-pop2names

            ifelse(length(thetaPos)==0,
                  posthoc[[r]]<-
data.frame(matrix(NA,ncol=Nposthoc,nrow=i)),
                  posthoc[[r]]<-
read.csv(paste(path,"model_output/posthoc",k,"rep",r,".csv",sep="")))
                  names(posthoc[[r]])<-posthocnames
            if(length(thetaPos)!=0)
                  posthoc[[r]]<-
posthoc[[r]][match(unique(posthoc[[r]]$ID),posthoc[[r]]$ID), ]

            posthoc.pkg[[r]]<-posthoc[[r]]
```

```
            if(length(thetaPos)!=0)
                  posthoc[[r]][ ,indparms]<-log(posthoc[[r]][ ,indparms])
            ifelse(length(thetaPos)==0,
                  posthocmean<-
data.frame(CLi=c(rep(NA,nABins)),Vi=c(rep(NA,nABins))),
                  posthocmean<- aggregate(posthoc[[r]][
,indparms],by=list(posthoc[[r]]$ABIN),mean,na.rm=T))
            ifelse(length(thetaPos)==0,posthocmean<-
posthocmean,posthocmean<- exp(posthocmean)[ ,indparms])
            posthocbias1<-((posthocmean-bankmean)/bankmean)*100
            posthocequiv1<-exp(log(posthocmean/bankmean))

            #allsim[[k]][[r]][ ,parms]<-log(allsim[[k]][[r]][ ,parms])
            simmean<-aggregate(allsim[[k]][[r]][
,parms],by=list(allsim[[k]][[r]]$ABIN),mean,na.rm=T)
            simmean<-exp(simmean[ ,parms])
            posthocbias2<-((posthocmean-simmean)/simmean)*100
            posthocequiv2<-exp(log(posthocmean/simmean))

            posthoc.pkg[[r]][ ,indparms]<-
                  posthoc.pkg[[r]][
,indparms]/c(((posthoc.pkg[[r]]$WT**ALLOCL)*(posthoc.pkg[[r]]$AGE/(postho
c.pkg[[r]]$AGE+A50))),posthoc.pkg[[r]]$WT**ALLOV)
            if(length(thetaPos)!=0)
                  posthoc.pkg[[r]][ ,indparms]<-log(posthoc.pkg[[r]][
,indparms])
            ifelse(length(thetaPos)==0,
                  posthocmean.pkg<-
data.frame(CLi=c(rep(NA,nABins)),Vi=c(rep(NA,nABins))),
                  posthocmean.pkg<- aggregate(posthoc.pkg[[r]][
,indparms],by=list(posthoc.pkg[[r]]$ABIN),mean,na.rm=T))
            ifelse(length(thetaPos)==0,
                  posthocvar.pkg<-
data.frame(CLi=c(rep(NA,nABins)),Vi=c(rep(NA,nABins))),
                  posthocvar.pkg<- aggregate(posthoc.pkg[[r]][
,indparms],by=list(posthoc.pkg[[r]]$ABIN),var,na.rm=T))
            ifelse(length(thetaPos)==0,
                  posthocsd.pkg<-
data.frame(CLi=c(rep(NA,nABins)),Vi=c(rep(NA,nABins))),
                  posthocsd.pkg<- aggregate(posthoc.pkg[[r]][
,indparms],by=list(posthoc.pkg[[r]]$ABIN),sd,na.rm=T))

            posthocse<- sqrt(posthocvar.pkg/Nbin)
            posthocUCI<-posthocmean.pkg+t*(posthocse)
            posthocUCI<-exp(posthocUCI)[ ,indparms]
            if(length(thetaPos)!=0) posthocmean.pkg<-
exp(posthocmean.pkg)[ ,indparms]
            posthocprec<-(posthocUCI/posthocmean.pkg)*100
            posthocRSE<-(posthocse[ ,indparms]/posthocmean.pkg)*100

            posthocsd.pkg<-(posthocsd.pkg)[ ,indparms]
```

```
            meanvar<-pop2[[r]][(Ncovar:(Ncovar+1)),pop2names[2]]
            meansdcl<-rep(sqrt(meanvar[1]),nABins)
            meansdv<-rep(sqrt(meanvar[2]),nABins)
            popsd<-cbind(meansdcl,meansdv)
            shrinkage<- (1-posthocsd.pkg/popsd)*100

            indest <-
cbind(agebin,posthocprec,posthocbias1,posthocbias2,posthocequiv1,posthoce
quiv2,shrinkage)
            posthocest[[k]][c(b:(nABins*r)),] <- indest
            indest2<-cbind(agebin,posthocmean,posthocRSE)
            indraw[[k]][c(b:(nABins*r)),] <- indest2
            b<-b+nABins
      }
      allposthoc[[k]] <- posthoc

      pass<-c()
      p.pass<-c()
      for(a in agebin) {
            for(p in 2:3) {
                  con<-
is.na(posthocest[[k]][posthocest[[k]]$AgeBin==a,p])
                  ncon<-length(con[con==F])
                  test1<-
ifelse(is.na(posthocest[[k]][posthocest[[k]]$AgeBin==a,p])==T,0,

      ifelse(posthocest[[k]][posthocest[[k]]$AgeBin==a,p]<=140, 1,0))
                  test2<-(length(test1[test1==1])/ncon)*100
                  pass[p]<-test2
                  }
            for(p in 4:7) {
                  pass[p]<-NA
                  }
            for(p in 8:11) {
                  pass[p]<-NA
                  }
            for(p in 12:13) {
                  pass[p]<-NA
                  }
            p.pass<-rbind(p.pass,pass)
      }
      p.pass[,1]<-agebin

      meanposthoc <- aggregate(posthocest[[k]][
,posthocestnames],by=list(posthocest[[k]]$AgeBin),mean,na.rm=T)
      posthocest[[k]][c((nrep*nABins+1):(nrep*nABins+nABins)),] <-
meanposthoc
      posthocest[[k]][c((nrep*nABins+nABins+1):(nrep*nABins+nABins*2)),]
<- p.pass
      posthocest[[k]][,c(PRECposthoc,BIAS1posthoc,BIAS2posthoc)]<-
round(posthocest[[k]][,c(PRECposthoc,BIAS1posthoc,BIAS2posthoc)],0)
```

```
      posthocest[[k]][,c(SHRINKposthoc,EQUIV1posthoc,EQUIV2posthoc)]<-
round(posthocest[[k]][,c(SHRINKposthoc,EQUIV1posthoc,EQUIV2posthoc)],1)
      posthocest[[k]]$REP <- rep(c(1:nrep,"MEAN","POWER"),each=nABins)
      posthocest[[k]]<- posthocest[[k]][
,c(ncol(posthocest[[k]]),1:ncol(posthocest[[k]])-1)]
      write.table(posthocest[[k]],
file=paste(path,"results/method2/",vari,"_Sparse_",nAdult,"ad_M2_output",
k,".csv",sep=""),
            sep=",", quote=F, row.names=F, na=".")

      meanraw <- aggregate(indraw[[k]][
,indrawnames],by=list(indraw[[k]]$AgeBin),mean,na.rm=T)
      indraw[[k]][c((nrep*nABins+1):(nrep*nABins+nABins)),] <- meanraw
      indraw[[k]]$REP <- rep(c(1:nrep,"MEAN"),each=nABins)
      indraw[[k]]<- indraw[[k]][
,c(ncol(indraw[[k]]),1:ncol(indraw[[k]])-1)]


      finalposthoc[f:(f+nABins*2-1), ]<-
posthocest[[k]][(nrep*nABins+1):(nrep*nABins+nABins*2), ]
      finalposthoc$SCENARIO[f:(f+nABins*2-1)]<-k
      f<-f+(nABins*2)
      k<-k+1
      }
}

finalposthoc<-finalposthoc[ ,c(ncol(finalposthoc),1:ncol(finalposthoc)-
1)]
write.table(finalposthoc,
file=paste(path,"results/method2/",vari,"_Sparse_",nAdult,"ad_M2_summary.
csv",sep=""),sep=",", quote=F, row.names=F, na=".")
```

NONMEM model

```
$PROBLEM sim_NM thru R ; truncated normal wrt t1/2, cl, v, ka
; PROGRAMMER=MALLIKA

$DATA inputk.csv IGNORE=@

$INPUT ID TIME AMT DV=CONC ABIN AGE WT SEX
;TIME=HRS, DV=CONC=ug/mL, AMT=DOSE=MG, ABIN=AgeBin=1-5, AGE=YRS, WT=KG,
SEX=1=M,2=F

$SIMULATION (12345678 NEW) ONLYSIM
SUBPROBLEMS = 1

$SUBROUTINE ADVAN2 TRANS2
;1 COMP. MODEL oral

$THETA
   3.2 FIX  ; CLTI (L/h/20kg)
   6.2 FIX  ; VTI  (L/20kg)
   2   FIX  ; KATI (/h)
   0.75 FIX ; ALLOCL
   1 FIX    ; ALLOV
   0.18 FIX ; A50 (years)

$OMEGA
   0.49 FIX ; CVCL
   0.49 FIX ; CVV
   0.16 FIX ; CVKA

$SIGMA
   0.01 FIX ; CVCP


$PK

 IF (ICALL.EQ.4) THEN

   CLTI   = THETA(1)
   VTI    = THETA(2)
   KATI   = THETA(3)
   ALLOCL = THETA(4)
   ALLOV  = THETA(5)
   A50    = THETA(6)

   ETCL   = ETA(1)
   ETV    = ETA(2)
   ETKA   = ETA(3)

   TVCL   = CLTI*((WT/20)**ALLOCL)*(AGE/(AGE+A50))
   TVV    = VTI*((WT/20)**ALLOV)
   TVKA   = KATI
```

```
TVKE   = TVCL/TVV
TVTHF  = 0.693/TVKE

    DLTACL  = 2*0.7        ; MUST BE 3.27*SQRT(CVCL)!
    DLTAV   = 2*0.7        ; MUST BE 3.27*SQRT(CVV)!
    DLTAKA  = 2*0.4
    DLTATH  = 2*0.7
    LNMUCL  = LOG(TVCL)
    LOCL    = EXP(LNMUCL-DLTACL)
    HICL    = EXP(LNMUCL+DLTACL)
    LNMUV   = LOG(TVV)
    LOV     = EXP(LNMUV-DLTAV)
    HIV     = EXP(LNMUV+DLTAV)
    LNMUKA  = LOG(TVKA)
    LOKA    = EXP(LNMUKA-DLTAKA)
    HIKA    = EXP(LNMUKA+DLTAKA)
    LNMUTH  = LOG(TVTHF)
    LOTHF   = EXP(LNMUTH-DLTATH)
    HITHF   = EXP(LNMUTH+DLTATH)


    CL=TVCL*EXP(ETCL)
    IF (CL.GE.LOCL.AND.CL.LE.HICL) THEN
       CLOK=1
    ELSE
       CLOK=0
    ENDIF

    V=TVV*EXP(ETV)
    IF (V.GE.LOV.AND.V.LE.HIV) THEN
       VOK=1
    ELSE
       VOK=0
    ENDIF

    KA=TVKA*EXP(ETKA)
    IF (KA.GE.LOKA.AND.KA.LE.HIKA) THEN
       KAOK=1
    ELSE
       KAOK=0
    ENDIF

    KE=CL/V
    THF=0.693/KE
    IF (THF.GE.LOTHF.AND.THF.LE.HITHF) THEN
       THFOK=1
    ELSE
       THFOK=0
    ENDIF

    DOWHILE (CLOK.EQ.0.OR.VOK.EQ.0.OR.KAOK.EQ.0.OR.THFOK.EQ.0)
       CALL SIMETA(ETA)
```

```
          ETCL   = ETA(1)
          ETV    = ETA(2)
          ETKA   = ETA(3)

          CL=TVCL*EXP(ETCL)
          IF (CL.GE.LOCL.AND.CL.LE.HICL) THEN
             CLOK=1
          ELSE
             CLOK=0
          ENDIF

          V=TVV*EXP(ETV)
          IF (V.GE.LOV.AND.V.LE.HIV) THEN
             VOK=1
          ELSE
             VOK=0
          ENDIF

          KA=TVKA*EXP(ETKA)
          IF (KA.GE.LOKA.AND.KA.LE.HIKA) THEN
             KAOK=1
          ELSE
             KAOK=0
          ENDIF

          KE=CL/V
          THF=0.693/KE
          IF (THF.GE.LOTHF.AND.THF.LE.HITHF) THEN
             THFOK=1
          ELSE
             THFOK=0
          ENDIF

       ENDDO
 ENDIF


S2 = V/1          ;S2 is scaling factor to comp. 2 (for oral);
Cp(t)=A(t)/S2

REP = IREP


$ERROR
ICP  = F
IRES = ERR(1)

Y = F + F*ERR(1)  ;proportional error model


$TABLE REP ID TIME AMT DV Y CL V KA THF ETCL ETV ABIN AGE WT SEX ICP IRES
```

SAS program

```
options mlogic mprint;

*delete all datasets in work;
proc datasets lib=work kill memtype=data;
quit;

*import data;
PROC IMPORT OUT= data
  DATAFILE="simdatakrepr.csv"
  DBMS=CSV REPLACE;
  GETNAMES=YES;
  DATAROW=2;
  RUN;
data dose; set data; if evid; dose=amt; keep id dose;run;
data data; merge data dose; by id; if evid=0; dv=conc; run;

*create the median age and wt combination within each age group for derived TVCL and TVV;
data newid; set data; by id ; if first.id; if id<=4; id=1000+id; dv=.;
if id=1001 then do; age=1 ; wt=9.7; end;
if id=1002 then do; age=4 ; wt=15.7; end;
if id=1003 then do; age=9 ; wt=29; end;
if id=1004 then do; age=14 ; wt=49.5; end;
run;
*attach newid to raw data;
data data; set data newid;run;

*fit model;
%let flag = 0;
%macro RunModel(pTVCL=,
pTVV=,
pALLOCL=,
pALLOV=,
pA50=,
ps2cl=,
ps2v=,
ps2=,
repeats=);

%let seedi = 18;
%let count = 0;
%let itvcl = &ptvcl;
%let itvv = &pTVV;
%let iallocl = &pALLOCL;
%let iallov = &pALLOV;
%let ia50 = &pA50;
%let is2cl = &ps2cl;
%let is2v = &ps2v;
%let is2 = &ps2;
%let tvclest = -1;
%let tvvest = -1;
```

```
%let alloclest = -1;
%let allovest = -1;
%let a50est = -1;
%let s2clest = -1;
%let s2vest = -1;
%let s2est = -1;

%do %while ((&flag = 0 or %sysevalf(&tvclest < 0) or %sysevalf(&tvvest < 0) or
%sysevalf(&alloclest < 0) or %sysevalf(&allovest < 0 ) or
  %sysevalf(&a50est < 0) or %sysevalf(&s2clest < 0) or %sysevalf(&s2vest < 0) or
%sysevalf(&s2est < 0 )) and &count < &repeats);

proc nlmixed data=data  QPOINTS=1;   *QPOINTS=1 is like Laplacian and METHOD=FIRO is like
FO in NONMEM;
    parms
TVCL=&iTVCL
TVV=&iTVV
ALLOCL=&iALLOCL
ALLOV=&iALLOV
A50=&iA50
s2cl=&is2cl
s2v=&is2v
s2=&is2;
   anchor = 20;
   TVKA = 0.6;
   s2ka = 0.09;
   F = 1;
      TVCLI= TVCL*((WT/anchor)**ALLOCL)*(AGE/(AGE+A50));
      CL = TVCLI*EXP(ETACL);
      TVVI=TVV*((WT/anchor)**ALLOV);
      V = TVVI*EXP(ETAV);
      KA = TVKA;
      KE = CL/V;
      TVKE = TVCL/TVV;
      pred= log(((F*dose*TVKA)/(TVV*(TVKA-TVKE)))*(exp(-TVKE*time)-exp(-TVKA*time)));
      ipred = log(((F*dose*KA)/(V*(KA-KE)))*(exp(-KE*time)-exp(-KA*time)));
      ldv=log(dv);
      model ldv ~ normal(ipred,s2);
      random ETACL ETAV ~ normal([0,0],[s2cl,0,s2v]) subject=id;
      *random ETACL ETAV ETAKA ~ normal([0,0,0],[s2cl,0,s2v,0,0,s2ka]) subject=id;
predict ipred out=ipred;
predict pred out=pred;
predict TVCLI out=TVCLI;
predict TVVI out=TVVI;
predict cl out=cl;
predict v out=v;
predict ka out=ka;
ods output  ParameterEstimates=para;
run;

*reset inital parameter est;
data _NULL_;
    set para;
```

```
    if _N_ = 1 then call symput("tvclest", Estimate);
    if _N_ = 2 then call symput("tvvest", Estimate);
    if _N_ = 3 then call symput("alloclest", Estimate);
    if _N_ = 4 then call symput("allovest", Estimate);
    if _N_ = 5 then call symput("a50est", Estimate);
    if _N_ = 6 then call symput("s2clest", Estimate);
    if _N_ = 7 then call symput("s2vest", Estimate);
    if _N_ = 8 then call symput("s2est", Estimate);
run;

data _NULL_;
    seed = &seedi;
    call ranuni(seed, rannum1);
    rannum = 1 + (rannum1 - 0.5) * 0.2;
    TVCL = &ptvcl * rannum;
    TVV = &ptvv * rannum;
    ALLOCL = &pallocl * rannum;
    ALLOV = &pallov * rannum;
    A50 = &pa50 * rannum;
    s2cl = &ps2cl * rannum;
    s2v = &ps2v * rannum;
    s2 = &ps2 * rannum;
    call symput("seedi", seed);
    call symput("itvcl", tvcl);
    call symput("itvv", tvv);
    call symput("iallocl", allocl);
    call symput("iallov", allov);
    call symput("ia50", a50);
    call symput("is2cl", s2cl);
    call symput("is2v", s2v);
    call symput("is2", s2);
run;

data _NULL_;
    set ipred;
    if _N_ ^= 0 and %sysevalf(&tvclest > 0) and %sysevalf(&tvvest > 0 ) and
%sysevalf(&alloclest > 0) and %sysevalf(&allovest > 0 ) and
     %sysevalf(&a50est > 0) and %sysevalf(&s2clest > 0 ) and %sysevalf(&s2vest > 0) and
%sysevalf(&s2est > 0 ) then
        call symput("flag", 1);
run;

%if &flag = 0 %then
%do;
    proc datasets library=work;
        delete ipred;
    quit;
%end;

%let count = &count + 1;
%end;
%mend RunModel;
%RunModel
```

```
(pTVCL=13.5,
pTVV=10.4,
pALLOCL=0.75,
pALLOV=1,
pA50=0.18,
ps2cl=0.09,
ps2v=0.09,
ps2=0.01,
repeats=20)

%macro printRes;

*tailoring output structure;

%if &flag=1 %then
%do;
data _NULL_;
title "Successful";
file print;
put "Successful";
run;

data tvcliout; set tvcli; if id>1000; keep id age wt Pred StdErrPred Upper CLbin SECL CVCL
UCICL; CLbin=Pred; SECL=StdErrPred; CVCL=StdErrPred/Pred*100; UCICL=Upper; run;
data tvviout; set tvvi; if id>1000; keep id age wt Pred StdErrPred Upper Vbin SEV CVV UCIV;
Vbin=Pred; SEV=StdErrPred; CVV=StdErrPred/Pred*100; UCIV=Upper; run;
data cliout; set cl; by id; if first.id; if id<1000; CLi=Pred; SECLi=StdErrPred;
UCICLi=Upper; run;    *pred in this file is the posthoc CL estimate for each id;
data viout; set v; by id; if first.id; if id<1000; Vi=Pred; SEVi=StdErrPred; UCIVi=Upper;
run;         *pred in this file is the posthoc V estimate for each id;
data kaiout; set ka; by id; if first.id; if id<1000; KAi=Pred; SEKAi=StdErrPred;
UCIKAi=Upper; run;    *pred in this file is the posthoc KA estimate for each id;
data pred; set pred; if id<1000; PPRED=exp(Pred); run;
data ipred; set ipred; if id<1000; IPRED=exp(Pred); run;
data bins; merge tvcliout tvviout; by id; keep id age wt CLbin SECL CVCL UCICL Vbin SEV CVV
UCIV; run;
data posthoc; merge cliout viout kaiout; by id; keep id abin age wt sex dose cli secli
ucicli vi sevi ucivi kai sekai ucikai; run;
data preds; merge pred ipred; by id; keep id time abin age wt sex dose dv ppred ipred; run;
data posthoc; merge posthoc preds; by id; run;

*output;
proc export data=bins outfile="binskrepr.csv" DBMS=CSV REPLACE; run;
proc export data=para outfile="parmskrepr.csv" DBMS=CSV REPLACE; run;
proc export data=posthoc outfile="posthockrepr.csv" DBMS=CSV REPLACE; run;
%end;
%mend printRes;
%printRes
run;
```

APPENDIX B

NONMEM model file used for simulations for warfarin pediatric model qualification (Chapter 5)

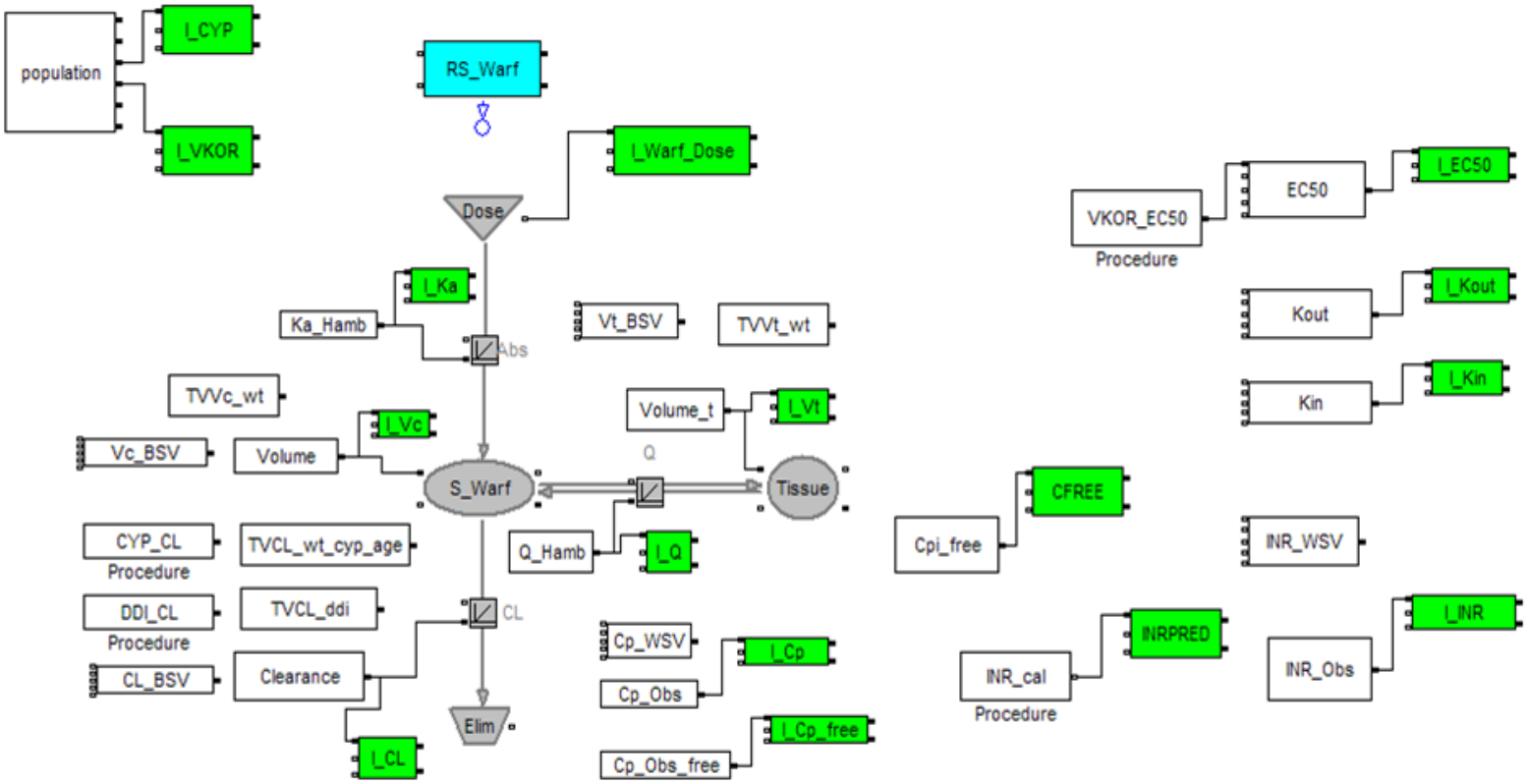
```
$PROB WARF PEDS PKPD MODEL VALIDATION

$INPUT C ID TIME DAY CMT AMT DV TYPE EVID MDV INRO AGE WT GENO CYP VKOR
TARG

$DATA nmdata3.csv IGNORE='C'

$SIMULATION (123456 NEW) ONLYSIM
SUBPROBLEMS = 150

$SUBROUTINE ADVAN6 TOL=6
;User defined model written as differential equations

$MODEL ; DEFINES THE NO. OF COMPARTMENTS IN THE MODEL

COMP = 1
COMP = 2
COMP = 3
COMP = 4


$THETA (0,0.01698,1)  ; TVKOUT
$THETA (0,0.019527,1) ; TVKIN
$OMEGA  0.0961        ;BSV CL
$OMEGA  0.0686        ;BSV V2
$OMEGA  0.9821        ;BSV V3
$OMEGA  0.000001      ;BSV EC50
$OMEGA  0.000025      ;BSV KOUT
$OMEGA  0.000025      ;BSV KIN

$SIGMA  0.3429        ;sd=0.585662


$PK

   TVKA = 2
   TVCL = 0.1207
```

```
   TVV2 = 3.45
   TVQ  = 0.05
   TVV3 = 1.65
   F1   = 0.5

   ETCL  = ETA(1)
   ETV2  = ETA(2)
   ETV3  = ETA(3)

   CLWT = TVCL*((WT/20)**0.75)*((0.821*AGE/(AGE+0.01))+0.21)*EXP(ETCL)
   CL   = CLWT
   IF (CYP.EQ.1)  CL = CLWT*0.685

   IF (CYP.EQ.2)  CL = CLWT*0.547

   IF (CYP.EQ.3)  CL = CLWT*0.28

   IF (CYP.EQ.4)  CL = CLWT*0.31

   IF (CYP.EQ.5)  CL = CLWT*0.148

   KA  = TVKA
   V2  = TVV2*((WT/20)**0.75)*EXP(ETV2)
   V3  = TVV3*((WT/20)**0.75)*EXP(ETV3)
   Q   = TVQ*((WT/20)**0.75)

   S2 = V2
; S2 is scaling factor to cmpt. 2 (for oral); Cp(t)=A(t)/S2 to get conc
in MG/L

TVKOUT = THETA(1)
TVKIN  = THETA(2)

ETEC50 = ETA(4)
ETKOUT = ETA(5)
ETKIN  = ETA(6)

TVEC50 = 0.003953
   IF (VKOR.EQ.1)  TVEC50 = 0.003075

   IF (VKOR.EQ.2)  TVEC50 = 0.002547

EC50 = TVEC50*EXP(ETEC50)
KOUT = TVKOUT*EXP(ETKOUT)
KIN  = TVKIN*EXP(ETKIN)
BSLN = KIN/KOUT         ; R0 baseline INR
F4   = BSLN

REP  = IREP


$DES
```

```
DADT(1) = -KA*A(1)
DADT(2) = (KA*A(1))+((Q/V3)*A(3))-((Q/V2)*A(2))-((CL/V2)*A(2))
DADT(3) = ((Q/V2)*A(2))-((Q/V3)*A(3))
CFREE = A(2)*0.01/S2
DADT(4) = KIN - KOUT*A(4)*(1-(CFREE/(CFREE+EC50)));
;INHIBITION OF OUTPUT SINCE INR IS RESPONSE INVERSELY RELATED TO PCA
(INHIBITORY INDIRECT RESPONSE MODEL)


$ERROR

IF (ICALL.EQ.4) THEN

   INR   = A(4)
   CFRE  = A(2)*0.01/S2
   IPRE  = INR
   RESID = ERR(1)

       LOINR = 1
       HIINR = 6

       Y = IPRE + RESID
       IF (Y.GE.LOINR.AND.Y.LE.HIINR) THEN
          INROK=1
       ELSE
          INROK=0
       ENDIF

       DOWHILE (INROK.EQ.0)
          CALL SIMEPS(EPS)
          RESID = ERR(1)
          Y = IPRE + RESID
          IF (Y.GE.LOINR.AND.Y.LE.HIINR) THEN
             INROK=1
          ELSE
             INROK=0
          ENDIF
       ENDDO
ELSE
   INR   = A(4)
   CFRE  = A(2)*0.01/S2
   IPRE  = INR
   RESID = ERR(1)
   Y = IPRE + RESID   ;ADDITIVE ERROR MODEL
ENDIF


$TABLE REP ID TIME DAY CMT AMT DV TYPE CFRE INR RESID Y INRO AGE WT GENO
CYP VKOR TARG CL EC50 BSLN KOUT KIN ETA1 ETA4 ETA5 ETA6
NOPRINT ONEHEADER FILE=sdtab8
```

APPENDIX C

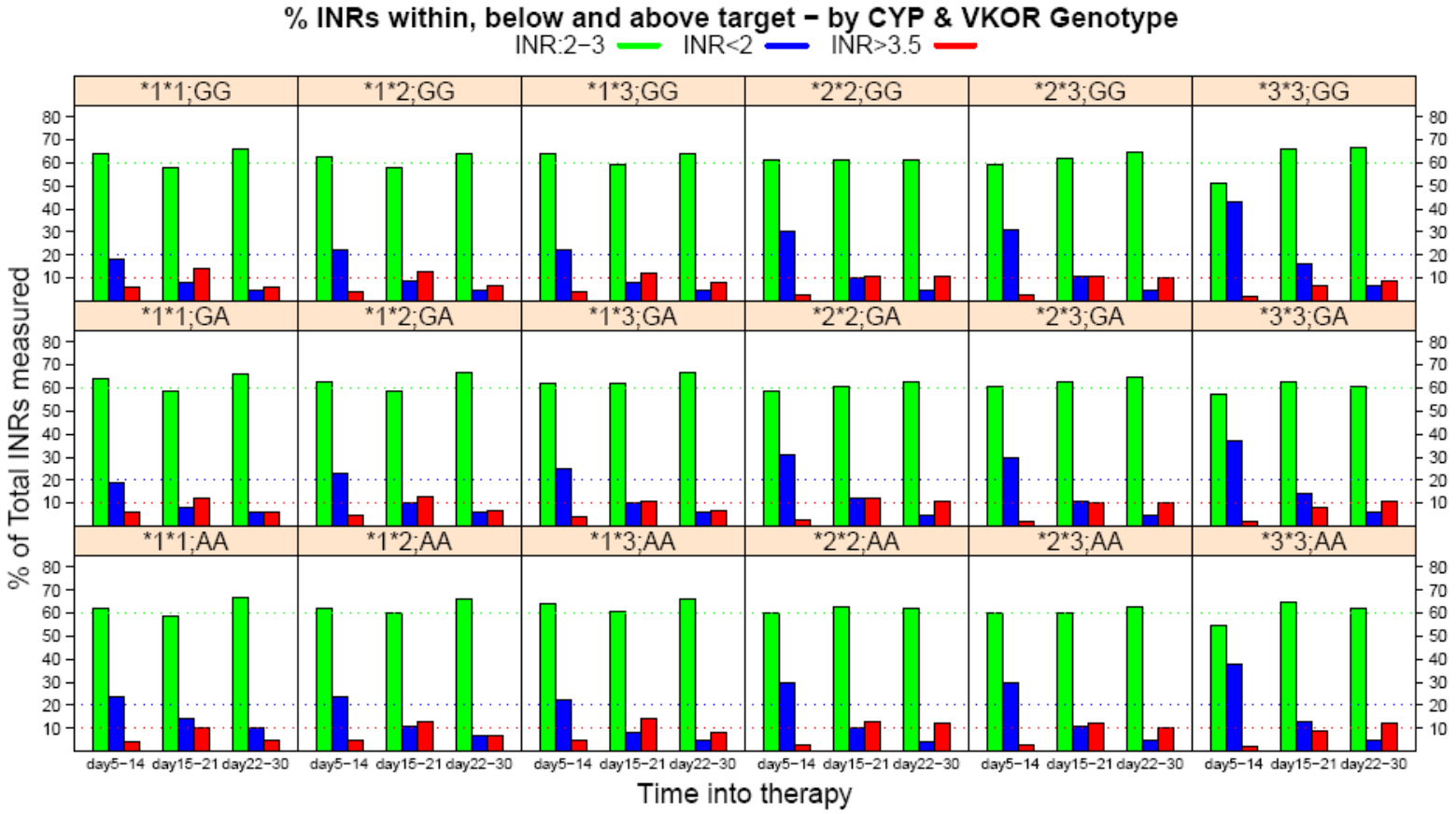
Drug Model set-up in Trial Simulator for optimizing pediatric warfarin dosing regimen (Chapter 5)



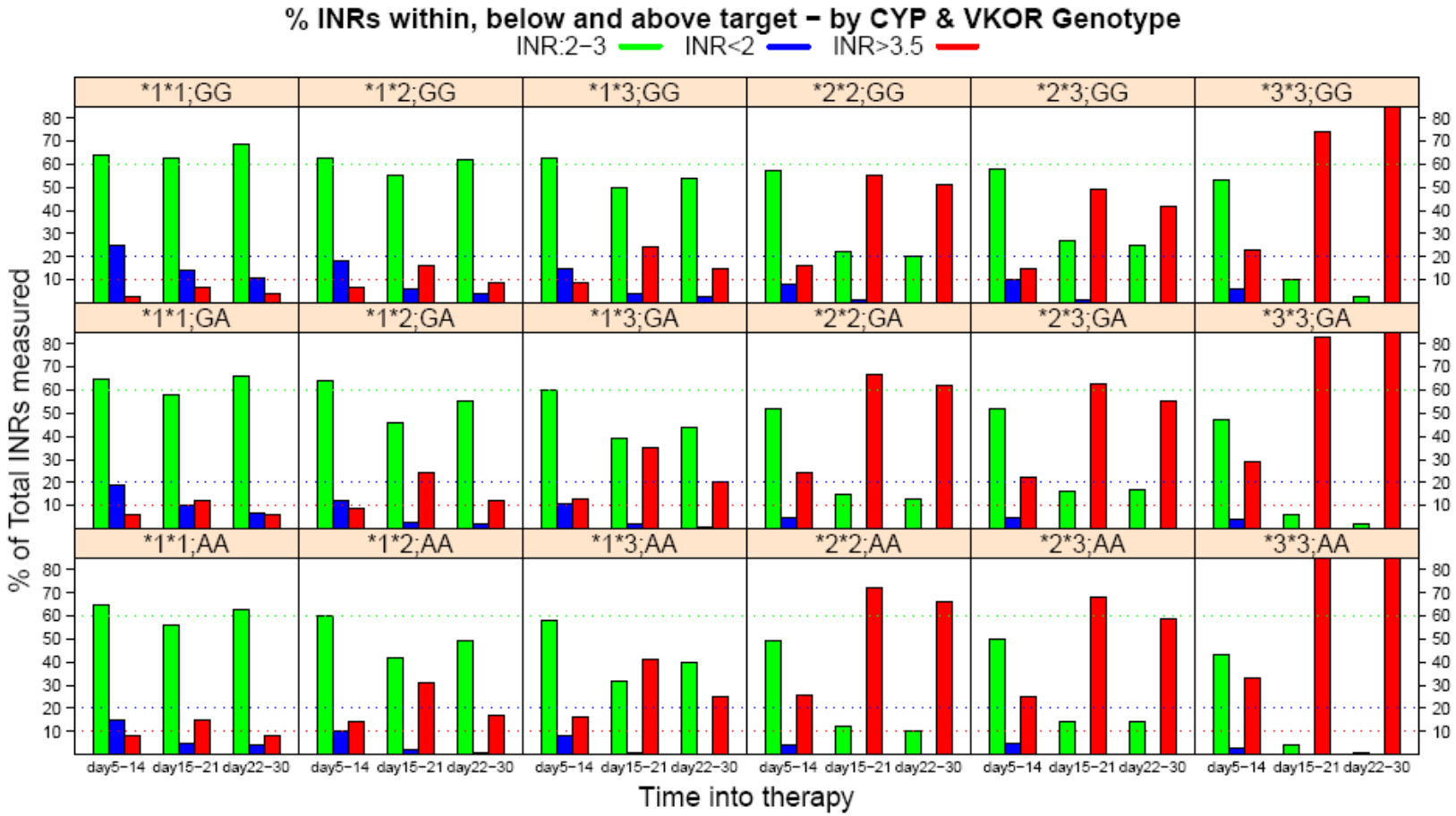
APPENDIX D

Target INR outcomes for all genotypes (Chapter 5)

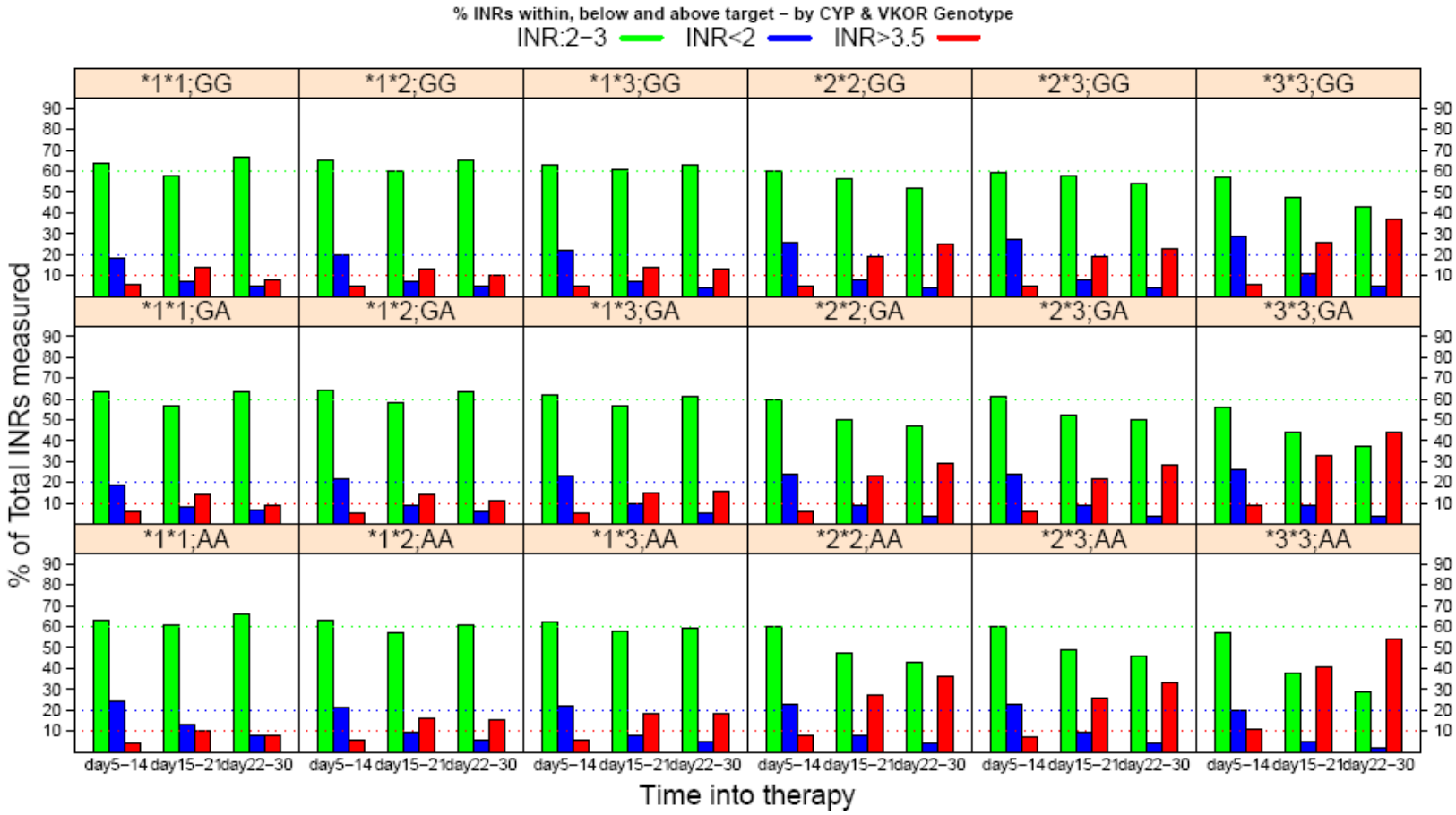
1. Proposed dosing regimen:



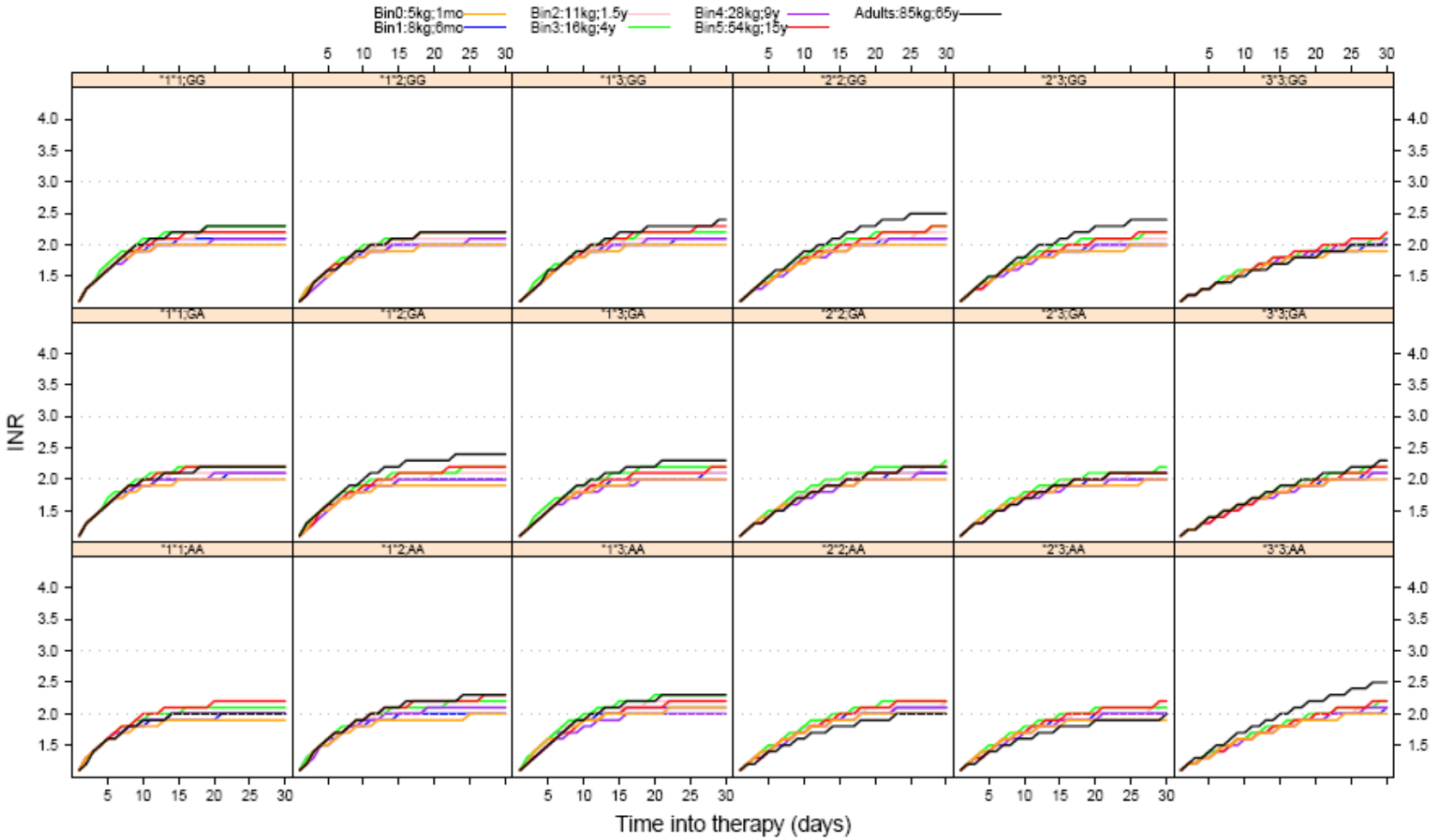
2. Genotype-independent dosing:



3. Proposed dosing regimen with formulation restrictions:



4. Initial starting dose estimates - INR-time profile in typical subjects:



APPENDIX E

Population Pharmacokinetic-Pharmacogenetic Analysis of Nevirapine in HIV-infected Populations in Uganda and the U.S. – A Covariate Exploration

ABSTRACT

The aims of this open-label, pharmacokinetic study were to characterize nevirapine pharmacokinetics in two geographically distinct populations of HIV- infected patients and to assess demographic and genetic covariates on drug exposures, focusing on the *CYP2B6, CYP3A4, CYP3A5*, and *MDR1*genes. A total of 46 HIV-infected adults underwent nevirapine sampling under steady state conditions. All data were analyzed using nonlinear mixed-effects modeling, and the population pharmacokinetic model was used to assess the effects of covariates. The following homozygous loss-of-function alleles, *CYP2B6 516G>T*, *CYP3A5*3* and *CYP3A4*1B,* were associated with 35%, 25% and 18% reductions in nevirapine clearance, respectively. These three genotypes in combination with body weight, explained 71% of the interpatient variability in nevirapine apparent clearance. Regardless of CYP genotype, all patients had trough nevirapine concentrations above the 3,000 ng/mL threshold. As previously noted by others, variability in apparent nevirapine clearance tended to be low and was heavily influenced by *CYP2B6 516G>T*.

INTRODUCTION

At the beginning of 2009, approximately 33.4 million people world-wide were infected with the human immunodeficiency virus (HIV); 22.5 million of these individuals live in sub-Saharan Africa (1). In recent years, significant progress has been made in providing antiretroviral therapy (ART) for HIV-infected patients residing in low and middle income countries. Expanded ART access has resulted in a 10-fold increase in the number of people receiving treatment in these underserved areas (2, 3). The availability of potent ART to developing nations has been largely driven by the manufacture and distribution of generic ART formulations. Of these, nevirapine has gained widespread use due to (a) its status as a recommended component of combination ART for treatment-naïve individuals who meet criteria for initiating therapy (4), (b) its ability to reduce mother-to-child transmission (MTCT) of HIV-1 (5) and (c) its availability as an affordable fixed-dose combination product (6).

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1, which binds directly to- and allosterically inhibits viral reverse transcriptase (RT) activity. Upon oral administration, the drug is rapidly absorbed (T_{max} = 2 h) with an absolute bioavailability of 90-93%. It has a long half-life of 25-30 hours following repeated dosing and is 60% bound to plasma proteins (7). Nevirapine undergoes oxidative metabolism by *CYP3A4* and *CYP2B6* enzymes, while the role of *CYP3A5* in nevirapine metabolism is not entirely clear (8). In addition to *CYP2B6* and *CYP3A*, nevirapine may also be a substrate for the *ABCB1* (*MDR1*) gene product and efflux transporter, P-glycoprotein (P-gp) (9). Genetic polymorphisms in the *CYP2B6, CYP3A4, CYP3A5*, and *ABCB1* genes may contribute to interindividual differences in nevirapine pharmacokinetics among different populations. Indeed, homozygous expression of the *CYP2B6 516TT* variant allele was found by us and others to result in higher nevirapine concentrations in Ugandan and Swiss populations, respectively (10, 11). Similarly, a population pharmacokinetic-pharmacogenetic study in Cambodian patients found that *CYP2B6 516TT* was associated with reduced nevirapine clearance compared with *CYP2B6 516GT* and *516GG* genotypes

(12). Nonetheless, there is still a general paucity of information regarding covariates influencing nevirapine pharmacokinetics among different populations.

The purpose of this pharmacokinetic-pharmacogenetic study was to characterize nevirapine pharmacokinetics in comparable non-Western (Uganda, Africa) and Western (United States) HIV-infected populations. A population approach was used to identify demographic and genetic factors that influence nevirapine disposition; genetic variability in *CYP2B6, CYP3A4, CYP3A5,* and *ABCB1* (*MDR1*) were targeted for this purpose. Non-linear mixed-effects modeling was used to characterize the influence of all covariates on nevirapine pharmacokinetic parameter values.

METHODS

Patients. Data from this study were sequentially acquired from two pooled cohorts of HIV-infected patients from Uganda Africa, and the United States, respectively. To be considered for study inclusion, candidates had to be HIV positive, $\geq$18 years old, and in good general health as determined by medical history, physical examination, and serum chemistry values. There were no minimum or maximum requirements with regard to CD4+ counts or HIV-RNA levels, although patients could not have any clinical or laboratory evidence of an active opportunistic infection. Exclusion criteria also included receipt of interleukin-2 within 3 months of study participation, receipt of any medications known or suspected to modulate CYP2B6 and/or CYP3A4/5 activity, active drug or alcohol abuse, pregnancy, chronic diarrhea or loose stools, fever > 38.5 ^{0}C within 7 days of screening, and a history of poor adherence to antiretroviral therapy. After the Ugandan cohort completed their portion of the study, a comparator group of U.S. subjects was selected from the National Institutes of Allergy and Infectious Diseases (NIAID) outpatient HIV clinic and included subjects who were matched by gender and BMI to their Ugandan counterparts.

The study was approved by the Joint Clinical Research Center Institutional Review Board, the Uganda National Council for Science and Technology, and the National Institute of Allergy and Infectious Diseases Institutional Review Board. All participants gave written informed consent, and clinical research was conducted according to guidelines for human experimentation as specified by the US Department of Health and Human Services.

Study procedures. Because the study was designed to characterize nevirapine pharmacokinetics in two different HIV-infected populations, a single nevirapine formulation (Viramune™) Boehringer Ingelheim) was administered to both groups to eliminate the possibility of a formulation effect on study results. As such, Ugandan participants who were stabilized on a generic nevirapine formulation for at least 28 days were switched to brand name nevirapine (Viramune™) 200 mg twice daily; the remainder of their antiretroviral regimen remained unchanged. Due to the unavailability of generic nevirapine formulations in the U.S., patients were already stabilized on a Viramune-containing regimen (200 mg twice daily) for at least 28 days. To this end, pharmacokinetic sampling for both groups occurred under steady state conditions for all study participants.

In the Ugandan cohort, subjects were admitted to the Clinical Research Center the night before scheduled pharmacokinetic sampling. The evening nevirapine dose was observed by study personnel for all subjects and the time of administration recorded. The next morning, after an overnight fast, an intravenous catheter was placed into the forearm vein of participants for the purposes of blood drawing. Just prior to taking their morning nevirapine dose (12 hrs after the previous night's dose), blood was collected into heparinized tubes for a time 0 hr nevirapine concentration. Blood was also collected into EDTA tubes for determination of *CYP2B6*, *CYP3A4/5*, and *MDR1* genotypes as described below. Next, subjects took their morning 200 mg dose of nevirapine with 100 mL of water and a standardized breakfast provided by the clinic. Four hours after taking nevirapine,

subjects were free to eat lunch. Adherence with antiretroviral medications was assessed by patient interview and pill counts.

Sampling and bioanalysis. Blood samples (15 mL) for the determination of nevirapine concentrations were collected in heparinized (green top) tubes immediately before (time 0), and 2 and 6 hours after dosing. Blood was centrifuged after collection and plasma was harvested and frozen until the time of analysis. Nevirapine concentrations in human plasma were measured using a high-performance liquid chromatography (HPLC) liquid–liquid extraction method. Percentage errors, as a measure of accuracy, were <10%, and the inter- and intra-assay coefficients of variation were 4.35 – 8.55% and 3.54 – 6.52% respectively (R^2 = 0.998), and the limit of detection was 25 ng/ml. Blood samples collected during the study were also used to determine *CYP2B6, CYP3A4/5*, and *MDR1*genotypes of the study subjects for further genetic analysis.

Genetic analysis. Venous blood samples were obtained from all subjects, and DNA was isolated from peripheral leucocytes with the Qiamp system (Qiagen Inc, Valencia, CA). CYP2B6 (516GG, 516GT and 516TT) and CYP3A4*1B genotypes, and/or the CYP3A5*3 null allele together with the MDR1 genotype at position 2677 were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) as previously described (23, 24, 25, 26). Based on CYP2B6, CYP3A4*1B and the CYP3A5*3 genotypes, subjects were phenotypically identified as “poor”, “intermediate” and “extensive” nevirapine metabolizers. No ultrarapid metabolizers were identified. Further, based on the MDR1 genotype, subjects were phenotypically identified as “poor”, “intermediate” and “extensive” nevirapine transporters.

Pharmacokinetic analysis. A population approach was used for the current pharmacokinetic analysis. Previously, population PK models for nevirapine have been published (13, 14, 15) that are consistent with regard to the base structural, one-compartment body model with first order absorption. The primary objective of this study

was to mainly update the covariate model. While we tested for effects of all available covariates, the focus was on the effects of four genes – *CYP2B6, CYP3A4, CYP3A5* and *MDR1*. All analyses were carried out using the non-linear mixed-effects modeling software NONMEM version VI. All graphics were generated using R version 2.9.1.

Prior base model. The available pharmacokinetic data were sparse such that we could not estimate the oral absorption parameters. We used prior information on the base model from a previous study (13) and fixed the parameter values for the first-order absorption rate constant (Ka) and its variability (Ω^2_{Ka}) accordingly. The prior model included a linear body weight effect parameter (WT_{eff}) on clearance, using a proportional model. Between-subject variability (BSV) was explained using a proportional error model while within-subject or residual variability (WSV) was accounted for using an additive error model. We adapted our base model from this prior as follows:

$$CL_i = TVCL \bullet \left(1 + WT_{eff} \bullet \left(WT_i - 70\right)\right) \bullet \left(1 + \eta_{CL_i}\right) \quad (2)$$

$$Vc_i = TVVc \bullet \left(1 + \eta_{Vc_i}\right) \quad (3)$$

$$Ka_i = TVKa \bullet \left(1 + \eta_{Ka_i}\right) \quad (4)$$

$$Cp_i = Cp_{pred} + \varepsilon_{Cp} \quad (5)$$

where; η_{CLi} is the difference between individual (CL_i) and population mean or typical value (TVCL) of clearance for a 70-kg individual, η_{Vci} is the difference between individual (Vc_i) and population mean or typical value (TVVc) of volume of distribution and η_{Kai} is the difference between individual (Ka_i) and population mean or typical value (TVKa) of absorption rate constant, while Cp_i is the individual observed plasma concentration and Cp_{pred} is the individual model predicted plasma concentration. η_{CLi}, η_{Vci} and η_{Kai} were all assumed to follow a normal distribution with mean of zero and variances of Ω^2_{CL}, Ω^2_{Vc} and Ω^2_{Ka} respectively, and with η_{CLi} and η_{Vci} having a correlated distribution. ε_{Cp} is the

residual error assumed to follow a normal distribution with mean of zero and variance of σ^2_{Cp}.

We fit the prior model, without modification, to our study data to determine if it describes the observed individual level data well. We also tested use of an exponential error model for explaining BSV on all model parameters rather than a proportional model. We explored estimating BSV on volume with and without a covariance with clearance. We also considered fixing the BSV on volume to the value from the prior model, or leaving this parameter out of the model altogether.

Covariate exploration. We first tested the inclusion of each genetic and demographic covariate individually into the model. Covariate significance was assessed based on mechanistic plausibility, decrease in model objective function value ($\Delta OFV \geq -4$), and graphical inspection of overall model fit and covariate plots. While testing for covariate effects, we estimated all model parameters other than mean Ka and its variability.

We explored retaining the weight effect parameter on clearance as per the prior proportional model and estimating a weight effect parameter. We also considered an allometric scaling model for weight effect. We then modified the clearance model as described in the *Results* section above. Based on mechanism, effects of the three CYP450 isoforms were tested on nevirapine clearance and that of the *MDR1* gene on bioavailability. We used a stepwise approach for inclusion of multiple gene effects into the model, based upon the significance of individual effects. This approach is outlined in **Table 3.**

Initially, we ran the model with no gene effect included. Upon inspection of covariate plots for the four genes (vs. predicted apparent clearance (CL/F) residuals) we observed gene effects only for the homozygous variant genotypes. Residuals were similar for the wild type ("extensive" metabolizer) and heterozygous ("intermediate" metabolizer) genotypes. Hence for analysis of all gene effects, subjects were categorized into 2

genotype groups – "extensive" metabolizers (or transporters in case of *MDR1*) including wild type and heterozygous genotypes or "poor" metabolizers (or transporters in case of *MDR1*) including only the homozygous variant genotype. Since the two *MDR1* genotypes (2677G>T/A and 3435C>T) on which data were available occur in linkage disequilibrium we focused our analysis for gene effects only on the *MDR1 2677* polymorphism since there were more subjects (n = 4) with this variation than for the silent mutation *MDR1 3435* (n = 2).

Although our study population was comprised of two cohorts, we considered the influence of covariate effects based on race (White vs. Black) rather than region (U.S vs. Uganda). Gender, age, height and BMI were the other covariates we considered for effects on both clearance and volume, although previously published work on nevirapine population pharmacokinetics only found either body weight or gender to be of significance.

RESULTS

Patients

A total of 46 patients met inclusion criteria and were included in the final analysis (24 and 22 subjects from the U.S. and Ugandan cohorts, respectively). Cohort demographics are provided in **Table 1.**

Table 1 Characteristics of 46 HIV infected participants in this population pharmacokinetic analysis from the U.S. and Uganda

	U.S. SUBJECTS (mean values and range)	**UGANDAN SUBJECTS** (mean values and range)	**Total**

N	22	24	46
AGE	37.23 (21-50)	35.75 (27 - 64)	36.9 (21-64)
HEIGHT (cm)	164.27 (145.7 - 184.8)	166.65 (155.3 - 195)	165.6 (145.7 - 195)
WEIGHT (kg)	72.18 (47.4 - 98.5)	65.33 (40 - 87.5)	68.5 (40-98.5)
BMI	26.82 (22.01 - 37.75)	23.57 (14.3 - 29.7)	25 (14.3 - 37.76)
GENDER			
F	14 (63.6%)	16 (66.7%)	30
M	8 (36.4%)	8 (33.4%)	16
RACE			
BLACK	9	24	
WHITE	13		
GENOTYPE			
CYP2B6-516			
Extensive metabolizers **GG**	11 (50%)	13 (54.2%)	24 (52%)
Intermediate metabolizers **GT**	8 (36.4%)	7 (29.2%)	15 (33%)
Poor metabolizers **TT**	3 (13.6%)	4 (16.7%)	7 (15%)
CYP3A4*1B			
Extensive metabolizers **AA**	12 (54.5%)	0	12 (26%)
Intermediate metabolizers **AG**	3 (13.6%)	15 (62.5%)	18 (39%)
Poor metabolizers **GG**	7 (31.8%)	9 (37.5%)	16 (35%)
CYP3A5*3			
Extensive metabolizers **AA**	6 (27.3%)	14 (58.3%)	20 (44%)

Intermediate metabolizers **AG**	5 (22.7%)	8 (33.3%)	13 (28%)
Poor metabolizers **GG**	11 (50%)	2 (8.3%)	13 28%)
MDR1-2677			
Extensive transporters **GG**	11 (50%)	23 (95.8%)	34 (74%)
Intermediate transporters **GT**	7 (31.9%)	1 (4.2%)	8 (17%)
Poor transporters **TT**	4 (18.2%)	0	4 (9%)

Pharmacokinetic analysis

Prior base model. The sparseness of the available pharmacokinetic data precluded estimation of oral absorption parameters. Hence, the base model was adapted from a previous study (13). The prior model, without modification, fit the individual observed data well. Using an exponential error model for between-subject variability **(**BSV) resulted in an increase in objective function value **(**OFV); hence we retained the proportional error model to describe BSV on all primary model parameters. We also found that estimating BSV on volume of distribution along with a covariance with clearance yielded the best fit in terms of OFV, standard error (SE) on mean volume estimate and predicted volume residual plots (data not shown).

Covariate exploration. We could not estimate a weight effect parameter for clearance since the effective body weight range in both cohorts was limited (60-90 kg). Nonetheless, removing the weight effect on clearance adversely impacted the overall model fit; hence we chose to retain this covariate in our model. However, we used a physiologically more

plausible allometric scaling model, with an exponent of 0.75 for body weight effect, rather than the proportional model used previously. Besides weight, we did not find any other demographic covariate to be of significance. Thus, our modified base clearance model is as follows:

$$CL_i = TVCL \bullet \left(\frac{WT_i}{70} \right)^{0.75} \bullet \left(1 + \eta_{CL_i}\right) \quad (1)$$

where; η_{CLi} is the difference between individual clearance (CL_i) and population mean or typical value (TVCL) of clearance for a 70-kg individual.

Genetic covariates were initially assessed individually for their effect on nevirapine clearance. The *CYP2B6* variant genotype was found to be most significant (31% reduction in clearance, Decrease in Objective Function Value (ΔOFV) = -8) followed by the *CYP3A5* variant genotype (19% reduction in clearance, ΔOFV= -4). As lone covariates, the *CYP3A4* and *MDR1* variant genotypes were not found to significantly affect nevirapine clearance.

Table 2 summarizes our findings of gene effects on nevirapine clearance. The plots of individual and population predicted vs. observed plasma concentrations are depicted in **Figure 1**. Improved model fits can be observed for the population predictions upon addition of significant gene effects. **Figure 2** shows the covariate plots (individual - population mean predicted apparent clearance (CL/F) residuals vs. covariate levels) for the relevant genotype effects.

Table 2 Step-wise inclusion of gene-effects into model

STEP	GENE	EFFECT* (on CL)	95%CI	OFV

1	NONE			247
2	*CYP2B6*	-31%	-18 to -44%	239
3	*CYP2B6*	-31%	-19 to -43%	
	CYP3A5	-19%	-5 to -32%	235
4	*CYP2B6*	-35%	-24 to -46 %	
	CYP3A5	-25%	-13 to -37%	
	CYP3A4	-18%	-5 to 31%	231
5	*CYP2B6*	-36%	-26 to -46%	
	CYP3A5	-21%	-6 to -36%	
	CYP3A4	-18%	-1 to -39%	
	MDR1	+22% (eff. on F)	-14 to +65%	230

Figure 1: Observed vs. predicted nevirapine plasma concentrations

(a) No gene effect included (b) CYP2B6, CYP3A5 and CYP3A4 gene effects included

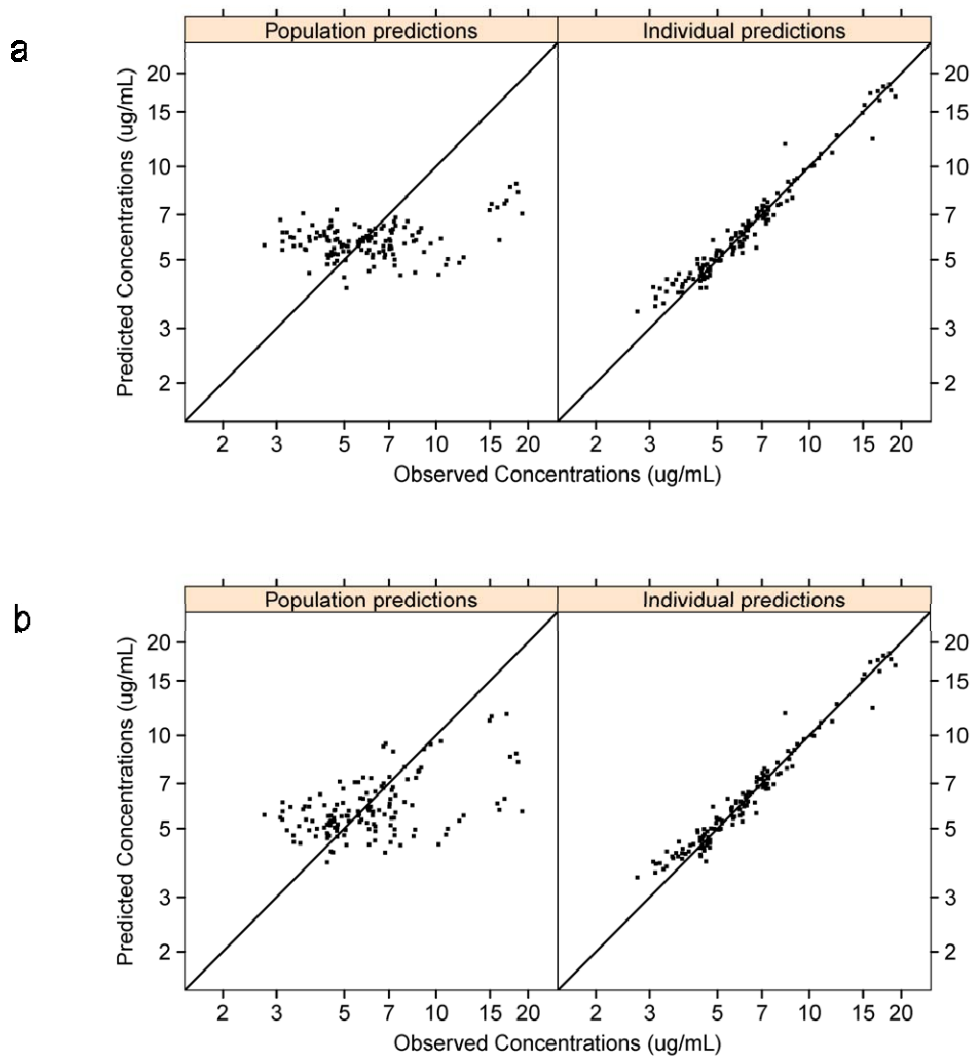


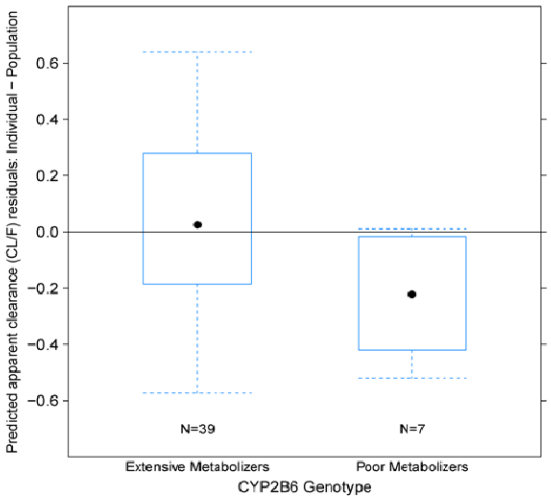
Figure 2: Covariate plots showing individual vs. population mean predicted apparent clearance (CL/F) residuals for genotype effects

(a) No gene effect included (b) CYP2B6 gene effect included

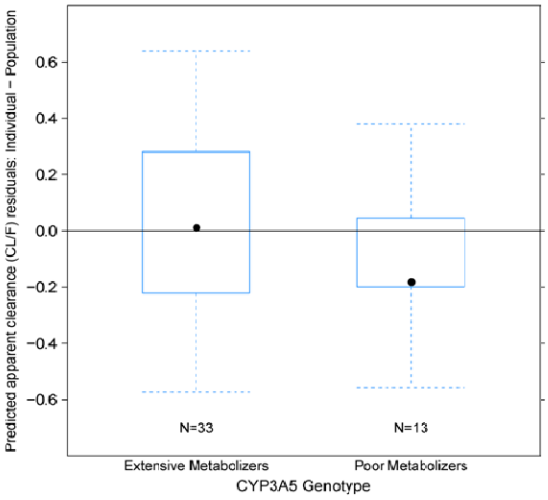
(c) CYP2B6 and CYP3A5 gene effects included (d) CYP2B6, CYP3A5 and CYP3A4 gene effects included

*Extensive metabolizers reflect pooled wild and heterozygous genotypes. Poor metabolizers reflect homozygous variant genotypes alone.

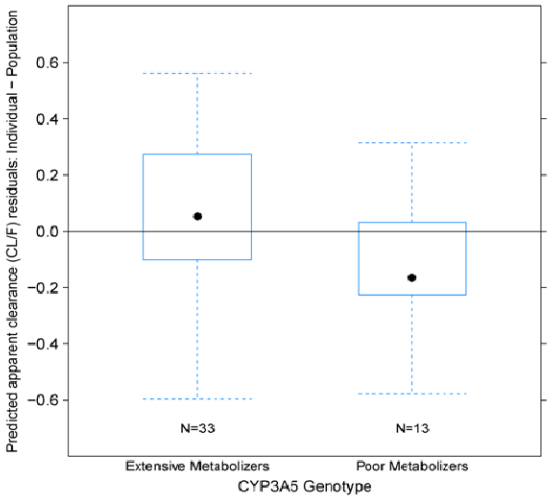
a1


Predicted apparent clearance (CL/F) residuals: Individual – Population
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
N=39
N=7
Extensive Metabolizers
Poor Metabolizers
CYP2B6 Genotype

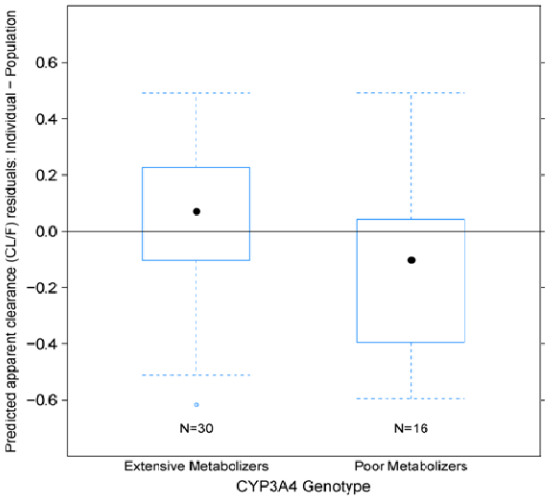
a2


Predicted apparent clearance (CL/F) residuals: Individual – Population
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
N=33
N=13
Extensive Metabolizers
Poor Metabolizers
CYP3A5 Genotype

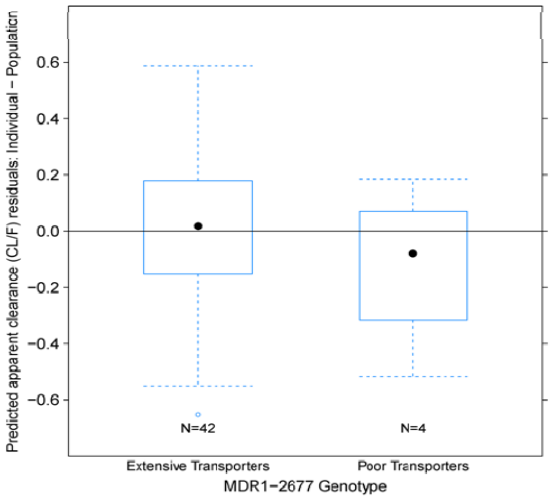
b


Predicted apparent clearance (CL/F) residuals: Individual – Population
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
N=33
N=13
Extensive Metabolizers
Poor Metabolizers
CYP3A5 Genotype

c


Predicted apparent clearance (CL/F) residuals: Individual – Population
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
N=30
N=16
Extensive Metabolizers
Poor Metabolizers
CYP3A4 Genotype

d


Predicted apparent clearance (CL/F) residuals: Individual – Population
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
N=42
N=4
Extensive Transporters
Poor Transporters
MDR1−2677 Genotype

In the absence of any gene effect in the model, the covariate plots were indicative of a genotype effect for *CYP2B6* and *CYP3A5* genes (**Figure 2a**). Hence, we first included the *CYP2B6* variant genotype effect on nevirapine clearance in our model and found statistical significance (ΔOFV = -8, **Table 2**) and improved graphical model fit. The average exposures (C_{avg}) were 8.63 ug/ml and 6.33 μg/ml in poor and extensive metabolizers respectively.

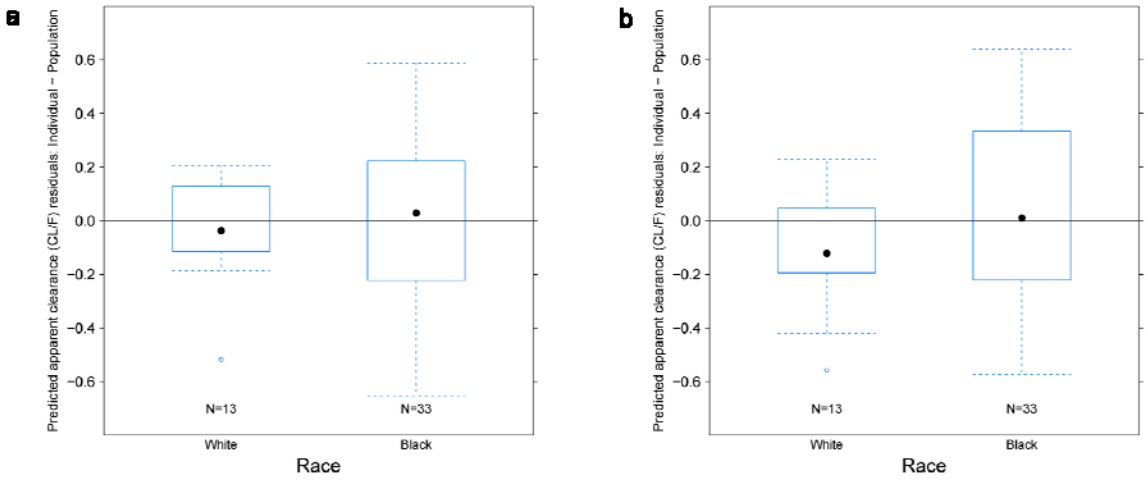
Following inclusion of *CYP2B6* in the model, the covariate plots were still indicative of a genotype effect for the *CYP3A5* gene **(Figure 2b).** We included the *CYP3A5* variant genotype effect next and found this covariate to be marginally statistically significant (ΔOFV= -4, **Table 2**). The covariate plots now revealed a *CYP3A4* genotype effect that was absent earlier **(Figure 2c)** and a mild, if any, *MDR1* genotype effect (not shown). Hence the third genetic covariate we added to our model was the *CYP3A4* variant genotype and again found it to be marginally statistically significant (ΔOFV= -4, **Table 2**). Finally, the *MDR1* genotype effect on bioavailability was tested, since the covariate plots still indicated a mild signal **(Figure 2d)**; however, the effect was not found to be statistically significant (ΔOFV= -1, **Table 2**) and did not improve the graphical model fit.

We considered differences in nevirapine clearance across race (White vs. Black). In absence of any gene effect in the model, the covariate plots indicate a potential 'race effect'. However, upon inclusion of the three CYP450 isoforms into the covariate model for clearance, this apparent race effect was no longer present in the covariate plots and the variability in apparent clearance residuals within each race was also relatively reduced **(Figure 3)**.

Figure 3: Covariate plots showing individual vs. population mean predicted apparent clearance (CL/F) residuals for race effects

(a) No gene effect included (b) CYP2B6, CYP3A5 and CYP3A4 gene effects included

*Extensive metabolizers reflect pooled wild and heterozygous genotypes. Poor metabolizers reflect homozygous variant genotypes alone.



Final model parameters are presented in **Table 3**. While our parameter estimate for volume of distribution (287 L) is higher when compared with previous models (70-210 L), of note is the fact that this parameter estimate appears to vary considerably between different models (12, 13, 14, 15). When mean volume was fixed to the prior model value (106 L) (13), there was bias seen in the predicted volume residual (η_{Vci}) plots. Estimating the mean volume parameter eliminated such bias yielding uniformly distributed residuals.

Table 3 Final model parameters

Parameter	Estimate (RSE %)	Variability (RSE %)
Mean Clearance (TVCL)	3.62 L/h/70kg (6.6)	29% (22.2)
Mean Volume (TVVc)	287 L (19.8)	46% (24.8)

Absorption rate constant (TVKa)	1.68 /h [fixed]	38% [fixed]
WTeff_CL	0.75 [fixed]	
CYP2B6 effect on CL	-35% (16.1)	
CYP3A5 effect on CL	-25% (24.8)	
CYP3A4 effect on CL	-18% (36)	
Residual error	0.63 ug/ml (43.9)	

RSE%: Relative Standard Error percentage

DISCUSSION

The base model parameters from our population model of nevirapine pharmacokinetics in this study are in agreement with previously published accounts (12, 13, 14, 15) and they represent the first covariate model to identify significant genotype effects on nevirapine disposition in an African cohort. Our data are in line with prior findings that have linked low body weight to an increase in nevirapine exposure (13, 15, 16). In fact, body weight was the only demographic covariate with a significant impact on drug exposure, as no differences in nevirapine pharmacokinetics were observed based on race (White vs. Black), when accounting for the effects of the CYP genotypes in the model. Thereby, any observed differences in nevirapine exposure among patients of different racial (or regional) backgrounds are likely due to differential distribution of variant genotypes among different races, particularly *CYP2B6* and *CYP3A5*. Highlighting this point, the *CYP2B6 516TT* genotype, which is associated with minimal metabolic activity of the CYP2B6 enzyme, was more prevalent among White subjects (24%) compared with Black subjects (12%), while the *CYP3A5*3* genotype, which is associated with minimal CYP3A5 activity, was predominant in White subjects (85%) and negligible among Blacks (6%). The *CYP3A4*1B* variant genotype was absent among Whites and 50% prevalent in the Black race.

In an initial study of nevirapine trough concentrations in the same Ugandan cohort reported herein, we observed a significant association between nevirapine pre-dose concentration

and *CYP2B6* genotype (10). Consistent with these findings, our population analysis revealed that the *CYP2B6* genotype was the most significant covariate of the population model, being associated with a 35% reduction in nevirapine clearance. After *CYP2B6*, *CYP3A5*3* and *CYP3A4*1B* variant genotypes were identified as secondary covariates, each of which explained 25% and 18% reductions in nevirapine clearance, respectively. The identification of *CYP2B6* and *CYP3A5* variant alleles as contributors to nevirapine clearance is consistent with previously published data (12). However, this is the first nevirapine population model that included *CYP3A4*1B*. Even though the reduction in variability in nevirapine clearance explained by *CYP3A4*1B* was relatively minor, inclusion of this polymorphism improved the fit of our model and may be considered in future studies assessing the influence of genetic covariates on nevirapine clearance.

Finally, our analysis indicated a non-significant increase of 20% in nevirapine bioavailability in patients with the *MDR1 2677TT* variant genotype, when tested in context of all genes. However, inclusion of the *MDR1* gene effect did not improve the graphical model fit. This is likely because nevirapine exhibits high oral bioavailability (> 90%), suggesting that drug concentrations in the gastrointestinal tract would likely exceed those necessary to saturate P-gp –assuming the nevirapine is in fact a P-gp substrate (17). To this end, any alteration in nevirapine bioavailability due to *MDR1* genotype would appear to carry little, if any, clinical significance.

Perhaps the most important concern with reduced nevirapine clearance is the risk of persisting subtherapeutic concentrations. This is particularly relevant to pregnant women who receive a single dose of nevirapine for the prevention of mother-to-child-transmission (MTCT) of HIV in Africa. Indeed single-dose nevirapine administered intrapartum, has been associated with detectable concentrations of the drug in plasma between 1-3 weeks after drug administration (18, 19, 20). Mothers with the *CYP2B6 516TT* genotype, which is associated with reduced nevirapine clearance, may be at particular risk for persisting nevirapine concentrations and development of NNRTI resistance mutations (K103N and

Y181C) (21). However, a recent investigation assessed the influence of *CYP2B6 516G>T* on nevirapine concentrations in HIV-infected Thai women and found that this polymorphism had only a minor impact on nevirapine concentrations following a single intrapartum nevirapine dose (22).

This study has several limitations. Our study design employed a limited sampling strategy of 3 samples per subject with one pre-dose trough sample (time 0), and two post-dose samples at 2 and 6 hours, respectively. As a result, our data was missing information on the absorption phase of nevirapine and we could not estimate oral absorption parameters. Hence we were unable to generate a complete model *de novo*, so we used a Bayesian-like approach where we adopted prior information on these base model parameters from previous nevirapine models. Notably, leveraging prior information where possible is considered good practice in model building. Next, we recognize that our sample size of 46 is comparatively smaller than other nevirapine population pharmacokinetic studies. However, our results are consistent with previously reported findings from nevirapine population pharmacokinetic studies, and the mechanistic reasoning for including the selected genetic covariates is based on solid scientific rationale. Lastly, since all of our HIV-infected patients were in good general health, we did not consider creatinine clearance or co-morbidities as factors potentially impacting nevirapine apparent clearance. Yet, absence of significant co-morbidities in our patient population allowed us to isolate the influence of the genetic polymorphisms on nevirapine disposition.
Despite these limitations, data from this study show that the three genetic polymorphisms, *CYP2B6, CYP3A4*1B* and *CYP3A5*3* and body weight collectively explained 71% of variability in nevirapine clearance.

Pharmacogenetic-pharmacokinetic data explaining the impact of genetic polymorphisms on nevirapine clearance have not been previously reported in a Ugandan - U.S. cohort. These data were largely consistent with those recently reported in HIV-infected Cambodian patients where *CYP2B6* also had the greatest impact on nevirapine clearance

(12). Also similar to patients from other studies, all of our patients had nevirapine trough concentrations in excess of 3,000 ng/mL compared to 95% of patients in the *Chou et al.* investigation (12). The main theoretical concern in individuals with the three genetic polymorphisms mentioned above, particularly the *CYP2B6 516TT* variant, is the potential for increased risk of nevirapine toxicity or development of nevirapine resistance due to higher and/or persisting plasma concentrations when the drug is used for prevention of MTCT. Further study into the pharmacogenetics and pharmacokinetics of nevirapine in diverse patient populations will likely illicit information that will allow clinicians to optimize the use of this agent in developing nations. Model-based dose adjustments may also be considered for future study in homozygous variant genotype individuals to avoid toxicity due to higher drug exposures.

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25. Tsuchiya, N. *et al*. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplantation* **78**, 1182–1187 (2004).

26. Kurata, Y. *et al*. Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin. Pharmacol. Ther.* **72**, 209–219 (2002).

VITA

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Current position

May 2011 **Virginia Commonwealth University (VCU/School of Pharmacy)** **Richmond, VA**

Ph.D. candidate in Dept. of Pharmacotherapy and Outcomes Science

June 2009 **Food and Drug Administration (FDA/CDER/OTS/Office of Clinical Pharmacology)**

to May 2011 **Silver Spring, MD**

Fellow in Division of Pharmacometrics - completing research for Ph.D. dissertation

- Research interests: pharmacometrics, pharmacogenetics, pediatric pharmacotherapy
- Dissertation work: Application of Pharmacometric Methods to Improve Pediatric Drug Development. Encompasses mainly two projects:
  1. Simulation-based methodology for designing pediatric clinical trials to meet the regulatory PK quality standard
     - Objective: to evaluate the feasibility and methodological challenges while designing a pediatric study to comply with a recent regulatory requirement.
  2. A Genetics-based pediatric warfarin dosing regimen
     - Objective: to derive the first ever dosing guidelines for pediatric warfarin use, including starting dose and titration scheme for dose individualization, focusing on CYP2C9 and VKORC1 genetic polymorphisms.
- Major co-advisers: Dr. Joga Gobburu (FDA) and Dr. Douglas Boudinot (VCU)
- Cumulative GPA : 4.0

Publications and Presentations

Manuscripts

- Population Pharmacokinetic-Pharmacogenetic Analysis of Nevirapine in HIV-infected Populations in Uganda and the U.S. – A Covariate Exploration

M Lala*, KM Vanevski*, JVS Gobburu, G Kabuye, P Mugyenyi, V Natarajan, RM Alfaro, H Masur, JJL Lertora and SR Penzak
*Denotes equal contribution
Original research paper - submitted to Clinical Pharmacology and Therapeutics, Feb. '11

- CLARIFICATION ON USING THE 20% SE CRITERIA WHEN DESIGNING PEDIATRIC PK STUDIES
  Wang Y, Lala M, Jadhav PR and Gobburu JVS

Short communication - submitted to Journal of Clinical Pharmacology, April '11.

- Covariate models – Do not center at values outside the data range.
  Lala M, Gobburu J and Wang Y

Short communication - submitted to Journal of Clinical Pharmacology, April '11.

- Pharmacometric Analyses Impact Pediatric Drug Approval and Dosing
  Mallika Lala and Pravin R Jadhav

Review article – to be submitted to Journal of Pediatrics, April '11.

- Simulation-based Method for using PK Quality Standard to Design Pediatric Trials in the Population Analysis Setting
  Mallika Lala, Yaning Wang, Pravin Jadhav, Joga Gobburu

Original research paper – to be submitted to Journal of Pharmacokinetics and Pharmacodynamics, April '11.

Book Chapter

- Pharmacometrics: Concepts and Applications to Drug Development
  Mallika Lala and Jogarao V. S. Gobburu

In process for publication in Immunotherapy in Transplantation: Principles and Practice by Wiley-Blackwell publishers, April '11.

Posters

- Implementation of a Pharmacokinetic Quality Standard to Improve Pediatric Trial Design. Presented at:
  - ACOP (American Conference on Pharmacometrics), April 2011
  - Research and Career Day, VCU School of Pharmacy, October 2010.
- Population Pharmacokinetic-Pharmacogenetic Analysis of Nevirapine in HIV-infected patients in Uganda and the U.S. – A Covariate Exploration. Presented at:
  - ACOP, April 2011.
  - Research and Career Day, VCU School of Pharmacy, October 2010.
- Covariate models – Do not center at values outside the data range. Presented at ACOP, April 2011.
- Cost-effectiveness Analysis of implementing warfarin dosing using a pharmacogenetic model. Presented at AACC annual conference, July 2009.
- Comparison of Pharmacogenetic Models for prediction of Indivdualized Warfarin Dosing. Presented at:

- AACC (American Association for Clinical Chemistry) annual conference, July 2008.
- Research and Career Day, VCU School of Pharmacy, October 2008.

Academic History

Aug. 2006 to date **School of Pharmacy, VCU** **Richmond, VA**

- Awarded full tuition waiver based on undergraduate academic profile and experience.
- SmartWarf Clinical study: PI - Dr. Bonny Bukaveckas
  - Objective: to build an adult warfarin-dosing model using retrospective multiple regression analysis of patient data including CYP2C9 and VKORC1 genetic polymorphisms, age, weight, gender, race and INR; and to test clinical effectiveness of dosing model over standard of care warfarin management in the anticoagulation clinic at MCV.
  - Contributions: involved with study design, patient recruitment and data collection and fully responsible for data archiving and analysis.
  - Study was terminated prematurely due to patient recruitment problems.
- Workshop on PK-PD Modeling conducted by Dr. Jurgen Venitz, Dept. of Pharmaceutics. Gained experiential training in modeling completed the course for credit with grade A.
- Workshop on Population PK Modeling conducted by Dr. Joga Gobburu, Dr. Pravin Jadhav, Dr. Christopher Tornoe and Dr. Yaning Wang from Division of Pharmacometrics, FDA. Conceptual and hands-on training in population modeling. Completed the course with a journal club presentation to the group from FDA.
- Pharmacoeconomic project on cost-effectiveness analysis of implementing warfarin dosing using a pharmacogenetic model under supervision of Dr. David Holdford, Dept. of Pharmacotherapy and Outcomes Science. Involved thorough understanding and implementation of cost-effectiveness studies, writing of project report and poster presentations.
- Departmental Research Seminars:
  - Spring ’07: “Individualized warfarin dosing, the time has come”. Literature review seminar, covering the past work done on warfarin pharmacogenetics, the current state of the field, its merits and limitations.
  - Spring ’08: “Individualizing warfarin dosing, building the right model”. Research design seminar, covering the hypothesis, specific aims and planned methods for the adult warfarin PGx project.
  - Spring ’09: “Pharmacogenetics: the present and the future, warfarin and beyond”. Literature review seminar, covering the current successful clinical applications of pharmacogenetics and future avenues, as well as a summary of the warfarin PGx project and the potential for newer methods, mainly pharmacometrics, to improve dosing of warfarin and other drugs.
  - Spring ’10: “Approaches to assess quality of PK data for designing pediatric trials”. Research design seminar, covering rationale, aims and methods for a simulations-based pediatric trial design project, proposed as part of dissertation research at FDA.

July '02 to May '06 **University of Mumbai, Institute of Chemical Technology (UICT)** **Mumbai, India**
Degree earned: **Bachelor of Pharmaceutical Sciences (B. Pharm. Sci.)**

- Cumulative grade: **Distinction** (**75%** aggregate score)
- **Research Seminar** on 'Estrogen as a neuroprotective'. The work entailed a comprehensive literature review and was presented at the Dept. of Pharmaceutical Sciences & Technology, UICT (Jan. '05).
- Member of the **TB Fact Card Project** group - the pioneer project of its kind in India initiated by the IPSF & IPA - for creating awareness about the course & gravity of the disease and helping in the implementation of proper drug therapy via co-ordination with various retail pharmacists all over the city of Mumbai. (May 2005-06)
- Participant in workshop on **Bioinformatics and Drug Design** at SIES-Institute of Environmental Management, Mumbai. (April 2005).

Employment Record

June '09 to date **FDA (Office of Clinical Pharmacology)** **Silver Spring, MD**
FELLOW in Division of Pharmacometrics

- Undertaken two major research projects as part of Ph.D. dissertation.
- NIH collaboration research project: derived a population pharmacokinetic-pharmacogenetic model for the drug Nevirpaine used as HIV treatment. Wrote and submitted manuscript on the project in collaboration with researchers from National Instititutes of Health (July'10-Feb'11).
- Conducted six pediatric IND reviews.
- Additional assignments by supervisors.

Aug. '06 to Aug. '09 **Virginia Commonwealth University (VCU)** **Richmond, VA**
TEACHING ASSISTANT in Dept. of Pharmacy

- Skills Labs: Trained 1st and 2nd year Pharm.D. students in pharmacy skills - role playing (patient counseling, tele- prescription filling with physician etc), services (measuring blood pressure, cholesterol, blood glucose etc), parenteral nutrition bag preparation under aseptic conditions.
- Courses and Labs: Prepared quizzes, posted quizzes to blackboard, administered paper quizzes and exams in class, administered clicker quizzes in class, graded quizzes, exams and homework assignments, both MCQ type and subjective essay-type, posted grades to blackboard.
- Assisted with overall conduct of classes and labs and co-ordination of course activities, maintained blackboard course websites and handled course, instructor, and peer evaluations.

June-July ’05 **Pfizer** **Mumbai, India**

STUDENT INTERN

- Exposure to the sophisticated working environment of a multinational pharmaceutical company.
- Overview training of operations in various departments of the industry viz. Production, Quality Control, cR&D Pilot Plant, Engineering & Maintenance, Pharmacy, Stores and P.P.I.C.
- Conducted a comprehensive study and presentation of how a large-scale pharmaceutical plant functions, to support the knowledge heretofore gained by coursework and small-scale laboratory work at school.

Technical Skills

- Data analysis and simulation software programs –
  NONMEM, R, SAS, **Trial Simulator, DATA** (cost-effectiveness modeling)
- Laboratory techniques for genetic testing –
  PCR and **Microarray**

Achievements

- Selected as **Phi Kappa Phi Graduate School Scholarship Recipient** from School of Pharmacy, VCU for two years – 2008 and 2010.
- Invited to membership of the **Honor Society of Phi Kappa Phi** for academic excellence - October 2007.
- Elected to the post of **Vice President** for the **Graduate Student Association** of the Dept. of Pharmacy, VCU School of Pharmacy - 2007.