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Utilisation et développement de techniques pharmacocinétiques avancées afin d'améliorer le développement de molécules pharmaceutiques

Improving drug development through the use and development of advanced pharmacokinetic techniques

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Résumé

Malgré le progrès technologique et nos connaissances pharmaceutiques et médicales croissantes, le développement du médicament demeure un processus difficile, dispendieux, long et très risqué. Ce processus mérite d'être amélioré pour faciliter le développement de nouveaux traitements. À cette fin, cette thèse vise à démontrer l'utilité de principes avancés et d'outils élaborés en pharmacocinétique (PK), actuels et nouveaux. Ces outils serviront à répondre efficacement à des questions importantes lors du développement d'un médicament, sauvant ainsi du temps et des coûts.

Le premier volet de la thèse porte sur l'utilisation de la modélisation et des simulations et la création d'un nouveau modèle afin d'établir la bioéquivalence entre deux formulations de complexe de gluconate ferrique de sodium en solution de sucrose pour injection. Comparé aux méthodes courantes, cette nouvelle approche proposée se libère de plusieurs présuppositions, et requiert moins de données. Cette technique bénéficie d'une robustesse scientifique tout en étant associée à des économies de temps et de coûts. Donc, même si développé pour produits génériques, elle pourra également s'avérer utile dans le développement de molécules innovatrices et « biosimilaires ».

Le deuxième volet décrit l'emploi de la modélisation pour mieux comprendre et quantifier les facteurs influençant la PK et la pharmacodynamie (PD) d'une nouvelle protéine thérapeutique, la pegloticase. L'analyse a démontré qu'aucun ajustement posologique n'était nécessaire et ces résultats sont inclus dans la monographie officielle du produit. Grâce à la modélisation, on pouvait répondre à des questions importantes concernant le dosage d'un médicament sans passer par des nouvelles études ni d'évaluations supplémentaires sur les patients. Donc, l'utilisation de cet outil a permis de réduire les dépenses sans prolonger le processus de développement. Le modèle développé dans le cadre de cette analyse pourrait servir à mieux comprendre d'autres protéines thérapeutiques, incluant leurs propriétés immunogènes.

Le dernier volet démontre l'utilité de la modélisation et des simulations dans le choix des régimes posologiques d'un antibiotique (TP-434) pour une étude de Phase 2. Des

données provenant d'études de Phase 1 ont été modélisées au fur et à mesure qu'elles devenaient disponibles, afin de construire un modèle décrivant le profil pharmacocinétique du TP-434. Ce processus de modélisation exemplifiait les cycles exploratoires et confirmatoires décrits par Sheiner. Ainsi, en se basant sur des relations PK/PD d'un antibiotique de classe identique, des simulations ont été effectuées avec le modèle PK final, afin de proposer de nouveaux régimes posologiques susceptibles d'être efficace chez les patients avant même d'effectuer des études. Cette démarche rationnelle a mené à l'utilisation de régimes posologiques avec une possibilité accrue d'efficacité, sans le dosage inutile des patients. Ainsi, on s'est dispensé d'études ou de cohortes supplémentaires coûteuses qui auraient prolongé le processus de développement. Enfin, cette analyse est la première à démontrer l'application de ces techniques dans le choix des doses d'antibiotique pour une étude de Phase 2.

En conclusion, cette recherche démontre que des outils de PK avancés comme la modélisation et les simulations ainsi que le développement de nouveaux modèles peuvent répondre efficacement et souvent de manière plus robuste à des questions essentielles lors du processus de développement du médicament, tout en réduisant les coûts et en épargnant du temps.

Mots-clés : modélisation de population, pharmacocinétique, pharmacodynamie, développement du médicament

Abstract

Despite the scientific and technological breakthroughs that have graced the last century, the path to bringing a drug to the market is fraught with risk and remains an expensive and time-consuming process. Significant improvements to this process are needed if patients are to continue to benefit from new therapies. The objective of this thesis is to demonstrate the use of modeling and simulations in this regard. It will show how advanced pharmacokinetic (PK) techniques can be used to answer critical questions that arise during the drug development process, and that their use can lead to cost and time savings.

The first part of this thesis shows how modeling and simulations, including the development of an innovative model, can be used for the relative bioequivalence assessment of a new sodium ferric gluconate complex intravenous formulation. Compared to traditional methods, this approach did not make inaccurate assumptions about drug characteristics, nor did it necessitate the enrollment of hundreds of subjects. Thus, this scientifically robust approach was associated with significant financial savings and economy of time. Although this research was conducted within the generic drug development, it could also be applied to innovator or biosimilar drug development.

The second section illustrates the use of modeling and simulations in the development of an innovative biological therapeutic agent, pegloticase. This approach allowed us to answer important questions pertaining to the factors influencing pegloticase's PK and pharmacodynamics (PD), confirming that no special dosing adjustments were required, thus contributing to the official product labeling. These questions were answered without conducting additional trials or performing supplementary assessments on patients, which resulted in significant cost and time savings. In the future, such PK/PD models could be used to better understand other biological agents, including their immunogenicity profiles which remain a concern for clinicians.

The third part of this thesis focuses on the use of modeling and simulations to select optimal dosing regimens for the Phase 2 study of the novel antibiotic TP-434. A PK model

for TP-434 was developed with Phase 1 data. This model was continuously updated and confirmed as new Phase 1 data became available, similar to the approach advocated by Sheiner's learn and confirm paradigm. Based on simulations performed with this model, and knowledge of PK/PD relationships for another compound, Phase 2 dosing regimens were recommended that were likely to show efficacy in the patient population. This rational approach to dose selection ensured that patients were not needlessly exposed to the drug, and that efficacy would likely be demonstrated in Phase 2. Consequently, it was unnecessary to dose additional cohorts or conduct additional trials, which would have lengthened the process and been expensive. Moreover, this was the first published account of Phase 2 dose regimen selection based solely on simulated Phase 1 data for an antimicrobial drug.

In summary, the research presented in this thesis illustrates how advanced pharmacokinetic techniques like modeling and simulations, including the creation of innovative models, can efficiently answer key drug development questions, leading to significant cost and time savings.

Keywords : population modeling, pharmacokinetics, pharmacodynamics, drug development

Table of contents

Résumé i
Abstract iii
Table of contents v
List of Tables ix
List of Figures xi
List of Abbreviations and Symbols xiii
Dedication xv
Acknowledgments xvi

Preface 1
Chapter 1 – Introduction 3
1. The Drug 3
2. Pharmacokinetic and Pharmacodynamic Principles 6
2.1 Pharmacokinetics 6
2.2 Pharmacodynamics 14
3. Quantification of Pharmacokinetic and Pharmacodynamic Properties 19
3.1 Noncompartmental Analysis 19
3.2 Compartmental Analysis 22
3.3 Differences Between Noncompartmental and Compartmental Approaches 32
4. The Drug Development Process 33
4.1 Pre-clinical 34
4.2 Clinical 34
4.3 Generic Product Development 37
5. Current Use of Modeling and Simulations in the DDP 41
5.1 Innovator Drugs 41
5.2 Generic Drugs 44
6. Challenges in the Drug Development Process 44
6.1 High Costs 45
6.2 High Attrition Rates 47

6.3 Room for Improvement 49
7. Research Hypotheses 53

Chapter 2 – Article #1 55
Novel Population Pharmacokinetic Method Compared to the Standard Noncompartmental Approach to Assess Bioequivalence of Iron Gluconate Formulations 55
2.1 Preface 56
2.2 Abstract 59
2.3 Introduction 60
2.4 Subjects and Methods 63
2.4.1 Study Design 63
2.4.2 Population 64
2.4.3 Ethics 64
2.4.4 Sample Handling and Bioanalytical Method 65
2.4.5 Population Pharmacokinetic Modeling 65
2.4.6 Statistical Analyses for Bioequivalence Assessment 67
2.4.7 Noncompartmental Analyses 68
2.5 Results 69
2.5.1 Population Characteristics 69
2.5.1 Population PK Approach 71
2.5.1 Noncompartmental Approach 79
2.6 Discussion 80
2.7 Conclusions 84
2.8 Abbreviations 85
2.9 References 87

Chapter 3 – Article #2 92
Population pharmacokinetic and pharmacodynamic analysis of pegloticase administered by intravenous infusion in two dose regimens to subjects with chronic gout 92
3.1 Preface 93
3.2 Abstract 96
3.3 Introduction 96
3.4 Methods 98
3.4.1 Study Design 98
3.4.2 Ethics 100
3.4.3 Inclusion and Exclusion Criteria 100
3.4.4 Analytical Methods 100
3.4.5 Population PK/PD Analysis 101
3.5 Results 103
3.6 Discussion 111
3.6.1 Pharmacokinetics 111
3.6.2 Pharmacodynamics 113
3.6.3 Antibodies 117
3.7 Conclusion 118
3.8 Conflict of Interest/Disclosure 119
3.9 References 119

Chapter 4 – Article #3 124
Optimizing Drug Development of TP-434, a Novel Fluorocycline, with Adaptive Learn & Confirm Cycles of Modeling & Simulation Using Single Ascending Dose Data 124
4.1 Preface 125
4.2 Abstract 128
4.3 Introduction 129
4.4 Materials and Methods 130
4.4.1 Study Design 130
4.4.2 Analytical Methods 131
4.4.3 Pharmacokinetic Analyses 132
4.4.4 Simulations 134
4.5 Results 135
4.6 Discussion 146
4.7 Conclusion 149
4.8 References 149

Chapter 5 – Article #4 152
Population Pharmacokinetic Modeling of TP-434 Following Multiple Dose Administration 152
5.1 Preface 153
5.2 Abstract 154
5.3 Introduction 156
5.4 Materials and Methods 157
5.4.1 Study Design 157
5.4.2 Analytical Methods 158
5.4.3 Pharmacokinetic Analyses 158
5.5 Results 161
5.6 Discussion 168
5.7 Conclusion 171
5.8 References 171

Chapter 6 – General Discussion 173

Chapter 7 - Conclusion 193

List of Tables

CHAPTER 1

Table 1. Comparison of Least-Squares Methods..... 25

Table 2. Advantages and Disadvantages of Noncompartmental and Compartmental Population Analyses..... 32

CHAPTER 2

Table 1. Demographic Traits of Subjects Included in the Population PK Analysis 70

Table 2. PK Model Discrimination.....71

Table 3. Population PK Parameter Estimates 74

Table 4. Secondary PK Parameters 77

Table 5. Summary of the Statistical Analyses for Ln-transformed PK Parameters 78

CHAPTER 3

Table 1. Sampling Schedule 99

Table 2. Summary Demographics of Subjects 104

Table 3. Population PK/PD Parameters of Pegloticase 109

Table 4. Bayesian Terminal Elimination Half-life Estimates 110

CHAPTER 4

Table 1. Subject Demographics.....136

Table 2. Structural Model Determination for TP-434 using Standard Two-Stage Analysis.....137

Table 3. Population Pharmacokinetic Parameter Estimates for TP-434.....141

Table 4. Predicted Secondary Pharmacokinetic Parameters for TP-434 142

Table 5. Secondary Simulated Steady-State Pharmacokinetic Parameters for TP-434 144

CHAPTER 5

Table 1. Subject Demographics 161

Table 2. Structural Model Determination for TP-434 using Standard Two-Stage Analysis 162

Table 3. Population Pharmacokinetic Parameter Estimates for TP-434 Following Repeated Administration 166

Table 4. Secondary Pharmacokinetic Parameters for TP-434 167

List of Figures

CHAPTER 1

Figure 1. Illustration of Typical Pharmacokinetic Processes 7
Figure 2. Relationship between Drug Pharmacokinetics and Pharmacodynamics 16
Figure 3. Indirect PD Models 18
Figure 4. Example of a Concentration Time Profile from Individual Compartmental Analysis 24
Figure 5. Example of Concentration Time Profiles from Population Compartmental Analysis 28
Figure 6. Phases of the Drug Development Process 33
Figure 7. Example of a Dose-Response Curve in a Therapeutic Equivalence Study 39
Figure 8. Biopharmaceutics Classification System 40
Figure 9. Capitalized Estimates (in 2009 Million US Dollars) of Drug Development Costs Over Time 46

CHAPTER 2

Figure 1. Final PK Model 73
Figure 2. Goodness-of-Fit Plots 75
Figure 3. Visual Predictive Checks 76
Figure 4. Mean Concentration Versus Time Profiles 79

CHAPTER 3

Figure 1. Final PK/PD Model for Pegloticase 107
Figure 2. Example of Predicted Profiles for One Patient 108
Figure 3a. Predicted (Simulated) Uric Acid Profiles by Treatment Group and Anti-pegloticase Antibody Category - Pegloticase Administered Every 2 Weeks 115
Figure 3b. Predicted (Simulated) Uric Acid Profiles by Treatment Group and Anti-pegloticase Antibody Category - Pegloticase Administered Every 4 Weeks 116

CHAPTER 4

Figure 1. Final Pharmacokinetic Model for TP-434 138
Figure 2. Predicted versus Observed TP-434 Concentrations 139
Figure 3. Mean Observed versus Predicted Plasma TP-434 Concentrations (Semi-log scale) 140
Figure 4. Predictive performance of the final model 143
Figure 5. Percentage of target attainment for different dosing regimens over a range of MIC values 145

CHAPTER 5

Figure 1. Final Pharmacokinetic Model for TP-434 163
Figure 2. Predicted versus Observed TP-434 Concentrations 164
Figure 3. Mean Observed versus Predicted Plasma TP-434 Concentrations (Semi-log scale) 165
Figure 4. Predictive performance of the final model 168

List of Abbreviations and Symbols

Abbreviation	Definition
AAG	α_1-acid glycoprotein
ABE	Average bioequivalence
AIC	Akaike information criterion
ANOVA	Analysis of variance
AUC	Area-under-the-curve
BCS	Biopharmaceutics classification system
BE	Bioequivalence
BIC	Bayesian information criterion
BID	Twice daily
CI	Confidence interval
Cmax	Maximal observed concentration
Cmax(pred)	Maximal predicted concentration
Cmin	Minimum observed concentration
CV%	Percent coefficient of variation
DDP	Drug development process
ε_i	Error associated with prediction for i^{th} observation
EBE	Empirical Bayes' estimate
F	Bioavailability
Frel	Relative bioavailability
f	Function
FDA	Food and Drug Agency
FO	First order
FOCE	First order conditional estimation
IV	Intravenous(ly)
kDa	Kilodalton
LSM	Least squares means
MAP	Maximum *a posteriori* probability

Abbreviation	Definition
mL	Milliliter
MLEM	Maximum likelihood expectation maximization
min	Minute
NCE	New chemical entity
NME	New molecular entity
NTBI	Non-transferrin bound iron
ϕ_j	Vector of model parameters for j^{th} subject
θ	Population (mean) model parameter estimates
OF	Objective function
PEG	Polyethylene glycol
PK	Pharmacokinetics
PD	Pharmacodynamics
POC	Proof-of-concept
QD	Once daily
R^2	Coefficient of determination
SAEM	Stochastic approximation expectation maximisation
SD	Standard deviation
SUPAC	Scale-up and post approval changes
TBI	Transferrin-bound iron
TE	Therapeutic equivalence
T_{Emax}	Time associated with maximal pharmacodynamic effect
TI	Total iron
TIBC	Total iron binding capacity
Tmax	Time of maximal concentration
W_i	Weighting factor
X_i	Vector of known quantities
Y_i	i^{th} observed value
$\hat{Y}_i$	i^{th} predicted value

This work is dedicated to my parents, Noel and Shirlie, who have fostered, nurtured and supported my desire to continue learning, and who continue to inspire me by showing through example that it is possible to learn at any age.

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"It was the best of times, it was the worst of times".

- *Charles Dickens, A Tale of Two Cities*

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Preface

In spite of the scientific and technical advances made by our society in the last century, drug development today remains a gruelling process. Not only is it expensive, time consuming, and a highly risky endeavour, but it is associated with a very low probability of success. This thesis will demonstrate how modeling and simulations, including the creation of innovative PK models, can help answer key questions within the drug development process (DDP), thereby improving its efficiency and decreasing some of the inherent risk.

To better illustrate the relevance and significance of the research presented in this thesis, it is necessary to first review some basic principles of drug action and describe the types of analyses typically conducted to better understand drug effects. It is also important to understand the drug development process, appreciate its strengths and more importantly underline some of its weaknesses.

After describing the context in which the research presented herein has evolved, the following articles will be presented individually:

1) Novel Population Pharmacokinetic Methods Compared to the Standard Noncompartmental Approach to Assess Bioequivalence of Iron Gluconate Formulations
2) Population pharmacokinetic and pharmacodynamic analysis of pegloticase administered by intravenous infusion in two dose regimens to subjects with chronic gout
3) Optimizing Drug Development of TP-434, a Novel Fluorocycline, with Adaptive Learn & Confirm Cycles of Modeling & Simulation Using Single Ascending Dose Data

4) Population Pharmacokinetic Modeling of TP-434 Following Multiple Dose Administration

Chapter 1 – Introduction

1. The Drug

The word "drug" evokes different associations in people, ranging from therapeutic aid to illicit activities. The Canadian government defines a drug as "any substance or mixture of substances manufactured, sold or represented for use in (a) the diagnosis, treatment, mitigation or prevention of a disease, disorder or abnormal physical state, or its symptoms, in human beings or animals, (b) restoring, correcting or modifying organic functions in human beings or animals, or (c) disinfection in premises in which food is manufactured, prepared or kept" (1). A similar definition is employed by the American "Food, Drug and Cosmetic Act", which defines a drug as: "(A) articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals (D) articles intended for use as a component of any article specified in clause (A), (B), or (C)" (2).

It is clear that drugs can be used to fulfill different therapeutic roles, ranging from the diagnosis to the treatment of disease. Although most clinicians are taught to favor non-medical treatments whenever possible (such as adopting lifestyle changes), the benefits of appropriate drug therapy are undeniable. Patients themselves recognize the importance of drug therapy. In a survey of 1000 patients in the United States, over 85% indicated that prescription medication was very or extremely valuable to their health and well-being (3).

While not solely attributable to drug therapy, in the span of the last 60 years, modern medicine has contributed to increasing our lifespan from 30 years up to 78 years in developed countries, which is a remarkable feat (4, 5). In the first half of the last century, medical advances such as vaccinations and antibiotics caused a significant decline in

infection-related mortality, which leveled off in the second half of the century (5). Drug and medical interventions also contributed to decreasing mortality rates in the second half of the century, especially those related to cardiovascular events (5). Clearly, the use of drug therapy has revolutionized modern medicine by providing viable treatment options when they did not exist before.

The poster child for drug therapy is without a doubt penicillin, which, even in its early commercial form was able to treat infections ranging from pneumonia to syphilis, including those that proved to be resistant to sulphonamide antibiotics. Although it was not the first antibiotic used to treat bacterial infections, more than 70 years after its discovery by Fleming, penicillin remains part of our modern therapeutic arsenal (6, 7). Its use, which began in World War II, serves as a reminder that while drug therapy as we know it is relatively new, it is a cornerstone of modern medicine.

As the benefits of drug therapy throughout the years have greatly evolved, so have our methods for discovering and developing new drugs. In the early days of drug discovery, fortuitous findings related to plants and other natural products were the key to developing new medicines (8). Some classic examples include the discovery that digitalis extracted from the foxglove plant had certain cardiac properties (9) and that extracts from moldy sweet clover hay could produce dicoumarol, a powerful anticoagulant compound (10). Since then, drug discovery has come a long way. Advances in disciplines such as analytical chemistry, biochemistry, microbiology, and more recently molecular biology and genomics have helped shape the process to its current state (11). Our improved understanding of chemistry, disease and pharmacology has allowed us to move away from reliance on serendipity to a more targeted approach to drug discovery.

Despite the contributions of drug therapy to the improvement of healthcare and the progression of drug discovery, unmet needs still remain. Although our lifespan has more than tripled in comparison with that of our cave-dwelling ancestors (5), there is still much for us to learn about the human condition. Mortality caused by infection or cardiovascular events have declined in the previous century, but they still remain among the top 15 causes

of death in North America (12, 13) and are among the ten leading causes of death in Canada (14). Added to this list are diseases such as cancer and Alzheimer's disease (12, 13), which were closer to the bottom of the list at the turn of the 20th century in the United States (15). Thus, with an increasingly aging population, we are faced with additional medical challenges that call for new uses for products already on the market, or for innovative new products.

It is important to remind ourselves that the ultimate goal of drug therapy will always be to improve lifespan as well as quality of life, and the aim of the drug development process is to create products that will help achieve these goals. In addition to developing products that are novel in their mechanisms of action (exemplified by pegloticase in Research Project #2), the drug development process can also produce therapies that are not necessarily different in terms of mechanism of action but that offer clinical advantages with respect to what is already on the market (such as developing anti-infective agents that are associated with less bacterial resistance, exemplified by TP-434 in Research Projects #3 and #4). Another important aspect of the drug development process is the development of generic drugs, which allow medication to be more accessible in terms of costs (as illustrated by sodium ferric gluconate complex in glucose in Research Project #1).

Before embarking upon an overview of the drug development process, the following section will describe some fundamental aspects of drug pharmacology that play a key role in the process. More details on the general pharmacology of the drugs and/or therapeutic classes of the drugs studied within this thesis can be found in Appendix 1.

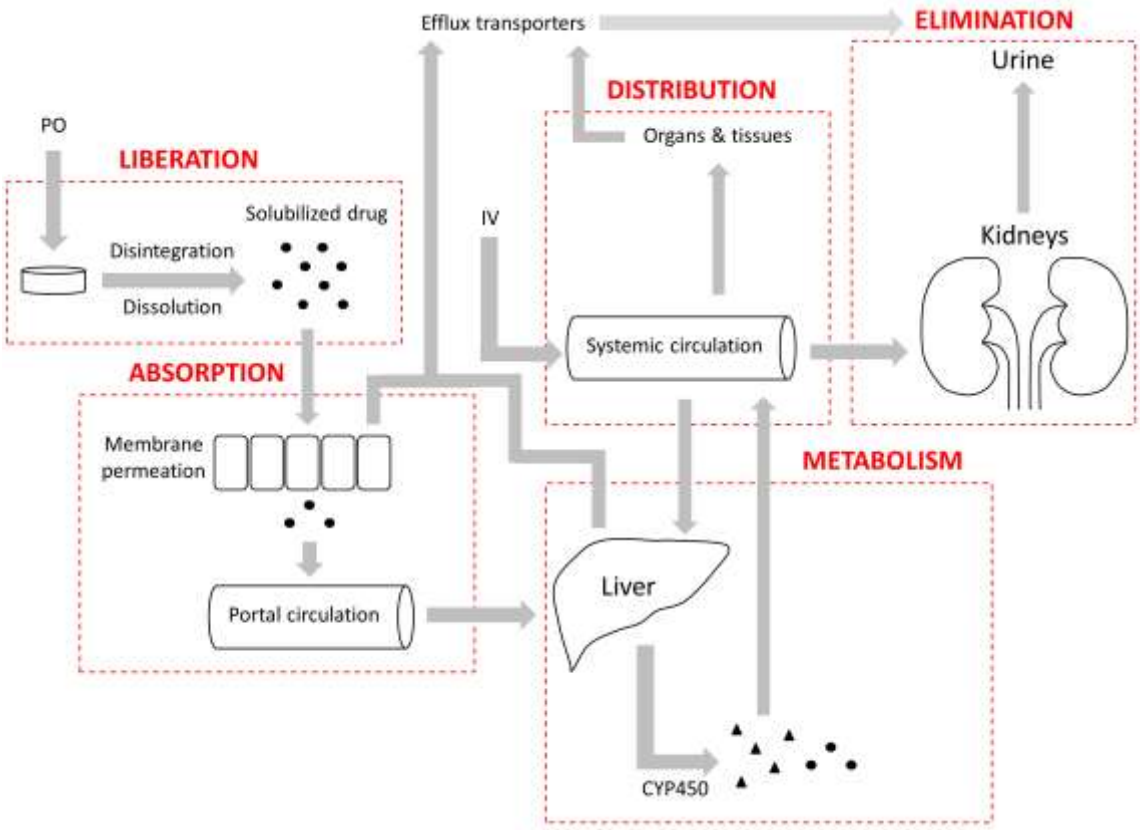
2. Pharmacokinetic and Pharmacodynamic Principles

In order to better appreciate why drug products have had, and continue to have, such an important impact on health and mortality, it is necessary to have a global understanding of "what the body does to the drug", otherwise known as pharmacokinetics (PK), and "what the drug does to the body", defined by the term pharmacodynamics (PD) (16).

2.1 Pharmacokinetics

For all drugs, pharmacokinetics encompasses different phenomena, which will each be discussed separately. These include liberation, absorption, distribution, metabolism and excretion, and are often referred to collectively as "LADME". Whether or not a drug's PK includes all of these steps depends on its route of administration. Drugs that are administered by the enteral route (i.e., they enter the gastrointestinal (GI) tract after being given orally) generally involve more processes than those that are administered by parenteral routes, such as intravenously (IV) administered products. An overview of all of the processes is depicted in Figure 1 and presented in more detail hereafter, with special attention being paid to intravenous drug products.

Figure 1. Illustration of Typical Pharmacokinetic Processes



2.1.1 Liberation

The liberation of a drug from its pharmaceutical form is a critical first step in a drug's disposition pathway. This process is inherently tied to drug formulation. For instance, an active ingredient that is administered as a solid form (tablet, capsule, etc.) must be dissolved into a solution before any of the other PK processes can occur (17). This also implies that the product must first disintegrate (break up into smaller particles) and disperse. Conversely, a drug that is administered as a solution (for either oral or injectable administration) does not require this dissolution step. Because the drugs studied in the context of this thesis were all administered intravenously (IV), liberation will not be described any further in this chapter.

2.1.2 Absorption

Once the drug has been solubilized, it is ready to be absorbed by the body and enter the bloodstream. Drugs administered intravenously bypass this step, since they are injected directly into the bloodstream, but most drugs that are given via other routes must necessarily undergo this process. Although the topic of drug absorption is beyond the scope of this thesis, it should be mentioned that it is a complex process that is governed in part by physicochemical drug properties (such as lipophilicity and molecule size), the presence of membrane transporters (both influx and efflux), and physiological states (such as intestinal transit time or food intake).

2.1.3 Distribution

Once the drug has entered the bloodstream (either directly or via passage through various membranes), it has the opportunity to enter different tissue or organs throughout the body. If the human body can be compared to a large city made up of dynamic components (people, animals, vehicles, etc.), and the drug is a person trying to reach a particular destination, then the bloodstream is analogous to the city streets that allow that person to

navigate through the city until the target building (representative of a tissue or organ) is attained. Depending on various factors (size, use of a vehicle, mobility), people are able to access multiple buildings or only certain ones, much like drugs whose distribution is controlled by different factors.

Protein binding plays a critical role in drug distribution, since generally only free (unbound) drug is able to cross membranes and reach specific tissues. In the cityscape analogy, plasma proteins are analogous to vehicles such as automobiles or buses which transport people (the drugs) to various locations. However, in order to enter buildings (reach target tissue), occupants must disembark from their vehicles. In human plasma, the most important transport proteins include albumin, α_1-acid glycoprotein (AAG) and lipoproteins (18, 19). Albumin is the most important plasma protein (accounting for roughly 60% of plasma protein (20)) and exhibits low affinity, high capacity binding. Conversely, AAG is less present in the plasma but is considered to have a high affinity, low capacity binding site (19). Most drugs that are acid or neutral bind to albumin while AAG is a major binding protein for basic drugs. Variations in albumin or AAG levels due to pathophysiological conditions can thus affect the concentrations of free (unbound) drug that are available to distribute to organs or tissue.

In order to reach target receptors in organs or tissues, drugs must cross membrane barriers. As with drug absorption, different types of transport mechanisms across membranes exist, such as paracellular transfer (passage between cells), passive diffusion (passage through cell lipid bilayers dependent upon a concentration gradient that follows Fick's law) (21), simple diffusion (drug transfer through aqueous pores of the cell that follows the concentration gradient), facilitated transport (drug transfer along the concentration gradient using a transporter protein) and active transport (drug transfer against the concentration gradient using a transporter protein and requiring energy expenditure). Therefore, many of the factors governing the membrane permeability during drug absorption will also influence drug distribution. For example, drug distribution is influenced by the size of the compound and its lipophilicity. Larger molecules ($\geq$ 15-30

kDa) such as biologicals tend to remain in the plasma compartment and/or extracellular space (22). More lipophilic molecules cross the membrane barrier by passive diffusion more readily than hydrophilic, charged molecules.

2.1.4 Metabolism

The human body is remarkably endowed with protective mechanisms, and the metabolic processes surrounding drugs generally aim to transform them into compounds that are more easily eliminated or excreted. Due to its size and the high content of metabolizing enzymes, one of the major organs involved in metabolism is the liver (23), which oversees two general types of chemical reactions that are known as Phase I and Phase II type reactions. Phase I reactions involve (but are not limited to) the oxydation, reduction and hydrolysis of compounds while some Phase II reactions include glucuronidation, sulfation and methylation, whereby groups (glucuronide, sulfate or methyl, for instance) are conjugated to the drug to facilitate its excretion (21, 24). Metabolites resulting from Phase I or Phase II reactions can either be active or inactive compounds. Some drugs, termed "pro-drugs", are specially formulated as inactive compounds which must be transformed into metabolites which possess the desired pharmacological activity.

Many enzymes are responsible for Phase I and II reactions. Some examples of Phase I enzymes include (but are not limited to) catalases, peroxidases, reductases, cholinesterases, dehydrogenases, while examples of Phase II enzymes are N-acetyltransferase, glucuronosyltransferases, glutathione-S-transferase, and sulfotransferases (25). However, one type of enzyme formerly known as cytochrome P450 (now referred to as CYP) is responsible for the metabolism of approximately 90% of all drugs (26). These enzymes are divided into different families (such as CYP1, CYP2, CYP3, etc.) which have amino acid sequences that are < 36% similar to each other. Each family is further divided into sub-families (such as CYP2A, CYP2B, etc.) where each member has an amino acid sequence that exhibits greater than 68% similarity to other members. Individual enzymes

are identified by a number following the letter, such as CYP2C9 or CYP2C19. The families CYP1, CYP2 and CYP3 are those which are mainly involved in drug metabolism while other CYPs are associated with the biosynthesis and catabolism of endogenous substances (27). Enzymes belonging to the CYP3A sub-family, especially CYP3A4, account for approximately 30% of all hepatic cytochromes (27) while they make up the majority of all intestinal CYP (21, 28). This family of cytochromes is responsible for most of the body's hepatic and intestinal metabolism (23), and is involved in the metabolism of approximately 50% of all drugs (26).

Although a comprehensive review of the topic is beyond the scope of this introduction, enzyme polymorphisms deserve a brief mention. Countless research has been devoted to better understand the genetic variations in metabolizing enzymes that frequently translate into starkly different drug responses between individuals. A classic example of polymorphisms that influence drug effect involves CYP2D6. Approximately 5 to 10% of Caucasians exhibit a poor metabolizer phenotype for CYP2D6, which can be attributed to a number of alleles. Compared to extensive metabolizers, these individuals have a limited capacity to inactivate drugs such as fluoxetine, and therefore have a greater risk of suffering from adverse events due to supratherapeutic levels of active drug. Conversely, drugs such as codeine, which must be metabolized into an active metabolite by CYP2D6, are mostly ineffective in such populations compared to extensive metabolizers (27).

Many drugs are subject to hepatic metabolism, but this is not true of all drugs. Compounds that are proteins undergo catabolic processes that allow the body to recycle the amino acids found in such proteins (22). In other words, proteins are degraded into their amino acid subunits through proteolytic processes, such as those in the ubiquitin-proteasome pathway (29). Polyethylene glycol (PEG)-modified recombinant mammalian urate oxidase (pegloticase) is an example of a biological product that is not metabolized (30). In addition, for many drug products, only a portion of the administered drug is metabolized while another remains unchanged.

As previously discussed, metabolism can occur in other organs, such as the intestine, where both Phase I and Phase II reactions take place (21). Other sites of drug metabolism include the kidneys, the lungs, skin and brain (27). The presence of hydrolyzing enzymes, such as cholinesterases, in the plasma compartment even allows metabolism to occur within the vascular space (31).

Metabolism of an orally administered drug can occur after systemic absorption, but it can also occur before a drug reaches systemic circulation. This pre-systemic metabolism is often called "first pass metabolism", and includes metabolism that may occur at the intestinal and hepatic levels (21). The fraction of a drug that is metabolized during its initial transit through an organ is referred to as the extraction ratio, which is also known as "first pass" or "pre-systemic" elimination. Examples of drugs that are subject to significant first pass metabolism in the intestine include cyclosporine (32), midazolam, nifedipine, quinidine, saquinavir and terfenadine (21).

2.1.5 Excretion

Drugs and their metabolites can be excreted via different organs such as the skin or lungs, but the main eliminating organs remain the kidney and the liver. Hepatic excretion involves biliary elimination of drugs and metabolites in fecal matter. ABCB1 transporters located on the canalicular membrane of hepatocytes are often involved in expulsing drugs into the bile (23). Renal excretion of unchanged drugs and/or their metabolites can involve glomerular filtration, tubular secretion, and reabsorption (23).

A key element of renal excretion involves the filtration of unbound (free) drug through the glomerulus. Another process that contributes to renal elimination is secretion to the proximal tubule, which is an active process involving transporters such as the organic anion transporter (OATP) and organic cation transporter (OCT). ABCB1 transporters located on the luminal brush-border membrane of renal cells also play a role in drug excretion by expulsing drugs into the urine (23). Finally, tubular reabsorption is a component of renal excretion that involves the reuptake of drugs by passive diffusion,

sometimes with the aid of endocytosis. Thus, the extent to which a drug is eliminated by the kidney depends on the relative importance of each of the processes involved.

Exceptionally, some compounds are not eliminated at all from the human body, such as iron. This mineral plays a vital role in many bodily functions, most notably involving the heme protein and oxygen transport (33, 34). Although the average human body contains 3 to 4 g of iron, only about 1 mg is lost daily (33, 35-37), which represents negligible elimination. Similarly, biotechnology derived proteins (biologicals) often undergo catabolism which generates amino acids that are re-used by the organism (22). This is the case for the biological product pegloticase.

2.1.6 Bioavailability

Extravascular routes of drug administration, in particular the oral route, are the generally preferred route of administration (38), which explains in part why over 60% of currently marketed products are for oral administration (39, 40). Although this route of administration is favoured, absorption and metabolism can be huge impediments to its use, since both phenomena can contribute to a decreased bioavailability (F).

Bioavailability is a contraction of the terms “biological availability” (41) and it is defined as “the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action” (42). In other words, bioavailability indicates the proportion of an administered drug dose that attains the site of action, after accounting for the fraction that is not absorbed as well as the fraction that undergoes metabolism in the gut or liver (21, 43).

The European Agency for the Evaluation of Medicinal Products (EMEA), Committee for Proprietary Medicinal Products, has also defined F in a similar manner, but has nuanced its description by replacing “site of drug action” by the phrase “general circulation” (44). The latter definition reflects the difficulties faced with measuring drug levels at the site of action, which can be highly impractical and extremely invasive. Instead, it is often assumed, as in this definition, that drug concentrations in the general

circulation (for instance as characterized by venous plasma concentrations) are indicative of drug levels at the site of action.

The use of plasma (or urinary) concentrations to calculate metrics for rate and extent of drug exposure is widely accepted as a surrogate for concentrations at the site of action, for drugs that reach the systemic circulation before or at the same time as they reach their site(s) of activity. Maximal observed serum or plasma concentration (Cmax) is associated with the rate of exposure, or how quickly drug concentrations attain their peak level, while area under the plasma/serum concentration time curve (AUC) is a measure of the overall extent of drug exposure. In Phase 1 studies, both parameters are calculated using noncompartmental PK analysis (45), which, is a robust approach when many plasma samples are available. This method of analysis is described in more detail in Section 3.1.

2.2 Pharmacodynamics

The drug development process is ultimately concerned with bringing a product to the market that will exert a desired effect or elicit a particular response. Such drug responses, otherwise known as pharmacodynamics, are intrinsically linked to PK and will be briefly described in this section. For a more detailed overview of the subject, the reader is referred to a book chapter previously published by our laboratory (46). A review of PK-PD modeling in a historical context (covering the 1960's until 2004) has also been published by Csajka and Verotta (47).

2.2.1 Classification of Effects

The pharmacological activity of a drug comprises both beneficial and harmful effects, the former generally being the reason for which the drug is administered. Although a drug may be administered with a specific purpose in mind, such as eradicating bacteria to treat an infection, other unwanted effects can occur, such as diarrhea. One of the challenges of drug therapy is finding the right balance between these pharmacodynamic effects, to help patients while avoiding undesirable effects as much as possible.

Pharmacological responses can be categorized broadly as quantal or continuous variables. Quantal responses are discrete categorical responses that do not belong to a continuum of responses. Such responses include dichotomous responses (awake/asleep) or polychotomous responses (grades of hematological toxicity) (48). Continuous responses can include laboratory values such as triglyceride levels or other clinical results such as heart rate.

PD responses can range from easily measured short-term responses (such as decrease in plasma glucose level or decrease in pain), to long-term outcomes (for instance increased survival or decrease of the risk of irreversible morbidity). A PD response can be considered to be a "clinical endpoint", which is defined as a characteristic or variable that reflects how a patient feels, functions or survives (49-51). When clinical endpoints are difficult to assess, surrogate endpoints that are a substitute for clinical endpoints provide an alluring alternative especially when there are severe time constraints. For instance, the desired clinical outcome of a drug may be a decrease in mortality and it may be difficult to wait for information on patient mortality. Therefore, it would be more practical to associate drug levels with a biomarker that is easily and quickly detectable (52, 53). By definition, a biomarker is "a characteristic that is objectively measured as an indicator of normal or pathologic, biologic or pathogenic processes, or pharmacological responses to a therapeutic indication" (49, 51). Biomarkers can server diagnostic purposes (for prognostic purposes or to guide therapeutic choices), they can measure disease activity, they can assess drug effect, or they can server as markers of drug kinetics (e.g., polymorphisms transporter proteins or in metabolizing enzymes) (50, 51). Biomarkers that are intended substitutes for clinical endpoints and that are expected to "predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence" are considered "surrogate endpoints" (49-51).

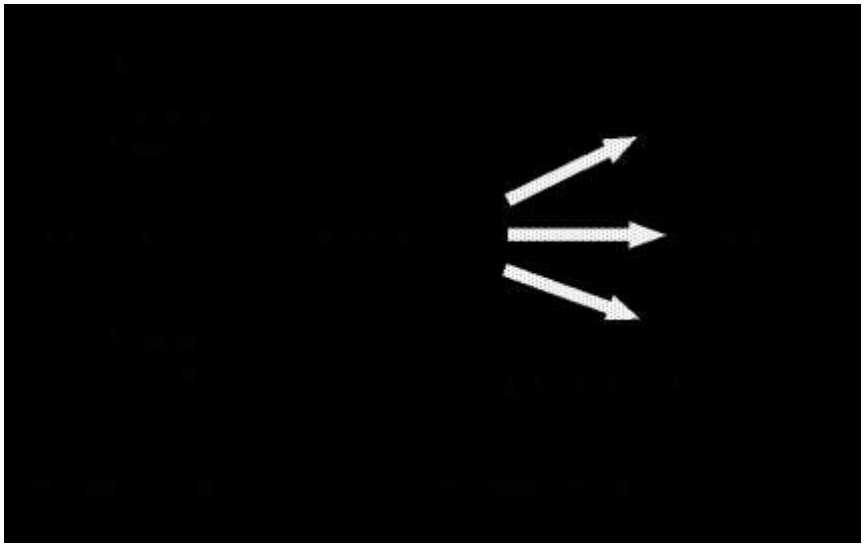
Cases have been reported where the use of a biomarker proved to be successful as a predictor of clinical outcome (e.g. HMG-CoA reductase inhibitors (54)) and could be used as a surrogate endpoint, while in other cases (e.g. antiarrhythmic agents (55, 56)), the

biomarker proved to be inadequate. However, caution must be employed with the use of surrogate endpoints because there is always the danger that they might fail to predict rare, negative side effects that can lead to overall negative clinical outcomes (54).

2.2.2 Types of PK-PD Relationships

A fundamental principle of clinical pharmacology is the link between the PK of the drug and its PD. Although this relationship may not be easily discernible, it is always present. This fundamental relationship is depicted in Figure 2.

Figure 2. Relationship between Drug Pharmacokinetics and Pharmacodynamics



Drugs can exert their effects in a direct or indirect manner. For instance, the drug bivalidurin (57) acts directly on thrombin to produce an anticoagulant effect, while a drug such as warfarin exerts an indirect anticoagulant effect on blood clotting by inhibiting the formation of clotting factors (58). In addition, effects can be reversible or irreversible, or in other terms, the effect will either dissipate or remain well after the drug is no longer present in the system. An example of a reversible drug effect is the bronchodilation caused by theophylline, while irreversible tumour cell death caused by an anticancer drug exemplifies irreversible effects (46).

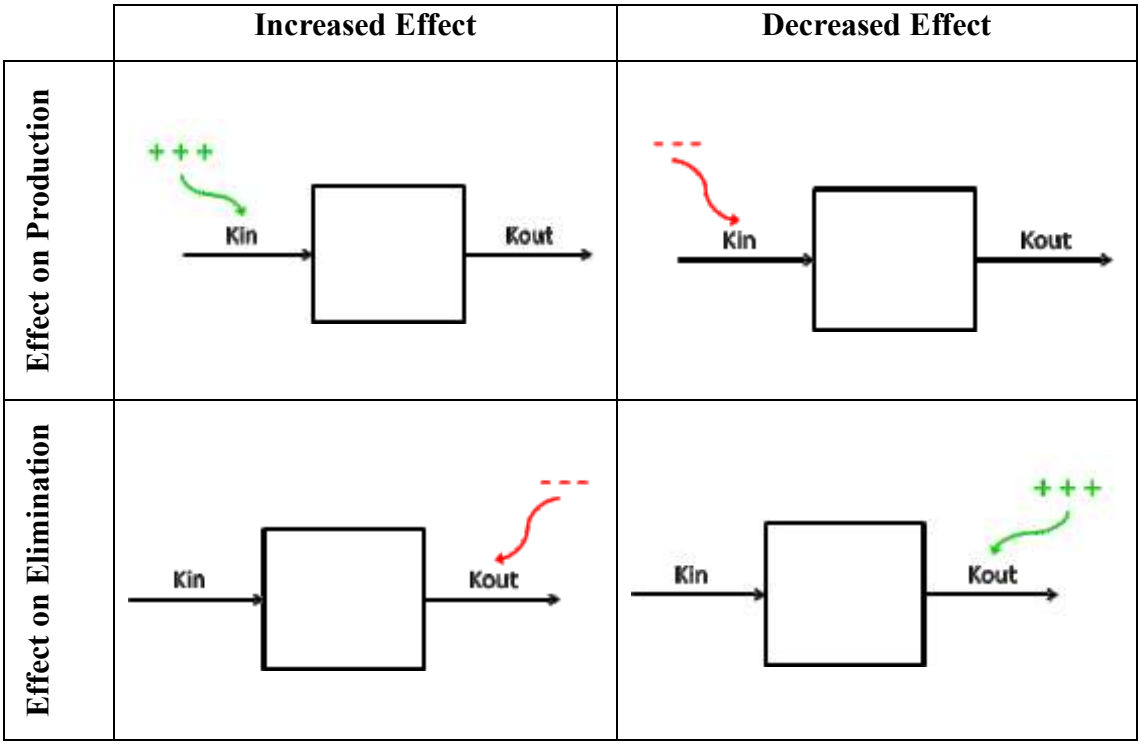
Direct response models can be used to relate a drug's PK to its PD using linear models (with a slope and intercept), Emax models or sigmoidal Emax models. The ordinary Emax model and sigmoidal Emax model are intuitively appealing because they indicate that there is no effect when drug levels are equal to zero and that, above a certain concentration threshold, increases in concentration cause no further increment in effect (59). Although the ordinary or sigmoidal Emax models are hyperbolic in nature, it must be pointed out that, within certain concentration ranges, the relationship between the concentration and response is linear. For the sigmoidal Emax model, there is a log-linear relationship between concentration and response between 20 to 80% of the maximal effect while below 20% or above 80% of the maximal response, the relationship is non-linear even on a log scale.

In many cases, there is a delay between the change in plasma drug concentrations and the associated drug response and/or the same drug concentration can be associated with different responses. To account for these time-dependent effects, many response models have been developed from the now classic effect compartment (or link) model to the more recent indirect modeling approach. The link model includes an artificial delay compartment where concentrations are in equilibrium with those in the biophase, which results from "collapsing" of the hysteresis curve. The link model attributes the delay in drug response to a process whereby the drug must attain the biophase from systemic circulation (47, 48, 60). As previously described, this process is influenced by many pharmacokinetic factors. Therefore, the observed delay between the pharmacological response and drug concentration levels are related to the time necessary for equilibration of concentrations in the plasma and the effect compartments (61). The drug concentrations in the effect compartment can then be related to the PD marker using direct models such as the Emax model or sigmoidal Emax model.

A major advance was made in our ability to relate the PK to the PD of drugs with the indirect modeling technique proposed by Jusko (60, 62). As the name suggests, indirect models relate drug concentrations to their pharmacological effect by an indirect

mechanism. In this approach, concentrations of drugs inhibit or induce the formation or the elimination of an effect. The basic indirect or turnover model includes K_{in} (the zero-order constant for production of the response) and K_{out} (which represents the first-order rate constant for loss of the response). An increase in response can be achieved by either stimulation of the response production (K_{in}) or by inhibition of the loss of response (K_{out}). Conversely, a decreased response can be attributed to an inhibition of K_{in} or a stimulation of K_{out}. These four situations, illustrated in Figure 3, correspond to the four basic indirect models (62, 63). Of course, response can be described by a combination of these four basic models. As with the link model and indirect model, drug effect on K_{in} or K_{out} can be described by an Emax or sigmoidal Emax function.

Figure 3. Indirect PD Models



3. Quantification of Pharmacokinetic and Pharmacodynamic Properties

The characterization of a drug's pharmacokinetic and pharmacodynamic behaviour is an essential component of the drug development process. The quantification of these properties allows researchers and drug developers to make informed decisions concerning doses, dosing regimens and clinical benefits, among other things. Improved understanding of a drug's PK and PD gained from post-marketing studies can also help clinicians use the drug in a more judicious manner.

Essentially, two approaches exist for quantifying the PK and PD of drugs: noncompartmental analysis and compartmental analysis. These analyses can be performed with data obtained from animals or humans, although only the latter will be discussed here. Both methods will be briefly outlined and contrasted in the following sections.

3.1 Noncompartmental Analysis

Noncompartmental analysis stems from statistical moment theory, where parameters describing PK or PD can be easily derived from concentration-time data (45). The rate of drug absorption or exposure is often characterized by the parameters Cmax and Tmax, which represent the maximal observed concentration (in a biological matrix such as blood, plasma or serum) over the time period studied, and time at which this concentration is observed, respectively. For PD parameters, maximal observed effect is often denoted as Emax, while T_{Emax} corresponds to the time associated with this maximal effect.

Following the administration of a single dose of a drug, exposure can be assessed by determining the area-under-the-concentration-time curve (AUC) using linear trapezoidal, log-linear trapezoidal or a combination of both linear and log-linear trapezoidal methods. Using the linear trapezoidal method, the AUC from time zero to time "t" (AUC_{0-t}) is calculated using Equation 1 while the AUC from time zero to infinity (AUC_{0-inf}) is

calculated using the formula in Equation 2. In both equations, a total of *n* concentrations are available, with the last detectable concentration (C_n) being associated with time t_n (45).

Equation 1 $$AUC_{0-t} = \sum_{i=1}^{n-1} \left(C_i + C_{i+1}\right) \cdot \left(t_{i+1} - t_i\right) \cdot 0.5$$

Equation 2 $$AUC_{0-\inf} = AUC_{0-t} + \frac{C_n}{Kel}$$

The second term in Equation 2 represents the extrapolated AUC from time t to infinity, and it requires the determination of Kel, or the terminal elimination rate constant. This parameter is calculated by ln-transforming the terminal portion of the concentration-time profile and estimating the slope of this transformed data using linear regression. In turn, this parameter can be used to calculate the terminal half-life ($T_{1/2}$), using Equation 3. $T_{1/2}$ represents the time necessary for the amount of drug in the organism to decrease by 50%. It should be noted that for PD analyses, the term AUC is replaced by the term AUEC to denote "area-under-the-effect-curve".

Equation 3 $$T_{1/2} = \frac{\ln(2)}{Kel}$$

The area-under-the-first-moment-curve (AUMC) also requires the determination of Kel. Equation 4 and Equation 5 can be used to calculate AUMC from time zero to time t ($AUMC_{0\text{-}t}$) and AUMC from time zero to infinity ($AUMC_{0\text{-}inf}$), respectively.

Equation 4 $$AUMC_{0-t} = \sum_{i=1}^{n-1} \left(t_i \cdot C_i + t_{i+1} \cdot C_{i+1}\right) \cdot \left(t_{i+1} - t_i\right) \cdot 0.5$$

Equation 5 $$AUMC_{0-\inf} = AUMC_{0-t} + \frac{C_n \cdot t_n}{Kel} + \frac{C_n}{Kel^2}$$

Other pharmacokinetic parameters can be calculated using the ones described above. Following the administration of a drug by the IV route, the mean residence time (MRT) can be calculated by dividing $AUMC_{0\text{-inf}}$ by $AUC_{0\text{-inf}}$ and subtracting half of the duration of infusion. MRT represents the average time spent by a molecule in the body, or the time necessary for 63.2% of an intravenously administered drug to be eliminated from the organism if the drug's PK is well described by a 1-compartment model (45).

The body's capacity to eliminate a drug is often characterized by clearance (CL). Clearance is often expressed as a volume per time unit (such as L/h), representing the volume of blood or plasma that is cleared of the drug per unit of time. For a drug administered intravenously, clearance is calculated as dose divided by $AUC_{0\text{-inf}}$. For an orally administered drug, the formula is similar except that it is an apparent clearance (CL/F) that is calculated, since the dose in the numerator is actually the bioavailable dose (dose x F), which is not necessarily the total dose. The total volume of distribution (Vss/F for a parenteral dose), which does not necessarily correspond to a true physiological volume, can also be determined by noncompartmental methods. This parameter should be viewed as a constant that relates the amount of drug in the body to the measured concentration at pseudo equilibrium, and it can be calculated as CL x MRT following single-dose intravenous administration of a drug. Should the drug be administered by an extra-vascular route, the volume of distribution can then be approximated in the terminal phase and is denoted by Varea/F or Vz/F. Generally, Vss/F is a much more meaningful parameter than Varea/F, but it should be noted that both parameters are equivalent if the PK of the drug follows a one-compartment model.

If concentration data obtained following IV and oral administration of a drug are available, it is possible to calculate the absolute bioavailability (F) of the orally administered product using noncompartmental analysis, as demonstrated by Equation 6. Similarly, the relative bioavailability (Frel) of two products can also be determined with AUCs using Equation 7.

Equation 6 $$F = \frac{Dose_{IV} \cdot AUC_{oral}}{Dose_{oral} \cdot AUC_{IV}}$$

Equation 7 $$F_{rel} = \frac{Dose_{ref} \cdot AUC_{test}}{Dose_{test} \cdot AUC_{ref}}$$

In order to perform noncompartmental analyses in a robust manner, it is necessary to include an important number of concentration values sampled at appropriate times. The trapezoidal method of calculating AUC actually provides an approximation of the true AUC (which could be determined by integrating the function describing the concentration as a function of time over the desired time interval), therefore more samples leads to a better approximation (45). In addition to requiring an important number of concentration values (biological samples), noncompartmental analyses should only be applied when certain assumptions hold true. The first assumption is that the drug in question displays linear pharmacokinetics (45, 64). In other words, exposure increases in proportion with increasing dose and PK parameters are stable through time. A second important assumption is that the drug is eliminated from the body strictly from the pool in which it is being measured, the plasma, for example (64, 65). Finally, this approach assumes that all sources of the drug are direct and unique to the measured pool (64). Examples of drugs which may not be candidates for noncompartmental analyses because they do not fulfill these criteria include iron supplements and thyroid hormones (66).

3.2 Compartmental Analysis

The essence of compartmental analysis is to create a model defined by integrated, matrix, or partial differential equations (equations that have derivatives with respect to more than one variable) that describe the PK or PD behaviour of a drug. Although this approach may not explain the true mechanisms underlying PK or PD behaviour, important correlations between covariates and parameters may point the way to further studies or provide deeper mechanistic understanding (67). Among other advantages of this method are its use in special populations (such as pediatric or hepatic impairment patients) and its

potential partitioning of variability into inter-individual, intra-individual, inter-occasion and residual sources (68).

Various types of compartmental analyses exist, ranging from individual analysis to population PK modeling including the naïve pooled data approach, the standard two stage approach, and non-linear mixed effect modeling that includes among others the iterative two stage, the First Order Conditional Estimation (FOCE) and the MLEM (Maximum likelihood Expectation Maximization) approaches (67, 69, 70). In these last approaches, all data is modeled simultaneously while retaining individual information, in order to obtain estimates of population mean and variance as well as quantify sources of variability (68, 71).

At the core of compartmental analyses is non-linear regression. In contrast with linear regression, where data is being fitted with a straight line defined by a slope and intercept, non-linear regression depends on equations whose partial derivatives (with respect to each of the parameters) involve other model parameters (72). Another important difference between the two types of regressions is that linear regressions have analytical solutions, such that the functions can be manipulated to obtain a specific equation for the solution, while only numerical solutions exist for non-linear regressions. For non-linear equations, approximate solutions to the equations can only be obtained through iterative processes that are described in further detail below. Since most biological processes are described by non-linear equations, linear regressions will not be examined any further.

3.2.1 Individual Analysis

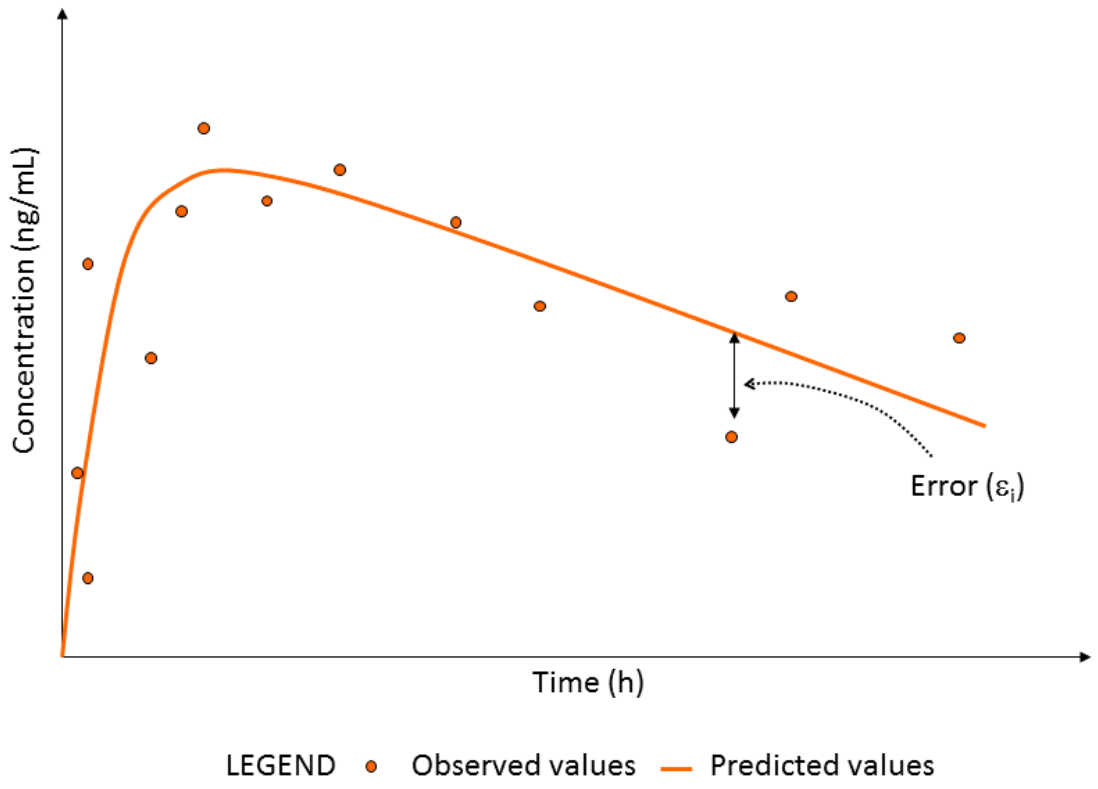
As its name implies, individual analysis involves the development of a model using data from one source (such as one human or one animal). Because of the error that is always inherent in data, whether it be related to the collection procedures themselves or to analytical assays, a model can never perfectly predict the observed data. The relationship between observed and predicted concentration values must therefore account for this error, as defined in Equation 8. In this equation, X_i represents a vector of known values (such as

dose and sampling times), C_i represents the vector of observed concentrations, ε_i represents the measurement errors, ϕ_j represents the vector of model parameters (in other words the pharmacokinetic parameters) and f_i is the function that relates C_i to ϕ_j and X_i. The subscript *i* represents the total number of observations or values.

Equation 8 $$C_i = f_i(\phi_j, X_i) + \varepsilon_i$$

The successful creation of a model might give rise to a semi-log concentration-time profile similar to the one in Figure 4.

Figure 4. Example of a Concentration Time Profile from Individual Compartmental Analysis



3.2.1.1 Numerical Approaches for Individual Analyses

The aim of PK compartmental analysis is to develop a model that is associated with predicted concentration values (or whatever observation is being studied) that are as close as possible to the observed values. In other words, the goal is to minimize the difference between the predicted and observed values (represented by ε_i in Equation 8), and generally the least-squares and maximum likelihood approaches are used to quantify these differences (73).

Various least-squares metrics (often termed "residual sum of squares") can be used to quantify these differences, and they are outlined in Table 1 below (72, 73).

Table 1. Comparison of Least-Squares Methods

Method	Objective Function Formula	Characteristics
Ordinary least squares (OLS)	$O_{OLS} = \sum_{i=1}^{n} (C_i - \hat{C}_i)^2$	No weighting
Weighted least squares (WLS)	$O_{WLS} = \sum_{i=1}^{n} W_i (C_i - \hat{C}_i)^2$	Model and parameters must be defined and stated empirically
Extended least squares (ELS) or Maximum Likelihood (ML)	$O_{ELS} = \sum_{i=1}^{n} \left[W_i (C_i - \hat{C}_i)^2 + \ln(\mathrm{var}(\hat{C}_i)) \right]$	Models can be defined, but parameters of the models are fitted within the procedure, e.g., $W_i = 1/\mathrm{var}(\hat{C}_i)$

$\hat{C}_i$ = predicted i^{th} concentration value, C_i = observed i^{th} concentration value, W_i = weighting factor, n = number of observations, var = variance

Although it is a simple formula, O_{OLS} is inherently biased because it tends to favour model estimates that provide better predictions for larger observations compared to smaller

ones. The WLS and ML/ELS approaches are an improvement over the OLS method since they account for the magnitude of observations (and their relative variability) by incorporating a weighting factor into their formulas. The ML/ELS approaches differ from the least-squares approach, because they deal with the probability of observing the actual data given the model and its parameter estimates. In these methods, the function that is being minimized is the log-likelihood (LL), or the probability of observing the actual concentration values given a set of model parameter estimates. The function for LL is presented in Equation 9. It should be noted that the only difference between ELS and ML is in the assumptions about the distribution of the variance parameters. In the ML approach, the distribution is assumed to be normal, while the ELS approach makes no such assumption (74).

Equation 9
$$LL(C|\theta) = -\frac{n}{2}Ln(2\pi) - \frac{n}{2}Ln\left[\frac{\sum\left(C_i - \hat{C}_i\right)^2}{n}\right] - \frac{n}{2}$$

Because it is easier to minimize a positive number rather than a negative one, the LL is often multiplied by -2 to obtain a positive number called the "-2LogLikelihood" (-2LL).

3.2.1.2 Algorithms for Numerical Problem Solving

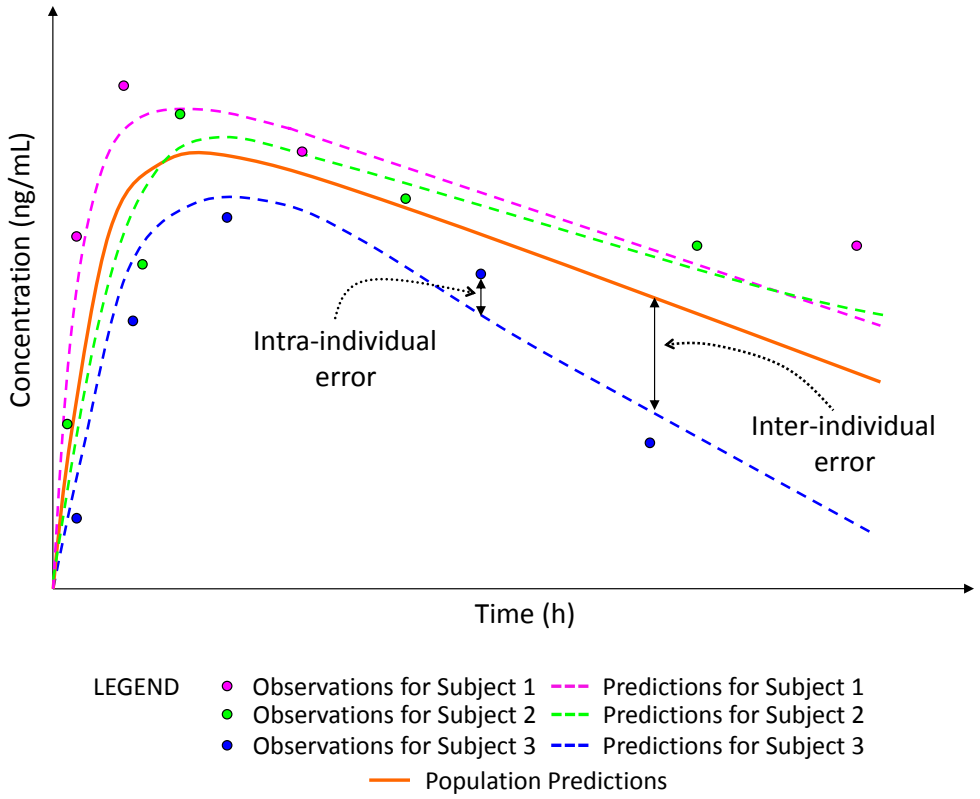
Since many combinations of parameter estimates must be evaluated in order to find the parameters that minimize one of the objective functions described previously, many algorithms have been developed to systematically do so. Some algorithms apply linearization techniques to approximate the model using linear equations. Cauchy's method employs a first-order Taylor series expansion, Newton or Newton-Raphson based methods utilize a second-order Taylor series expansion while the Gauss-Newton method iteratively uses multiple linear regressions via first-order Taylor series expansion. The Levenberg-Marquardt method is another algorithm which includes a modification of the Gauss-

Newton method. Finally, in contrast with the algorithms previously described, the Nelder-Mead simplex approach does not involve linearization procedures. This technique involves the examination of the response surface (in order to find the lowest point) using a series of moving and contracting or expanding polyhedra (three dimensional objects composed of flat polygonal faces joined by vertices) (73).

3.2.2 Population Analysis

Population analysis can be viewed as an extension of individual analysis, since it attempts to develop a model that predicts concentration data associated with different individuals or animals. The general concept is similar to that embraced by individual analysis, except that the model must also take into consideration inter-individual variability. The resulting model is therefore able to predict concentration values for each individual within the population, but it also provides an "overall" (mean or population) set of predictions. In other words, the model describes the behaviour of the whole population as well as the behaviour of each individual within this population. This concept is illustrated by Figure 5, where the coloured circles represent observed concentrations from different individuals, and the dotted lines of the same colour represent the predicted concentrations for that specific individual. The solid orange line passing through the middle of the figure represents the population predicted values.

Figure 5. Example of Concentration Time Profiles from Population Compartmental Analysis



Observed concentrations must therefore be ascribed to specific subjects, as defined in Equation 10 which is analogous to Equation 8. In this equation, X_{ij} represents a vector of known values (represented by i) for the j^{th} subject, C_{ij} represents the vector of observed concentrations for the j^{th} subject, ε_{ij} represents the measurement errors for the j^{th} subject, ϕ_j represents the vector of model parameters for the j^{th} subject and f_{ij} is the function that relates C_{ij} to ϕ_j and X_{ij}.

Equation 10 $$C_{ij} = f_{ij}\left(\phi_j, X_{ij}\right) + \varepsilon_{ij}$$

Each individual has a distinct set of PK model parameters (ϕ_j) that will provide the best predicted values for that individual's observed data. However, as previously

mentioned, there is also a typical profile of "population predictions" that is associated with population PK model parameters (θ) that can be regarded as mean values. The relationship between the mean PK parameters and individual PK parameters is described by Equation 11, where g is a known function that relates ϕ_j to θ using the individual's characteristics such as height or weight, denoted by z_j. The last term, η_j, represents random (unexplained or uncontrollable) variability that also causes ϕ_j to deviate from θ.

Equation 11 $$\phi_j = g(\theta, z_j) + \eta_j$$

3.2.2.1 Numerical Approaches for Population Analyses

The numerical approaches described for individual compartmental analysis are also used in population compartmental analyses. In population compartmental analyses, the goal is also to minimize some sort of objective function.

3.2.2.2 Various Approaches to Population Compartmental Analyses

The most basic type of population compartmental analysis is the "naïve-average data" method, where the average concentration value at given time points are computed from the entire dataset, and then a model is developed using these average values. A similar method is the "naïve-pooled data" approach, where data from different individuals are treated as though they were obtained from a single individual, and then analyzed using the individual approach.

The two-stage approach to population compartmental analyses offers some improvement over the previous ones. In essence, data from each subject is first fitted individually (in other words using the individual approach), and in the second step, population parameter estimates are obtained. Different types of two-stage approaches exist, such as the standard two-stage (STS) approach, the global two-stage (GTS) approach, and finally a mixed effect modeling approach known as the iterative two-stage approach (IT2S or ITS). In the STS approach, the population parameters estimates (for mean and variance)

are determined by calculating the mean and variance of the individual PK parameters, while the GTS approach actually estimates expectations for the mean and variance through an iterative process. The ITS method is a non-linear mixed effect modeling technique that uses a more refined iterative approach utilizing a mixture of ML and MAP (Maximum *a posteriori* probability) techniques. Within each population iteration, prior values are used to estimate individual PK parameters in the first step, while individual values are then used in the second step to recalculate a newer, more probable set of population parameters. Steps one and two are subsequently repeated until there is little to no difference between the new and old prior distributions (e.g., until the algorithm "converges").

In contrast with the iterative two-stage approach, other types of non-linear mixed effect modeling techniques proceed by first fitting the data in a reverse manner so they obtain population mean estimates followed in a second step with individual data estimates (therefore called "post-hocs"). The fixed effects (variables that can be controlled, such as dose or pharmacokinetic parameters) and random effects (uncontrollable factors like inter-occasion variability) are fitted simultaneously as it regards to population mean and variability estimates as well as the residual variability.

3.2.2.3 Algorithms for Numerical Problem Solving

Some of the algorithms used in the context of population compartmental analyses include the first order (FO) method, first order conditional estimation (FOCE) approach, and the maximum likelihood expectation maximization (MLEM) method. In both the FO and FOCE algorithms, the minimum objective function is sought out by linearization of the model through a series of first order Taylor series expansions of the error model. The difference between the FO and FOCE algorithms is that in the former, inter-individual variability for PK parameters is estimated using estimates of the population mean and variance in a *post hoc* step, while in the latter, inter-individual variability is estimated simultaneously with the population mean and variance (75). In other words, the FO algorithm uses a linearization technique that first assumes $\eta = 0$, contrary to the FOCE

algorithm which uses the posterior mode of η (that relies on conditional estimates) (73). Furthermore, the FO method assumes that inter-individual variability follows a constant coefficient of variation model, even if it is coded for a log-normal model. This is due to the Taylor expansion, whose first term is the same for the log normal model and the constant coefficient of variation model. A modification of the FOCE algorithm, known as the Laplacian FOCE method, exists whereby a second order Taylor series is performed instead of the first order expansion (75).

The MLEM algorithm is different from the previous methods because it does not rely on any linearization techniques (76). This algorithm involves maximizing a likelihood function through an iterative series of two steps which are repeated until convergence. In the first step, termed the expectation step or "E-Step", the conditional mean and covariance for each individual's data are computed and the expected likelihood function associated with these parameters is obtained. In the second step, the maximization step or "M-step", the population mean, covariance and error variance parameters are updated to maximize the likelihood from the previous step (73, 76).

3.2.3 Software Available for Compartmental Analyses

Although many computer programs are available to perform both individual and population pharmacokinetic analyses, only the two that were used in the context of this thesis (NONMEM® and ADAPT 5®) will be described briefly. One of the first software developed for this purpose was NONMEM®, which was created by Sheiner and Beal in the early 1980's for mainframes and personal computers (77-79), and many consider it to be the gold standard even today. Many versions of NONMEM® have been developed over the years, incorporating various types of algorithms, including FO, FOCE, FOCE Laplacian and most recently, ITS and MLEM. The second software is ADAPT 5®, the fifth version of the ADAPT-II® software developed by D'Argenio and Schumitzky in 1982 (76). Different algorithms are also available in ADAPT 5®, such as STS, ITS and MLEM.

3.3 Differences Between Noncompartmental and Compartmental Approaches

Noncompartmental and compartmental analyses are both excellent methods that can be used to characterize the PK and/or PD of a drug, when used in their appropriate context. The disadvantages of each method highlight the advantages of the other method, but when utilized correctly, each approach has its own merits. Table 2 summarizes the key advantages and disadvantages of each approach (68, 80).

Table 2. Advantages and Disadvantages of Noncompartmental and Compartmental Population Analyses

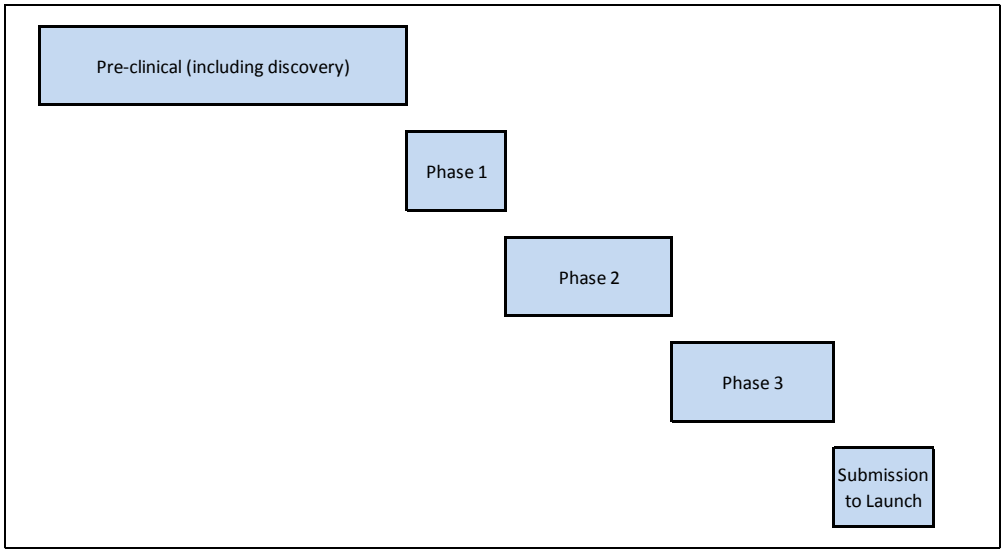
	Advantages	Disadvantages
Noncompartmental Analysis	- Easy and quick to perform - No special software is needed - Robust and easily reproducible	- Requires rich sampling - Makes assumptions regarding linearity
Compartmental Population Analysis	- Can be performed with rich or sparse data - Can be performed using data from heterogeneous sources or special populations - Quantifies inter-subject variability - Can perform covariate analyses - Can deal with both linearity and nonlinearity	- Requires experienced analyst - Time-consuming and labour intensive - Software is not user-friendly

4. The Drug Development Process

Now that we have examined some of the pharmacokinetic and pharmacodynamic principles that underlie drug development, we can turn our attention to the process itself, in order to better introduce the benefits of modeling and simulations within this process that will be demonstrated throughout this thesis.

Although each phase of the drug development process is important, in the context of this thesis, more emphasis will be placed on the clinical pre-marketing phases (Phases 1 to 3) of the process. Generally the process is viewed as a series of sequential (and sometimes overlapping) phases, as illustrated in Figure 6 (81). The whole process can take anywhere from 10 to 20 years to complete (82, 83).

Figure 6. Phases of the Drug Development Process



The subsections below present an overview of each of the phases of the DDP. Additionally, a description of the drug development process for generic products is also provided.

4.1 Pre-clinical

Once promising drug compounds are identified and a suitable formulation is developed, pre-clinical tests are performed. At this stage, compounds are screened for efficacy and safety through *in vitro* and animal studies, to make sure that they will be potentially effective and reasonably safe to administer to humans (84). Four general types of studies are conducted during this phase. The first type of studies (often called the "pharmacological screen") aim to detect drug effects that are not the intended ones. The second series of tests are performed to characterize the PK of the compound in relevant animal species, while the third type of test includes toxicology assessments in the same species, to evaluate the effects of single-dose and repeated administration of the drug in target animals. Toxicology tests in animals are actually pursued throughout clinical development, in order to assess potential long term toxicity (85). Tests are also conducted to assess the compound's teratogenicity (*in vivo*) and mutagenicity (*in vitro*) (86).

During this phase, the maximal tolerated dose and no-observed-adverse-event-level (lower dose level where no adverse events are observed) will be determined in at least two different species (85). Using this information, a safe dose that can be administered to humans for the first time is then selected.

4.2 Clinical

4.2.1 Phase 1

The studies undertaken during Phase 1 aim to assess the safety and tolerability of the drug in humans, and they offer the first opportunity to characterize the PK/PD of the compound in humans (82, 87). Phase 1 studies are generally conducted in a small group of healthy volunteers, with the exception of some drugs (such as oncology drugs) which are administered to patients instead (82). A small number of healthy volunteers, ranging from 20 to 100 (85, 88), are normally included in these studies.

The types of studies encountered in Phase 1 are generally randomized, placebo-controlled studies which may include a single, ascending dose administration study and a multiple (repeated administration), ascending dose study. Ultimately, these studies should help determine a range of doses that are safe and tolerated in humans (85). In addition to the ascending dose studies, Phase 1 studies may also include drug-drug interaction studies, studies to assess the effect of food on PK, special population studies such as in renally or hepatically impaired subjects, a thorough QTc study, and others (82).

4.2.2 Phase 2

In Phase 2 studies, the goal is to obtain preliminary evidence of efficacy and safety by administering the drug to patients who suffer from the targeted disease or condition (85). A relatively small number of patients are included in these studies, often numbering in the hundreds (85, 88). In addition to providing insight into the drug's efficacy, these studies can also indicate what type of short term adverse events may occur in this population.

Phase 2 studies are normally randomized, placebo-controlled trials. They are sometimes divided into two parts, Phase 2a and Phase 2b, but occasionally the two trials are combined into a larger trial. In Phase 2a, a range of doses of the compound is administered to a small group of patients (twelve to one hundred) to prove the drug's short term efficacy (and safety) (85). This is what is known as "Proof-of-concept" (POC). POC has also been described as "the earliest point in the drug development process at which the weight of evidence suggests that it is 'reasonably likely' that the key attributes for success are present and the key causes of failure are absent" (89), and therefore POC studies can also be performed within Phase 1. POC might require demonstrating proof of mechanism, identifying novel endpoints, confirming PK-PD behaviour or assessing safety.

The subsequent Phase 2b trial administers a range of doses to patients for a longer period of time, starting from doses deemed to be sub-therapeutic to maximally tolerated ones, in order to establish a dose-response relationship. This is done to find the minimal effective dose or dosing regimen that will be used in subsequent stages of the DDP (82).

In addition to determining the minimal effective dose to be further investigated in Phase 3, Phase 2 is often a period during which other issues must be taken into consideration before proceeding any further. As always, the safety and tolerability of the dose in question remains a concern. In addition, the efficacy of the product with respect to competitor compounds must also be addressed, as well as the probability of technical and regulatory success, and potential market share (82).

4.2.3 Phase 3

This phase of the DDP involves even more patients from the target population than in Phase 2. The number of patients recruited for these trials can range from several hundred to several thousand (82, 85, 88). Though they vary in design, they are frequently conducted as multi-center trials. They aim to confirm the efficacy and safety that was demonstrated in Phase 2 and to uncover side effects that may be infrequent (87). In addition, they serve to confirm the dose and dosing regimen selected based on the Phase 2 results.

The drug product that is tested at this stage is generally the one that the company is planning to market in terms of composition, formulation and strength (85).

Generally, two successful, pivotal Phase 3 trials are required by regulatory authorities. A placebo arm is generally included in these studies, but it is not uncommon to include treatment arms where other treatments (currently on the market) are administered, especially if a "gold-standard" is available or if a placebo arm is not ethical. In this way, the efficacy of the new compound can be compared to that of the current treatment(s), with the hopes of demonstrating non-inferiority or superiority. Non-inferiority could be targeted when the drug being studied possesses other attributes (such as a superior safety profile or simplified dosing regimen) that make it an appealing therapeutic alternative to what is already on the market. At a minimum, the drug's efficacy and safety must be compared to that of the placebo (82, 85) if it is ethical to administer one.

4.2.4 Phase 4

Once a drug is on the market, it is still the object of investigations often called "post-marketing studies" or "pharmacovigilance". The overall objective of these studies is to continue to monitor the drug's efficacy but especially its safety and tolerability (85). Now that the drug has found its way to the market, it is being exposed to a broader audience than the population tested in Phase 3 studies, therefore Phase 4 studies may reveal the presence of rare but dangerous adverse events that were not previously noted. Phase 4 studies may also have pharmacoeconomic objectives, or may seek to study additional indications (85).

4.3 Generic Product Development

Although the development of generic drug products must adhere to stringent rules and regulations, bringing a generic product to market is generally a shorter, less risky and easier process than bringing an innovator product to market (90). Unlike the DDP for innovator products, companies that develop generics are not usually required to submit clinical data that establishes their product's safety and efficacy. This is because regulatory authorities rely on the already-approved safety and efficacy profile of the innovator reference product (88). Furthermore, no pre-clinical data are normally submitted for review (91).

A key concept in generic drug product development is termed bioequivalence (BE), where the relative bioavailability of two drug products are judged to be equivalent, with the underlying assumption that drug levels in systemic circulation reflect levels, or will eventually be linked to levels, at the site of action. The U.S. FDA, Health Canada, and European Agency describe products as being bioequivalent if administration of the same molar dose of the active ingredient or moiety under identical conditions leads to similar bioavailability, or bioavailability that is not significantly different.

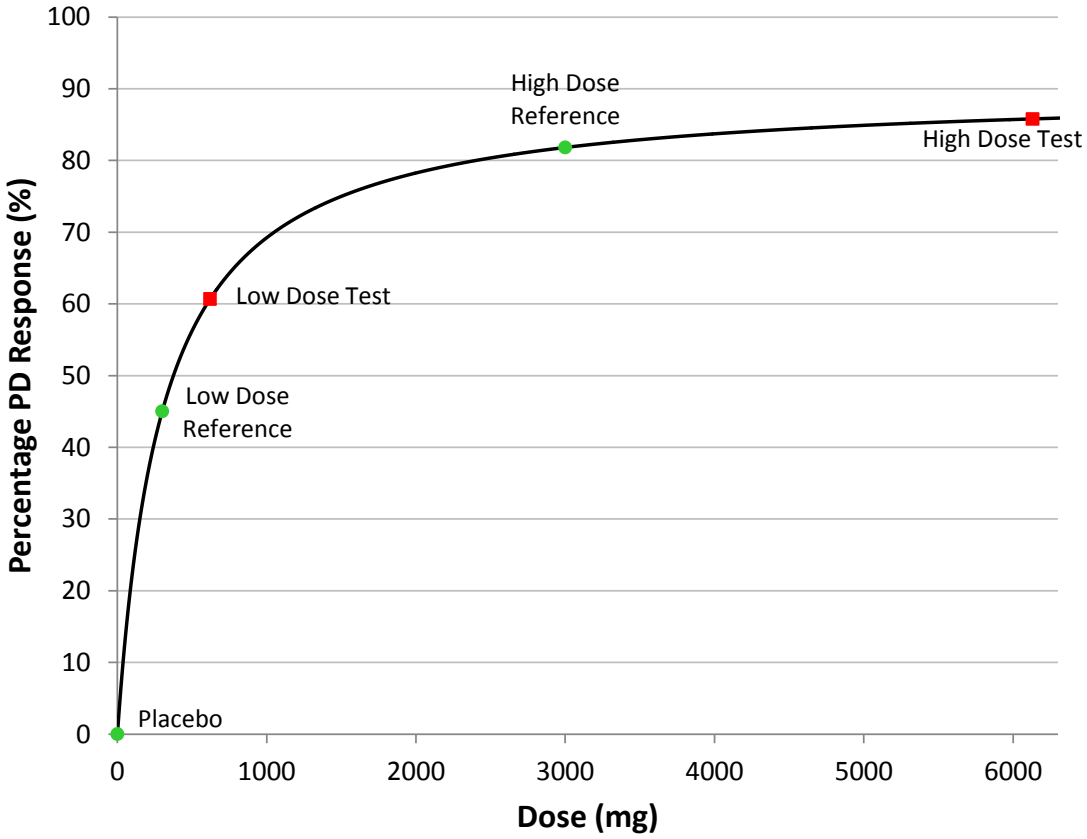
BE can be assessed by various means, such as PK studies, PD studies, clinical studies and *in vitro* studies, although European, Canadian and U.S. regulatory authorities

favour PK studies in general (91, 92). PK studies typically involve conducting *in vivo* trials in healthy volunteers. Specific BE guidelines outlining the ideal *in vivo* study design and statistical approaches that are preferred have been emitted by various regulatory (government) agencies (93-96), but for most drug products, BE is generally assessed by comparing the average relative bioavailability parameters (Cmax and AUC) of two products (91). Study designs tend to be two-way, two-treatment, two-sequence crossovers conducted in healthy volunteers, to minimize variability by administering the test and reference products to the same subjects (91). Using the ln-transformed PK parameters calculated for each subject, analyses of variance (ANOVA) are then performed and least square mean (LSM) ratios of test to reference PK parameters are obtained. Pre-defined confidence interval (CIs) limits, which can be viewed as target goalposts, are then used to determine BE by assessing whether or not LSM ratios and CIs fall within the targeted range.

Pharmacodynamic methods can be employed when it is not possible to measure systemic concentrations, such as in the case of locally-acting products like bronchodilators or topical creams, which have limited systemic absorption. This approach can also be adopted when systemic concentrations are not reflective of concentrations at the purported site of action. For such products, bioequivalence may be evaluated using an appropriate PD endpoint instead of PK parameters (91). When PD endpoints are employed instead of PK parameters in what is referred to as a Therapeutic Equivalence (TE) study, it is normally necessary to establish a dose response curve (depicted by the black line in Figure 7), by administering a placebo, a low dose of both test and references treatments and a high dose of the reference product (illustrated by the green circles in Figure 7). (The inclusion of a high dose test product is not necessary, and it is only included in the figure to illustrate the importance of selecting the right dose, as described in more details below.) The approach of projecting the PD effect on the dose scale in order to construct a ratio and a 90% CI is known as the dose-scale approach (97). The inclusion of a placebo will confirm that the low dose of the test or reference product is more efficacious than a placebo, and the administration of the high dose of the reference product will demonstrate that the

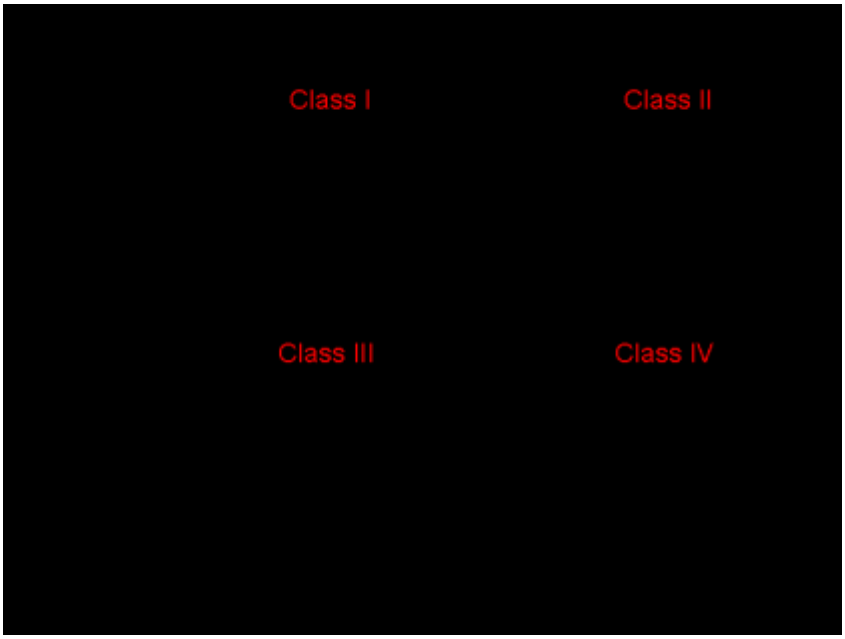
comparison between products at the low dose is truly discriminatory. In other words, by establishing a complete dose-response curve, it can be shown that the test and reference product meet BE criteria because they are truly BE and not because their responses are both at the upper end (plateau) of the dose-response curve or at the bottom end where no difference with placebo is seen. In the example depicted in Figure 7, the red squares depict test doses that are more than 2 times greater than the corresponding references doses (green circles). At a low dose, the difference in percentage PD response (45% vs. 61%) is more apparent and reflects the true difference in doses (300 mg vs. 620 mg). However, at higher doses which are located on the plateau portion of the dose-response curve, the similarity in percentage PD response (82% vs. 86%) masks the true difference in doses (3000 mg vs. 6100 mg). This highlights the importance of selecting appropriate doses when undertaking this type of study.

Figure 7. Example of a Dose-Response Curve in a Therapeutic Equivalence Study



In some cases, PK or PD studies can be replaced by adequate and well-controlled clinical trials (91). Furthermore, there are situations where the conduct of *in vivo* BE studies for immediate-release products can be waived entirely, based on the principles set forth by the "biopharmaceutic drug classification" system (BCS). In the mid 1990's, Amidon and coworkers published a seminal paper on the BCS that categorized drugs according to their solubility and permeability (98). Drugs belonged to one of four classes, more specifically I: high solubility and high permeability drugs, II: low solubility and high permeability drugs, III: high solubility and low permeability drugs or IV: low solubility and low permeability drugs, as depicted in Figure 8. It was suggested that *in vivo* bioavailability of products could be predicted from this classification system. Based on this premise, the FDA adopted the BCS in their 2000 guidance entitled "Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System" (99). According to this document, *in vivo* bioavailability and bioequivalence studies may be waived for immediate-release formulations of drugs belonging to Class I. Health Canada has also adopted a similar guidance, but their guidance includes drugs belonging to Class I and Class III (100).

Figure 8. Biopharmaceutics Classification System



It should be noted that BE studies do not only fall within the domain of generic drug development, but that innovator products require the conduct of BE equivalence studies to bridge the different formulations that they have used during their clinical trials. For instance, formulations typically evolve and change during the development process or after approval by regulatory authorities has been granted. BE studies must be performed to ensure that the changes that were made do not impact the drug's PK properties and call into question the associated efficacy and safety (91). In other words, BE studies can be performed to bridge results obtained with different drug formulations at various stages of the DDP.

A description of the generic drug product development would not be complete without mentioning "supergenerics", although a detailed description of the topic is beyond the scope of this thesis. These products differ from traditional generics because rather than being pure copies of the innovator drug, they offer an improvement over the latter, with regards to drug delivery system or formulation, among other possibilities (90). Some "added value" that could be provided by a supergeneric drug include modification of a dosing form to improve patient compliance or improvement of a compound's safety margins (90).

5. Current Use of Modeling and Simulations in the DDP

The utility of PK/PD modeling and simulations throughout the drug development process has been the focus of many reports and discussions (53, 80, 84, 101-111). Although it is not being used to its full potential, some of its current applications in the development of both innovator and generic drug products are described herein.

5.1 Innovator Drugs

Performing PK/PD modeling using animal (pre-clinical) data can provide some insight into the nature of the exposure-response shape as well as estimates of potency (EC_{50}) or maximal effect (Emax) (108). Information on PK properties (absorption,

distribution, metabolism, elimination) that could impact pharmacological response can also be obtained at this stage. Modeling can also make use of existing clinical data on similar compounds. Despite its utility in screening potential drug candidates, one of the main uses of PK/PD modeling at the nonclinical stage remains the selection of doses for first-time human administration (102). Different modeling approaches can be used for dose range projection including population PK/PD analyses of sparse nonclinical data, allometric scaling to predict human PK and efficacy scaling, or physiological-based PK modeling (105).

The PK/PD model developed at the pre-clinical stage can be further refined using data obtained from Phase 1 studies conducted in small numbers of healthy volunteers. A model can actually be developed to link animal and human data. In this first phase of clinical development, a PK/PD model can describe complex exposure-response relationships, such as those which involve non-linearity, as well as make use of scarce data (related to sparse sampling or assay limitations) (112). PK/PD modeling can make use of data available from all Phase 1 studies in order to get robust parameter estimates. Modeling can also reveal if there are any deviations from dose-proportionality, and can describe possible time-related phenomena such as tolerance or sensitization.

Phase 2 studies are conducted in a larger number of subjects and information can therefore be obtained to better understand inter-subject variability. Another important application of PK/PD modeling at this stage is that it can support proof-of-concept (POC) claims by demonstrating that the drug acts on its targeted mechanism, thereby leading to the desired short-term outcome. PK/PD models can, in fact, be used to support claims of efficacy throughout drug development (101). As in Phase 1 studies, PK/PD modeling can be used for further evidence of POC as well as in the selection of dosing regimens, sampling schedules and study design. Information gleaned from Phase 2 studies can be also used to optimize the design of future trials, as well as further enrich the understanding of a compound's attributes that will distinguish it from competitor drugs (105). For example, based on a developed PK/PD model, several dosing schemes can be simulated in

order to select the range of doses or types of regimens that could provide optimal response (113).

In later stages of clinical development, PK/PD models can be used to understand the impact of covariates on drug response. Since Phase 3 studies are conducted in larger numbers of patients from the target population compared to Phase 2 trials, they provide key information regarding the effect of patient characteristics and different pathological states on drug response.

Models developed during Phase 3 can further confirm the dose-exposure-response relationship in the target population. In some cases, when surrogate markers are available, the use of exposure-response information coupled with a single pivotal clinical trial can be sufficient evidence of effectiveness (101). This demonstrates how a thorough understanding of the exposure-response relationship can even obviate the need for additional studies. In addition to developing PK-PD models, disease-drug models can be developed at this stage.

Modeling and simulations are not only being used and further developed by the pharmaceutical industry or academia, but from a regulatory perspective, they have also been used to enhance decision-making and contribute to product labeling (pertaining to dosage and administration, safety or clinical pharmacology) (114). In some submissions to the FDA, drug companies benefitted from modeling and simulations performed by reviewers, who were able to extract information from the data that had not otherwise been presented (114, 115). Over an eight year period studied (2000 to 2008), modeling and simulations contributed to the approval of 64% of products while it influenced the labeling of 67% of products (116). However, although modeling and simulations are being used more frequently by regulatory reviewers as an aid to decision-making, this tool remains under-utilized by drug developers and the research presented in this thesis will demonstrate other ways in which it can be applied or further developed.

5.2 Generic Drugs

While the use of modeling and simulations in the development of innovator drugs is gaining greater acceptance, its use in the world of generic drug development is still in its infancy. Indeed, the generic drug industry still relies heavily on simple noncompartmental methods to assess PK and PD.

One exception to this is when BE or TE studies require Emax calculations such as those for topical corticosteroids (117) or certain locally acting products (118, 119) for the U.S. FDA. Many times equivalence can be calculated with the dose-scale approach where a dose-response relationship is developed for the drug under study, and modeling (i.e., data fitting) is often relied upon to establish this relationship. An example of an equation that could be used to describe the dose-response relationship is provided in Equation 12.

Equation 12
$$\text{Response} = \frac{E\max \cdot Dose \cdot F_{rel}}{Dose \cdot F_{rel} + ED_{50}}$$

By fitting the overall response data to this equation, it is then possible to obtain mean estimates for the parameters Emax (maximal response), F_{rel} (relative bioavailability) and ED_{50} (the dose associated with half of the maximal response). But, as previously mentioned, modeling and simulation is still in its infancy for generic drugs because even though this equation would be better solved using mixed-effect modeling, at this time U.S. regulators are still recommending the use of a naive pooled data approach to sponsors. Once parameter estimates are found, a non-parametric bootstrap can then be performed to establish a bias-corrected and accelerated 90% confidence interval (120) around the estimated F_{rel}.

6. Challenges in the Drug Development Process

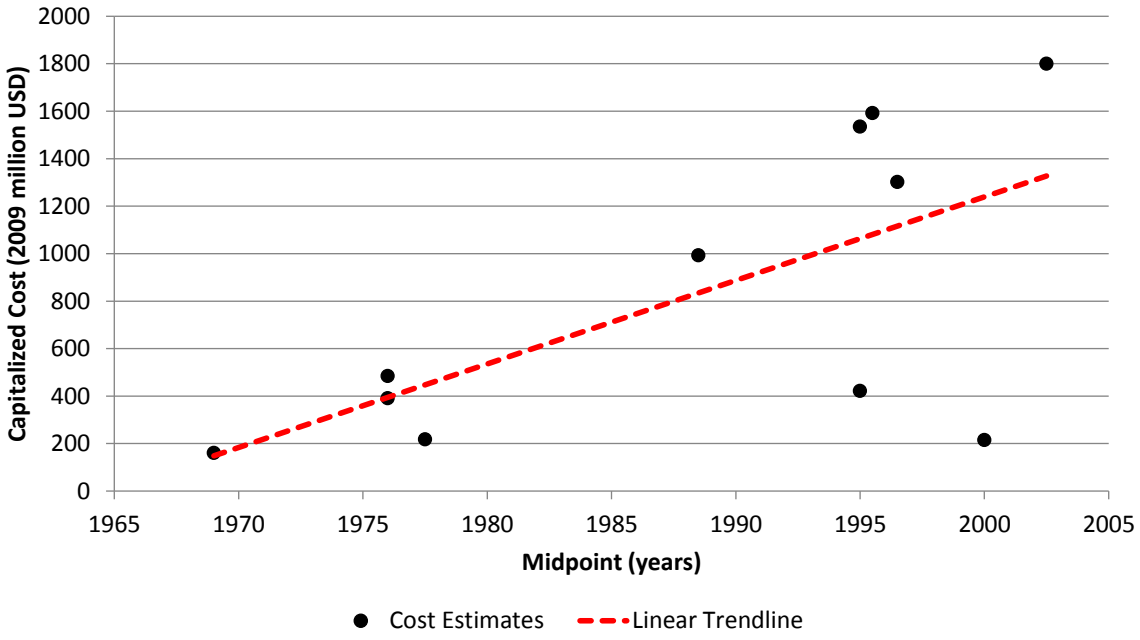
While there are undeniable benefits to drug therapy, the process of discovering a new chemical entity (NCE) and bringing it to market, whether it be a small or large molecule, is marred by several obstacles. Indeed, two major issues faced by drug

developers are the staggering costs associated with the DDP and the extremely low rate of success. In the following subsections, each of these problems will be addressed in more detail. Finally, some reflections on how the DDP can be improved with the use of modeling and simulations are presented in the last sub-section.

6.1 High Costs

Bringing a drug to the market is far from being inexpensive, and costs associated with the DDP have risen dramatically within the last 40-year period. Over the years, researchers have attempted to calculate the cost associated with drug development, using various methodologies. The findings of some of the more prominent studies are illustrated in Figure 9 (81, 87, 121-123). In this figure, the year associated with each cost represents the mid-point of the range of years studied by the researchers. These estimates represent the cumulative costs of all stages of the DDP. They take into consideration the revenues that are invested (and ultimately wasted) on compounds that never made it to the market, and they also account for the time involved in the DDP by increasing the cost by a certain percentage that represents the potential return if the funds had been invested elsewhere (hence the term capitalized estimate).

Figure 9. Capitalized Estimates (in 2009 Million US Dollars) of Drug Development Costs Over Time



This figure shows that despite differences in methodologies, data sources and studied timeframes, there is a trend towards increase costs associated with the entire DDP. This trend is confirmed when comparing the results of studies conducted at different times but by the same research group (121). The increase may actually even be exponential in nature, as suggested by others (124, 125).

The rising cost of drug development can be attributed to many factors, but one of the important ones is the increase in the number and magnitude of clinical trials in response to increased regulatory requirements (83). Indeed, although the cost of pre-clinical research has also increased over the years, the overall increases can mostly be attributed to costs associated with increased human trials rather than pre-clinical efforts (87).

An increase in the number and size of clinical trials also adds to the development time, which also impacts the DDP costs (83). Over the past decade, the time required for research and development has increased by 12 to 15 months (126). The old adage "time is money" also holds true in the pharmaceutical industry, where it has been estimated that a

delay of six months to launch a product translates to a loss of 100$ million in net present value (126).

Although the majority of drug development costs (approximately 63%) are associated with clinical phases (Phases 1 to 3) (81), these costs are not evenly divided between the different phases, and they increase with each phase. According to one study, mean costs associated with pre-clinical, Phase 1, Phase 2, and Phase 3 studies were 5.2, 15.2, 23.5, and 86.3 millions (dollars in the year 2000), respectively (87). These figures were even higher in 2012, with mean costs rising to 35.7, 34.3, 82.1 and 245.1 millions for pre-clinical, Phase 1, Phase 2, and Phase 3 studies , respectively (127). The elevated cost associated with Phase 3 studies is not surprising, as these studies can include thousands of patients from different sites, and can span many years.

6.2 High Attrition Rates

What makes drug development such a high-stakes venture is that in addition to the staggering costs associated with the process, the chances of success are very slim. Estimates of the probability of successfully bringing a drug to the market (starting from pre-clinical stages) have ranged from 11.7% to 30.2% (81, 87, 121-123). Even with the most optimistic of these probabilities, it is clear that the likelihood of success is marginal. These numbers can also be viewed from the perspective of the number of tested candidates that eventually make it to the market. Some analyses suggest that for every new molecular entity that makes it to the market, 8 others will have been pursued but eventually discarded (81). Similarly, other authors have reported that only 1 in 9 or 10 drugs that are tested in clinical trials will make it to the market (82, 128).

In the past, a drug's inability to make it to market was mostly related to pharmacokinetic causes (poor characterization of pharmacokinetic properties or low bioavailability) (128). However, improvements in our understanding of pharmacokinetics and in the quality of the tools used for pharmacokinetic analyses have led to less pharmacokinetic-related failure. Now, at the beginning of the twenty-first century, attrition rates remain high, but rather than being attributable to pharmacokinetics, failure is often

related to safety and efficacy outcomes revealed at the end of the drug development process (Phase 3). What is striking is that Phase 3 attrition rates remain high and have not decreased since the last decade despite advances in technology and our increasing knowledge (124).

Failure of drugs in Phases 1 or 2 of the DDP can be broadly categorized as being due to poor drug characteristics (either PK or PD) or due to poor choices made by researchers. In the former, the drug may not act on its target as anticipated, or it may cause unacceptable adverse events. In the latter, Phase 2 attrition may be caused, among other reasons, by the selection of the wrong patient population, the administration of an inappropriate dose or by the inadequacy of the measured endpoint to detect the desired effect (89). One analysis of Phase 2 failures occurring between 2008 and 2010 suggests that 51% of all Phase 2 failures could be attributed to a lack of efficacy while 19% were due to safety or toxicology concerns (129). Despite technological advances and increased medical knowledge, the attrition rate for drugs entering Phase 2 of the drug development process can be as high as 62% (128). Nevertheless, if a drug is destined to fail, it would be better to do so in Phase 2 than during or after costlier Phase 3.

In two analyses conducted in 1991 and 2003, DiMasi and colleagues used similar methodologies to estimate the failure rates of drug compounds in different phases of drug development. Interestingly, there was an increased failure rate in Phase 1 studies (from 32.5% to 37%) and a decreased failure rate in Phase 3 (17.1% to 12.6%), while rates for Phase 2 studies remained stable. This shows that companies today may be more proficient at weeding out less promising compounds earlier in the drug development process, leading to lower failure rates later in the process (87, 122).

In an ideal world, drugs in Phase 3 of the DDP would only fail due to the occurrence of unforeseen and extremely rare adverse events. However, this is not the case and the attrition rate for drugs entering Phase 3 can be as high as 45% (128). The reasons for failure in late-stage Phase 3 studies are numerous, including the underpowering of studies or even selecting the wrong dose or dosing regimen (85). One report suggests that

up to 30% of Phase 3 failures can be attributed to lack of efficacy, while safety concerns led to another 30% of Phase 3 failures (128). An even higher failure percentage of 66% due to efficacy reasons has been suggested by two separate analyses (130, 131). In either case, the figure is high and signals a problem. Indeed, the repeating of failed pivotal trials represents a significant waste of time, resources, monetary investment, and corporate energy, which is why the pharmaceutical industry seeks to avoid this situation (85).

Many have argued that the generally high attrition rates currently seen are also due to an increased stringency in regulatory demands (83, 128). Indeed, in light of recent post-approval withdrawal of high-profile drugs, this precautionary attitude is entirely understandable. Additional reasons for such elevated failure rates may include the attempt to treat diseases or conditions that are more complex than in the past (128). In other words, therapies for "easier" disease targets have already been developed. In addition, standards of care have improved considerably over the years, making it even more difficult to prove that a drug can provide added benefits compared to what is currently available (128) because efficacy trials will be compared to an active, currently marketed treatment instead of a placebo. This has led people to discuss the "better than the Beatles" argument, whereby regulatory agencies are exceedingly demanding of companies by requiring new medications to be better than everything else, instead of allowing drugs to come onto the market when they are efficacious even though they would not appear from the Phase III trial to necessarily be the best product (132). The argument is not without merit, as of course if we were to prevent music from being marketed unless proven to be better than the Beatles' music, then this would lead to severe attrition of new commercialized music. In addition, the true benefit of a drug is often not realized or found during the DDP but later when it is marketed, supporting the argument that the regulatory bar for new medicines may currently be too high.

6.3 Room for Improvement

The constantly increasing costs associated with drug development, as well as the high chance of failure, have led to a decrease in the number of new therapies that are

marketed yearly. Since the 1990's, the top 50 pharmaceutical companies have seen a decline in their productivity (defined as the number and quality of new chemical entities brought to market) (133). This trend is reflected in the number of new molecular entities (NMEs) that have been submitted to the FDA over a fifteen year period (from 1996 to 2011), which have been declining steadily. In 1996, 45 applications were submitted while in 2010, only 23 submissions were filed (134).

The high risks and rising costs associated with drug development may also foster other problems, by encouraging drug companies to develop products that are deemed to be "higher selling" (to obtain a better return on investment), which are normally drugs that act against proven targets. This means that there will be less impetus to develop drugs with novel mechanisms of action or products to treat less common conditions (86). Despite incentives such as the "Orphan drug status", which offers companies fast-track review and in the US a seven-year market exclusivity for targeting diseases that affect less than 200 000 people, costs remain prohibitively high for these specialized markets (82). Thus, if cutting the cost of drug development can encourage researchers to develop products for all unmet needs, without favouring the more lucrative options, then this is certainly something that cannot be ignored.

These disturbing issues and trends have not gone unnoticed and led the FDA to publish its oft-cited "Challenge and Opportunity on the Critical Path to New Medical Products", sometimes called the "Critical Path document" (134). This document has been viewed somewhat as a "call to arms" for the pharmaceutical industry, to spur researchers into reshaping their ways. In it, the FDA re-iterates that the DDP is an expensive, time-consuming process that has a very low success rate and that high clinical failure rates can generally be attributed to safety problems and lack of effectiveness. Throughout the document, they highlight the need for a new set of tools (such as assays, standards, computer modeling techniques, biomarkers, and clinical trial endpoints) that will help make the process more efficient and effective. Some suggested areas of improvement mentioned in this document were the making use of proteomics and toxicogenomics, developing new

tools to assess heart rhythm abnormalities, expanding the knowledge base for pediatric studies and finding new biomarkers or surrogate endpoints.

Among the items that they list as opportunities for improvement, the FDA included *in silico* (computer) modeling, which could enhance development knowledge management and decision-making with regards to both safety and efficacy. However, even before the publication of the FDA's infamous "Critical Path Initiative", others had recognized the need for change and the role that modeling and simulations could play in bringing about such change. In his 1997 paper, Carl Peck suggests that modeling and simulations should play a more prominent role in the DDP, as they do in other industries such as the aerospace or automotive industries. He also suggests that data should be analyzed to gain insight on the dose-response relationship, rather than simply relying on empirical hypothesis testing (135).

The concept of model-based drug development described in the FDA's white paper was also largely inspired by Sheiner's "learn-confirm" concept first presented in 1997 (136). In this new paradigm for drug development, the phases of drug development are viewed as successive learn-confirm cycles. In the learning phases, the goal of study design is to glean as much information as possible about exposure-response relationships and mechanisms of action. The confirmatory portions of the cycle serve to provide further evidence to support specific claims, for instance regarding risk/benefit ratios or optimal doses. Thus, Phase 1 studies are studies designed to learn about tolerated doses and explore dose-response relationships while Phase 2a studies are meant to confirm that selected doses are associated with the desired response. The learn-confirm cycle begins anew with Phase 2b studies where researchers learn about the drug's behaviour in the intended patient population. Finally, Phase 3 and 4 studies aim to confirm the dose and dosing regimen selected based on the Phase 2b studies as well as provide further supportive evidence on the drug's postulated efficacy.

It is clear that many opportunities exist for improving the current drug development process, and it is the hope that these modifications will allow the pharmaceutical industry to

continue to provide new therapies to patients which are safe, reliable and effective. Furthermore, the changes brought about should allow the industry to do so in a timely and more cost effective manner. Indeed, cost analyses have shown that improvements in the development process can lead to significant savings. For example, reducing the lengths of clinical phases by 25% is associated with a 16% reduction in capitalized costs (roughly 129$ million year 2000 dollars). Furthermore, increasing the success rate from 21.5% to 33.3% would save 221 to 242 million dollars (year 2000 dollars) per NCE, which is not negligible (137). According to one model, decreasing Phase 2 attrition rates from 66% to 50% would yield a 25% in the cost of bringing a NME to market. Similarly, a decrease in Phase 3 attrition rates from 30% to 20% would be associated with cost savings of 12% (81). Thus, there is a clear economic incentive to decrease failure rates.

If humanity is to continue to benefit from advances in drug therapy, it is imperative that old methods be cast off and replaced with different ones. This thesis will therefore focus on approaches that can contribute to the improvement of the drug development process.

7. Research Hypotheses

In light of the elevated costs and risks that plague the drug development process, changes must be made if these are to be decreased. While it is not always possible to shorten the time period devoted to clinical trials (because a minimum amount of data, especially pertaining to long-term safety and efficacy, must be collected), and thereby reduce costs, it is possible for researchers to use their time more efficiently by employing some of the methods described earlier. Similarly, it is not within our power to change the stringent regulatory requirements, nor would it be advisable to reduce safety standards, but there are certainly methods that can be devised or processes that can be improved so that these demands are met in a more timely and efficient manner.

As previously described, one tool that could render different aspects of the drug development more efficient is modeling and simulations. More specifically, risks can be decreased by using modeling and simulations to answer questions that are a key part of decision-making which will ultimately influence how (and if) a drug will make it on the market. Importantly, modeling and simulations can provide quantitative answers that are more objective than "gut-feelings" and that do not rely upon a researcher's underlying desire to "see the project through". These answers can ultimately ensure that subsequent studies are better designed to succeed and that they are conducted in a timely manner.

In its white paper, the FDA has cited a need for "new tools to get fundamentally better answers" (134), and we hypothesize that modeling and simulation is one such tool. Thus, this thesis will demonstrate how modeling and simulations, including new tools in the form of innovative models, can be used to answer the following key questions that may arise during drug development:

1) How to prove that two different intravenous formulations of iron are equivalent using a new innovative approach instead of relying on standard approaches that contradict iron's known pharmacology?

2) How to determine the factors that may influence the PK of a drug in patients and select a more refined dosing regimen accordingly?
3) How to predict the dose of a drug that could be administered to patients when no data is available yet in that population?

The articles presented hereafter will each present the utility of creating innovative models within modeling and simulations to answer the above key questions. In addition, it will be shown that the use of such tools to answer these critical questions can significantly improve the drug development process by relying on data from smaller trials, eliminating the need for additional trials and planning trials that have a higher probability of success.

Chapter 2 – Article #1

Novel Population Pharmacokinetic Method Compared to the Standard Noncompartmental Approach to Assess Bioequivalence of Iron Gluconate Formulations

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2.1 Preface

The first research project undertaken for this thesis aimed to demonstrate how advanced pharmacokinetic techniques, such as modeling, can be used to improve the development of generic products. Compartmental modeling is not a technique that has been entirely embraced in this particular subset of drug development, yet this article will show that it can be instrumental in answering important questions related to generic drugs, such as "Are two products bioequivalent?".

In a society that is already burdened by healthcare costs and an increasingly aging population, the savings afforded by generic drug products are an excellent incentive towards their continued, and increased, usage. Indeed, the cost savings associated with the use of generic products are substantial. One study reports that only 12 months after the introduction of a generic product to a therapeutic class, overall reductions in daily cost of therapy were noticeable. For lipid regulators and biphosphonates, the cost decreased by 32%, while it was 42% for selective serotonin reuptake inhibitors and 20% for calcium-channel blockers (138).

Accordingly, the use of generic drugs is on the rise, accounting for 51% of prescriptions in 2002 and 67% in 2007 (138). A recent estimate indicates that by the end of 2010, generic drugs accounted for 78% of all retail prescriptions dispensed in the U.S. (139). Similarly, in Canada, patented drugs have experienced a decline in overall drug sales between 2003 and 2012, suggesting that the sale of generic drugs has increased over the same time period (140). With the expiration of more and more patents, and with the increased economic pressure faced by third-party payers and patients, these figures can be expected to increase steadily.

Because of this increased use of generic drug products, it is important to consider how the manufacturers of such products can continue to provide quality drugs in a timely manner. In truth, the challenges faced by the pharmaceutical industry not only apply to the development of innovative new products, but they also affect the development of generic drugs. Although developing a generic drug product entails less risks and costs compared to

a brand-name product, generic drug companies must also be cost-efficient because of lower profit margins and increased competition with other generic manufacturers (141). Effective use of time is also an issue in generic drug development, and efforts are also made to perform the necessary assessments in a timely and effective manner. Thus, this research project aimed to address some of these challenges through the use of modeling and simulations.

In order to address drug development issues specific to the generic drug industry, the FDA emitted a document entitled "Critical Path Opportunities for Generic Drugs", 3 years following the publication of their original "Critical Path Initiative" (142). In this document, the FDA lists four main areas of opportunity specific to generic drug development: 1) improve the science of quality by design for the development and manufacture of generic drug products 2) improve the efficiency of current methods for assessment of BE of systemically acting drugs (including complex ones or those that employ novel methods of drug delivery 3) develop methods for the assessment of bioequivalence of locally acting drugs 4) develop methods for characterizing complex drug substances and products.

Some ideas proposed by the FDA to improve the science of quality by design include the use of modeling and simulations (such as absorption models or *in vitro-in vivo* correlation models) to better formulate products. Various methods are also suggested to improve the efficiency of current methods for evaluating the BE of systemic compounds, including expanding biowaivers based on BCS criteria to include categories II and III. Additionally, new approaches could be developed to assess the BE of drugs with novel delivery technologies (such as transdermal patches) or highly variables drugs. Improvements could also be made to the BE assessments of locally acting and targeted delivery drugs, such as inhaled products, nasal sprays, topical dermatological products and liposome products. Finally, complex drug substances could benefit from improved analytical methods for identification and from better statistical methods to compare profiles.

By addressing some of the issues raised by the FDA, it may be possible to develop generic products for innovator drugs that are either too complex to mimic, or difficult to characterize properly. It has been estimated that for a drug with no generic counterpart earning $500 million dollars per year, the introduction of a generic product could result in cost savings equivalent to hundreds of millions of dollars (141). Therefore, using improved tools to develop generic equivalents for products that only exist under innovator form could translate to significant savings. An improved process would not only lead to the creation of new generic products, but would also be worthwhile for the generic industry as a whole. Indeed, simulation results have also suggested that "sizable cost savings can also be attained by increasing generic efficiency rates" (138).

It is therefore important to examine ways in which the development of generic drugs can be improved. As demonstrated by the results of this first research project, one tool that has been used more considerably in the development of innovator products, but that can also be applied in the development of generic drugs, is modeling and simulations. Although the FDA mentions some of the advantages that this technique could confer at the drug formulation stage, modeling and simulations could also be used later on in the development process.

One of the potential utilities of modeling and simulations within the generic context is as a tool to assess bioavailability for further assessment of bioequivalence. This approach could be particularly useful when traditional methods of estimating bioavailability (based on PK studies and noncompartmental analyses) are not appropriate or robust. As previously mentioned, although they are relatively simple to conduct, noncompartmental analyses make certain assumptions which do not always hold true with all compounds. Iron complexes are examples of such compounds, as they are not significantly eliminated from the body and exhibit non-linear PK behaviour. Furthermore, it is not possible to directly measure iron that is bound to complexes. Therefore, data obtained from iron complexes are ideal candidates for compartmental analyses, which, as previously explained, can take into consideration all types of elimination (or lack thereof) as well as non-linearity. Compartmental analyses can also be used to describe the disposition of iron

associated with complexes in an indirect manner, by modeling the PK of analytes that are detectable (such as total iron or transferrin-bound iron).

Thus, the following article will demonstrate how population PK (compartmental) analyses can be used to determine if two formulations of iron complexes are bioequivalent. It will also illustrate that properly conducted population PK analyses can replace larger, traditional PK studies that rely on noncompartmental methods, thereby resulting in time and cost savings.

2.2 Abstract

Purpose: Iron-containing products are atypical in terms of their pharmacokinetic properties because iron is only removed by plasma sampling and is non-linear. This study aims to present a novel way of assessing the relative bioavailability of two sodium ferric gluconate complex (SFGC) formulations and compare this approach to a standard previously published noncompartmental approach.

Methods: Data were from open-label, randomized, single-dose studies (Study 1 was parallel whereas Study 2 was crossover). Subjects with low but normal iron levels were infused IV SFGC in sucrose by GeneraMedix Inc. and/or Ferrlecit® Injection (Watson Laboratories Inc.). In Study 1 (n=240), 125 mg was infused over 10 minutes. In Study 2 (n=29), 62.5 mg was infused over 30 minutes. Samples were assayed for total iron (TI) and transferrin-bound iron (TBI) over 36 hours (Study 1) or 72 hours (Study 2) post-dose. Studies 1 and 2 used standard noncompartmental analysis. Study 2 also used population PK (PPK) analyses with ADAPT 5®. The final model predicted SFGC area-under-the-curve (AUC_{pred}) and maximal concentration ($Cmax_{pred}$). Analyses of variance was conducted on ln-transformed PK parameters. Ratios of means and 90% confidence intervals (CIs) were estimated. Bioequivalence was demonstrated if values were within 80-125%.

Results: For Study 1, ratios and 90% CIs for TI baseline-corrected Cmax and AUC_{0-36} were 100.4 (96.5 – 104.5) and 99.7 (94.2 – 105.5). For TBI, results for TI baseline-

corrected Cmax and AUC_{0-36} were 86.8 (82.7 – 91.1) and 92.4 (85.6 – 99.7). For Study 2, a multi-compartmental model simultaneously described the PK of TI, TBI and SFGC. Ratios and 90% CIs for SFGC $Cmax_{pred}$ and AUC_{pred} were 89.9 (85.9 - 94.0) and 89.7 (85.7 - 93.9), while ratios and 90% CI obtained from the noncompartmental analysis of Study 2 did not meet BE criteria because of low power.

Conclusions: Both the standard and PPK modeling approach suggested bioequivalence between the iron products. However, with the PPK method, less subjects were required to meet study objectives compared to the standard noncompartmental approach which required considerably more subjects (29 vs 240).

2.3 Introduction

Iron plays several important roles in the human body, by participating in transmembrane transport, electron transfers, DNA synthesis and acting as a co-factor in enzymatic reactions (especially those involving heme) (1,2). In addition to these functions, iron plays a vital part in the transport of oxygen via the heme molecule, a porphyrin ring structure with a central iron atom (1,2). It is also a component of enzymes such as peroxidase, myeloperoxidase, amino acid hydroxylase and 5-lipoxygenase (2).

Because of its vital importance to so many bodily functions, severe iron deficiency is often treated with intravenous administration of iron. Parenteral iron was first administered over a century ago, and since then various intravenous formulations of iron have been developed (3). Although the different iron nanoparticles (iron dextran, iron sucrose, sodium ferric gluconate, ferumoxytol) exhibit various characteristics, once the iron is internalized, it is taken up by the reticuloendothelial system (RES) composed of monocytes and macrophages in the liver, spleen and bone marrow (2,4,5). Subsequently, it is bound to transferrin, either intracellularly in pools or extracellularly for transport to erythrocytes. It should be noted that a small fraction of iron likely binds to extracellular transferrin directly from the plasma (4). Only a small amount of iron is excreted daily in the

urine and feces (around 1 mg or 0.03% of the average body's total stores), as there is no true excretion pathway for iron, and the loss from desquamation of skin cells and sweat is negligible (1,5,6,7). Thus, iron requirements for erythropoiesis are generally met through the recycling of iron from senescent erythrocytes (1,5). An overview of iron metabolism is presented in Appendix 1.

The pharmacokinetics (PK) of iron appears to be non-linear, as demonstrated by the saturable plasma clearance of iron dextran, which appears to reach a plateau after doses exceeding 500 mg (8). This non-linearity is thought to occur between the distribution of iron taken up by the RES that subsequently binds to transferrin. In addition, it is virtually not eliminated from the body, as it is only lost through phenomena such as blood donations/sampling or through blood loss and hemorrhagic events (2,9). Because of these particular PK characteristics, iron does not lend itself well to noncompartmental PK analyses (10). Indeed, it violates certain basic assumptions of noncompartmental analysis such as linearity and constant elimination from the sampling compartment (10,11,12). In addition, the endogenous baseline concentration of iron is not constant and changes significantly after iron dosing simply because iron is not eliminated. All of this can pose problems when using the noncompartmental approach to derive baseline adjusted parameters such as the maximal observed serum or plasma concentration (Cmax) and the area under the plasma/serum concentration time curve (AUC) as this method assumes linearity in the PK of iron and in its baseline levels. These PK parameters are often used to assess the relative bioavailability (BA) between two formulations, a process that compares "the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action" (13). This comparison is central to the bioequivalence (BE) assessment, which aims at determining if administration of the same molar dose of the same active ingredient or moiety under identical conditions leads to similar BA, or BA that is not significantly different.

Specific BE guidelines outlining the ideal study design and statistical approaches that are preferred have been published by various regulatory (government) agencies (14,15,16,17), and for most drug products, BE is generally assessed by comparing the

average BA parameters (Cmax and AUC) of two products. Study designs tend to be two-period, two-treatment, two-sequence crossovers conducted in healthy volunteers, to minimize variability by administering the test and reference products to the same subjects. Using the ln-transformed PK parameters calculated for each subject, analyses of variance (ANOVA) are then performed and least square mean (LSM) ratios of test to reference PK parameters and 90% confidence intervals (CIs) associated with the ratios are obtained. Pre-defined bioequivalence limits, which can be viewed as target goalposts, are then used to determine BE by assessing whether or not LSM ratios and CIs fall within the targeted range.

Although such BE guidelines are generally appropriate for most drugs, the BE assessment of drugs with particular PK characteristics remains challenging. For example, the presence of endogenous levels for drugs such as iron or levothyroxine can make it difficult to distinguish between drug concentrations that come from exogenous sources and those already present in systemic circulation, especially if baseline levels account for a large portion of the observed concentrations (18,19). Furthermore, endogenous substances are frequently subject to processes not typically associated with non-endogenous products (feedback mechanisms (20,21,22), saturable transport or elimination, etc.) (23).

For iron products, the Office of Generic Drugs (OGD) of the US Food and Drug Administration (FDA) has recommended to sponsors of generic submissions that bioequivalence be assessed on baseline-adjusted Cmax and AUC_{0-t} of total serum iron and of transferrin-bound iron. Considering the relative bioavailability of two iron formulations *theoretically* cannot be determined reliably by noncompartmental methods using total serum iron (TI) and transferrin-bound iron (TBI) because of the changing baseline following iron administration and its non-linear PK, other approaches for calculating AUC and Cmax and subsequently establishing the bioequivalence of iron products should be considered. Thus, we postulated that compartmental analyses, rather than noncompartmental analyses, could be more powerful (e.g., would present better statistical power for the same number of subjects) to use to determine the PK parameters necessary for the assessment of the relative bioavailability of two formulations of intravenously-

administered Sodium Ferric Gluconate Complex in Sucrose (SFGC) simply because the non-linear characteristics of iron and its changing baseline could be addressed with that method. In other words, the aim of this analysis was to assess the relative bioavailability of two IV formulations of iron by using the compartmental approach. Standard noncompartmental analyses were also conducted so that statistical power would be compared, including results from a previously published study.

2.4 Subjects and Methods

2.4.1 Study Design

This study was an open label, randomized, single dose, two-treatment, two-period, two-sequence, crossover study. A four week washout period was observed between doses. Subjects received both the test product (Sodium Ferric Gluconate Complex in Sucrose Injection, 62.5 mg/5 mL) and the reference product (Ferrlecit® Injection by Watson Laboratories Inc., 62.5 mg/5 mL) at a dose of 62.5 mg as an intravenous infusion over 30 minutes. Both treatments were diluted in 50 mL of 0.9% NaCl.

Subjects were advised to fast for at least 10 hours before dosing, and until at least 4 hours after dosing. Water was permitted ad libitum. Standardized meals (with no specific restrictions on iron content) were served at about 4 (breakfast), 10 (lunch) and 14 (dinner) hours post-dose on Day 1 and at 24 (breakfast), 28 (lunch) and 33.5 (dinner) hours post-dose on Day 2.

Subjects were confined to the clinical facility from at least 10 hours before Day 1 dosing in each period and were required to stay until 36 hours thereafter. Subjects returned to the clinical site for the 48 (± 1) and 72 (± 1) hour post-dose blood sample collection.

During both periods, twenty one (21) PK samples were collected at the following times based on the start of the infusion: -0.5, -0.25 and -0.083 hour (to establish baseline values); 0.25 hour (mid-point of infusion), 0.5 hour (immediately at the end of the infusion); and 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, 36, 48 and 72 hours after the start of the infusion. An additional 12.5 mL of blood was obtained from subjects pre-

infusion and an additional 8.5 mL of blood was obtained from subjects at 4, 16, 48 and 72 hours post-infusion start for the measure of hematocrit (only pre-infusion), ferritin, transferrin saturation and total iron binding capacity. A total of about 420 mL of blood was obtained from each subject over the course of the study for analysis.

Adverse events and vital signs were monitored throughout the study. Subjects were closely supervised and remained within sight of study personnel for four hours after receiving their initial dose. In addition, seated blood pressure and heart rate were measured prior to dosing and at 12, 24 and 36 hours post-dose.

2.4.2 Population

The study population was comprised of healthy male and non-pregnant female volunteers between the ages of 18 to 55 years old, inclusively, with low but normal iron levels. Ferritin levels had to be between 22 and 100 ng/mL, inclusively, for men, and between 10 and 100 ng/mL, inclusively, for women prior to first dosing. All subjects were required to have a body mass index of 18-32 kg/m^2 as well as an acceptable medical history, laboratory evaluation and physical examination before study entry. The laboratory tests included screens for biochemistry, hematology, urinalysis, cotinine, drugs of abuse, hepatitis B and C, and HIV as well as beta-human chorionic gonadotropin (if applicable) and follicle-stimulating hormone (if applicable).

2.4.3 Ethics

The protocol, protocol amendments and informed consent forms were approved by an institutional review board before any study-related procedures were initiated. Written, informed consent was obtained from volunteers prior to their participation in this study. This study was conducted in accordance with ethical principles outlined in the Declaration of Helsinki, and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans as well as Canadian Regulatory requirements and guidelines.

2.4.4 Sample Handling and Bioanalytical Method

Samples were sequentially collected by direct venipuncture or catheter and processed in a timely manner. Samples were allowed to clot at room temperature for a minimum of 30 minutes and for a maximum of 45 minutes. The tubes were then centrifuged at 3000 RPM and 4 ± 1° C for 10 minutes and then placed at room temperature for a maximum of 15 minutes. A minimum of 1 mL of serum was transferred into duplicate 5 mL polypropylene tubes and maintained in the ice bath or cooling device until frozen. Samples were stored at approximately -20°C (between -10 and -35°C) until transfer or shipment to the bioanalytical laboratory. The time between sample collection and freezer storage did not exceed 1.5 hours.

A validated analytical colorimetric method was used to assay total iron (TI) and transferrin bound iron (TBI). TI and TBI concentrations were measured within the validated standard curve range of 50 to 2000 mcg/dL. Assays were performed by Cetero Research.

2.4.5 Population Pharmacokinetic Modeling

Datasets included subjects who completed the study and who had measurable concentrations of TI and TBI. Actual doses, infusion durations and sampling times were used to create the datasets, and iron lost through blood sampling was also taken into consideration. For each subject, the hematocrit value used to account for iron lost during blood sampling was calculated as an average of the hematocrit values taken before dosing in either Period 1 or 2. Concentration values that were below the limit of quantitation (BLQ) were treated as missing values.

Compartmental analyses were performed using the software ADAPT 5® (24), first using the maximum likelihood method to obtain initial estimates and subsequently using the iterative two stage (ITS) approach. This is a fully automated mixed effect modeling approach using both maximum likelihood and maximum a posteriori (MAP) modeling approaches. Briefly, the first probable population PK parameters and variance estimates (e.g., residual variability) were found by using maximum likelihood. Then a population

analysis is undertaken where population, individual and residual variability PK parameters are calculated and updated with ever more probable values at every new population iteration. The mixed modeling approach was eventually stopped when it had converged (last iteration preceded by 10 consecutive iterations having a similar objective function (within 3%)) and the most probable and stable results were found.

The base model used for model discrimination was a previously published multicompartmental model that simultaneously described the time courses of total iron, transferrin-bound iron and iron bound to sodium ferric gluconate complex (SFCG-I) (25). The primary PK parameters estimated by this model included: CL_1 (the clearance of SFGC-I to the RES), CL_2 (clearance of SFGC-I directly to transferrin), CL_3 (clearance of iron entering and exiting the marrow and red blood cell compartment), CL_4 (clearance of TBI to the RES), Km (Iron concentration associated with half of the maximal rate of exchange between the RES and TBI compartments), Vss (the apparent steady-state volume of distribution of SFGC-I), V_RES (volume of distribution associated with the RES), V_RBC (marrow and red blood cell compartment), V_TBI (volume of distribution associated with TBI), and Vmax (maximal rate of exchange between the RES and TBI compartments).

All iron concentrations were fitted using weighting procedures of Wj = 1/σj2 where the variance σ_j^2 was calculated for each observation using the equation $S_j^2=(a+b\cdot Y_j)^2$ where a and b are the intercept and slope of each variance model. The slope is the residual variability proportional to each concentration and the intercept is the additional component of the residual variability. Inter-subject variability was also estimated for each PK parameter estimated by the model.

Secondary PK parameters that were derived from the primary PK parameters included the following: CL (total clearance for SFGC-I, calculated as the sum of CL_1 + CL_2), AUC_{pred} (area under the serum-time curve of SFGC-I, from the beginning of the infusion to infinity, calculated as dose divided by CL), $Cmax_{pred}$ (maximum predicted serum concentration of serum SFGC-I over the 72-hour sampling period), and $T_{1/2}$ (apparent first-order terminal elimination half-life of SFGC-I).

In addition to standard metrics used to evaluate goodness of fit, visual predictive checks were performed. With the final model estimates for both population PK parameters and variability, concentration-time profiles for 1000 subjects were simulated, and median concentrations along with 95% confidence intervals were established using the predicted concentrations. Observed concentration values and predicted confidence intervals were then overlaid graphically.

2.4.6 Statistical Analyses for Bioequivalence Assessment

Analyses of variance (ANOVA) were performed on the natural logarithm of $Cmax_{pred}$ and AUC_{pred} for SFGC-I obtained from the compartmental analysis. The ANOVA model included group, sequence, period nested within group and formulation as fixed effects and subject nested within group*sequence as a random effect. The group*formulation interaction was tested at a 5% level of significance and removed from the model if it was not significant. Sequence was tested using subject nested within group*sequence as the error term. A 10% level of significance was used to test the sequence effect. Each analysis of variance included calculation of least-squares means, the difference between adjusted formulation means and the standard error associated with this difference. The above statistical analyses were conducted using the appropriate SAS® procedure.

In agreement with the two one-sided test for bioequivalence (26), 90% confidence intervals for the difference between drug formulation least-squares means (LSM) were calculated for AUC_{pred} and $Cmax_{pred}$ obtained from the compartmental analysis, using the data transformed to their natural logarithm. The confidence intervals were expressed as a percentage relative to the LSM of the reference formulation.

Ratios of means were calculated using the LSM for the above mentioned ln-transformed AUC_{pred} and $Cmax_{pred}$ obtained from the compartmental analysis. The geometric mean values were reported and ratios of means were to be expressed as a percentage of the LSM for the reference formulation.

Bioequivalence was to be declared if the Test/Reference ratios of geometric means of $Cmax_{pred}$ and AUC_{pred} and their complete 90% confidence intervals were to be contained within the bioequivalence interval 80.00 to 125.00% for iron bound to the SFGC.

2.4.7 Noncompartmental Analyses

Baseline-adjusted PK parameters AUC_{0-t}, AUC_{inf}, Cmax and Tmax were calculated for TI and TBI. Baseline adjustments were performed by subtracting each individual's baseline value (which was the average of all 3 pre-dose values) from each of their post-dose concentration value. ANOVA were conducted on ln-transformed PK parameters AUC_{0-t}, AUC_{inf} and Cmax for TI and TBI using the same statistical model as the one employed for the parameters obtained from the compartmental analysis. Similarly, ratios of LSM and 90% CI were calculated for each parameter.

Results derived from noncompartmental analyses were also obtained from a previously published study (27). This study was an open-label, randomized, single-dose, parallel-group study conducted in 240 healthy volunteers under fasting conditions. Subjects received 125 mg of the test (Nulecit™, Watson Pharmaceuticals) or reference (Ferrlecit™, A. Nattermann & Cie. GmbH.) SFGC formulation infused intravenously over 10 minutes. Samples for TI and TBI analysis were collected prior to dosing and at 0.0833, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, and 36 hours after the start of the infusion. Samples were also collected at 24, 18, 12, 6, and 0 hours before dosing to determine baseline levels. Validated spectrophotometric assays were used to assay TI and TBI (refer to original publication for more details).

Post-dose concentration values were adjusted using the average of all 5 baseline values for each individual, and PK parameters AUC and Cmax were determined by standard noncompartmental methods with these baseline-adjusted concentrations assuming a stable baseline. ANOVA was conducted to compare ln-transformed PK parameters between formulations. Treatment, group and group-by-treatment were used as classification variables. The group-by-treatment interaction and group terms were removed if they were deemed non-significant at an alpha level of 5%. Geometric mean treatment ratios and the

corresponding 90% CIs were determined and BE was declared if the ratios and 90% CIs were contained within 80 and 125% (27).

2.5 Results

2.5.1 Population Characteristics

A total of 32 subjects were enrolled in the study, and 29 completed both periods of the study. Subjects were dosed in two groups, where Group 1 (Subjects 1 to 16) was dosed on February 5, 2008 (Period 1) and March 4, 2008 (Period 2) and Group 2 (Subjects 17 to 32) was dosed on February 8, 2008 (Period 1) and March 7, 2008 (Period 2). Table 1 summarizes the demographic characteristics of the subjects enrolled in the study (both groups combined).

Table 1. Demographic Traits of Subjects Included in the Population PK Analysis

Characteristic	**Number (%)**
Sex	
Female	24 (75%)
Male	8 (25%)
Race	
Caucasian	20 (62.5%)
African American	7 (21.9%)
Asian	4 (12.5%)
Native American	1 (3.1%)

Characteristic	**Mean ± SD (CV%)** **Median (Minimum – Maximum)**
Age (years)	37.3 ± 9.29 (24.9%) 37.5 (22 – 51)
Height (cm)	164.4 ± 7.20 (4.38%) 164.5 (149.5 – 180.0)
Weight (kg)	67.8 ± 10.3 (15.1%) 65.5 (47.1 – 89.1)
Body mass index (kg/m^2)	25.0 ± 3.00 (12.0%) 24.8 (20.4 – 31.8)
Hemoglobin at screening (g/dL)	13.4 ± 1.09 (8.13%) 13.2 (11.2 – 15.4)
Hematocrit at screening (%)	40.3 ± 3.35 (8.32%) 39.9 (33.9 – 46.1)

CV% : Percent coefficient of variation; SD : Standard deviation

Of the 3 subjects who did not complete the clinical phase of the study, 2 withdrew before Period 2 because of adverse events deemed unlikely to be related to the

investigational product and one subject was withdrawn by the Investigator because of a positive drug screen test (amphetamines) at Period 2 check-in.

Subjects included in the previously published study were predominantly White (comprising around 91% of the subjects receiving the generic formulation and 85% of the subjects receiving the reference formulation). Around 7% and 13% of subjects in the generic and reference treatment arms, respectively, were Black or African American and other races (American Indian/Alaskan, Native Hawaiian/Pacific Islander, multirace subjects) accounted for less than 2.5% of subjects in both groups. In terms of ethnicity, over 90% of subjects in each arm were not Hispanic or Latino. In both treatment arms, around 50% of the subjects were male. Mean (± SD) age, height, weight and body mass index in the generic cohort were 30.8 ± 9.6 years, 171 ± 9.5 cm, 75.1 ± 15.0 kg and 25.5 ± 3.77 kg/m^2, respectively. Mean (± SD) values for age, height, weight and body mass index were similar in the reference cohort, at 29.9 ± 8.5 years, 172 ± 8.7 cm, 77.5 ± 13.4 kg and 26.0 ± 3.50 kg/m^2, respectively.

2.5.1 Population PK Approach

A total of 2413 concentrations of TI and TBI were included in the analysis. Two basic models were evaluated. In the first model, it was assumed that the test and reference product had the same values for CL_1, CL_2 and Vss but with a different relative bioavailability factor (Frel). In other words, CL_1, CL_2 and Vss between formulations only differed by the same factor Frel. In the second model, different values for CL_1, CL_2 and Vss were estimated for the test and reference products. Results from the STS analysis performed with these 2 models are presented in Table 2.

Table 2. PK Model Discrimination

Model	BIC	AIC	OF	R^2		Residual variability (%)	
				TI	TBI	TI	TBI
Model 1	990.742	945.014	453.507	0.927	0.808	21.0	17.2
Model 2	998.591	948.050	453.025	0.930	0.829	20.1	16.0

AIC – Akaike information criterion; BIC – Bayesian information criterion; OF – Objective function; R^2 – Coefficient of determination; TI – Total iron; TBI – Transferrin-bound iron;

Based on the model discrimination criteria, especially as indicated by the Bayesian and Akaike information criterion, as well as the graphical indicators of goodness of fit, the first model was superior. Two additional parameters were estimated: C_NTBI_P1 and C_NTBI_P2. These parameters estimated the concentrations of non-transferrin bound iron in Periods 1 and 2, respectively. Differential equations describing the final model were:

$$\frac{dX(1)}{dt} = R(1) - \frac{CL_1 + CL_2}{Vss} \cdot X(1)$$

$$\frac{dX(2)}{dt} = \frac{CL_1}{Vss} \cdot X(1) + \frac{CL_3}{V\_RBC} \cdot X(4) + \frac{CL_4}{V\_TBI} \cdot X(3) - \frac{V_{max}}{Km \cdot V\_RBC + X(2)} \cdot X(2)$$

$$\frac{dX(3)}{dt} = \frac{CL_2}{Vss} \cdot X(1) + \frac{V_{max}}{Km \cdot V\_RBC + X(2)} \cdot X(2) - \frac{CL_4}{V\_TBI} \cdot X(3) - \frac{CL_3}{V\_TBI} \cdot X(3)$$

$$\frac{dX(4)}{dt} = \frac{CL_3}{V\_TBI} \cdot X(3) - \frac{CL_3}{V\_RBC} \cdot X(4) - K0 \cdot R(2)$$

Where X(1), X(2), X(3) and X(4) represent the amount of iron in the serum, RES, TBI and red blood cell (marrow) compartments. R(1) represents the SFGC infusion rate while R(2) is an on/off switch that accounts for the iron loss associated with blood sampling. K0 was the rate of iron loss, which was calculated as the product of the hematocrit (for each subject at each period) and the approximate blood volume extracted per blood draw (7 mL).

The parameter Vmax was defined as Vmax = (CL3 + CL4) x (Km + TBIbase), where TBIbase represents the observed baseline TBI concentration for each subject before dosing in each period. This equation was determined from the assumption that prior to the administration of SFGC, iron levels are at an equilibrium between the RES, TBI and RBC (marrow) compartments.

Observed concentrations for total serum iron and TBI, parameterized as Y(1) and Y(2), respectively, were fitted according to the following equations, where C_NTBI was different for Periods 1 and 2, as previously described.

$$Y(1) = \frac{X(1)}{Vss} + \frac{X(3)}{V\_TBI} + C\_NTBI$$

$$Y(2) = \frac{X(3)}{V\_TBI}$$

The final model is depicted in Figure 1 and the PK parameter estimates from the final model are presented in Table 3.

Figure 1. Final PK Model

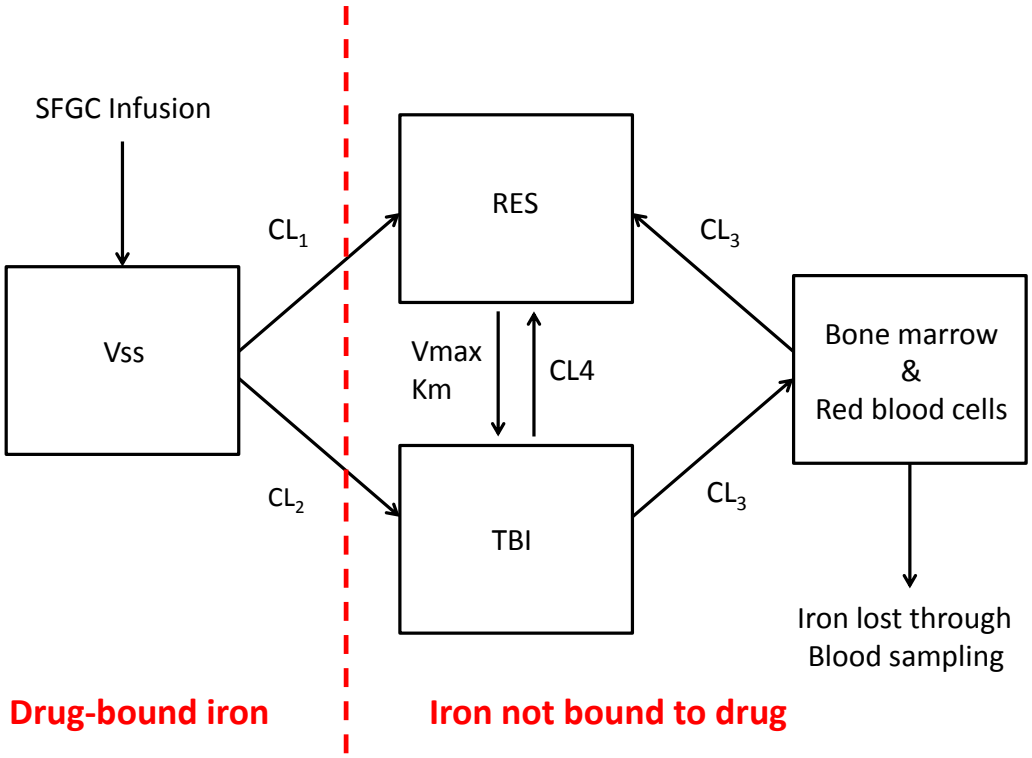

Table 3. Population PK Parameter Estimates

PK Parameter	Mean (CV%)	Median (Minimum- Maximum)
CL_1 (L/h)	2.25 (32.0%)	2.03 (1.46 – 4.17)
CL_2 (L/h)	0.0458 (11.8%)	0.0455 (0.0357 – 0.0571)
Vss (L)	4.41 (14.5%)	4.53 (3.32 – 5.50)
V_RES (L)	1220 (0.175%)	1220 (1214 – 1226)
V_TBI (L)	0.589 (5.62%)	0.595 (0.522 – 0.637)
CL_3 (L/h)	0.000000213 (59.5%)	0.000000267 (4.01E-11 – 3.48E-07)
V_RBC (L)	0.000288 (55.0%)	0.000369 (0.00000539 – 0.000451)
CL_4 (L/h)	0.0313 (23.2%)	0.0315 (0.0170 – 0.0439)
Km (mcg/dL)	36.1 (37.0%)	38.8 (3.61 – 59.7)
Frel	0.905 (13.9%)	0.926 (0.644 – 1.19)
C_NTBI_P1 (mcg/dL)	44.3 (52.8%)	45.5 (5.18 – 83.1)
C_NTBI_P2 (mcg/dL)	75.8 (50.2%)	73.6 (0.00 - 147)

CL_1: Clearance of SFGC-I to the reticuloendothelial system (RES) compartment; CL_2: Clearance of SFGC-I directly to transferrin; Vss: the apparent steady-state volume of distribution of SFGC-I; V_RES: volume of distribution associated with the RES; V_TBI: volume of distribution associated with TBI; CL_3: clearance of iron entering and exiting the marrow and red blood cell compartment; V_RBC: marrow and red blood cell compartment; CL_4: clearance of TBI to the RES; Km: Iron concentration associated with half of the maximal rate of exchange between the RES and TBI compartments; Frel: Relative bioavailability factor; C_NTBI_P1: Concentration of non-transferrin-bound iron during Period 1; C_NTBI_P2: Concentration of non-transferrin-bound iron during Period 2; CV%: Percent coefficient of variation

Plots of goodness of fit are presented in Figure 2 while visual predictive checks are presented in Figure 3.

TOTAL IRON **TRANSFERRIN BOUND IRON**

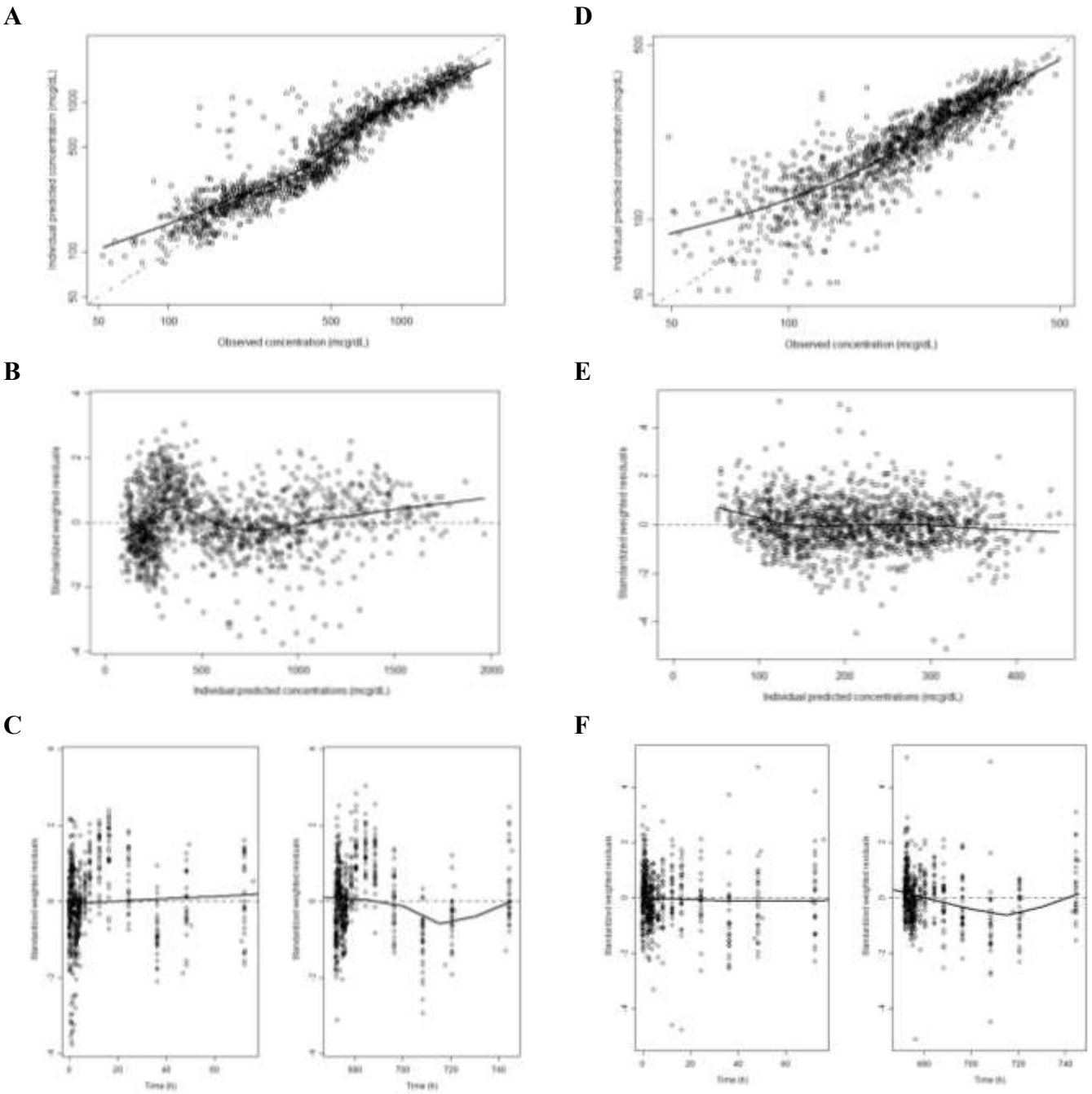

Figure 2. Goodness-of-Fit Plots Individual observed versus individual predicted total iron concentrations on a log scale (A); Standardized residuals versus individual predicted total iron concentrations (B); Standardized residuals versus time for total iron concentrations (C); Individual observed versus individual predicted transferrin-bound iron concentrations on a log scale (D); Standardized residuals versus individual predicted transferrin-bound iron concentrations (E); Standardized residuals versus time for transferrin-bound iron concentrations (F); Legend: Circles = observed concentrations, Dotted line = reference line (unity or zero), Solid black line = Loess curve (span = 0.5, degree = 1)

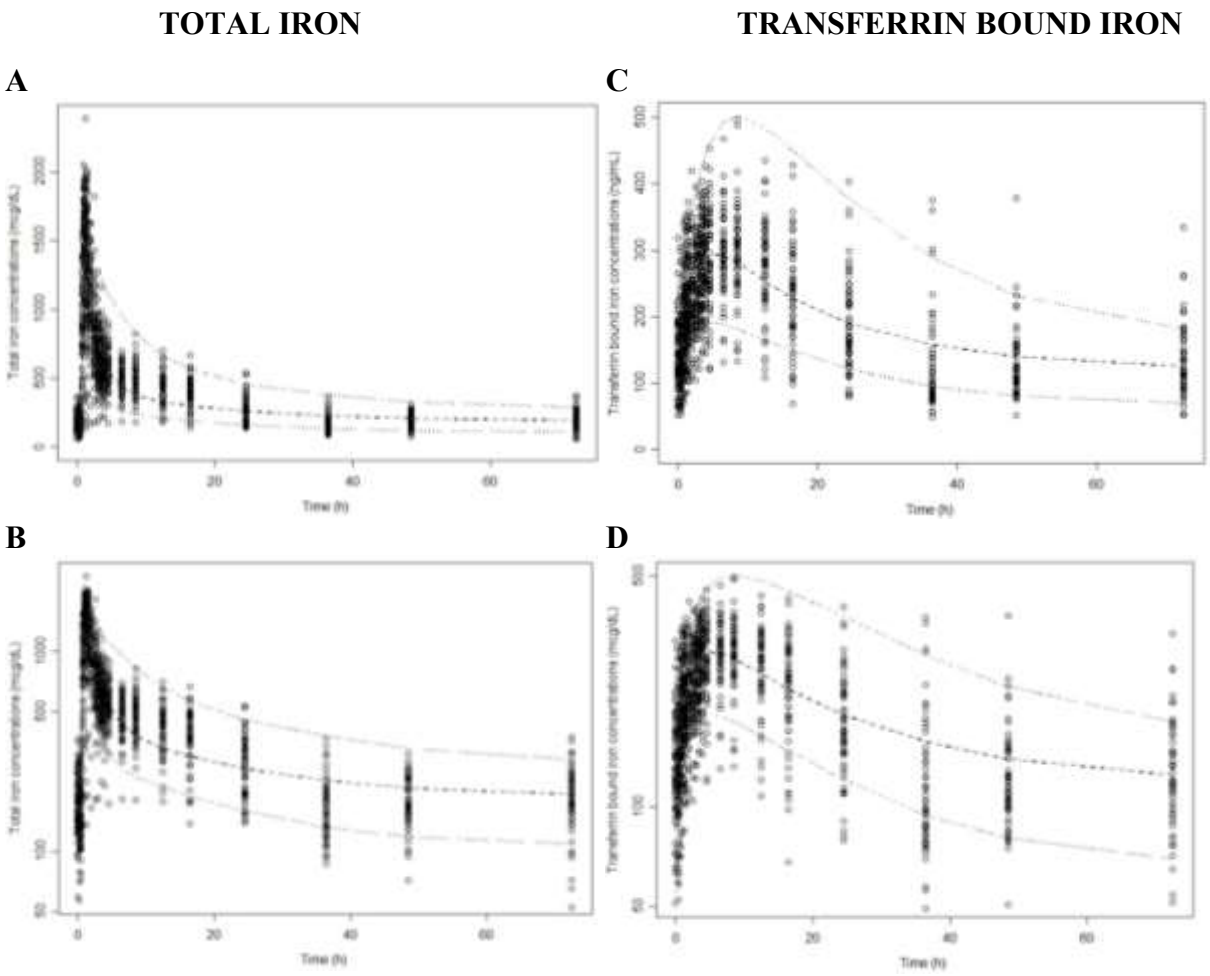

Figure 3. Visual Predictive Checks Total Iron Concentration Time Profiles (Linear Scale) (A); Total Iron Concentration Time Profiles (Semi-Log Scale) (B); Transferrin Bound Iron Concentration Time Profiles (Linear Scale) (C); Transferrin Bound Iron Concentration Time Profiles (Semi-Log Scale) (D); Legend: Circles = observed concentrations; Dotted line = 95% confidence intervals; Dashed line = median predicted concentration

As demonstrated by the goodness-of-fit plots and visual predictive checks, the model adequately describes all observed concentrations of total iron and transferrin-bound iron. Predicted versus observed concentrations were randomly scattered around lines of identity. No important trends were observed with respect to the standardized weighted residuals or with respect to time. In addition, the residual variability, which includes the intra-individual variability, variability from the bioanalytical measurement, all experimental error and all errors from the modeling itself, was only 23.0% and 17.2% for total iron and transferrin-bound iron, respectively.

Secondary PK parameters estimated from the final model are presented in Table 4. The median estimated terminal elimination half-life for both the test and reference products was 1.63 hours (ranging from 0.785 to 5.92 hours).

Table 4. Secondary PK Parameters

Parameter	Mean (CV%) Median (Range)	
	Test	**Reference**
Vss (L)	4.96 (19.3%) 5.20 (3.38 – 6.41)	4.41 (14.5%) 4.53 (3.32 – 5.50)
CL (L/h)	2.60 (34.4%) 2.30 (1.55 – 4.77)	2.30 (31.4%) 2.07 (1.51 – 4.22)
AUC_{pred} (mcg*h/dL)	2662 (30.8%) 2714 (1400 – 4033)	2927 (24.9%) 3017 (1481 – 4152)
$Cmax_{pred}$ (mcg/dL)	1150 (20.5%) 1100 (854 – 1654)	1270 (14.7%) 1227 (946 – 1649)

AUC_{pred}: Area under the serum-time curve of SFGC-I, from the beginning of the infusion to infinity, calculated as dose divided by CL; CL: Total clearance for SFGC-I, calculated as the sum of $CL_1 + CL_2$; $Cmax_{pred}$: Maximum predicted serum concentration of serum SFGC-I over the 72-hour sampling period; CV%: Percent coefficient of variation; Vss: Apparent steady-state volume of distribution of SFGC-I

ANOVA results demonstrated no statistically significant sequence effect at a 10% level, although there was a statistically significant group effect for ln-transformed $Cmax_{pred}$ (p=0.0104). Because the sizes of Groups 1 and 2 were similar (n=15 and n=14, respectively), the equality of variances test was not performed since ANOVA is robust to the violation of the equality of variance assumption when groups are equally sized and larger than 5 (28). Statistical analyses on the ln-transformed $Cmax_{pred}$ and AUC_{pred} parameters for SFGC-I are presented in Table 5.

Table 5. Summary of the Statistical Analyses for Ln-transformed PK Parameters

Study	Analysis Type	Analyte	Cmax (mcg/dL)			AUC (mcg·h/dL)[d]			Statistical Power to Prove BE
			Test[a]	Reference[a]	Ratio[b] (90% CI)	Test[a]	Reference[a]	Ratio[b] (90% CI)	
Study 1 (n=29)	Compartmental	SFGC-I	1127	1256	89.9 (85.9 - 94.0)	2534	2828	89.7 (85.7 - 93.9)	> 80%
	Noncompartmental[c]	TI	1338	1279	104.6 (86.3 – 127.0)	8807	9071	97.1 (74.1 – 127.2)	< 40%
		TBI	171.1	178.4	95.9 (83.4 – 110.2)	3364	2811	119.7 (20.5 – 698.6)	
Study 2 (n=240)	Noncompartmental[c]	TI	3106	3098	100.4 (96.5 – 104.5)	11101	11033	99.7 (94.2 – 105.5)	> 80%
		TBI	NA	NA	86.8 (82.7 – 91.1)	NA	NA	92.4 (85.6 – 99.7)	

NA – Information not available in publication
[a]Geometric mean for Study 1 and arithmetic mean for Study 2 (geometric mean not reported for Study 2)
[b]Ratio of geometric means (Test/Reference)
[c]Baseline-adjusted PK parameters
[d]AUC_{inf} for Study 1 and AUC_{0-36} for Study 2

2.5.1 Noncompartmental Approach

Mean concentration-time profiles for TI and TBI associated with each of the studies are presented in Figure 4. ANOVA results obtained from noncompartmental analyses of both studies are summarized in Table 5.

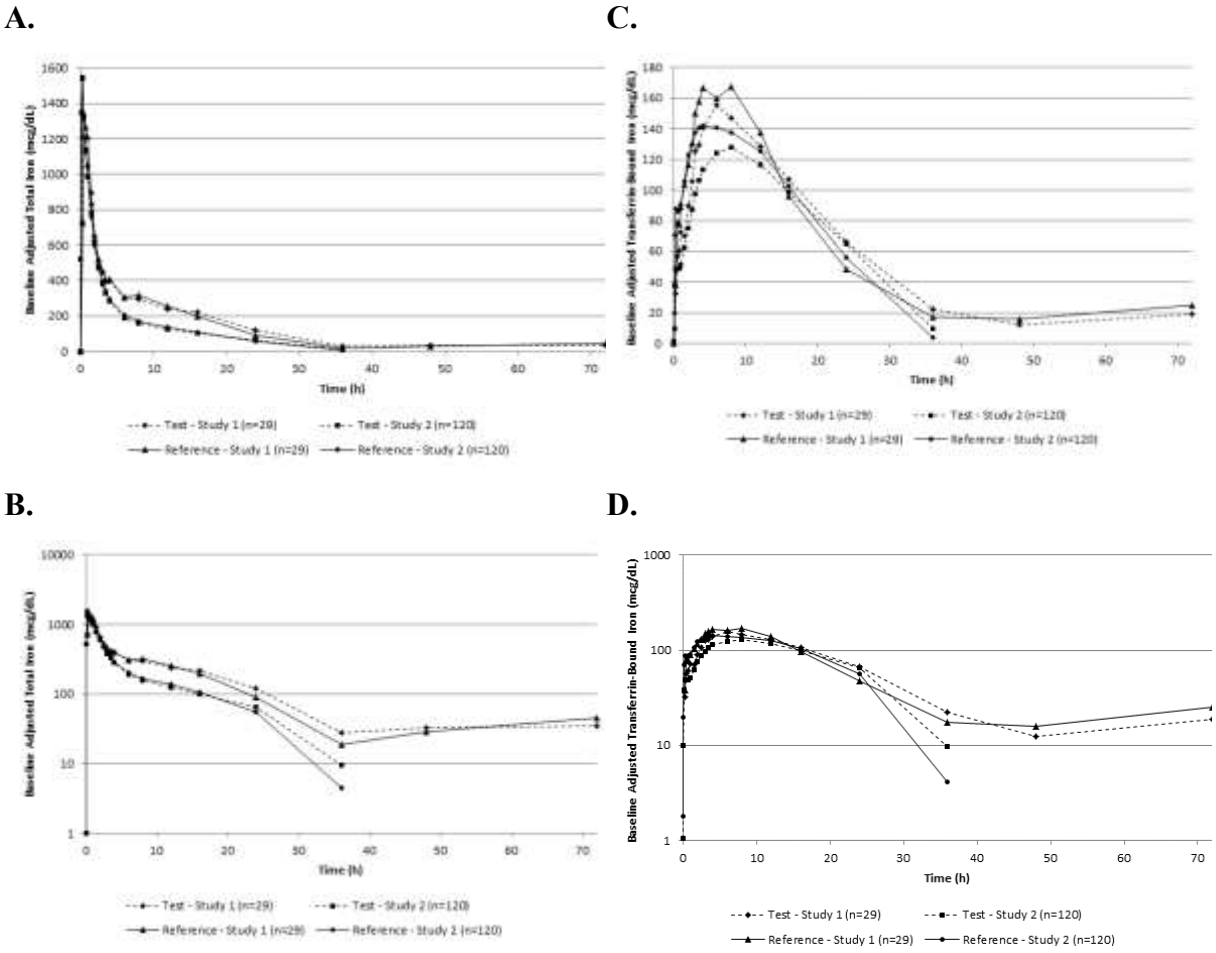

Figure 4. Mean Concentration Versus Time Profiles (A) Baseline-adjusted total iron profiles (linear scale) (B) Baseline-adjusted total iron profiles (semi-log scale) (C) Baseline-adjusted transferrin-bound iron profiles (linear scale) (D) Baseline-adjusted transferrin-bound iron profiles (semi-log scale); Legend: Dashed lines represent the test product while solid lines represent the reference product. Diamond and triangle symbols

represent data associated with Study 1 while squares and circles are associated with Study 2.

2.6 Discussion

Both the standard, noncompartmental approach to BE assessment (only when n = 240) as well as the innovative population PK approach (n = 29) demonstrated BE between the iron formulations that were tested. To our knowledge, this is the first published account of compartmental analyses being used to demonstrate BE with pharmacokinetic endpoints for iron products. This unconventional approach was favoured over the traditional noncompartmental method of calculating Cmax and AUC because of the particularities of iron pharmacokinetics. Noncompartmental analyses are robust when certain assumptions hold true, and iron violates many of these assumptions. The first assumption is that the drug in question displays linear pharmacokinetics (10,12). In other words, exposure increases in proportion with increasing dose. A second important assumption is that the drug is eliminated strictly from the body from the pool in which it is being measured, the plasma, for example and in a continuous fashion (11,12). Finally, this approach assumes that all sources of the drug are direct and unique to the measured pool, and that consequently baseline levels remain constant (12). In contrast, the compartmental method employed in the current analysis does not require the drug under study to meet such assumptions as the model included all of these iron PK characteristics. Similar approaches could be applied to analyze other difficult drugs from a PK point of view. Examples may include enzymes or metabolites that are metabolized intra-cellularly (29).

Another challenge for assessing SFGC pharmacokinetics is the inability to assay the iron associated with SFGC directly. Although it is possible to assay total serum iron and transferrin-bound iron, it is impossible to distinguish endogenous iron from the iron provided by SFGC supplementation. One approach that is often used to work around this problem involves subtracting transferrin-bound iron levels from total serum iron levels, thus assuming that the resulting concentration differences represent iron from the SFGC. In contrast, the compartmental model used in the current analysis estimates iron levels coming

from both endogenous and exogenous sources without performing this simple subtraction and does not assume that total iron is only composed of TBI and SFGC-I.

In order to apply compartmental analysis in a BE context, the first step was the determination of a compartmental model that described the pharmacokinetics of iron in all its forms (total, bound to transferrin and associated with SFGC). Based on a previously published model (25), a model describing both formulations of iron was established. Overall, it explained the data very well. Although twelve parameters were estimated by the model, the model was not deemed overparameterized since two analytes were being fitted, each demonstrating two visible exponentials in their disposition with known nonlinearity (30). In addition, over 80 concentrations were fitted per subject, signifying that 6 samples were available per PK parameter, which represents a clear rich sampling scenario for the PK model (sparse sampling can be defined when less than 1 sample is available per fitted PK parameter). This PK model accounted for serum iron, iron bound to transferrin, and stores in the reticuloendothelial system and bone marrow (red blood cells). The model also took into consideration iron lost during each blood sample, as well as iron not bound to transferrin. Although the administration of intravenous iron is not associated with the generation of detectable or dialyzable free iron (3,31), there is evidence that points to the existence of non-transferrin bound iron (NTBI) that is biologically active and labile (9,31,32). This NTBI may even be bound to albumin (32). The levels of NTBI estimated in our population were about 46 mcg/dL before any treatment (Period 1) and about 74 mcg/dL before Period 2, which are equivalent to roughly 0.001 and 0.002 μM and which are well under the 1 μM levels normally seen in healthy subjects (1).

The PK parameters for volume of distribution and clearance estimated with our model were slightly lower than what has been reported in the literature. The Vss and CL estimated for SFGC-I from our analysis were 4.41 L and 2.3 – 2.6 L/h, whereas they were 5.72 L and 3.87 L/h in the previous study for which a compartmental analysis was used (25). Conversely, the average SFGC-I half-life in our study was 1.63 h whereas it was closer to 1 h in the other study (25). The differences observed between the PK parameter

estimates from our analysis and those in the literature may result from the differences in study populations. In the current study, enrolled subjects had ferritin levels between 22 ng/mL (10 ng/mL for women) and 100 ng/mL, inclusively, whereas levels were less than or equal to 20 ng/mL in the other study. This means that the subjects in this analysis had a less profound iron deficiency than those studied by Seligman et al., considering ferritin is a measure of bodily iron stores (9). Degree of deficiency has been shown to influence iron pharmacokinetics, particularly its rate of transfer from RBC to RES (4). Patients who are more iron-deficient incorporate iron faster into the RES, which could explain why the terminal elimination half-life of SFGC-I determined by others was shorter than in the current study. In addition, the proportion of women was different in each study. The study population in the current study was composed of 75% women whereas women only made up 43% of the other population. As iron storage and loss are different between men and women (9), it is possible that the different gender compositions led to slightly different pharmacokinetic parameter estimates.

By using a compartmental approach to assess the BE of two formulations of SFGC, problems associated with the noncompartmental method were altogether avoided. Indeed, because SFGC-I Cmax and AUC were calculated by model-based methods, iron's non-linear behaviour, non- continuous elimination (e.g., only through the specific blood samples), unstable baseline, and continual recycling were no longer issues as they were directly addressed by the model. All the specificity of iron pharmacokinetics such as non-transferrin bound iron and iron lost through blood draws were specifically incorporated. The absence of a stable baseline for both total iron and TBI also became a non-issue as the model fitted all the analytes specifically and allowed for the fact that the iron administration in the two periods naturally raised the levels of TBI and total serum iron. The overall result is that all of this unaccountable variability in the baseline adjusted concentrations of total serum iron and TBI are not present in the population compartmental analysis, therefore demonstrating BE with a much lower number of subjects because of this lower unaccountable variability.

The population PK modeling approach described in this article shows that BE can be demonstrated with a relatively small sample size for iron products. Although BE was concluded in the study described by Baribeault (27), it was necessary to dose 240 subjects in a parallel-group design. Moreover, when noncompartmental analyses were conducted with data from our study, only one parameter (Cmax of baseline-adjusted TBI) met bioequivalence criteria. Post-hoc analyses also revealed that the study lacked the power to show equivalence at ± 20% with an alpha error of 5% (i.e., power was less than 40%). Overall, this suggests that in order to demonstrate BE between SFGC formulations using traditional noncompartmental methods, a very large number of subjects must be dosed. In contrast, the compartmental approach was adequately powered to show equivalence with a sample size of 29 subjects. This highlights again the strengths of the compartmental approach used in this study, as it is able to demonstrate bioequivalence with a significantly smaller number of subjects in a crossover design. In other words, this approach is not only scientifically sound, but it decreases the number of subjects who are exposed to the study drugs, resulting in a more cost-efficient and time-efficient study.

Other researchers have shown, through the analysis of simulated data or data obtained from real clinical trials, that the compartmental approach can be used to assess bioequivalence (33,34,35,36,37,38,39,40,41). However, the PK models employed in previously published analyses were relatively simple ones (one or two compartment models). The population PK model developed in the current analysis is obviously more complex, but it allowed us to simultaneously characterize the PK of two different analytes (TI, TBI) and present concentration time profiles for what was directly administered ferrlecit-bound-iron or SFGC.

The analysis presented here demonstrates how the compartmental approach can be used to perform BE assessments for drugs that do not meet assumptions necessary to employ more traditional, noncompartmental approaches. These drugs may or may not be highly variable drugs (i.e., drugs for which within-subject variability of AUC or Cmax are greater than 30%). Thus, for highly variable drugs that do not lend themselves to

traditional analyses, the compartmental approach described here can be adopted. This would first entail developing a model that simultaneously describes the PK of both the test and reference drugs, which could be based on models already described in the literature. Specific model development criteria must be established a priori in the protocol and ideally in a population PK analysis plan. For highly variable drugs that meet the criteria associated with standard noncompartmental analyses, use of the compartmental approach can also be used but other types of analyses, such as the reference-scaled average bioequivalence method (42,43), should also be considered.

Although there were many advantages to adopting this non-traditional approach to evaluating BE, the development of a PK model for iron was more time-consuming and labour-intensive than the noncompartmental approach from an analysis perspective. The advantages and disadvantages of using a compartmental PK approach for a highly variable drug that meets the assumptions associated with standard analyses must be weighed in comparison with the reference-scaled average bioequivalence approach. Despite this, results show that this compartmental approach to BE assessment should be seriously considered for iron and potentially other rare, complicated drugs from a PK point of view for which traditional methods are unsuitable.

2.7 Conclusions

A compartmental analysis approach was applied successfully to demonstrate the BE between two formulations of sodium ferric gluconate complex in sucrose. BE was also concluded for the same products in a separate, much larger, parallel-design study employing traditional, noncompartmental methods of analysis. The results of this study suggest that alternative methods, such as the population compartmental analysis proposed here, should also be considered for assessing BE of drugs that are complicated from a PK point of view and for which the standard approach becomes artificially variable thereby necessitating the enrollment of too many subjects.

2.8 Abbreviations

Term	Definition
AIC	Akaike information criterion
ANOVA	Analysis of variance
AUC_{pred}	Area under the serum-time curve of SFGC-I, from the beginning of the infusion to infinity, calculated as dose divided by CL
AUC_{0-36}	Area-under-the-concentration-time-curve from time zero to 36 hours
BA	Bioavailability
BE	Bioequivalence
BIC	Bayesian information criterion
BLQ	Below the limit of quantitation
CI(s)	Confidence interval(s)
CL_1	Clearance of SFGC-I to the RES
CL_2	Clearance of SFGC-I directly to transferrin
CL_3	Clearance of iron entering and exiting the marrow and red blood cell compartment
CL_4	Clearance of TBI to the RES
CL	Total clearance for SFGC-I, calculated as the sum of $CL_1 + CL_2$
$Cmax_{pred}$	Maximum predicted serum concentration of serum SFGC-I over the 72-hour sampling period
C_NTBI_P1	Concentration of non-transferrin-bound iron during Period 1
C_NTBI_P2	Concentration of non-transferrin-bound iron during Period 2
CV%	Percent coefficient of variation
FDA	Food and Drug Administration
Frel	Relative bioavailability
ITS	Iterative two-stage
Km	Iron concentration associated with half of the maximal rate of exchange between the RES and TBI compartments

Term	Definition
LSM	Least squares mean
MAP	Maximum a posteriori
NTBI	Non-transferrin bound iron
OGD	Office of Generic Drugs
PK	Pharmacokinetics
PPK	Population pharmacokinetics
RBC	Red blood cells
RES	Reticuloendothelial system
SD	Standard deviation
SFGC	Sodium ferric gluconate complex
SFCG-I	Iron bound to sodium ferric gluconate complex
STS	Standard two-stage
$T_{1/2}$	Apparent first-order terminal elimination half-life of SFGC-I
TBI	Transferrin-bound iron
TI	Total iron
Vmax	Maximal rate of exchange between the RES and TBI compartments
V_RBC	Marrow and red blood cell compartment
V_RES	Volume of distribution associated with the RES
Vss	Apparent steady-state volume of distribution of SFGC-I
V_TBI	Volume of distribution associated with TBI

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Chapter 3 – Article #2

Population pharmacokinetic and pharmacodynamic analysis of pegloticase administered by intravenous infusion in two dose regimens to subjects with chronic gout

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3.1 Preface

The previous article demonstrated that the innovative use of modeling and simulations in the development of a generic drug could provide a scientifically sound and robust approach to bioequivalence assessment when other traditional approaches were inappropriate. In addition, this method was able to answer the study objective without relying upon a large and lengthy clinical study, therefore it proved to be a cost-efficient and time-efficient approach. Modeling and simulation can also be applied to develop innovator products, as the current article will demonstrate. Indeed, in the present article, advanced pharmacokinetic techniques are used to better understand factors that could influence the PK and PD of a first-in-class biological agent that was being developed to treat gout. The drug in question has since been approved by regulatory agencies and is available on the market, which is further evidence that these approaches can contribute to a product's success. This article is also the first published population PK/PD analysis for a drug belonging to the urate-oxidase drug class conducted on a large cohort of Phase 3 gout patients.

Therapeutic biological agents are essentially proteins or peptides that are derived from biotechnology. In other words, the technology used to produce these agents generally involves recombinant DNA and organisms such as bacteria, yeast or mammalian cells (22, 143). Some well known examples of biologics include insulin and rituximab. A classification system has been proposed that categorizes biological therapeutics into different groups based on their functions (143). Group 1 includes agents with enzymatic or regulatory activity, meaning that these biologics replace absent or dysfunctional enzymes, modulate existing pathways or provide novel functions. This group includes drugs such as recombinant erythropoietin and rasburicase. Group 2 therapeutics target specific activities or act as delivery agents for other compounds or proteins, and includes products such as infliximab and trastuzumab. In Group 3 are protein vaccines (for instance the Hepatitis B vaccine) and in Group 4 are proteins used for diagnostic purposes (such as the recombinant purified protein derivative used to diagnose tuberculosis exposure).

Biologics are two to three times larger than most “small molecule” drugs which are synthesized chemically (22). Other differences between biologics and small molecules include their route of administration (small molecules can often be administered orally while biologics must often be given parenterally), immunogenicity (biologics can be highly antigenic compared to small molecules), and pharmacokinetic properties (22, 144). Some particularities regarding the PK of biologics are that they often reach systemic circulation via the lymphatic system, they are poorly distributed outside of the vasculature and they are not metabolized into active or inactive metabolites (rather, they are catabolized to endogenous amino acids).

Given these fundamental differences between biologics and small molecules, it is understandable that there are some drug development issues that are specific to biologics. Although the phases of drug development are the same regardless of the source of the drug (biological or chemical), some challenges are unique to biologic therapeutics. Primary among them is immunogenicity. Even for agents that are endogenous or from “natural sources”, the possibility that the organism mounts an immune response against the therapeutic agent is always an underlying concern. The consequences of antibody binding to the protein can vary, ranging from a complete absence of clinical effect to increased activity or elimination (144). In some cases, immune responses leading to harmful effects can even occur (145), which is why special attention is paid to the immunogenicity of these compounds. In addition, due to their heterogeneous nature, developing reliable and accurate analytical methods to assay protein therapeutics can prove to be difficult (143, 144).

Beyond the goal of developing safe and effective drugs, one concern that is shared by developers of both small molecules and biologics is cost effectiveness. DiMasi and colleagues have shown that developing biologics is just as expensive as developing a small molecule, with similar estimated total capitalized costs between the two ($1241 million 2005 dollars for biologics and $1318 million 2005 dollars for small molecules) (123). It

must be noted, however, that initial out-of-pocket costs were 24% higher in total for biologics, which was offset by shorter clinical development times (123).

Despite the challenges in bringing a biologic to market, as of 2008, there were at least 130 biological therapeutic agents available on the U.S. market (143). The number of biologics submissions have continued to rise since 1982 (146), and they will no doubt continue to do so. Protein therapeutics can provide treatments for diseases that cannot always be treated by small molecules, and they can also be complimentary to certain small molecule therapies (146). There is clearly an interest in continuing to develop such products and to render the process more efficient.

Some have suggested that a better understanding of PK and PD during the biologic drug development process can lead to many improvements (144). More specifically, it was suggested that the application of an integrated PK/PD database throughout the drug development process could save time and money, reduce the number of unnecessary studies, generate pivotal information to influence key decisions, and improve the overall odds of demonstrating safety and efficacy. A thorough understanding of PK/PD can also be useful in making decisions at the regulatory level, such as those pertaining to product labeling. Because an overwhelming amount of PK/PD information can be gathered during the course of preclinical and clinical trials, it is important to find a way to integrate all of this information and to build upon previously acquired knowledge. One way of managing all of these PK/PD findings is through model building. The construction of PK/PD models leads to clear dose-response relationships which have the flexibility of incorporating past results with more recent ones, in a continual and ongoing basis.

Thus, the following article demonstrates how the development of a PK/PD model for a biological product was able to answer a key question regarding potential factors that could influence the PK and PD of the drug. This product was the first of its class that was developed to treat gout, and because of its novelty, clinical studies and specific analyses were necessary to better understand the drug and answer this question. (Additional information concerning the drug class can be found in Appendix 1.) In the end, the PK/PD

model that was created answered the question efficiently and contributed to the eventual product labeling.

3.2 Abstract

Two identical Phase 3 studies were conducted in persistently hyperurecemic patients with treatment refractory chronic gout. Subjects received placebo or 8 mg pegloticase intravenously infused over approximately 2 hours every 2 or 4 weeks for 24 weeks. Samples for pegloticase, uric acid and antibody assays were collected from baseline through 2 weeks after the last dose. Population pharmacokinetic (PK) and pharmacodynamic (PD) analyses were conducted with data from 163 subjects using NONMEM VI. Covariates tested were weight, height, body surface area (BSA), anti-pegloticase antibody level (Ab), creatinine clearance, age and sex. Pegloticase PK was best-described by a 1-compartment model with linear elimination with BSA and Ab exhibiting covariate effects on Vc and CL. An indirect model described the PD of pegloticase, where the depletion rate of uric acid was influenced by pegloticase and Ab levels.

Keywords: Urate oxidase, uricase, population pharmacokinetics, population pharmacodynamics, uric acid, PEG

3.3 Introduction

Gout is the most common inflammatory arthritis in developed countries, occurring in 1 to 3% of people in most developed countries and in 3.9% of the US population, predominantly men.(1, 2) The hallmark biochemical marker of gout is hyperuricemia, although hyperuricemia alone is insufficient to diagnose gout. When the concentration of serum uric acid (SUA) is above the biochemical limit of solubility, 6.8 mg/dL, monosodium urate (MSU) crystals precipitate in tissues. (3) It is the biological response to MSU crystals in tissues, and not to circulating urate, that causes the signs and symptoms of the disease. These include the occurrence of episodic acute inflammation in and around a

joint or joints (a gout flare), the formation of gout tophi, gouty arthritis, and uric acid nephropathy (including uric acid renal stones). (4, 5)

Approximately 3 – 5.9 million patients in the US diagnosed with gout receive treatment to lower serum uric acid, and ≥ 90% of these patients are treated with allopurinol (a xanthine oxidase inhibitor, XOI), making allopurinol the standard conventional urate-lowering therapy. (3-13) However, for a subset of chronic gout patients, XOIs either cannot control hyperuricemia with the maximum medically appropriate dose or is contraindicated. (3, 14) Compliance can also be an issue. These treatment refractory chronic gout patients tend to have advanced disease characterized by frequent gout flares, multiple gout tophi, and chronic painful joints (gouty arthritis), and also may have urate nephropathy. (1, 4, 15)

Almost all mammals, except for humans and great apes, express an enzyme that catalyzes the conversion of urate, the by-product of purine metabolism, to allantoin. (4) Allantoin, unlike urate, is easily eliminated by renal excretion. The administration of exogenous uricase to control urate levels is therefore an attractive alternative for chronic gout patients who cannot benefit from existing therapies. Pegloticase (a PEGylated recombinant modified mammalian urate oxidase), which was developed to provide this therapeutic option, was approved by the US FDA in September 2010 and received marketing authorization in Europe in January 2013.

Pegloticase was shown to effectively decrease plasma uric acid (PUA) to well below the solubility limit in early clinical development. Phase 1 and 2 clinical studies suggested that 8 mg of pegloticase by intravenous (IV) infusion can maintain PUA levels below the clinical target of 6.0 mg/dL. (16, 17) Additionally, these Phase 1 and 2 studies revealed a long half-life (~17 days), low pegloticase concentration associated with 50% of the maximal effect and sustained PUA suppression over dosing intervals, which supported the use of a long dose administration interval (two weeks). Population pharmacokinetic (PK) and pharmacodynamic (PD) analysis of Phase 2 data suggested that no dose adjustment is required to account for covariates such as age, sex, race, body weight, ideal

body weight and antibody levels, since no covariates had a significant effect on the PD of the drug. (18)

The current parallel, randomized multi-center placebo-controlled pivotal Phase 3 studies were designed to determine the safety and efficacy of pegloticase and to further characterize the PK/PD of pegloticase using population PK methodology. This article presents the population PK/PD results from these studies of pegloticase for the treatment of patients with chronic gout refractory to conventional therapy.

3.4 Methods

3.4.1 Study Design

These were two Phase 3 multicenter, randomized, double-blind, placebo-controlled, three-arm, parallel treatment group studies conducted in patients suffering from gout and hyperuricemia. A total of 212 patients were dosed and 157 subjects completed the study per protocol. Patients were administered placebo or 8 mg pegloticase IV over approximately 2 hours, every 2 or 4 weeks for 24 weeks. Samples for PK/PD analyses were collected at various timepoints, as depicted in Table 1. In the event of an early termination, a sample was also collected at that time.

Table 1. Sampling Schedule

Week	Dose	PK sample	PD sample	Ab sample
1	1	X[a,b]	X	X
3	2	X	X	X
5	3	X[b]	X	X
7	4	X	X	
9	5	X[a,b]	X	X
10	.	X		
11	6	X[a]	X	
12	.	X		
13	7	X	X	X
15	8	X	X	
17	9	X	X	X
19	10	X	X	
21	11	X[a,b]	X	X
22	.	X		
23	12	X[a]	X	
24	.	X		
25	.	X	X	X

[a]Including a 2-hour post-dose collection

[b]Including a 24-hour post-dose collection

Each subject was expected to participate in the study for approximately 27 weeks, including a 2-week screening period, the 24 week treatment period and a 1 week post-treatment follow-up. Patient safety was monitored throughout the study. This included the monitoring of adverse events (such as infusion reactions and gout flares), vital signs, clinical laboratory tests (hematology, serum chemistry, and urinalysis), pegloticase

antibodies, total hemolytic complement function, electrocardiograms, and physical examinations.

3.4.2 Ethics

Prior to initiation of the study, the protocol, informed consent form and other pertinent information were reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee. Written, informed consent was obtained from all subjects before they participated in any protocol-related activities.

3.4.3 Inclusion and Exclusion Criteria

Subjects included in the study were: outpatients of either sex; ≥ 18 years; hyperuricemic (screening SUA ≥ 8 mg/dL); subjects with symptomatic gout (at least 3 gout flares experienced in the 18 months prior to entry, or at least 1 gout tophus, or gouty arthritis); subjects in whom conventional therapy was contraindicated or had been ineffective (i.e., history of hypersensitivity or of failure to normalize SUA with at least 3 months treatment with allopurinol, the only marketed XOI when the studies were conducted, at a maximum medically appropriate lower dose based on dose-limiting toxicity or dose limiting comorbidity).

3.4.4 Analytical Methods

Bioanalytical assays were conducted at Charles River Laboratories Preclinical Services Montreal Inc. Pegloticase in serum, anti-PEG antibodies and anti-pegloticase antibodies were measured by enzyme-linked immunosorbent assay, with pegloticase as the capture antigen and horseradish peroxidase-conjugated as secondary antibody. PUA was measured in trichloroacetic acid-precipitated chilled plasma, using a similar validated method. Analytical methods have been described in detail elsewhere. (18)

3.4.5 Population PK/PD Analysis

Data from the two Phase 3 studies was pooled in these analyses. Subjects who received active treatment and who had at least one detectable post-dose pegloticase concentration were included in the PK/PD analyses. Actual doses, infusion rates, dosing times and sampling times were used.

Using NONMEM VI 2.0, various structural PK models were tested, such as one- and two-compartment models with linear and non-linear elimination processes. (30, 33) Quality of fit and selection of the final model was determined using objective function (OF), Akaike information criterion, residual variability, and by visual inspection of pertinent graphs (e.g., fitted and observed concentrations versus time, weighted residuals versus fitted values).

After selecting the final structural PK model, various covariates were tested for potential inclusion in the model. The impact of covariates was assessed graphically and using a generalized additive model implemented in Xpose Release 3.104 with S-Plus® 8.0 for Windows. (34) Covariates suggested by the previous step were entered into the model one by one using forward stepwise regression. The most significant covariate was retained at each step, and subsequent covariates were added to this model. Covariates were added to the model based on their reduction of the OF (using the log-likelihood ratio test at $\alpha = 0.05$), and based on their physiological and pharmacological plausibility. Once all significant covariates were added to the model, backward stepwise deletion was performed to avoid redundancy and to ensure that all covariates remained significant despite the presence of all covariates. In this process, each covariate was removed one by one from the complete model. If its deletion resulted in a decrease in OF that was statistically significant ($\alpha = 0.01$), then it was excluded from the final model.

Covariates investigated for inclusion in the model were: weight, height, body mass index, body surface area (BSA), ideal body weight, age, sex, presence of tophi, creatinine clearance (calculated using the Cockcroft-Gault equation), (35) baseline SUA level, number of gout flare ups, antibody levels (against PEG and pegloticase), the presence of co-

morbidities (such as hypertension and diabetes) and allergy or gastrointestinal intolerance to allopurinol. Concomitant medications were not investigated as covariates since no drug-drug interactions were anticipated to affect the PK/PD of pegloticase.

Antibody response against pegloticase and PEG were treated as categorical variables. Subjects were categorized as having no increase or an increase (either low, moderate or high). No increase included subjects who were negative at baseline, Month 3 and Month 6 (no titer), or who were positive at baseline but with no increase from baseline in titer during Months 3 and 6 (no increase in titer); low increase if titer was > 0 and ≤ 810 at Month 3 and/or Month 6; moderate increase if titer was > 810 and $\leq 7{,}290$ at Month 3 and/or Month 6; high increase if titer $> 7{,}290$ at Month 3 and/or Month 6. Antibody isotyping data were not included in the covariate analysis since the data were deemed incomplete. No subjects demonstrated antibody neutralization of pegloticase, therefore, this covariate was not included in the covariate analysis.

For the PK analysis, antibody response against pegloticase was treated as a dichotomous categorical variable, where subjects either had no increase or an increase (either low, moderate or high). Antibody response was originally categorized as "no increase", "low increase", "moderate increase" or "high increase", however covariate analysis using these four categories did not produce pharmacologically plausible results. For the PD covariate analysis, the anti-pegloticase antibody level was separated into 5 categories ("no increase", "low increase", "moderate increase", "high increase" or "information unavailable"), as it was possible to stratify the effect of pegloticase based on the magnitude of the increase in anti-pegloticase antibody level. Missing results were due to analytical reasons or to subject dropout prior to antibody determination in Month 3.

After selecting the final PK model, a PD model was evaluated using the previously described model discrimination criteria and covariate analysis process. The same covariates were tested as in the PK analysis. To estimate the PD effect, final individual PK parameters were used to predict plasma pegloticase concentrations for developing the PD model. Different models were tested, including the direct model (PD effect is related to predicted pegloticase concentrations), effect compartment model (PD effect is related to

pegloticase concentrations at the site of action) and indirect model (pegloticase concentrations exert their PD effect by influencing the rate of formation or elimination of PUA). In the indirect model, the rate of uric acid presentation to plasma was estimated as K_{in}, which was influenced by the production rate of uric acid by the body and/or the release of uric acid from crystalline deposits within the body. The rate of uric acid depletion from plasma was estimated as K_{out}. The elimination of uric acid from plasma was increased directly by plasma pegloticase concentrations through a linear relationship.

All serum pegloticase concentrations were fitted using a weighting procedure where the weighting function is inversely proportional to the estimated variance. Inter-individual variability was assumed to be centered around a mean value of zero with a variance of ω^2. Intra-individual variability for pegloticase was modeled as a combination of additive and proportional error, while the error model for PUA was additive. When it was possible, inter-occasion variability (assuming normal distribution of error) was also modeled.

3.5 Results

In all, 163 subjects were included in the PK/PD analysis. The demographic characteristics of these patients are summarized in Table 2. Overall, a total of 1,176 serum pegloticase concentrations and 3,358 PUA concentrations obtained from this patient population were fitted with the PK/PD model.

Table 2. Summary Demographics of Subjects

Trait	Mean (CV%)	Median (Range)
Age (years)	55.6 (26.0%)	57 (23 - 89)
Body Weight (kg)	99.1 (25.1%)	96.2 (48.2 – 191)
Height (cm)	174 (6.28%)	175 (145 - 193)
Body mass index (kg/m^2)	32.8 (22.5%)	31.5 (15.0 - 65.9)
Body surface area (m^2)	2.12 (13.3%)	2.12 (1.44 - 2.88)
Ideal body weight (kg)	68.2 (16.3%)	70.5 (38.6 - 86.8)
Screening creatinine clearance (mL/min)*	92.4 (55.4%)	84.5 (17 - 264)
Screening serum uric acid (mg/dL)	10.07 (14.0%)	9.9 (8.0 - 14.9)
Number of acute gout flare ups in the past 18 months (n=161)	9.70 (113%)	6 (0 - 90)
	Number of Patients	**%**
Sex		
Male	131	80.4
Female	32	19.6
Presence of tophi	122	74.8
Diabetes mellitus	41	25.2
Hypertension	119	73.0
Allergy or gastrointestinal intolerance to allopurinol	96	58.9
Overall anti-pegloticase antibody level		
No increase	16	9.8
Low increase [$0 < \text{titer} \leq 810$]	49	30.1
Moderate increase [$810 < \text{titer} \leq 7,290$]	33	20.2
High increase [$7,290 < \text{titer}$]	50	30.7
Unknown change	15	9.2

*Creatinine clearance was calculated using the Cockcroft-Gault equation. (147)

The PK of pegloticase was best described by a one compartment model with a linear elimination process. The model was parameterized in terms of volume of distribution (Vc) and clearance (CL), as described by Equation 1, where C represents serum pegloticase concentration.

Equation 1 $$\frac{dC}{dt} = -\frac{CL}{Vc} \cdot C$$

An indirect model was selected to describe the PD of pegloticase. (19) Steady-state PUA levels prior to pegloticase dosing were defined as the ratio of K_{in} to K_{out}, as described by Equation 2. The rate of change of PUA and the effect of pegloticase on K_{out} are described by Equation 3 and Equation 4, respectively.

Equation 2 $$IC = \frac{K_{in}}{K_{out}}$$

Equation 3 $$\frac{dPUA}{dt} = K_{in} - \left[K_{out} \cdot (1 + Stim)\right] \cdot PUA$$

Equation 4 $$\mathrm{Stim} = \mathrm{Slope} \cdot \mathrm{C}$$

C = serum pegloticase concentration; IC = initial condition (steady-state uric acid level prior to pegloticase dosing); PUA = plasma uric acid concentration; Stim = Stimulation factor; Slope = variable representing the linear relationship between serum pegloticase and stimulation of Kout

Thus, according to this model, as the serum concentration of pegloticase reaches its peak, the stimulation (represented by Stim) also reaches its peak. Conversely, when serum pegloticase levels decrease to zero, the stimulation drops to zero and normal physiologic urate elimination remains.

Significant covariates included in the PK model were BSA and anti-pegloticase antibody level on Vc and CL. For each antibody category, a different population value (θ)

was estimated with identical inter-subject variability (η). An example of how these covariates were coded (for Vc) can be found in Equation 5 and Equation 6.

Equation 5 $$\theta_{individual\_no\_Ab\_increase} = \left[\theta_{Vc\_population\_no\_Ab\_increase} \cdot \left(\frac{BSA}{2.12} \right)^{\theta_{BSA\_Vc}} \right] \cdot e^{\eta_{Vc}}$$

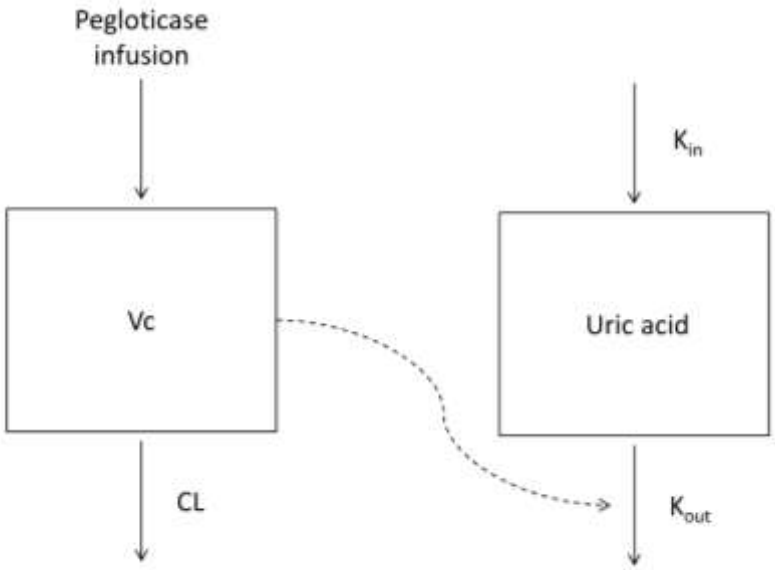
Equation 6 $$\theta_{individual\_Ab\_increase} = \left[\theta_{Vc\_population\_Ab\_increase} \cdot \left(\frac{BSA}{2.12} \right)^{\theta_{BSA\_Vc}} \right] \cdot e^{\eta_{Vc}}$$

The only covariate included on PD parameters was the overall anti-pegloticase antibody level on slope, the variable representing the linear relationship between serum pegloticase and stimulation of PUA elimination. For each category, a different θ and η were estimated.

An inter-occasion variability (IOV) factor was also added to the PK parameters Vc and CL to improve the quality of fit. This improved the model in a statistically significant manner and enhanced other goodness-of-fit criteria. The IOV term accounted for intra-subject variability from one occasion to another, where the first occasion was the first 3 months of the study period (Weeks 1 to 12), the second occasion was between months 3 and 6 (Weeks 13 to 24), and the third occasion was after the sixth month (Week 25).

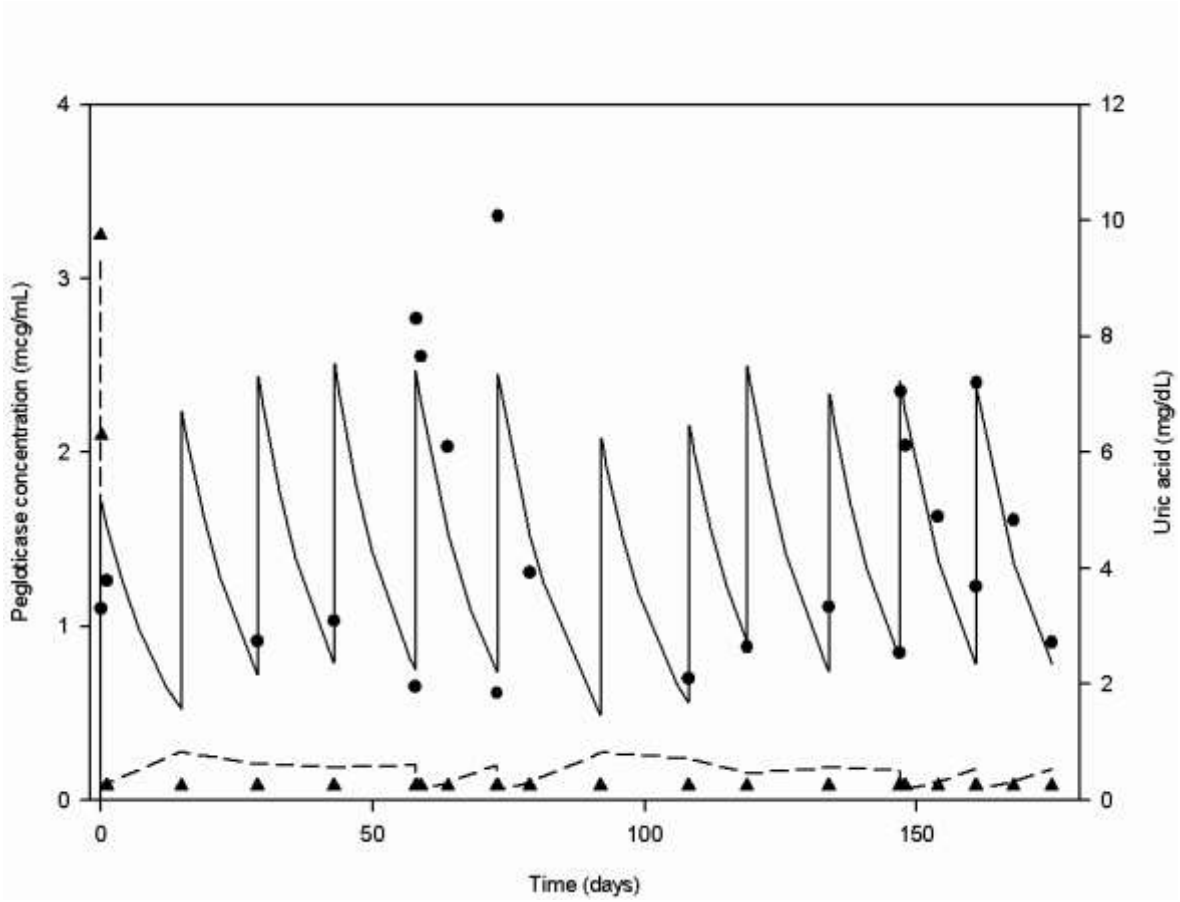
The final structural PK/PD model is depicted in Figure 1, while typical predicted pegloticase and PUA profiles are presented in Figure 2. Population PK/PD parameters and estimated half-life values are presented in Tables 3 and 4, respectively.

Figure 1. Final PK/PD Model for Pegloticase



CL = pegloticase clearance, Dashed arrow = stimulatory effect of pegloticase, K_{in} = rate of uric acid presentation to plasma, K_{out} = rate of uric acid depletion from plasma, Vc = pegloticase volume of distribution

Figure 2. Example of Predicted Profiles for One Patient



LEGEND: Circles = observed pegloticase concentrations, Triangles = observed uric acid concentrations, Solid line = predicted pegloticase concentrations, Dashed line = predicted uric acid concentrations

Table 3. Population PK/PD Parameters of Pegloticase

Parameter		Mean	Coefficient Variation (%) Inter-subject	Inter-occasion
Vc if no increase in anti-pegloticase antibodies (L)		4.73	24.7	18.2
Vc if increase in anti-pegloticase antibodies (L)		5.93		
Exponent for BSA on Vc		1.73	Not estimated	
CL if no increase in anti-pegloticase antibodies (L/h)		0.0145	39.6	17.0
CL if increase in anti-pegloticase antibodies (L/h)		0.0191		
Exponent for BSA on CL		1.12	Not estimated	
K_{in} (mg/dL/h)		0.727	14.6	Not estimated
K_{out} (1/h)		0.079	9.34	Not estimated
Slope (mL/mcg) if no increase in anti-pegloticase antibodies		3.93	112	Not estimated
Slope (mL/mcg) if low increase in anti-pegloticase antibodies		1.60	236	Not estimated
Slope (mL/mcg) if moderate increase in anti-pegloticase antibodies		0.578	163	Not estimated
Slope (mL/mcg) if high increase in anti-pegloticase antibodies		0.0526	39.8	Not estimated
Slope (mL/mcg) if information unavailable on increase in anti-pegloticase antibodies		0.380	Not estimated	Not estimated
Residual variability (%)	Pegloticase	30.8		
	Uric acid	64.0		

Table 4. Bayesian Terminal Elimination Half-life Estimates

Subgroup	Half-life (hours)		
	Mean	**Median**	**Range**
All subjects	221	217	123 – 452
Subjects with no increase in anti-pegloticase antibody levels	234	216	123 – 444
Subjects with an increase in anti-pegloticase antibody levels	220	217	124 – 452

3.6 Discussion

3.6.1 Pharmacokinetics

The one-compartment model with linear elimination that was chosen as the base PK model for this Phase 3 analysis was identical to the structural model determined by analysis of Phase 2 data.(18) The selection of a 1-compartment model suggests that pegloticase is mostly confined to the intravascular space, which can be explained by its size of approximately 545 kDa. With hydration of the PEG moieties, the apparent molecular weight is even larger. Other therapeutic biologics also tend to cross blood capillaries slowly (20, 21).

The inclusion of anti-pegloticase antibody response on Vc and CL indicates that the presence of antibodies is associated with an increased Vc as well as an increased CL. Increased CL is consistent with results published by Sundy and colleagues, who found that the presence of antibodies was associated with an increase in the clearance of IV pegloticase. (17) Since antibodies were non-neutralizing, the presence of antibodies likely facilitates the clearance of pegloticase, by a mechanism related to the formation of immune-complexes between the antibodies and pegloticase, which are possibly captured by the reticuloendothelial system. (22) Although anti-pegloticase antibody response was initially tested as a categorical covariate comprised of 4 categories, the use of 4 categories did not produce pharmacologically plausible results. More specifically, the presence of increased quantities of antibodies was associated with an increase in terminal elimination half-life rather than a decrease in half-life. Therefore, antibody response was classified as a dichotomous variable.

The increase in Vc associated with the presence of antibodies could also be explained by the formation of immune-complexes. Anti-pegloticase antibodies could increase the uptake of immune-complexes by phagocytes of the reticuloendothelial system,

thereby increasing the intracellularly-bound fraction of pegloticase. With increasing cellular uptake of pegloticase, the quantity of pegloticase in serum naturally decreases. Since Vc is a variable that relates serum pegloticase concentration with the amount of pegloticase in the central compartment, the decrease in pegloticase concentration is reflected by an increase in Vc.

The only demographic covariate that was retained in the final model was BSA, suggesting that increasing body size is associated with increasing Vc and CL. Indeed, it is not surprising that a person who is taller and heavier than another individual would have a larger Vc since it not only encompasses the intravascular space, but possibly tissue volume. Such a person might have a greater hepatic mass, further enhancing the elimination of pegloticase via the uptake of antibody-pegloticase complexes. (13) Although it has been suggested that BSA does not correlate strongly with hepatic metabolic capacity, the elimination of pegloticase does not involve metabolism, therefore the inclusion of BSA on CL in the model is reasonable. (2)

Although BSA was retained as a covariate in the PK model, this does not suggest that pegloticase dosing adjustments are required with respect to BSA. It simply indicates that part of the inter-individual variability in PK parameters can be attributed to BSA (i.e., differences in pegloticase concentrations between patients can be partially explained by differences in BSA). Moreover, patients had similar PD responses to pegloticase regardless of their BSA. Thus, based upon analysis of the data using the PK/PD model, dosing adjustments to correct for BSA are unnecessary.

Although the population PK approach was ideal to analyze the sparse data collected from this study, one of the analysis limitations was the variability of the data. Significant variability in the pegloticase concentrations was observed, which could explain the residual variability value of 30.8% (representing the variability that is not explained by the model, including intra-individual variability, the experimental "noise" of the analytical method and errors arising from the pharmacokinetic modeling itself), as well as the elevated inter-

individual variability associated with PK parameters. Such variability of the measured pegloticase concentrations is not unexpected, considering that multiple clinical sites were involved and that subjects were not confined to a controlled clinical setting, with the exception of visits to clinical sites for dose administration.

3.6.2 Pharmacodynamics

The use of PUA as a PD marker was deemed appropriate, as it is the direct target of pegloticase and it correlates well with clinical outcomes. (23) Indeed, low levels of urate are associated with dissolution of MSU crystals (leading to a decrease in gouty flares and tophi) (24, 25) and a decrease in the urate pool. (26, 27) Furthermore, although pegloticase was measured in serum, PUA was selected as a PD marker because it avoided the possibility of *ex vivo* SUA degradation by circulating pegloticase. In addition, statistical analyses demonstrated excellent agreement of PUA and SUA levels. (23)

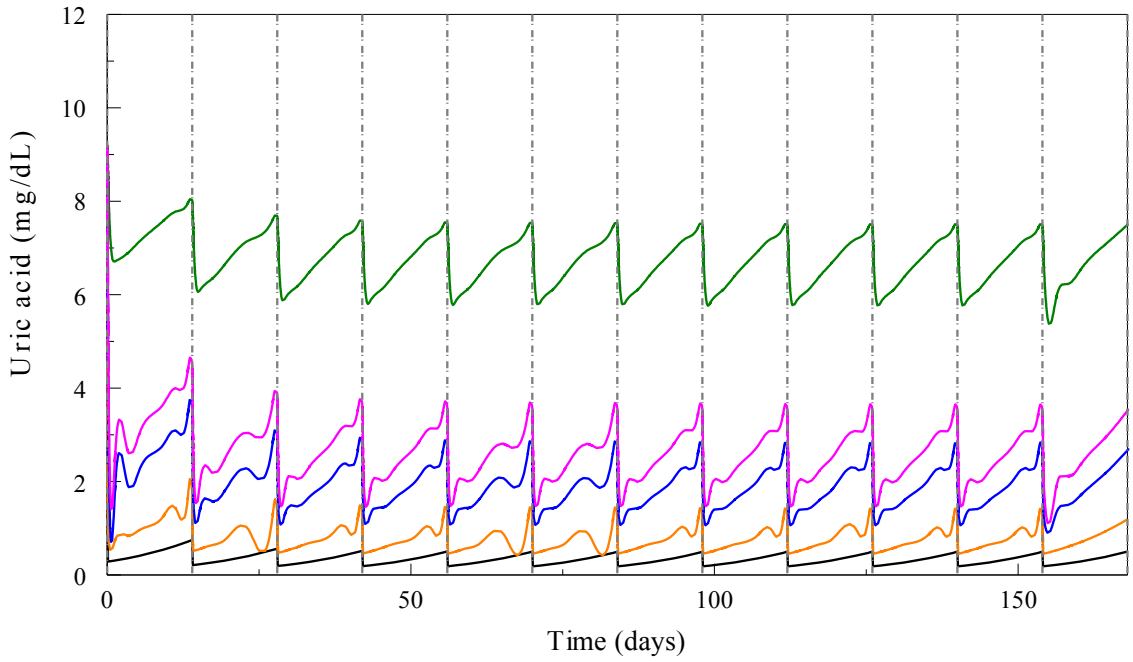
Pegloticase was designed to catalyze PUA metabolism, and a model reflecting its pharmacologic action with stimulation of the rate of urate elimination was selected as the best model. In this model, pegloticase affects the rate of PUA depletion (elimination) from the plasma to produce the observed decreases in PUA levels. Although several other structural models were tested, such as the effect compartment model and direct inhibitory E_{max} models, (28, 29) the indirect PD response model with stimulation of K_{out} provided the best overall fit to the observed data. Although a direct inhibitory Emax model was previously used to describe the PD effect of pegloticase based on Phase 2 data (18), only one dose of pegloticase was administered in Phase 3 (compared to Phase 2 where 3 different doses were administered), which made it more difficult to characterize an Emax curve in the current analysis.

Following covariate analysis, only overall anti-pegloticase antibody level had a significant effect on the slope (the variable representing the linear relationship between serum pegloticase and stimulation of PUA elimination). Subjects with large increases in

overall anti-pegloticase antibody level had a much smaller slope value than those who had little or no increase in overall anti-pegloticase level. A small slope value suggests that large amounts of pegloticase are required to effect a small stimulation in the elimination of uric acid. Conversely, large slope values suggest significant stimulation of uric acid elimination with small pegloticase levels. This means that in the presence of anti-pegloticase antibodies, more pegloticase is required to stimulate the elimination of urate, as illustrated by Figure 3. This finding is consistent with the known effects of antibodies to reduce efficacy of therapeutic agents.(22) However, even in subjects with high anti-pegloticase antibody levels, pegloticase elicits a 10.6% increase in the rate of PUA elimination at estimated average therapeutic levels (2 mcg/mL). In addition, despite the detection of antibodies in 89% of patients, 42% of patients receiving biweekly pegloticase were persistent responders (patients with PUA less than 6.0 mg/dL for 80% of the time or longer during months 3 and 6), for whom PUA levels remained well below the solubility limit throughout the inter-dosing period (30).

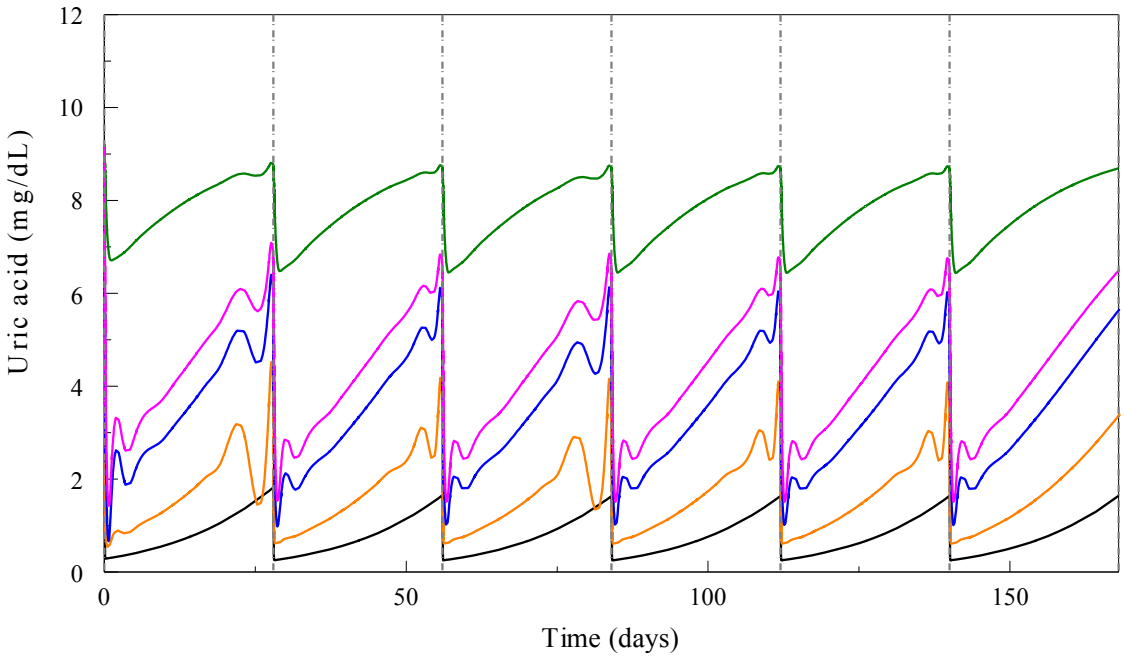
Figure 3. Predicted (Simulated) Uric Acid Profiles by Treatment Group and Anti-pegloticase Antibody Category

Figure 3a. Pegloticase Administered Every 2 Weeks



LEGEND: Black line = no increase in anti-pegloticase antibodies; Orange line = low increase in anti-pegloticase antibodies; Blue line = moderate increase in anti-pegloticase antibodies; Green line = high increase in anti-pegloticase antibodies; Pink line = information unavailable on increase in anti-pegloticase antibodies (n=1); Dashed, horizontal gray line = Dosing time

Figure 3b. Pegloticase Administered Every 4 Weeks



LEGEND: Black line = no increase in anti-pegloticase antibodies; Orange line = low increase in anti-pegloticase antibodies; Blue line = moderate increase in anti-pegloticase antibodies; Green line = high increase in anti-pegloticase antibodies; Pink line = information unavailable on increase in anti-pegloticase antibodies (n=1); Dashed, horizontal gray line = Dosing time

In humans, PUA is normally eliminated from the body via the kidney as urate, while pegloticase allows it to be eliminated renally as allantoin. One might therefore expect the K_{out} parameter (which incorporates the transformation of uric acid into allantoin and the subsequent urinary elimination of the latter) to be correlated with renal function and creatinine clearance to be retained as a statistically significant covariate. However, the results of this analysis suggest that baseline creatinine clearance was not a significant covariate of the effect of pegloticase on PUA levels. Creatinine clearance reflects mostly glomerular filtration while uric acid (urate) undergoes glomerular filtration, tubular

reabsorption, secretion and post-secretory reabsorption. (31, 32) It may be that creatinine clearance, as calculated by the Cockcroft-Gault formula, is not a variable that completely characterizes the renal processes involved in urate elimination, even though for most drugs creatinine clearance represents total tubule activity. Finally, hyperuricemia often involves an under-excretion of PUA, (31, 32) therefore the lack of correlation between baseline renal function and baseline PUA elimination may simply be a reflection of the abnormal PUA excretion.

Overall, the PD model appears to describe the PUA concentrations well, despite the significant amount of variability in the observed data. The between-subject variability is large, particularly for the slope parameter when anti-pegloticase antibody levels are low, suggesting that there are large differences in the responses between subjects. The residual variability (64%) is reasonable for this PK/PD analysis, considering that the PK profiles were quite variable, as previously mentioned.

3.6.3 Antibodies

The effect of antibodies on PK and PD parameters was assessed in a previously published Phase 2 study, but results failed to demonstrate any influence of antibodies on pegloticase. (18) In contrast, this Phase 3 analysis revealed a significant effect on pegloticase PK and PD. The larger sample size of the current study (n = 163 vs. n = 40) and the longer treatment period (6 months vs. 3 months) may have enabled the detection of an antibody effect in comparison with the previous study.

The results of the PD model with respect to the effect of anti-pegloticase antibodies concord with the efficacy findings of the Phase 3 studies, as measured by the percentage of subjects achieving PUA levels < 6 mg/dL for at least 80% of the time during months 3 and 6 combined. Indeed, 100% of subjects initially responded to pegloticase treatment with lowering of PUA to below the therapeutic target within 24 hours of receiving the first pegloticase infusion. However, persistent response was observed in 42% and 35% of

subjects receiving 8 mg every 2 weeks or 8 mg every 4 weeks, respectively and anti-pegloticase antibody responses were observed in 89% of subjects. (23)

While the inclusion of anti-pegloticase antibody titer category helped explain the variability in the PK/PD model, the titer itself has limited utility in the clinical setting. Indeed, only the highest anti-pegloticase antibody observed for each individual was incorporated into the model, as it was not possible to co-model antibody levels as a function of time. Therefore, the PK/PD model cannot be used to monitor loss of effect in a clinical setting.

3.7 Conclusion

The PK model that best described pegloticase was a 1-compartment model with a linear elimination process. For the first time, the influence of antibodies on the PK/PD of pegloticase was quantified. An increase in anti-pegloticase antibodies was associated with an increase in Vc and CL. BSA was also a significant covariate affecting the PK parameters Vc and CL. In other words, inter-individual variability seen in PK parameters could be attributed to differences in BSA as well as the presence of antibodies.

An indirect model was used to describe the PD of pegloticase, where the depletion rate of PUA was directly influenced by pegloticase levels. The stimulation of PUA elimination by pegloticase was influenced by antibody levels, such that in the presence of increased anti-pegloticase antibodies, greater pegloticase levels were necessary to stimulate uric acid metabolism. No other covariates (BSA, weight or creatinine clearance) influenced the PD parameters of pegloticase. Therefore, based upon the analysis of the data using the PK/PD model, dosing adjustments to correct for BSA are unnecessary.

Overall, both dosing regimens were able to reduce urate levels to well below the targeted 6 mg/dL. However, there was a substantial effect of anti-pegloticase antibodies on the pharmacodynamics of pegloticase in a subset of these patients, as indicated by the

modeling results. This is consistent with clinical laboratory results indicating that subjects with no or low anti-pegloticase antibody levels had a sustained urate-lowering response to pegloticase treatment while subjects who developed high levels of anti-pegloticase antibody titers lost their urate control over the course of the study. However, caution must be exercised with respect to the clinical utility of antibody levels, since a model could not be developed to predict loss of effect as a function of time-dependent antibody titers.

3.8 Conflict of Interest/Disclosure

At the time when the pegloticase clinical studies were performed and analyzed, W Huang, ZD Horowitz, AN Maroli, RW Waltrip and D Wright were employees and shareholders of Savient Pharmaceuticals Inc. LZ Benet was a member of the Science and Development Advisory Board and a shareholder of Savient Pharmaceuticals Inc.

The other authors declare no conflict of interest.

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Chapter 4 – Article #3

Optimizing Drug Development of TP-434, a Novel Fluorocycline, with Adaptive Learn & Confirm Cycles of Modeling & Simulation Using Single Ascending Dose Data

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4.1 Preface

Modeling and simulations is a tool that can enhance the drug development process by answering key questions in a timely and efficient fashion, and this applies as much to generic drugs as it does to innovator products. The first article presented in this thesis highlighted the role of PK modeling in the bioequivalence assessment of an iron complex, which was a novel approach within the generic drug development context. It was also an approach that was less costly and time-consuming than the traditional approach. The second article dealt with the innovator product environment for a biologic agent, pegloticase, which despite being different from the world of generic drug development, also benefitted from the use of modeling. PK/PD modeling was able to answer critical questions about potential pegloticase dosage adjustments, and the results of the research were even incorporated into the product labeling. Indeed, modeling made significant contributions to the eventual approval and successful marketing of pegloticase.

In the present article, another utility of modeling and simulations is demonstrated in the context of innovator drug development. The findings in this research project highlight the role of modeling and simulations in the selection of appropriate doses and dosing regimens, making use of available information and incorporating new data as it became available. In contrast with the previous article, the compound being studied here is a small molecule belonging to the antibiotic drug class, but as it will be shown, modeling and simulations can be used to enhance the development of a wide array of products.

The decline in the overall productivity of the pharmaceutical industry has affected many therapeutic areas, and antimicrobials are no exception. Since the early 1980's, there has been a steady decrease in the number of small molecule anti-infective agents that have received FDA approval (146, 148). Indeed, compared to other therapeutic classes (such as anesthetics/analgesics, antineoplastics, cardiovascular, endocrine, central nervous system, gastrointestinal, immunologic and respiratory) which have seen an increase in the number

of approvals over the last 3 decades or a mixture of increased and decreased numbers, the number of approved anti-infective agents has been on the decline since the 1980's (148). One of the reasons for this trend may be the slew of anti-infective agents already on the market, making it a highly competitive area of development (148). Moreover, the rising presence of generic products adds more pressure to those who wish to develop anti-infectives, because the drug must possess a truly novel mechanism of action or significant added benefit to justify its expense.

Drug resistance is both a hurdle to the development of antibiotics as well as an opportunity to improve current therapies. Increased resistance of bacteria to antibiotics has even led some pundits to warn of a return to a pre-antibiotic era (149). Indeed, antibiotics have been used for the last 60 years and their extensive use, and often misuse, has contributed to the growing resistance (149). The mechanisms for resistance are numerous, and include the modification of drug targets, the pumping out of the drug from the cell via efflux pumps, and the metabolism of drugs via bacterial enzymes (149). Our understanding and appreciation of these mechanisms has greatly improved, which helps drug developers chemically modify compounds that can bypass these resistance mechanisms. However, resistance remains inevitable, reminding us that antibiotic use must be well-controlled to slow down the onslaught of resistance as much as possible, while researchers continue to develop products to overcome this continuing problem.

Despite the challenges in bringing new anti-infective agents to market, the quest to add new anti-infectives to our therapeutic arsenal is still being pursued by some. One molecule currently under development is TP-434, a novel fluorocycline belonging to the class of tetracyclines. (A general overview of this drug class can be found in Appendix 1.) This IV product offers the possibility of eradicating bacteria that are resistant to antibiotics that are currently on the market. Some targets of TP-434 include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* and penicillin-resistant strains of *Streptococcus pneumonia*.

Although it has become harder and harder to identify promising anti-infective agents due to the increasing bacterial resistance, among other factors, modeling and simulations can help promising agents, such as TP-434, reach the market in a more effective and timely manner. For instance, modeling and simulation can help design optimal dosing regimens and aid in the planification of future trials (150). In one drug development example cited by the FDA, a more thorough analysis of the first of three trials that were conducted with the drug could have led to better planification of the subsequent trials, and may have shortened development time and improved the probability of approval (114). In other words, the subsequent trials (and the entire DDP for this drug) could have benefitted from a better understanding of the results obtained in the first trial. In contrast to this example described by the FDA, modeling and simulations was embraced during the DDP of TP-434, which made the process more efficient by continuously improving the understanding of the drug's PK and by applying this knowledge to dosing regimen selection.

Because the decisions made during the drug development process can have important ramifications, it is important that prior information be leveraged. For example, pre-clinical results can be included in PK/PD models to predict effects in humans. In the case of antimicrobials, PK/PD indices have been established for various classes of drugs. The minimum inhibitory concentration (MIC) represents the lowest concentration that inhibits visible growth of an organism, and this parameter has been correlated with various PK parameters to predict outcomes for different antibiotics. It has been shown for beta-lactams that time over MIC (over a 24 hour interval) greater than or equal to 40% correlates well with efficacy (151, 152). For aminoglycosides, ratios of Cmax to MIC ranging from 10 to 12 are good predictors of efficacy (153), while ratios of AUC to MIC ranging from 87 to 175 seem to predict the efficacy of fluoroquinolones (154-156). Modeling and simulations provide an excellent framework in which this information can be used, therefore it can play an important role in the development of antibiotics.

Thus, the current article illustrates how modeling and simulations, in conjunction with previously acquired knowledge, served to select appropriate TP-434 dosing regimens for further investigation. Importantly, this approach allows future protocols to be modified prior to their initiation to include dosing regimens that may not initially have been envisaged. This builds upon the learn and confirm paradigm advocated by Sheiner (136), by permitting learning and confirming to occur within different studies in the same phase of the DDP and further demonstrates how modeling and simulation can enhance the drug development process.

4.2 Abstract

Background: TP-434 is a novel fluorocycline being developed. A first in human, single ascending dose (SAD) study was conducted. TP-434 was infused IV over 30 minutes at doses of 0.1 to 3 mg/kg. This analysis aimed to describe the pharmacokinetics (PK) of TP-434 following a single dose and to determine the dosing regimens for a multiple ascending dose (MAD) study.

Methods: Population PK analyses were conducted with ADAPT 5 after the completion of each cohort using plasma & urinary data. The best model was chosen using standard model discrimination criteria. Simulations were performed with the model to predict clinical endpoints associated with various dosing regimens, such as AUC/MIC (area under the concentration-time curve/ minimal inhibitory concentration). MAD regimens were proposed using these endpoints.

Results: Seven cycles of modeling and simulation were conducted with data from 42 subjects. The model was improved with each successive analysis. The final model was a 4-compartment model with linear elimination. Mean parameters were Vc = 10.8 L, CLnr = 11.5 L/h, Vp1 = 16.1 L, CLd1 = 44.3 L/h, Vp2 = 132 L, CLd2 = 6.95 L/h, Vp3 = 103 L,

CLd3 = 26.9 L/h and CLr = 2.34 L/h. Inter-individual variability ranged from 8.7 to 41%. Based on AUC/MIC, simulations suggested that a minimum of 1.5 mg/kg QD for 10 days would be efficacious for organisms with an MIC_{50} = 0.5 μg/mL.

Conclusion: Using model-based simulations, dosing regimens originally proposed for the MAD study (1.0 mg/kg) were modified to evaluate more appropriate regimens (≥1.5 mg/kg daily).

4.3 Introduction

Antibiotic resistance is a phenomenon that continues to challenge clinicians and researchers who strive to find new treatments for different infections. This problem has touched many classes of antibiotics, if not most, and the tetracyclines have certainly not been spared. Resistance to tetracyclines, caused by the presence of efflux pumps or ribosomal protection, has resulted in the decreased use of these antibiotics (16). However, the relatively recent emergence of a new type of tetracycline which is effective against resistant organisms, tigecycline, has renewed interest in the class (2).

Another new type of tetracycline that belongs to the fluorocycline sub-group is TP-434, which is currently being developed by Tetraphase Pharmaceuticals, Inc. (5). It is a novel antibiotic that exhibits *in vitro* activity against a wide array of gram-positive and gram-negative pathogens, such as nosocomial and community-acquired methicillin-susceptible or -resistant *Staphylococcus aureus* strains (MRSA), vancomycin-susceptible or -resistant *Enterococcus faecium* and *Enterococcus faecalis*, and penicillin-susceptible or -resistant strains of *Streptococcus pneumoniae* (8, 17-18). In addition, it is active in animal models of infection (11).

Although in vitro and pre-clinical results were promising, the pharmacokinetics (PK) and safety of TP-434 in humans had yet to be determined. To this effect, a single

ascending dose (SAD) study was conducted in healthy volunteers. In this study, TP-434 was administered as a single intravenous (IV) infusion over 30 minutes, at a dose of either 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, or 3 mg/kg. In each cohort, 6 subjects received active treatment while 2 subjects received placebo. Serial blood and urine samples were collected pre-dose until 96 hours post-dose.

We hypothesized that the modeling of data from this SAD study, together with our knowledge of antimicrobial pharmacokinetic and pharmacodynamic (PD) relationships, would allow us to gain more insight into the PK of TP-434 during the conduct of the study and help us select more appropriate dosing regimens for a multiple dose study, before the initiation of the latter. Indeed, exposure response relationships for a similar compound, tigecycline, demonstrated that the ratio AUC_{SS}/MIC (the area under the concentration-time curve over 24 hours at steady state divided by the minimal inhibitory concentration) was a good predictor of clinical efficacy (1, 3, 4, 10). Because data was available after the completion of each successive cohort, it would be possible to create a PK model and improve upon the model with the inclusion of new results. Our objective was therefore to develop a PK model to describe the disposition of TP-434 after the administration of each dose and to select repeated dose regimens using simulations performed with this model.

4.4 Materials and Methods

4.4.1 Study Design

The study was a randomized, double-blind, parallel-group, placebo-controlled study. Healthy men and women, between 18 to 50 years of age inclusively, were included in the study. Good health was assessed by the Principal Investigator based on lack of clinically significant abnormalities in health assessments such as vital signs, electrocardiograms (ECG), laboratory tests, body mass index. Women included in the

study must have been surgically sterile (by tubal ligation, bilateral oophorectomy, or hysterectomy) for at least 6 months prior to study initiation.

Seven dose levels were studied: 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg and 3 mg/kg. In each cohort, 6 subjects received active treatment while 2 subjects received placebo. TP-434 was administered by IV infusion over 30 minutes. Dose escalation only proceeded in the absence of dose-limiting adverse events or clinically relevant safety laboratory parameters such as (but not limited to) alanine aminotransferase levels and absolute reticulocyte counts.

Subject safety was monitored throughout the study. Blood samples for TP-434 assay were collected prior to dosing and at the following times post-dose: 0.25, 0.5, 0.583, 0.75, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours. Urine samples were obtained before dosing and between 0 to 8 hours, 8 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours after the start of the infusion. Subjects were asked to void their bladders completely prior to dosing.

Protocol approval was obtained from the local institutional review board prior to the commencement of the study. In addition, written informed consent was obtained from study participants before conducting any study-related procedures.

4.4.2 Analytical Methods

Bioanalytical sample analysis for TP-434 was performed by Tandem Labs (Salt Lake City, UT, US). TP-434 in plasma was assayed using a validated liquid chromatography/mass spectrometry (LC/MS/MS) method with a quantitation range of 5.00 ng/mL to 1000 ng/mL. Assay precision (%CV) varied from 2.8% to 6.8% while accuracy (% bias) ranged from -0.1% to 3.0%. Similarly, TP-434 in urine was assayed using a validated LC/MS/MS method with a quantitation range of 5.00 ng/mL to 1000 ng/mL.

Assay precision (%CV) varied from 4.6% to 8.5% while accuracy (% bias) ranged from -0.8% to 3.3%.

4.4.3 Pharmacokinetic Analyses

Population PK analyses were performed using clinical and analytical data from all subjects receiving active treatment. Actual dose, drug infusion times, PK sampling times (for plasma and urine) as well as urinary volume were collected and used. Both plasma and urinary TP-434 concentrations were included in the analysis. Dataset preparation was performed using Microsoft Excel® 2003 and S-Plus® 8.0 for Windows.

Two, three and four-compartment models with linear elimination were first tested using the standard two-stage (STS) option in ADAPT 5® (6). The best model was selected based on standard discrimination techniques such as the minimization of the Akaike information criterion test, the residual variability, the maximization of the coefficient of determination, and based on graphical representation of the goodness of fit (e.g. observed versus predicted concentrations, weighted residuals versus predicted concentrations). All plasma and urinary concentrations were simultaneously fitted and explained by the model.

Following the determination of the final structural pharmacokinetic model using STS, a population pharmacokinetic analysis was performed using the maximum likelihood expectation maximization (MLEM) method within ADAPT 5®. This is a fully automated mixed effect modeling approach using both maximum likelihood and sampling-based methods. Briefly, the first probable population PK parameters and variance estimates (e.g., residual variability) were found by the STS approach using maximum likelihood. Then a population analysis was undertaken where population, individual and residual variability PK parameters were calculated and updated with more probable values at every new population iteration. The procedure was stopped when convergence was achieved (PK

mean and variance estimates were stabilized after over 1000 iterations were run) and the most probable and stable results for the population and individuals were determined.

All TP-434 concentrations were fitted using weighting procedures of $Wj=1/\sigma_j^2$ where the variance σ_j^2 was calculated for each observation using the equation $S_j^2=(a + b \times Y_j)^2$ where a and b are the intercept and slope of each variance model. The slope is the residual variability proportional to each concentration and the intercept is the additional component of the residual variability. These parameters were estimated continuously at each population iteration step as noted above using the MLEM approach.

Using the individual PK parameter estimates from the final model, secondary PK parameters were calculated for each subject. Cmax and Cmin were the maximal and minimal predicted concentration values for each subject (Cmin was the trough concentration at 24 hours post-dose). The area-under-the-curve from time zero to infinity (AUCinf) was calculated as dose divided by total clearance (CL_T), which was the sum of renal and non-renal clearances. Secondary PK parameters were calculated using Microsoft Excel® 2003 and S-Plus® 8.0 for Windows.

The model's predictive performance was assessed using the secondary predicted PK parameter AUCinf, as the overall predicted exposure would be used for exposure-response correlations. The predicted AUCinf values were visually compared to AUCinf values that were determined by noncompartmental methods (linear trapezoidal) using observed concentrations (7). Bias was determined as the percentage difference between the observed (noncompartmental) and predicted parameter, relative to the observed value, while precision was calculated as the absolute value of the bias (15). Equations for bias and precision are presented below, where $AUCinf_{obs}$ represents the AUCinf determined by noncompartmental methods while $AUCinf_{pred}$ is the AUCinf predicted by the model.

$$Bias = \left(\frac{AUC\inf_{obs} - AUC\inf_{pred}}{AUC\inf_{obs}} \right) \times 100$$

$$\Pr ecision = \frac{\left| AUC\inf_{obs} - AUC\inf_{pred} \right|}{AUC\inf_{obs}} \times 100$$

4.4.4 Simulations

Monte Carlo simulations (n=1000) were performed using the final model, in order to predict PK parameters in a population and associated with various dosing regimens that were either included in the MAD protocol or being considered for further investigation. The following 10-day dosing regimens were simulated for an average patient weighing 70 kg:

- 0.5 mg/kg IV administered over 30 minutes QD
- 0.75 mg/kg IV administered over 30 minutes QD
- 1.0 mg/kg IV administered over 30 minutes QD
- 1.0 mg/kg IV administered over 60 minutes QD
- 1.5 mg/kg IV administered over 30 minutes QD
- 1.5 mg/kg IV administered over 60 minutes QD
- 2 mg/kg IV administered over 30 minutes QD
- 1 mg/kg administered over 30 minutes BID

Using predicted concentrations from the simulated subjects in each of the dosing regimens, the PK parameters $AUC_{tau(ss)}$ (AUC over the dosing interval tau at steady-state), $Cmax_{(ss)}$ (maximal concentration at steady state) and $Cmin_{(ss)}$ (minimum concentration at

steady state) were calculated. $AUC_{tau(ss)}$ was calculated as dose divided by CL_T, while $Cmax_{(ss)}$ and $Cmin_{(ss)}$ were the maximal and minimal predicted concentration values for each subject over the dosing interval. The clinical endpoint AUC/MIC associated with various dosing regimens was also estimated. In addition, the percentage of subjects achieving a target AUC/MIC value was also determined for each dosing regimen. These target values were calculated from total (bound and unbound) tigecycline concentrations, and because TP-434 has a similar percentage of protein binding (~40%) (2), no adjustment for free fraction was deemed necessary. For complicated skin and skin-structure infections (caused by *S. Aureus* and *Streptococcus sp.*), the targeted AUC/MIC value was 17.9 while it was 12.96 for complicated intra-abdominal infections (caused by *Escherichia coli*, *Klebsiella sp.*, and *Enterobacteriacea*) (4, 10). These endpoints were obtained from studies conducted in patients who received tigecycline monotherapy. The AUC/MIC targets for community-acquired pneumonia (*S. pneumoniae*) and hospital-acquired pneumonia (*Escherichia coli* and MRSA) were 64 and 5.75, respectively (3).

Predicted PK parameters $AUC_{tau(ss)}$, $Cmax_{(ss)}$, and $Cmin_{(ss)}$ were calculated using Microsoft Excel® 2003 and S-Plus® 8.0 for Windows.

4.5 Results

A total of 56 subjects were enrolled in the study, with 42 subjects who received active treatment. Fifty-two subjects completed the clinical portion of the study (two subjects were withdrawn due to potential influenza infections). A summary of subject demographics is presented in Table 1.

Table 1. Subject Demographics

Characteristic	**n (%)**
Sex	
Male	49 (87.5%)
Female	7 (12.5)%
Race	
Asian	3 (5.4%)
Amerindian	1 (1.8%)
African-american	5 (8.9%)
Caucasian	47 (84%)
Characteristic	**Mean ± Standard deviation**
Age	28.1 ± 8.65
Weight (kg)	80.2 ± 12.5
Height (cm)	176 ± 7.71

Results associated with 2-, 3-, 4- and 5-compartment structural models that were investigated are presented in Table 2. The model with the lowest AIC value was the 4-compartment model.

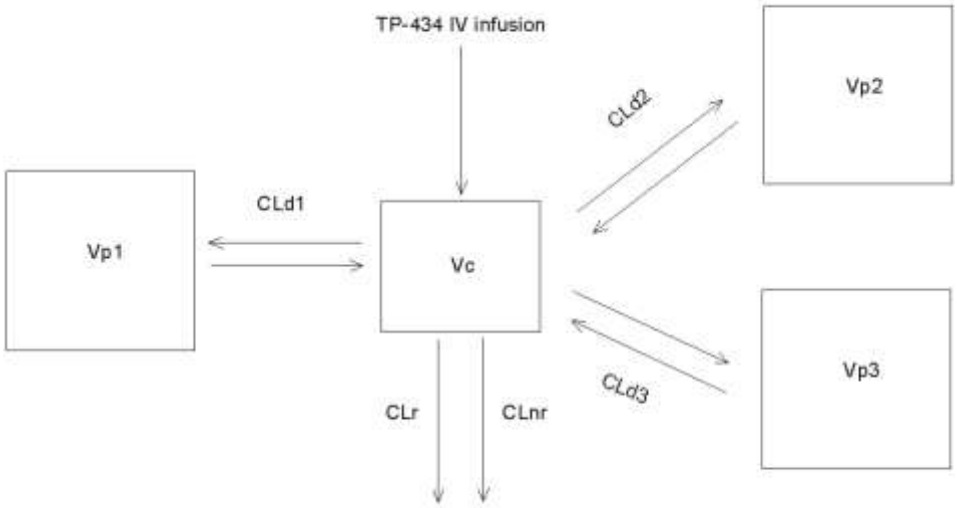
Table 2. Structural Model Determination for TP-434 using Standard Two-Stage Analysis

Model Description	Negative Log likelihood	AIC	BIC	R^2		Residual variability (%)	
				Plasma	Urine	Plasma	Urine
2-compartments, linear elimination	106.25	230.5	239.2	0.924	0.994	33.1	13.9
3-compartments, linear elimination	82.94	187.9	198.3	0.983	0.996	11.8	11.9
4-compartments, linear elimination	74.30	174.6	186.9	0.996	0.995	8.9	10.8
5-compartments, linear elimination	77.65	185.3	200.2	0.996	0.993	8.4	16.1

AIC: Akaike information criterion, BIC: Bayesian information criterion, R^2: Coefficient of determination

The final model selected to describe the plasma PK of TP-434 was a 4-compartment model with a linear (first order) elimination process, as depicted in Figure 1. The model was parameterized with a central volume of distribution (Vc), peripheral volumes of distribution (Vp1, Vp2 and Vp3), renal clearance (CLr), non-renal clearance (CLnr) and distributional clearances (CLd1, CLd2 and CLd3).

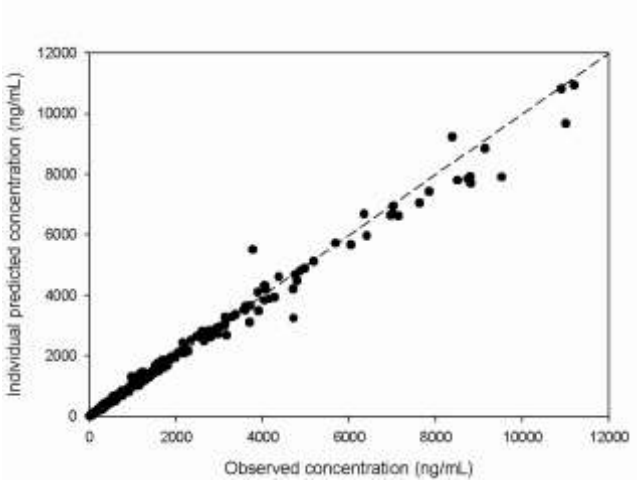
Figure 1. Final Pharmacokinetic Model for TP-434



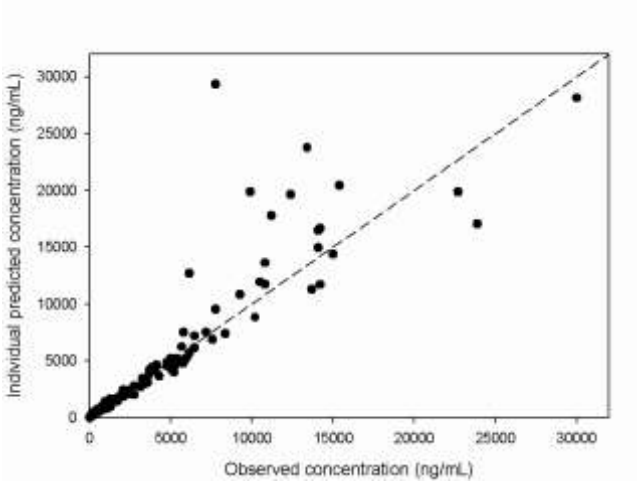
A total of 578 plasma concentrations and 209 urinary concentrations were simultaneously fitted by the model. Residual variability for plasma was 9.42% while it was 19.2% for urine. An example of a goodness-of-fit plot is presented in Figure 2. Plots of goodness of fit for weighted residuals (as a function of predicted concentrations or time) demonstrated no trends or biases in the quality of fit. Mean observed and predicted plasma concentration-time profiles are presented in Figure 3.

Figure 2. Predicted versus Observed TP-434 Concentrations

a) Plasma Concentrations

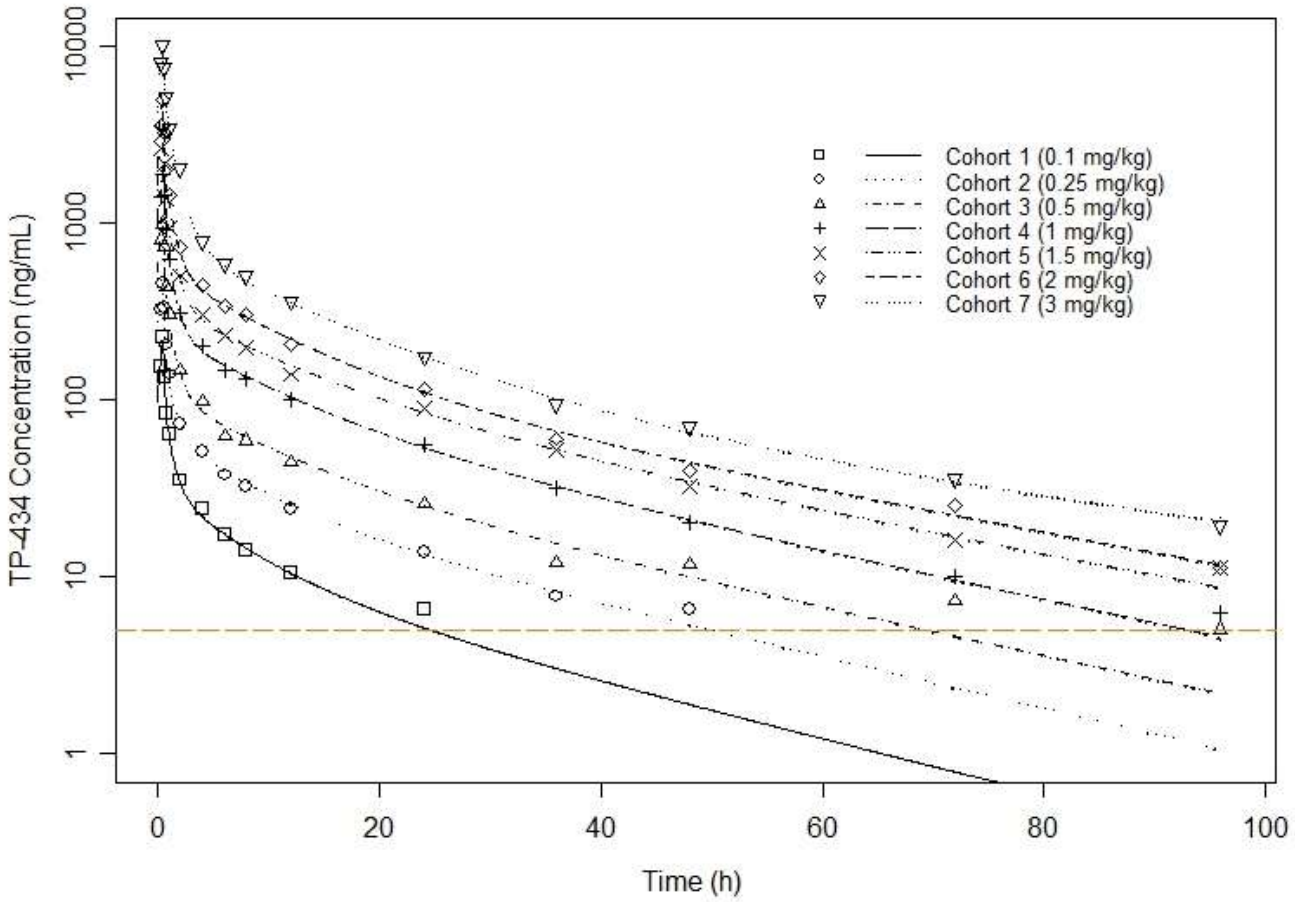


a) Urinary Concentrations



LEGEND: Dark circles represent concentrations while the dotted line represents the line of identity.

Figure 3. Mean Observed versus Predicted Plasma TP-434 Concentrations (Semi-log scale)



Note: Orange dashed line represents the lower limit of quantitation

Population PK parameter estimates and their precision are presented in Table 3 and a summary of secondary PK parameters by treatment group are presented in Table 4.

Table 3. Population Pharmacokinetic Parameter Estimates for TP-434

Parameter	Mean		Inter-subject	
	Estimate	%RSE	Estimate as CV%	%RSE
Vc (L)	10.8	12.1	20.6	41.2
CLnr (L/h)	11.5	6.74	19.5	29.1
Vp1 (L)	16.1	16.9	23.8	68.1
CLd1 (L/h)	44.3	11.1	8.69	181
Vp2 (L)	132	9.96	20.2	49.1
CLd2 (L/h)	6.95	20.9	40.8	51.4
Vp3 (L)	103	13.2	25.1	46.1
CLd3 (L/h)	26.9	14.2	30.8	49.7
CLr (L/h)	2.34	6.41	18.4	37.2

CLd1: distributional clearance between central compartment and first peripheral compartment; CLd2: distributional clearance between central compartment and second peripheral compartment; CLd3: distributional clearance between central compartment and third peripheral compartment; CLnr: non-renal clearance; CLr: renal clearance; %RSE: standard error as a percent of the corresponding maximum likelihood estimate; Vc: central volume of distribution; Vp1: first peripheral volume of distribution; Vp2: second peripheral volume of distribution; Vp3: third peripheral volume of distribution;

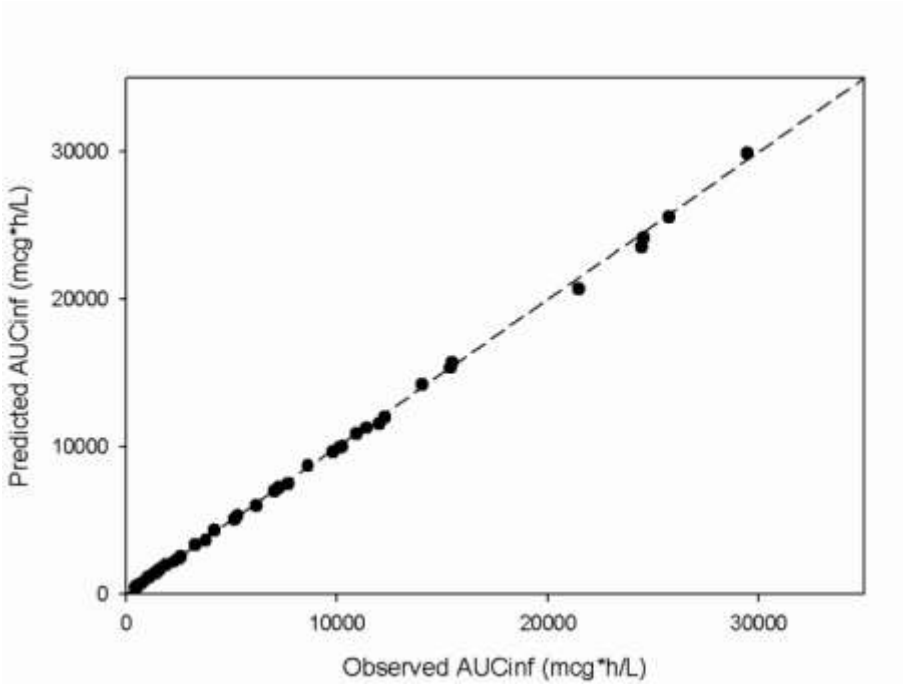
Table 4. Predicted Secondary Pharmacokinetic Parameters for TP-434

Dose	Mean ± SD (CV%)		
(mg/kg)	AUCinf (mcg*h/L)	Cmax (mcg/L)	Cmin (mcg/L)
0.1	560 ± 122 (21.7%)	212 ± 38.3 (18.1%)	5.16 ± 1.42 (27.6%)
0.25	1363 ± 203 (14.9%)	503 ± 79.7 (15.9%)	13.4 ± 2.63 (19.7%)
0.5	2680 ± 660 (24.6%)	1067 ± 207 (19.4%)	25.2 ± 6.84 (27.2%)
1.0	5546 ± 1085 (19.6%)	2027 ± 450 (22.2%)	53.4 ± 12.3 (23.1%)
1.5	8896 ± 1563 (17.6%)	3287 ± 656 (20.0%)	84.3 ± 14.1 (16.7%)
2.0	12376 ± 2010 (16.2%)	4694 ± 787 (16.8%)	110 ± 17.6 (16.0%)
3.0	23235 ± 4773 (20.5%)	9855 ± 1530 (15.5%)	175 ± 36.5 (20.8%)

The mean half-life of TP-434 in plasma (all cohorts confounded) was 26.2 h, while the median value was 23.1 h. Renal elimination of TP-434 accounted for approximately 17.1 ± 2.65 % of overall elimination.

Predictive performance is depicted in Figure 4, which suggests that AUCinf is well predicted by the model since all results are closely aligned with the line of identity (dashed line). Additionally, average bias and precision were 0.27% and 2.6% for AUCinf.

Figure 4. Predictive performance of the final model



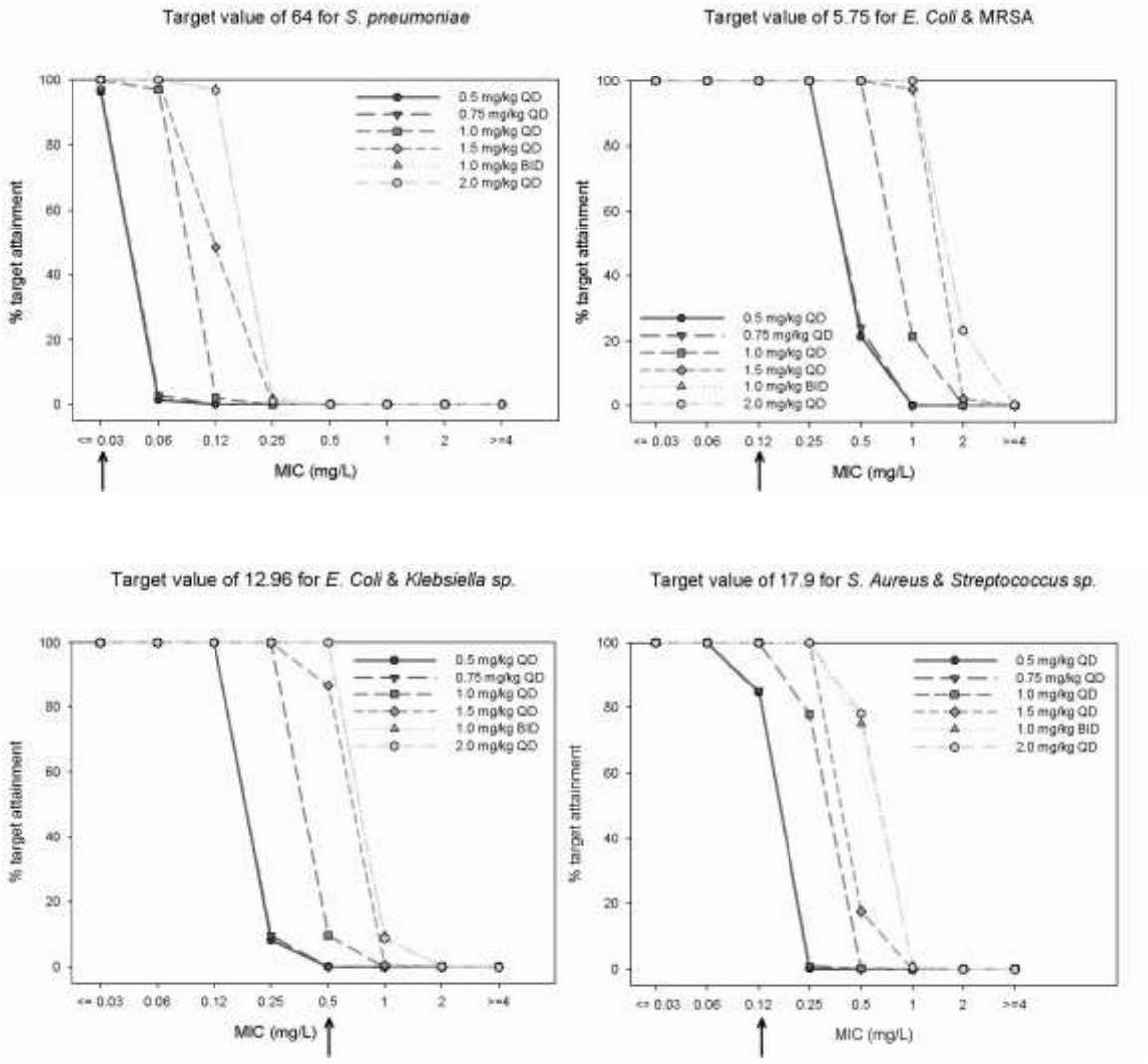
LEGEND: Dark circles represent AUCinf values while the dotted line represents the line of identity.

Mean secondary parameters estimated from the simulated profiles are summarized in Table 5, while Figure 5 displays the percentage of target attainment for various dosing regimens and different values of AUC/MIC. Target AUC/MIC values were taken from published tigecycline results in different patient populations (3, 4, 10). The arrow along the x-axis in each figure indicates the MIC value associated with TP-434 for the organism(s) being targeted. Therefore, this MIC value indicates the TP-434 clinical response associated with different TP-434 regimens for each indication.

Table 5. Secondary Simulated Steady-State Pharmacokinetic Parameters for TP-434

Dosing regimen (mg/kg)	Mean ± SD (CV%)		
	$AUC_{tau(ss)}$ (mcg*h/L)	$Cmax_{(ss)}$ (mcg/L)	$Cmin_{(ss)}$ (mcg/L)
0.5 mg/kg QD over 30 minutes	2575 ± 459 (17.8%)	1025 ± 147 (14.4%)	42.0 ± 15.5 (37%)
0.75 mg/kg QD over 30 minutes	2611 ± 498 (19.1%)	1556 ± 225 (14.4%)	64.1 ± 25.3 (39.5%)
1 mg/kg QD over 30 minutes	5163 ± 920 (17.8%)	2051 ± 293 (14.3%)	84.0 ± 31.0 (36.9%)
1 mg/kg QD over 60 minutes	5167 ± 938 (18.2%)	1405 ± 214 (15.3%)	84.4 ± 31.7 (37.6%)
1.5 mg/kg QD over 30 minutes	7814 ± 1389 (17.8%)	3106 ± 436 (14.0%)	127 ± 45.7 (35.9%)
1.5 mg/kg QD over 60 minutes	7725 ± 1393 (18%)	2078 ± 293 (14.1%)	126 ± 47.5 (37.8%)
2 mg/kg QD over 30 minutes	10386 ± 1967 (18.9%)	4120 ± 586 (14.2%)	168 ± 65.9 (39.4%)
1.0 mg/kg BID over 30 minutes	5182 ± 989 (19.1%)	2218 ± 318 (14.3%)	217 ± 69.6 (32.0%)

Figure 5. Percentage of target attainment for different dosing regimens over a range of MIC values



LEGEND: Arrows indicate the MIC value associated with TP-434 for the organism(s) being targeted. Simulated dosing regimens are represented as follows: dark circles with a solid line: 0.5 mg/kg QD; dark, inverted triangle with large dashes: 0.75 mg/kg QD; square with medium dashes: 1.0 mg/kg QD; diamond

with short dashes: 1.5 mg/kg QD; pale triangle with dotted line: 1.0 mg/kg BID; pale circle with dotted and dashed line: 2.0 mg/kg BID;

4.6 Discussion

This is the first published account describing the pharmacokinetics of TP-434 in humans. Before the study had even dosed, allometric scaling was performed with pre-clinical data in order to estimate possible PK parameters in humans. The analysis suggested that in humans, one could expect a CL of approximately 46 L/h, a Vc of around 20 L, a Vss of around 13 L/kg and a terminal elimination half-life close to 13 hours. Preliminary dose estimations suggested that a minimal daily dose of 1.5 mg/kg would be necessary for clinical efficacy against complicated skin and skin structure infections and possibly higher doses would be necessary for monotherapy against *S. Aureus*.

After the first cohort (receiving 0.1 mg/kg TP-434) had completed the clinical phase of the study, the plasma and urinary data that was obtained was used to develop an initial PK model. A 3-compartment model was selected at this stage. Following the completion of the next dose group (0.25 mg/kg TP-434) and the availability of their data, the model discrimination process was repeated to include data from both cohorts. The best model was also a 3-compartment model, and revised parameter estimates were obtained. This process was repeated following the completion of each treatment group, such that more data was being analyzed each time. By the time that data for the fourth cohort (1 mg/kg) was included in the analysis, the best model became a 4-compartment model, and this model was retained for all subsequent analyses.

The model development process that we used exemplifies the "learn and confirm" paradigm proposed by Sheiner nearly a decade and a half ago (14). Our initial attempt to learn about the PK of TP-434 in humans was through allometric scaling using preclinical data. This learning stage was further pursued when data from the first SAD cohort was

available and a first PK model was created. The PK parameters estimated in humans were different from those predicted by allometric scaling, therefore this analysis contributed significantly to our understanding of TP-434 PK. The PK results gathered at this stage were then confirmed with the availability and analysis of data from cohort 2, therefore this represented the first step in the "confirming" process. Subsequent analyses reinforced the "learn & confirm" cycle, improving the PK model and parameter estimates with each iteration.

The law of parsimony requires that the simplest model that describes the PK of a compound be used preferentially over a more complicated model, if the standard model discrimination criteria are similar between both models. In our case, the best model was a 4-compartment model according to the model discrimination criteria applied to the two-stage analysis results. The 4-compartment model was associated with lower AIC and residual variability values compared to the 2- and 3-compartment models, while the 5-compartment model did not improve these values any further.

The number of parameters that can be calculated for a given compartmental model depends on many factors, notably the number of visible exponentials in plasma disposition, the number of excretory pathways measured, the number of tissue spaces analyzed as well as the number of visible nonlinear features in the data (9). In the current analysis, 3 exponentials were visible and concentrations were measured in both plasma and urine. With this in mind, the rich plasma and urinary sampling scheme in this study (approximately 16 samples per subject) provided sufficient information to robustly estimate the nine PK parameters associated with the 4-compartment model (CLnr, CLr, Vc, CLd1, Vp1, CLd2, Vp2, etc.).

The residual variability associated with the final PK model (9.42% for plasma and 19.2 % for urine) represents the variability that is not explained by the model, including intra-individual variability, the experimental "noise" of the analytical method and errors

arising from the pharmacokinetic modeling itself (model misspecification). The low residual variability associated with our model confirms that the 4-compartment model was able to properly capture the PK of TP-434.

The current analysis demonstrates that TP-434 had a population mean CL_T of 13.9 L/h and a Vss of approximately 262 L (around 3.3 L/kg). The total clearance value is slightly lower than what was previously reported for tigecycline. The PK of tigecycline in healthy volunteers was also described by a multi-compartment model (19). For this drug, a 3-compartment model with zero-order input and first-order elimination was chosen to describe its PK. A population mean CL of 16.3 L/h was estimated, with a Vss of 749 L or approximately 10 L/kg.

Total clearance of TP-434 did not change with increasing doses, thus results of the current analysis indicate that TP-434 exhibits dose-proportional pharmacokinetics over the dose range studied. In addition, renal clearance estimated by the model (17.1% of total clearance) confirms that the elimination of TP-434 is mainly through non-renal pathways. This is similar to what was observed in chimpanzees, where urinary excretion was roughly 20% of total elimination (12).

Simulations revealed that for most target organisms, all regimens were associated with a probability of meeting the target AUC/MIC that exceeded 80%. However, for treating complicated intra-abdominal infections caused by *E. Coli* and *Klebsiella sp.*, doses inferior to 1.5 mg/kg TP-434 per day were not associated with much success. Indeed, for these dosing regimens, less than 10% of subjects achieved the desired AUC/MIC. Thus, prior to initiation of the MAD study, the protocol was modified in order to include a treatment regimen of 1.5 mg/kg administered once daily. Initially, the second dosing group of the MAD study was scheduled to receive 1.0 mg/kg QD, but the protocol was amended such that the 1.0 mg/kg QD was replaced by the 1.5 mg/kg dose group. In addition, single doses of 1.5 mg/kg appeared to be well tolerated by the subjects in the SAD study, and

simulations did not suggest significant accumulation following repeated administration. Finally, a twice-daily (BID) dosing regimen was added to the MAD study (1.0 mg/kg BID), in light of the simulation results and the tolerability of a single dose of 2.0 mg/kg in the SAD study.

4.7 Conclusion

The final model was not only used to describe the PK of TP 434 following single dose administration, but was also used to predict plasma TP-434 concentrations associated with various multiple dose regimens. These predictions were used to further refine the regimens tested in a multiple dose setting, prior to study initiation, and demonstrated the utility of modeling and simulations in early stages of drug development.

4.8 References

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Chapter 5 – Article #4

Population Pharmacokinetic Modeling of TP-434 Following Multiple Dose Administration

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5.1 Preface

The research that was presented in the first three manuscripts demonstrated how modeling and simulations can be used to answer key questions that are frequently encountered during the drug development process. In the first article, modeling and simulations played a critical part in an innovative approach to bioequivalence assessment, while in the second article, population modeling was used to better understand the PK and PD of a biologic drug and enhance the product labeling. The third article illustrated how PK/PD concepts used in conjunction with a population PK model could be useful as early as in Phase 1 of clinical development, in a single ascending dose study, and that such a model could lead to more rational dose and dosing regimen selections for subsequent studies. The current (fourth) article demonstrates how pharmacokinetic models can be continually refined as more information becomes available, such that it incorporates all current knowledge to allow for more informed decision-making.

As described in the third manuscript, the antibiotic TP-434 is being developed to treat various types of infections caused by highly resistant organisms. A first-in-man, single ascending dose study was conducted in healthy volunteers and the data collected from this study was incorporated into a population PK model that was used to select dosing regimens for investigation of repeated dosing. The current study describes the first repeat-dose study of TP-434 in man, which benefitted from previous population PK analyses (described in Article #3) with the addition of dosing regimens that were not originally envisaged. More specifically, a twice daily dosing regimen was evaluated instead of a once daily dosing regimen. Population PK analyses were also undertaken with data obtained from this multiple ascending dose study of TP-434 and they served to broaden our understanding of the PK of TP-434, as well as confirm the previously defined model. Thus, the research presented in this manuscript was conducted in the spirit of “learn and

confirm" advocated by Sheiner (136) and demonstrates how it can be applied to data obtained within the same phase of drug development. It shows how the continual use of modeling and simulation throughout the drug development process can increase confidence levels when important decisions must be made, thereby decreasing some of the risk inherent to bringing a drug to market. Dosing regimens that were administered in this multiple ascending dose study, and that had been tested purely based on the results of Article #3, were ultimately selected for administration to patients in Phase 2. Thus, this approach ensured that dosing regimens for the target population were assessed following repeated administration in healthy patients, prior to the initiation of Phase 2, which would not have occurred without the use of modeling and simulations.

5.2 Abstract

Background: A multiple ascending dose study was completed for TP-434. Placebo or TP-434 (either 0.5 or 1.5 mg/kg QD over 30 minutes, or 1.5 mg/kg QD or 1 mg/kg BID over 1 hour) was administered for 10 days. Plasma and urine samples were collected throughout. This analysis aimed to describe the pharmacokinetics (PK) of TP-434, confirm previously determined PK & confirm dosing regimens for further investigation.

Methods: Population PK analyses were done with ADAPT 5® using plasma and urinary data. Two-, three- and four-compartment models were tested and standard model discrimination criteria were used to select the best model. Results were compared to those obtained from single dose (SD) data.

Results: TP-434 was well described by a 4-compartment model with linear elimination. Mean parameters were Vc = 12.2 L, CLnr = 11.5 L/h, Vp1 = 16.6 L, CLd1 = 29.9 L/h, Vp2 = 188 L, CLd2 = 4.90 L/h, Vp3 = 103 L, CLd3 = 21.2 L/h & CLr = 2.05 L/h. Inter-

individual variability ranged from 2.3 to 47%. Steady-state volume of distribution was 320 L and mean half-life was ~ 48 hours. Results were similar to those of SD and confirm predictions made from SD data. Exposure associated with daily doses ≥ 1.5 mg/kg was expected to be efficacious against organisms with minimum inhibitory concentrations (MIC) ≤ 2 μg/ml.

Conclusion: Multiple dose PK of TP-434 was described by a 4-compartment model, with results similar to those following SD. Observed and predicted exposure confirmed the doses predicted to be efficacious in Phase 2.

5.3 Introduction

A novel antimicrobial agent belonging to the fluorocycline class, TP-434, is being developed by Tetraphase Pharmaceuticals, Inc (4, 7). Although this antibiotic is a member of the tetracycline family, *in vitro* tests have demonstrated activity against organisms that are generally resistant to tetracyclines, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium* and penicillin-resistant *Streptococcus pneumoniae* (12, 13). Thus, this molecule could be an excellent addition to a therapeutic arsenal that is becoming more and more limited by bacterial resistance.

A first-in-man, single ascending dose (SAD) study was undertaken with TP-434 to evaluate its safety and pharmacokinetics (PK) in humans. The drug was administered at doses ranging from 0.1 mg/kg to 3.0 mg/kg. Based on the results of the SAD study, a 4-compartment model was developed to describe the PK of the drug. Simulations performed with the final model were used to determine which dosing regimens should be tested in a multiple ascending dose (MAD) study, prior to study initiation (C. Seng Yue, J. A. Sutcliffe, P. Colucci, C. R. Sprenger, and M. P. Ducharme, submitted for publication). A key principle underlying this analysis was the relationship between clinical efficacy and the parameter AUC/MIC (the area under the concentration-time curve over 24 hours at steady state divided by the minimal inhibitory concentration) (1-3, 8). Thus, according to the simulation results, a minimum daily dose of a least 1.5 mg/kg would be necessary in order to achieve target AUC/MIC values for more resistant pathogens, such as *Escherichia coli* and *Klebsiella sp*.

The MAD study was designed to evaluate the safety, tolerability and PK of TP-434 following multiple dose administration. In this study, multiple ascending doses of TP 434 were administered to 4 separate cohorts. Subjects received TP-434 for a ten day period, at a dose of either 0.5 mg/kg infused over 30 minutes QD, 1.5 mg/kg infused over 30 minutes

QD, 1.5 mg/kg infused over 60 minutes QD or 1.0 mg/kg infused over 60 minutes BID. In each cohort, 6 subjects received active treatment while 2 subjects received placebo.

The objectives of this analysis were to describe the PK of TP 434 following repeated administration and to validate dosing regimens to be administered in Phase 2 studies. In addition, this analysis sought to confirm the PK of TP-434 previously predicted using SAD data.

5.4 Materials and Methods

5.4.1 Study Design

The study was a randomized, double-blind, parallel-group, placebo-controlled study. Healthy men and women, between 18 to 50 years of age inclusively, were included in the study. Good health was assessed by the Principal Investigator based on lack of clinically significant abnormalities in health assessments such as vital signs, electrocardiograms (ECG), laboratory tests, body mass index. Women included in the study must have been surgically sterile (by tubal ligation, bilateral oophorectomy, or hysterectomy) for at least 6 months prior to study initiation.

Four (4) ten-day dosing regimens were studied: 0.5 mg/kg IV infused over 30 minutes QD, 1.5 mg/kg IV infused over 30 minutes QD, 1.5 mg/kg IV infused over 60 minutes QD, and 1.0 mg/kg IV infused over 60 minutes BID. In each cohort, 6 subjects received active treatment while 2 subjects received placebo. Dose escalation only proceeded in the absence of dose-limiting adverse events or clinically relevant safety laboratory parameters.

Subject safety (laboratory results, electrocardiograms, vital signs, adverse events) was monitored throughout the study. Blood samples for TP-434 assay were collected on Days 1 and 10 prior to dosing and at the following times post-dose: 0.25, 0.5, 0.583, 0.75,

1, 2, 4, 6, 8, and 12 hours. In addition, pre-dose samples were collected on Days 2 to 9, and on Days 11-14, samples were collected 24, 36, 48, 72, and 96 hours after the start of the infusion on Day 10. Urine samples were obtained on Days 1 and 10 before dosing and between 0 to 8 hours, 8 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours after the start of the infusion of each of these days.

Protocol approval was obtained from the local institutional review board prior to the commencement of the study. In addition, written informed consent was obtained from study participants before conducting any study-related procedures.

5.4.2 Analytical Methods

Bioanalytical sample analysis for TP 434 was performed by Tandem Labs (Salt Lake City, UT, US). TP-434 in plasma was assayed using a validated liquid chromatography/mass spectrometry (LC/MS/MS) method with a quantitation range of 5.00 ng/mL to 1000 ng/mL. Assay precision (%CV) varied from 2.8% to 6.8% while accuracy (% bias) ranged from -0.1% to 3.0%. Similarly, TP-434 in urine was assayed using a validated LC/MS/MS method with a quantitation range of 5.00 ng/mL to 1000 ng/mL. Assay precision (%CV) varied from 4.6% to 8.5% while accuracy (% bias) ranged from -0.8% to 3.3%.

5.4.3 Pharmacokinetic Analyses

Population PK analyses were performed using clinical and analytical data from all subjects receiving active treatment. Actual dose, drug infusion times, PK sampling times (for plasma and urine) as well as urinary volume were used. Both plasma and urinary TP-434 concentrations were included in the analysis.

Two, three and four-compartment models with linear elimination were first tested using the standard two-stage (STS) option in ADAPT 5® (5). The best model was selected

based on standard discrimination techniques such as the minimization of the Akaike information criterion test, the residual variability, the maximization of the coefficient of determination, and based on graphical representation of the goodness of fit (e.g. observed versus predicted concentrations, weighted residuals versus predicted values).

Following the determination of the final structural pharmacokinetic model using STS, a population pharmacokinetic analysis was performed using the maximum likelihood expectation maximization (MLEM) method within ADAPT 5®. This is a fully automated mixed effect modeling approach using both maximum likelihood and sampling-based methods. Briefly, the first probable population PK parameters and variance estimates (e.g., residual variability) were found by the STS approach using maximum likelihood. Then a population analysis was undertaken where population, individual and residual variability PK parameters were calculated and updated with more probable values at every new population iteration. The procedure was stopped when convergence was achieved (PK mean and variance estimates were stabilized after over 1000 iterations were run) and the most probable and stable results for the population and individuals were determined.

All TP-434 concentrations were fitted using weighting procedures of $Wj=1/\sigma_j^2$ where the variance σ_j^2 was calculated for each observation using the equation $S_j^2=(a + b \times Y_j)^2$ where a and b are the intercept and slope of each variance model. The slope is the residual variability proportional to each concentration and the intercept is the additional component of the residual variability. These parameters were estimated continuously at each population iteration step as noted above using the MLEM approach.

Using the individual PK parameter estimates from the final model, secondary PK parameters were calculated. These included Vss, terminal elimination half-life and total clearance. Peak and trough concentrations were also determined using predicted concentrations on Day 1 and Day 10. On Day 1, Cmax and Cmin were the maximal and minimal predicted concentration values for each subject over the dosing interval. For

subjects dosed twice daily, these parameters were calculated for the morning dose. On Day 10, the maximal and minimal predicted concentrations over the dosing interval were labeled $Cmax_{(ss)}$ and $Cmin_{(ss)}$. In addition, the area-under-the-curve over the dosing interval at steady-state ($AUC_{tau(ss)}$) was calculated as dose divided by total clearance (CL_T) while $AUC_{0\text{-}24}$ for Day 1 was calculated with the linear trapezoidal method using all predicted concentrations during the dosing interval (6).

Predictive performance was assessed using the secondary predicted PK parameter $AUC_{tau(ss)}$, as the overall predicted exposure was critical for exposure-response correlations. The predicted $AUC_{tau(ss)}$ values were visually compared to $AUC_{tau(ss)}$ values that were determined by noncompartmental methods using observed concentrations (6). Bias was determined as the percentage difference between the observed (noncompartmental) and predicted parameter, relative to the observed value, while precision was calculated the absolute value of the bias (11). Equations for bias and precision are presented below, where $AUCinf_{obs}$ represents the AUCinf determined by noncompartmental methods while $AUCinf_{pred}$ is the AUCinf predicted by the model.

$$Bias = \left(\frac{AUC\inf_{obs} - AUC\inf_{pred}}{AUC\inf_{obs}} \right) \times 100$$

$$\Pr ecision = \frac{\left| AUC\inf_{obs} - AUC\inf_{pred} \right|}{AUC\inf_{obs}} \times 100$$

Dataset preparation and secondary PK parameter calculations were performed using Microsoft Excel® 2003 and S-Plus® 8.0 for Windows.

5.5 Results

A total of 32 subjects were enrolled in the study, with 24 subjects who received active treatment. All 32 subjects completed the clinical portion of the study. A summary of subject demographics is presented in Table 1.

Table 1. Subject Demographics

Characteristic	**n (%)**
Sex	
Male	31 (96.9%)
Female	1 (3.1)%
Race	
Asian	1 (3.13%)
Amerindian	1 (3.13%)
African-american	1 (3.13%)
Caucasian	29 (90.6%)

Characteristic	**Mean ± Standard deviation**
Age	30.8 ± 9.66
Weight (kg)	79.4 ± 13.1
Height (cm)	175 ± 8.05

Results associated with 2-, 3-, 4- and 5-compartment structural models that were investigated are presented in Table 2. The model with the lowest AIC value was the 4-compartment model.

Table 2. Structural Model Determination for TP-434 using Standard Two-Stage Analysis

Model Description	Negative Log likelihood	AIC	BIC	R^2		Residual variability (%)	
				Plasma	Urine	Plasma	Urine
2-compartments, linear elimination	275.97	569.9	586.2	0.891	0.951	43.4	17.0
3-compartments, linear elimination	244.36	510.7	530.6	0.975	0.948	13.3	20.3
4-compartments, linear elimination	234.50	495.0	518.5	0.984	0.949	12.3	21.6
5-compartments, linear elimination	234.55	499.1	526.2	0.984	0.948	12.3	21.1

AIC: Akaike information criterion, BIC: Bayesian information criterion, R^2: Coefficient of determination

The final model selected to describe the plasma PK of TP-434 was a 4-compartment model with a linear (first order) elimination process, as depicted in Figure 1. The model was parameterized with a central volume of distribution (Vc), peripheral volumes of distribution (Vp1, Vp2 and Vp3), renal clearance (CLr), non-renal clearance (CLnr) and distributional clearances (CLd1, CLd2 and CLd3).

A total of 812 plasma concentrations and 237 urinary concentrations were simultaneously fitted by the model. Residual variability for plasma was 14.0% while it was 21.5% for urine. An example of a goodness-of-fit plot is presented in Figure 2. Plots of goodness of fit for weighted residuals (as a function of predicted concentrations or time) demonstrated no trends or biases in the quality of fit. Mean observed and predicted plasma concentration-time profiles are presented in Figure 3.

Figure 1. Final Pharmacokinetic Model for TP-434

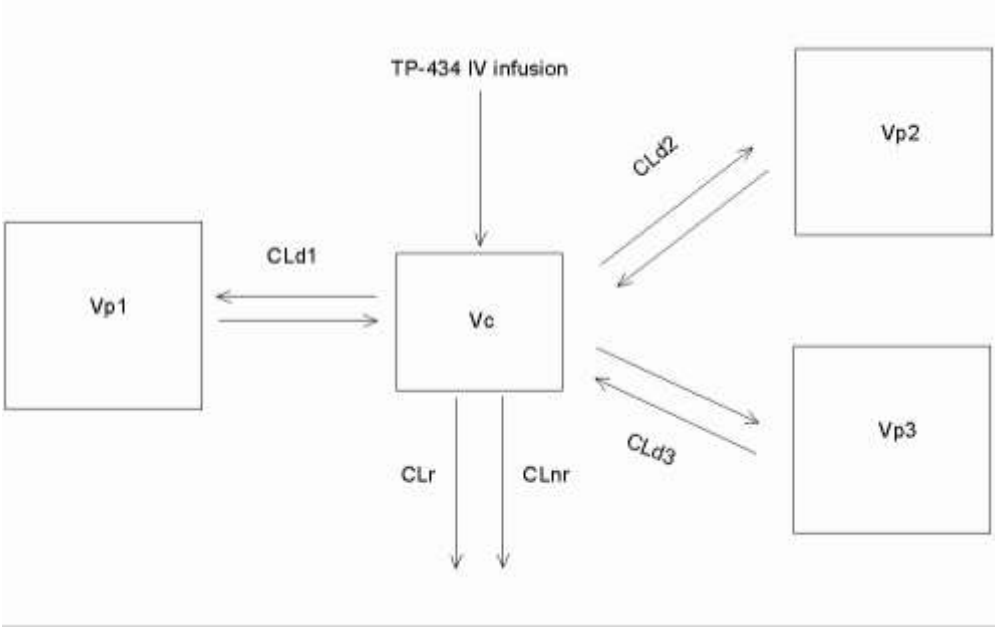
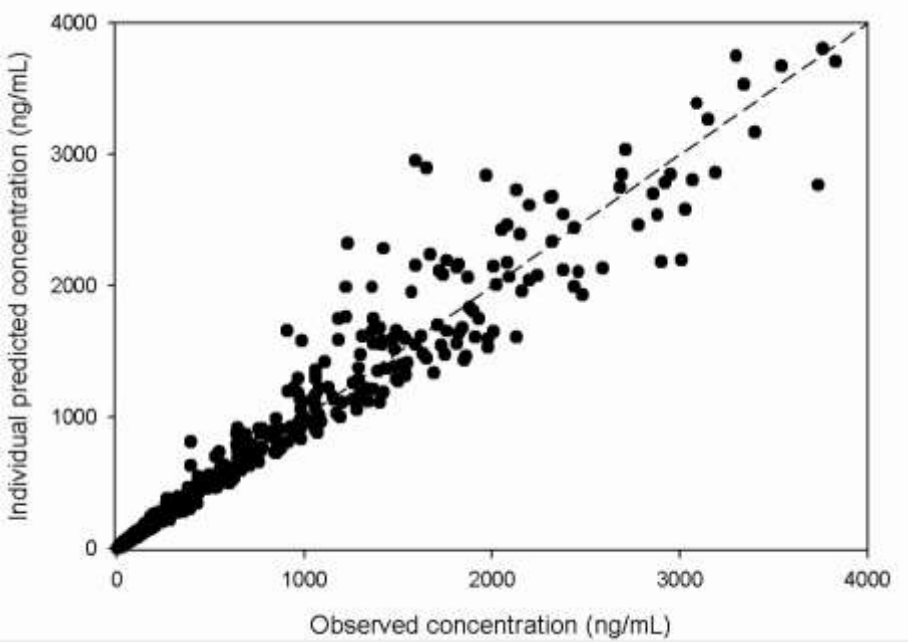
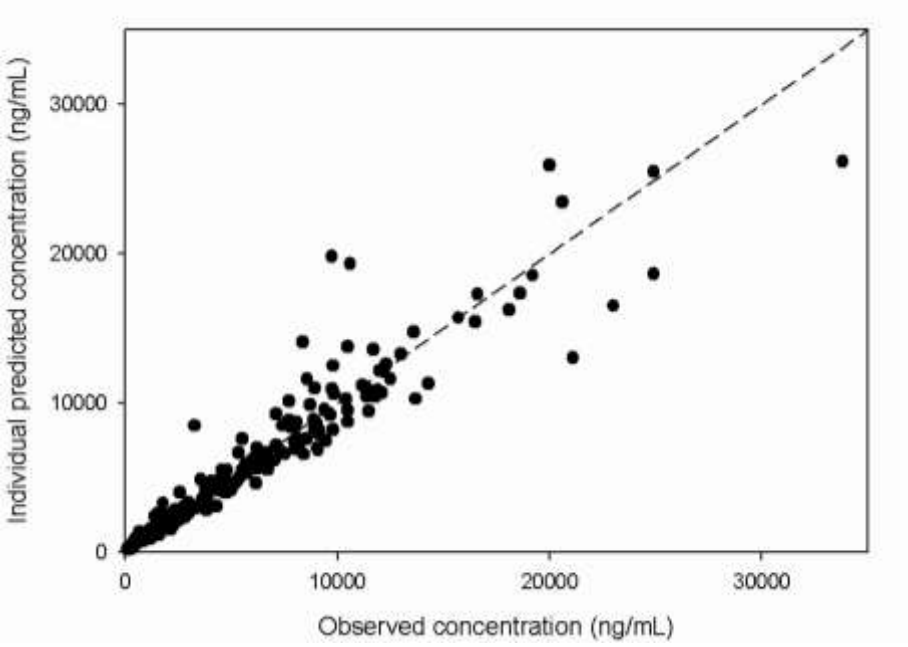


Figure 2. Predicted versus Observed TP-434 Concentrations

a) **Plasma Concentrations**

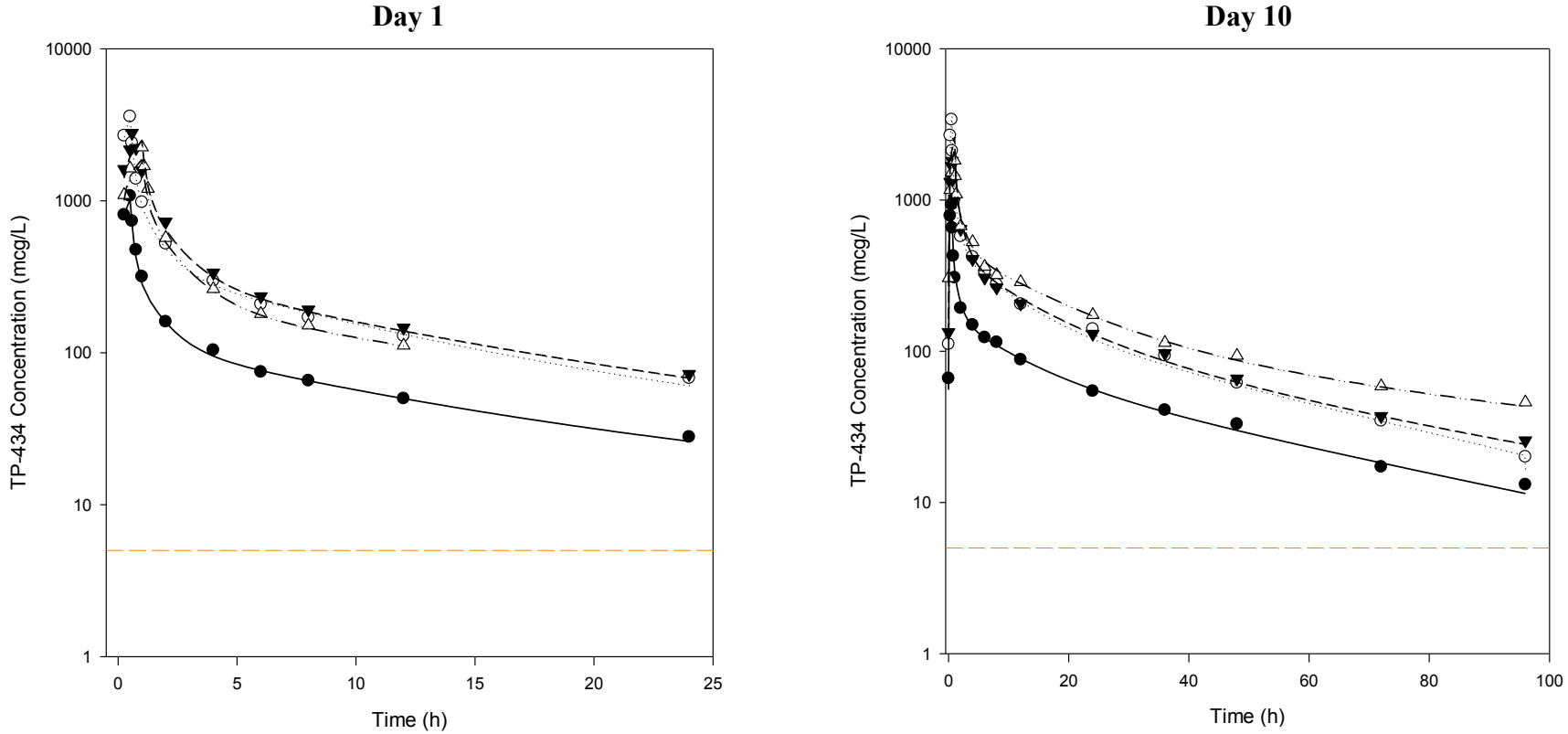


b) **Urinary Concentrations**



Legend: Dark circles represent concentrations while the dotted line represents the line of identity.

Figure 3. Mean Observed versus Predicted Plasma TP-434 Concentrations (Semi-log scale)



LEGEND: Dark circle = observed concentrations for Cohort 1 (0.5 mg/kg), white circle = observed concentrations for Cohort 2 (1.5 mg/kg over 30 minutes), Dark triangle = observed concentrations for Cohort 3 (1.5 mg/kg over 1 hour), White triangle = observed concentrations for Cohort 4 (1.0 mg/kg over 1 hour BID), Solid line = predicted concentrations for Cohort 1 (0.5 mg/kg), Dotted line = predicted concentrations for Cohort 2 (1.5 mg/kg over 30 minutes), Dashed line = predicted concentrations for Cohort 3 (1.5 mg/kg over 1 hour), Dashed and dotted line = predicted concentrations for Cohort 4 (1.5 mg/kg over 1 hour BID), Orange dashed line = lower limit of quantitation

Population PK parameter estimates and their precision are presented in Table 3 and a summary of secondary PK parameters by treatment group are presented in Table 4.

Volume of distribution at steady-state was approximately 320 L. The mean half-life of TP-434 in plasma (all cohorts confounded) was 47.7 h, while the median value was 35.3 h. Renal elimination of TP-434 accounted for approximately 15.5 ± 2.37 % of overall elimination.

Predictive performance is depicted in Figure 4, which suggests that $AUC_{tau(ss)}$ is well predicted by the model since all results are well aligned with the line of identity (dashed line). Additionally, average bias and precision were -5.8% and 7.1% for $AUC_{tau(ss)}$.

Table 3. Population Pharmacokinetic Parameter Estimates for TP-434 Following Repeated Administration

Parameter	Mean		Inter-subject	
	Estimate	%RSE	Estimate as CV%	%RSE
Vc (L)	12.2	19.4	10.9	186
CLnr (L/h)	11.5	12.4	23.0	52.2
Vp1 (L)	16.6	36.8	2.28	902
CLd1 (L/h)	29.9	69.0	21.2	300
Vp2 (L)	188	10.2	15.4	81.4
CLd2 (L/h)	4.90	49.2	46.7	61.1
Vp3 (L)	103	17.1	9.56	356
CLd3 (L/h)	21.2	39.0	29.4	81.8
CLr (L/h)	2.05	10.9	15.7	62.6

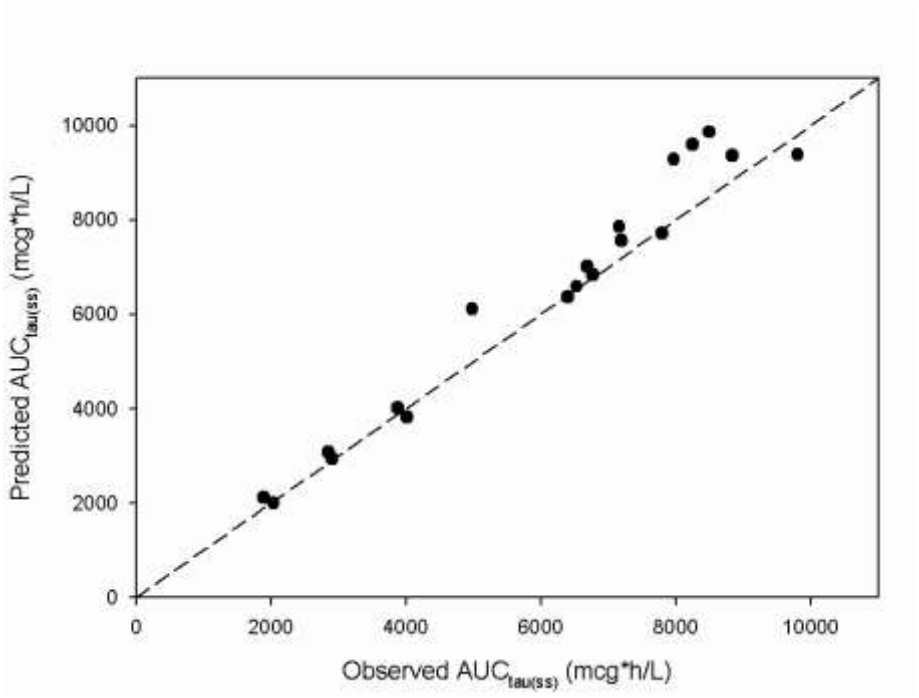
CLd1: distributional clearance between central compartment and first peripheral compartment; CLd2: distributional clearance between central compartment and second peripheral compartment; CLd3: distributional clearance between central compartment and third peripheral compartment; CLnr: non-renal clearance; CLr: renal clearance; %RSE: standard error as a percent of the corresponding maximum likelihood estimate Vc: central volume of distribution; Vp1: first peripheral volume of distribution; Vp2: second peripheral volume of distribution; Vp3: third peripheral volume of distribution;

Table 4. Secondary Pharmacokinetic Parameters for TP-434

Dosing Regimen (mg/kg)	Mean ± SD (CV%)					
	Day 1			Day 10		
	$AUC_{0\text{-}tau}$ (mcg*h/L)	Cmax (mcg/L)	Cmin (mcg/L)	$AUC_{0\text{-}tau(ss)}$ (mcg*h/L)	$Cmax_{(ss)}$ (mcg/L)	$Cmin_{(ss)}$ (mcg/L)
0.5 mg/kg QD over 30 minutes	1991 ± 405 (20.3%)	1020 ± 132 (13.0%)	26.1 ± 9.46 (36.2%)	2992 ± 838 (28.0%)	1075 ± 149 (13.9%)	55.9 ± 23.5 (42.1%)
1.5 mg/kg QD over 30 minutes	5903 ± 553 (9.4%)	3582 ± 316 (8.8%)	57.8 ± 10.5 (18.2%)	7803 ± 928 (11.9%)	3596 ± 295 (8.2%)[a]	121 ± 32.2 (26.7%)[a]
1.5 mg/kg QD over 60 minutes	6449 ± 898 (13.9%)	2486 ± 354 (14.2%)	68.0 ± 11.2 (16.5%)	8671 ± 1391 (16.0%)	2605 ± 376 (14.4%)	129 ± 28.6 (22.1%)
1.0 mg/kg BID over 60 minutes	3937 ± 462 (11.7%)	1815 ± 180 (9.9%)	104 ± 19.1 (18.3%)	6667 ± 668 (10.0%)	2142 ± 200 (9.3%)[b]	280 ± 48.2 (17.2%)[b]

[a]n=3; [b]n=4

Figure 4. Predictive performance of the final model



Legend: Dark circles represent AUCtau(ss) values while the dotted line represents the line of identity.

5.6 Discussion

The analysis presented here embodies Sheiner's "learn and confirm" cycle (10). Previous modeling work shed light upon the PK of TP-434 following single dose administration, but before this study had been completed, no information was available about disposition of multiple-dose TP-434. Therefore, this study allowed us to gather additional information and learn more about TP-434. At the same time, the PK analysis confirmed the selection of the 4-compartment model that was applied to single-dose data. Indeed, the pharmacokinetics of TP-434 following repeated administration was best described by a 4-compartment model with linear elimination, which was the same model used to describe its disposition after single dose administration. Consequently, this analysis served both "learning" and "confirming" roles.

Although certain residual figures suggested the presence of outlier concentration data, all concentration data was retained for the final analysis. When outlier concentrations

were removed from the analysis (C. Seng Yue, J. A. Sutcliffe, P. Colucci, C. R. Sprenger, and M. P. Ducharme, unpublished data), mean population values did not change significantly and there was only a minimal improvement in inter-subject variability, therefore they were kept in the analysis.

The residual variability associated with the final PK model (14.0% for plasma and 21.5 % for urine) represents the variability that is not explained by the model, including intra-individual variability, the experimental "noise" of the analytical method and errors arising from the pharmacokinetic modeling itself (model misspecification). The low residual variability associated with our model was similar to the residual variability in the SAD study, and further confirms that the 4-compartment model was able to properly capture the PK of TP-434.

The current analysis demonstrated that TP-434 had a population mean CL_T of 13.5 L/h and a Vss of approximately 320 L (around 4.2 L/kg). These values are similar to what was obtained in the SAD study (13.9 L/h and 262 L). Renal clearance values were also similar in the SAD and MAD population PK analyses (2.34 L/h and 2.05 L/h, respectively). In addition, the total clearance value is similar to what was previously reported for tigecycline. The PK of tigecycline was also described by a multi-compartment model (14). For this drug, a 3-compartment model with zero-order input and first-order elimination was chosen to describe its PK. A population mean CL_T of 16.3 L/h was estimated, with a Vss of 749 L or approximately 10 L/kg.

The mean terminal elimination half-life value estimated from the MAD data was approximately 48 hours (median 35.3 hours) and individual values ranged from 27.6 to 108.9 hours. No relationship was observed between individual half-life values and administered doses, further confirming the linear PK of TP-434. The mean half-life value estimated in the MAD study was longer than the one estimated using the SAD data (mean of 26.2 hours, median of 23.1 hours). This could be attributed to greater inter-individual

variability in the volume of distribution estimates (Vc, Vp1, Vp2 and Vp3) in the SAD study. It is also possible that the longer sampling schedule and the greater number of samples per subject associated with the MAD study allowed a better characterization of the terminal elimination half-life, since the collection period spanned over 300 hours (at least 6 times the half-life). Another possibility is that with repeated dosing, the rate of TP-434 penetration into certain compartments is increased, thereby increasing its half-life. Indeed, the ratio of the rate constants K_{12} and K_{21} (describing transfer between the central and second peripheral compartment) increased by approximately 26% following multiple dosing, in comparison with single dose administration. This suggests that the net transfer from the central compartment into the second peripheral compartment increases with repeated administration. Despite the differences between the terminal elimination half-life estimated separately with SAD and MAD data, both population PK analyses indicated that TP-434 has a long half-life.

Results of the current analysis indicated that TP-434 exhibits dose-proportional pharmacokinetics over the dose range studied, similar to what was found with the SAD analysis. Total clearance of TP-434 did not change with increasing doses or with the administration of multiple doses. In addition, renal clearance estimated by the model (15.5% of total clearance) confirms that the elimination of TP-434 is mainly through non-renal pathways. This is similar to what was observed in the SAD study, as well as in chimpanzees, where urinary excretion was roughly 20% of total elimination (9).

Finally, secondary PK parameters $AUC_{tau(ss)}$, $Cmax_{(ss)}$ and $Cmin_{(ss)}$ from the MAD study were similar to the values that were predicted using the model based on the SAD study. This provides further confirmation that the 4-compartment model used to describe the PK of TP-434 after both single and multiple dosing is adequate. Thus, simulation results that were previously obtained, suggesting that a minimal daily dose of 1.5 mg/kg of TP-434 would be efficacious against multidrug-resistant gram-negative aerobic and

facultative bacilli and gram-positive pathogens, remained accurate (C. Seng Yue, J. A. Sutcliffe, P. Colucci, C. R. Sprenger, and M. P. Ducharme, submitted for publication). Consequently, dosing regimens selected for further investigation in Phase 2 studies included 1.5 mg/kg administered once daily and 1.0 mg/kg given twice daily.

5.7 Conclusion

The same model describes the PK of TP-434 regardless of the number of doses that are administered, and this model can predict the PK of TP-434 under varying dosing conditions. Results from this multiple ascending dose study confirmed the dosing regimens that had been previously selected for administration to patients in Phase 2 studies.

5.8 References

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Chapter 6 – General Discussion

The four research projects presented within the context of this thesis all demonstrate how advanced pharmacokinetic techniques, such as modeling and simulations and the development of innovative new models, can be used to enhance decision-making within the drug development process. These articles highlight the versatility and flexibility of such techniques, since each article presents a different, critical question that is efficiently answered by modeling and simulations. Thus, the findings from each article fully support the hypothesis that this tool can be a significant asset in drug development.

As previously described, the drug development process is in drastic need of an overhaul, as evidenced by soaring costs which do not necessarily translate into an increased number of drugs submitted to regulatory agencies, let alone the number of drugs that eventually reach the market. Furthermore, those who wish to bring a drug to market undertake important risks, because the success rate is so low. The repercussions associated with a suboptimal drug development process can be felt on many levels. On a more direct level, the failure to successfully bring a product to market can mean that significant money is wasted, especially if attrition occurs during later phases of the process and expensive clinical trials have already been completed. Another important resource that is spent on the failed endeavour includes manpower, or the time and efforts of a diverse array of people who are involved in drug development. The energy that was invested in the failed product could conceivably have been invested elsewhere with a potentially greater return, such as in the development of another product that might have been successfully marketed.

The difficulties encountered in bringing a drug to market make new therapies even less accessible for the patients who use them in the end. This manifests itself in two ways. Firstly, so much time and money have been invested in bringing a drug to market that once regulatory approval has been received and market access is attained, new drugs are often

extremely costly, and prohibitively so for some patients. Secondly, due to the multiple challenges faced by drug developers, there are simply less new drugs being marketed, as demonstrated by the declining submission rate over the years (134).

Many aspects of the drug development process could benefit from some form of improvement. For example, better tools could be developed to predict toxicity, which currently relies on animal models that are not always highly predictive of toxicity in humans. The manufacturing aspect of drug development could also be enhanced by adopting state-of-the-art technology into its processes, and by creating software that could predict the effect of formulation or manufacturing process changes on drug performance in humans (134). While these aspects of drug development must not be neglected, the focus of the research presented within this thesis has been on the utility of modeling and simulations at later stages of the drug development process. The four articles have shown that advanced pharmacokinetic techniques, such as modeling and simulations, can be used to answer key questions frequently encountered during Phase 1 and Phase 3 of drug development.

The first research article presents an innovative approach to proving whether or not two different intravenous formulations of iron are equivalent. This question is normally answered by analyzing study data using a noncompartmental approach, but our research proposes a flexible and innovative model developed using a compartmental population approach that does not necessitate the enrollment of a large group of subjects. Furthermore, this method makes no assumptions about linearity, nor does it require the drug under study to meet any assumptions regarding elimination.

Despite the advantages that the compartmental method has to offer, it is rarely used in the context of bioequivalence assessments. In the area of BE, *in silico* modeling is most often associated with biowaivers of drugs belonging to certain BCS classes (157-163), however some researchers have studied its applications in proving BE from *in vivo* studies.

In 2005, Panhard and Mentré compared traditional noncompartmental bioequivalence assessments to a population compartment approach using data from simulated theophylline studies with varying designs (164). They evaluated two global tests and two tests based on empirical Bayes' estimates (EBEs). Compared to standard tests based on noncompartmental methods, the global tests were associated with inflated Type I error while the tests based on EBEs produced results that were similar to standard tests. Dubois and colleagues performed a similar comparison using simulated theophylline data, however they used the stochastic approximation expectation maximisation (SAEM) algorithm rather than the FOCE algorithm, and they also used a different statistical approach (linear mixed effects model to determine treatment effect rather than paired t-tests) (165). Their findings showed that sample means estimated by the noncompartmental approach were generally more biased than with the compartmental approach, but for both methods, root mean square error increased as the number of samples per subject decreased. With sparse sampling, type I error was greater with the compartmental approach, because of shrinkage which caused EBEs to tend towards the mean, making it easier to declare bioequivalence even when it is not the case. However, when small sample sizes or elevated residual error were present, both approaches were flawed. Another study performed by the same group also analyzed simulated theophylline data using SAEM with Wald tests to evaluate bioequivalence (166). They showed that in the case of rich sampling with the compartmental approach, type I errors were close to the nominal value of 5%. They also applied their proposed approach to the comparison of two formulations of somatropin, and showed that bioequivalence Wald tests performed using ln-transformed PK parameters obtained from both methods yielded similar results. Their overall conclusion was similar to the earlier study, in that compartmental analysis could be an alternative to traditional BE approaches except when sample size was small or when the drug was highly variable.

While some researchers performed their analyses with simulated data, others have used compartmental analyses to analyze real data from bioequivalence trials. Due to the

small sample size (difficult patient recruitment because of the low disease prevalence) and limited blood sampling, Keizer and collaborators opted to use compartmental analyses to compare two drug formulations that differed in manufacturing processes (167). Their analyses showed that this approach was superior to the standard one. Kaniwa *et al*. performed bioequivalence assessments for various drugs (phenytoin, flufenamic acid, indomethacin, nalidixic acid, metronidazole and griseofulvin) using both standard and compartment approaches (with NONMEM) and demonstrated that similar ratios and confidence intervals were obtained with both methods (168). Combrink and collaborators determined relative bioequivalence using noncompartmental and compartmental methods for two formulations of ibuprofen that were administered in a single dose, open-label, crossover study in healthy volunteers (169). They found that relative bioavailability estimates obtained with the compartmental analysis were not biased by outlier data, contrary to the noncompartmental approach. In addition, confidence intervals associated with the compartmental approach, which were determined using two different methods (one which used the standard error of the estimate taken directly from NONMEM and the other which relied on a log-likelihood procedure), were similar in width to the one obtained with the standard method. However, these results were obtained with data that was not very variable, therefore the authors could not extrapolate these conclusions to more variable data. Fradette and colleagues also compared both methodologies (standard noncompartmental versus population compartmental) in the bioequivalence assessment of two formulations of cyclosporine administered to a group of patients (170). They demonstrated that both approaches led to similar conclusions of bioequivalence. Other researchers have also reached similar conclusions for the bioequivalence assessment of chlorthalidone (171), tiludronate (172), somatropin and epoetin-α (173).

In light of the paucity of published BE studies that employ non-traditional compartmental methods to meet their objectives, the first article presented in this thesis makes a significant contribution to the analysis of BE trials and to furthering our

knowledge about the use of advanced PK techniques within the field of generic drug development. Contrary to previously published articles (164, 165, 167-172, 174, 175), this article relies on an innovative, complex and sophisticated PK model that fits multiple analytes simultaneously and also includes a non-linear component. Although one other model incorporated non-linearity (PK model for epoetin-α) (173), the other published models are simpler (one- and two-compartment models with linear elimination), therefore this article provides additional insight into the use of more elaborate PK models in the world of BE evaluation. Furthermore, our analyses were conducted with highly variable data, thereby demonstrating that this approach is not only limited to homogeneous data, but that it can be used to better understand more variable data as well. This article is also the first published account which uses the software ADAPT 5® to conduct a bioequivalence assessment.

Importantly, the first article demonstrates that the population PK approach can result in considerable savings, from both a monetary and time perspective. Findings demonstrated that conclusions derived from this novel approach were comparable to those obtained from a traditional method applied to a sample size that was more than 8-fold greater (n=29 vs n=240). This suggests that less subjects need to be dosed if a non-traditional approach is favoured, which means that less money and time are invested in the clinical trial and less subjects are needlessly exposed to an investigational drug. This has important ramifications for the drug development process of generic products, which is subject to important time constraints and financial concerns.

The research presented in the first article in this thesis contributes significantly to the progress of bioequivalence assessments, which have greatly evolved since the last century (176). From a historical perspective, the concepts related to bioavailability were introduced in the mid 1940's, but interest in bioequivalence and its assessments only date back to the early 1960's, when there was rising concern on the bioavailability of generic

products with respect to innovator products (177). People were worried that generics might not provide equivalent exposure to innovators and that patients would suffer from these differences. Ideas and approaches evolved over the subsequent decades in what can be viewed as four phases. In the first phase, from the 1970's to the early 1980's, regulatory agencies such as the FDA began demanding proof of bioavailability in drug submission applications, and statistical discussions regarding bioequivalence assessments began to take place. In 1984, the "Drug Price Competition and Patent Term Restoration Act" was passed by the U.S. Congress, which allowed the FDA to approve generic drug products based on bioavailability and bioequivalence data. During the second phase, from 1984 to 1992, issues relating to the statistical aspect of bioequivalence testing were examined, culminating in the publication of the FDA guidance entitled "Statistical Procedures for Bioequivalence Studies Using a Standard Two-Treatment Crossover Design". This approach is commonly referred to as the "Average Bioequivalence" (ABE) approach. In the third phase, from 1992 to the beginning of the new millennium, discussions arose on many topics such as post-approval changes, racemate products, locally acting products, and BCS, leading to new guidances such as the skin blanching assay for topical corticosteroids (117), the BCS biowaiver option (99), as well as the scale-up and post-approval changes (SUPAC) recommendations (178-180). In addition, the individual bioequivalence approach was proposed as a replacement for ABE, but it was eventually abandoned (181, 182). The start of the fourth phase was marked by the FDA's publication of their guidance document "Statistical Approaches to Establishing Bioequivalence" (183), which was followed by guidance documents published in Canada, Japan and European nations (44, 92, 94, 184). During this phase, concerns arose with evaluating the bioequivalence of highly variable drugs (those possessing a within-subject coefficient of variability of at least 30%), as proving BE between two highly variable drugs using traditional ABE criteria necessitated the enrollment of many subjects, and this seemed unwarranted for drugs that generally had a large therapeutic index (185, 186). The scaled average bioequivalence

approach emerged from the discussions that ensued as being the most promising approach, because it allowed BE limits to be scaled according to the within-subject variability of the reference product (obtained through a replicate design study). Furthermore, with this approach, the mean difference between the test and reference product was still required to fall between the traditional limits of 80 to 125% (186). Our research, which supports the use of advanced pharmacokinetic modeling for certain bioequivalence assessments, will help usher in the newest phase in the evolution of bioequivalence approaches.

While our research has shown that compartmental analyses can be a valuable tool for assessing the relative BE between two products, it is an approach that also has its limits and challenges. Developing a pharmacokinetic model can be a difficult and time-consuming process, and very often more than one model can be used to describe the same set of data. This makes the results obtained by compartmental modeling more difficult to reproduce than the results obtained by traditional noncompartmental methods. The creation of a pharmacokinetic model also requires the skills of an experienced pharmacometrician, whereas noncompartmental analyses are easily performed. Therefore, for most drugs which exhibit linear pharmacokinetics, are detectable and are eliminated from the body, noncompartmental analyses should be more than sufficient to evaluate relative bioequivalence. However, compartmental analyses should be considered as an alternative for drugs which do not meet these criteria.

In addition to their use in evaluating BE between two drug products, the compartmental approach advocated by the results of the first research project could also be used to perform meta-analyses of bioequivalence trials to assess the switchability of different generic formulations of the same drug. Although generic drugs are approved based on bioequivalence with the established reference product, little research is done to compare generic products to one another. In other words, if a patient is currently being treated with an innovator product, bioequivalence trials have shown that it is acceptable to

replace the innovator product with an approved generic formulation, but it is unclear whether or not this generic formulation can be substituted with another. This may not be an issue for drugs with a wide therapeutic index and large safety margin, but it could pose a problem for certain drugs. Chow and colleagues have described methods for performing such meta-analyses (187, 188), but this is an area of research that could also benefit from compartmental modeling. Indeed, compartmental analysis can easily accommodate data derived from different studies (with different study designs). Furthermore, population PK models developed to compare multiple generic formulations can estimate different inter- and intra-subject variability for each study or formulation, in contrast to previously published methods which assume that they are similar across studies (187). In addition, the use of compartmental analysis could also be useful in the development of biosimilars, or large molecule therapeutic proteins that are similar but not identical to innovator proteins.

Our research has shown that advanced techniques such as modeling and simulation can improve the development of generic compounds, such as SFGC, and that this approach also has wider applications, such as studying the interchangeability of different generic formulations. However, such methods can also be used to develop innovator products. Recently, we used a modeling and simulation approach to better understand the pharmacokinetics of a novel oncology drug being developed for acute myeloid lymphoma, elacytarabine. The use of advanced PK methods, including the creation of an innovative model, allowed us to test certain hypotheses with respect to the pathways involved in the formation of an active metabolite and this improved understanding could not have been achieved through traditional techniques (189). Additional applications of modeling and simulations for the development of innovator drugs are also demonstrated by the remaining articles presented in this thesis.

There is a clear need to bring to market more innovator products, as there are fewer and fewer first-in-class products being developed. In 2008, of the 21 new drugs approved

by the FDA, only 29% were first-in-class while this number decreased the following year. In 2009, the FDA approved 24 new drugs, 17% of which were first-in-class (81). As shown in the second article of this thesis ("Population pharmacokinetic and pharmacodynamic analysis of pegloticase administered by intravenous infusion in two dose regimens to subjects with chronic gout"), modeling can help to develop first-in-class medicine for the treatment of refractory gout, by helping to better understand the PK and PD of new products (since we cannot rely on previous knowledge).

Pegloticase is a PEGylated recombinant modified mammalian urate oxidase that currently is the only member of the uricase class that is indicated for the treatment of chronic refractory gout (190). It acts by converting uric acid into the more easily excreted allantoin, while other treatments for gout exert their urate-lowering effect by reducing its production or its renal tubular re-absorption (191). Although another recombinant uricase enzyme is available on the market (rasburicase, commercialized as Fasturtec® and Elitek®), it is not indicated for the treatment of refractory gout. Rather, it is indicated for the treatment and prophylaxis of hyperuricemia caused by tumour lysis syndrome in pediatric and adult cancer patients (192). Although rasburicase is effective in decreasing uric acid levels in cancer patients (193-197), it is associated with a shorter terminal elimination half-life and persistent immunogenicity (191, 198). Therefore, despite some publications that have described its efficacy in treating refractory tophaceous gout patients (199-204), this remains an off-label indication for rasburicase.

The second article presented in this thesis represents the first population PK/PD analysis for a drug belonging to the urate-oxidase drug class conducted on a large cohort of Phase 3 patients. The only other published population PK/PD analysis for a urate-oxidase also involved pegloticase data, but subjects included in the analysis were from a smaller cohort (n = 40) of a Phase 2 study (205). Furthermore, the PD model developed with the Phase 2 data (direct inhibitory Emax model) was different from the model developed using

the Phase 3 data (indirect model), since the range of doses administered in both studies was very different. Fewer dosing regimens and doses were administered in Phase 3, which is why it was not possible to apply an Emax model to the data. Many articles have been published on population PK and/or PD analyses conducted on other biological agents, such as darbepoetin alfa (206-209), recombinant human erythropoietin (210, 211), alpha interferon (212), filgrastim (213, 214), PEGylated thrombopoietin (215), desonumab (216), abatacept (217), bevacizumab (218), cetuximab (219), infliximab (220, 221), rituximab (222), and trastuzumab (223). However, to our knowledge, this is the first large-scale population PK/PD analysis conducted on a urate-oxidase therapeutic protein. Thus, the findings from our research make important contributions to our knowledge about this drug class, by not only describing the PK and PD of pegloticase, but by quantifying its inter-subject variability and identifying factors (demographic and other) which could be influential. Among the important factors that affected PK parameters were BSA and anti-pegloticase antibody levels (for Vc and CL), while the PD of pegloticase was only influenced by anti-pegloticase antibody levels.

The results of the research presented in the second article not only improved our understanding of pegloticase disposition and effect, but they also played a critical role in determining whether or not dosage adjustments would be necessary. Indeed, they showed that body surface area and anti-pegloticase antibody levels influenced both the volume of distribution and clearance of pegloticase. Anti-pegloticase antibody levels also appeared to diminish the effect of pegloticase on the elimination of uric acid. Although anti-pegloticase antibody categories helped to explain the variability in pegloticase PK and PD responses, it was not necessary to perform any dosage adjustments to take this into account since pegloticase managed to elicit therapeutic responses even in patients with high levels of antibodies. Furthermore, the analysis revealed that no dosing adjustments were necessary for renal insufficiency, as estimated creatinine clearance (which ranged from 17 mL/min to 264 mL/min in the cohort) was tested as a potential covariate in the model and was not

retained as a significant covariate. Importantly, the population PK and PD analyses conducted on pegloticase contributed to the product labeling (monograph) (190) and ultimately enhanced the drug's development process.

The advanced pharmacokinetic techniques employed to better understand pegloticase were the ideal tool to help meet the analysis objectives, as compartmental analyses do not require rich sampling as is the case for noncompartmental analyses. Indeed, in Phase 3 trials such as the ones that were analyzed for the second article, it is often impractical or unfeasible to collect many blood samples from patients, who are already burdened by their disease and often multiple co-morbidities. Compartmental analyses also have the flexibility of incorporating data from different sources, such as trials with different study designs or sampling schedules. Moreover, by performing compartmental analyses on the data collected from these two pivotal Phase 3 studies to answer specific questions pertaining to pegloticase PK and PD, it was possible to avoid the conduct of additional clinical trials designed specifically to study such questions. For example, it was not necessary to conduct a clinical trial with renally-impaired patients, since the results of this analysis demonstrated that creatinine clearance was not a factor that influenced pegloticase pharmacokinetics or pharmacodynamics. Thus, advanced modeling allowed us to incorporate prior knowledge about pegloticase, gained from modeling of Phase 2 data, with data obtained from larger Phase 3 studies in order to extract the maximum amount of information from the available data and answer critical drug development questions, such as "Do dosing regimens need to be adjusted to account for demographic traits such as weight or body surface area?".

Modeling and simulations could also prove to be useful in further broadening our understanding of pegloticase, more particularly with respect to its immunogenicity. Although pegloticase was designed to be less immunogenic than rasburicase and non-recombinant uricase, by virtue of its PEG conjugates (224-227), immunogenicity remains

an important concern. Infusion reactions (systemic, localized or acute hypersensitivity) are an immune-response manifestation (228, 229), and in the Phase 3 studies from which data was obtained to conduct the population PK/PD analyses (230), infusion-related reactions were the second most common adverse event (the first one being gout flare). Serious infusion reactions occurred in 5 to 8% of patients, but all of them resolved completely. These types of immune responses do not only occur with pegloticase, but they can occur with any type of therapeutic protein or biological agent (228, 231, 232). Significant immune responses that affected clinical efficacy were reported for drugs such as salmon calcitonin (233), gonadotropin-releasing hormone (234), and granulocyte macrophage colony stimulating factor (235).

In light of the concerns surrounding biological agents and their immunogenicity, using the pegloticase PK/PD model that was developed as part of this thesis, it would be possible to build a model (or extend the current one) to predict the onset of immunogenic reactions. In the Phase 2 studies included in the PK/PD model, it was observed that over 90% of infusion reactions in the 8 mg biweekly cohort were preceded by uric acid levels exceeding 6 mg/dL, while this was the case for approximately 71% of infusion reactions in the 8 mg monthly cohort (230, 236). Indeed, there may be a link between loss of pegloticase effect (as reflected by rising uric acid levels) and the occurrence of infusion reactions and it has been suggested that discontinuing pegloticase treatment when uric acid levels are $\geq$ 6 mg/dL can avoid most infusion reactions (230, 236). The mechanism behind this potential relationship is unknown and although this was not a question that arose at the time when the pegloticase PK/PD was being developed, with modeling and simulations it would have been possible to confirm the validity of such a threshold uric acid level and its relationship with infusion reactions. Covariate analysis, such as the one conducted in the second article, could also help identify other factors related to the patient or the disease itself that could contribute to the occurrence of such adverse events. Factors such as a patient's genetic background, the type of disease, and the manufacturing process are some

of the factors known to influence the immunogenicity of therapeutic proteins (228), and population PK/PD analyses could help identify the specific factors which affect pegloticase's immunogenicity. Ultimately, developing such a model could help patients benefit from pegloticase treatment while minimizing the risk of unwanted effects.

Another potential application of modeling and simulations stems from both its utility in the characterization of biological agents, as illustrated by the second article of the thesis, as well as its use in bioequivalence assessments, as demonstrated in the first article of the thesis. As is the case with small-molecule innovator products, once the period of market exclusivity and patent protection have expired for biological agents, there is the possibility of developing similar biological agents. Unlike small molecule drugs, where generic drugs are considered identical copies of their innovator counterparts, biological agents cannot be copied strictly speaking, due to their inherently heterogeneous nature and complex, unique manufacturing process (237-241). These "similar" biological agents (sometimes labeled biosimilars, follow-on biologics or subsequent entry biologics) cannot simply rely on pharmaceutical equivalence and bioequivalence to demonstrate their comparability to the innovator product, unlike small molecule generics (237, 242, 243). Often, biosimilars must show that they are comparable to their innovator counterpart through *in vitro* and *in vivo* tests that provide evidence of similar quality (purity and potency), efficacy and safety, including immunogenicity (238, 240, 241, 243-245). Therefore, those who wish to develop biosimilar products are faced with challenges that are unique to these types of molecules.

Biological agents inherently possess traits that do not always make them candidates for noncompartmental analyses, making compartmental analyses an attractive alternative to demonstrate comparability. As previously mentioned, some underlying assumptions for noncompartmental analysis include linearity and elimination from the sampling compartment, and these do not always hold true for all drugs, as shown for iron in the first

article. Like iron, the PK of biological agents can often be non-linear, because therapeutic proteins can undergo target-mediated drug disposition, such that the drug's PK is influenced by its PD (specifically by its binding to target receptors). In such instances, PK is often non-linear because of this saturable process (246). Non-linearity can also be caused by immune-mediated clearance or receptor-mediated uptake into hepatocytes for subsequent elimination (232). Furthermore, numerous biological agents are also eliminated in peripheral compartments via proteolysis, which violates the assumption that elimination occurs solely from the central compartment (231). The accurate characterization of the PK and PD of therapeutic proteins using traditional methods can also be complicated by feedback mechanisms or loops that actively modify drug levels, or simply by the presence of endogenous counterparts (232). Although the use of population compartmental modeling to compare biological agents has been described in a handful of publications (166, 167, 247, 248) and even advocated by the FDA (241), this remains an area that could benefit greatly from the application of advanced pharmacokinetic techniques such as the ones described in this thesis, including the development of innovative new models.

Key questions arising during drug development, such as "Are two formulations of the same drug are bioequivalent?" or "What factors influence a drug's PK and PD?", can be efficiently answered by advanced pharmacokinetic techniques such as modeling and simulations, as shown in the first two articles presented within this thesis. Others have shown that such methods can also be used to halt drug development, as was the case for a cholesterol absorption inhibitor (107) and PEG-modified interferon alfa-2a (249), thus preventing more unnecessary time and money from being invested in a product that was unlikely to be successful. Although these examples show how modeling and simulations can answer "go or no-go" questions, it is often just as important to be able to answer questions that pertain to subsequent steps in the drug development process. Accordingly, the third and fourth articles presented in this thesis illustrate the applicability of advanced pharmacokinetic techniques in the planning of future studies. While the analyses of SFGC

and pegloticase demonstrated that modeling and simulations can be used to answer key drug development questions, the analyses of TP-434 take this one step further by answering critical questions that influence the design of subsequent studies.

The third article presented in this thesis demonstrates how the careful and timely use of modeling and simulations based on data obtained from a single, ascending-dose Phase 1 study was pivotal in the selection of dosing regimens for Phase 2 patient studies. Timeliness is an important consideration with the use of such a tool to answer these questions, because decision-making must occur prior to the initiation of subsequent studies. Indeed, as noted by Peck, "tardy analysis of a trial guarantees that the results cannot influence subsequent trials" (135). Thus, the findings from the third article show that modeling and simulations can not only answer important questions that arise during drug development, but they can do so in an efficient manner and within a reasonable timespan to modify, if necessary, the course of ongoing or future studies.

The more quantitative approach to selecting doses for a Phase 2 study, based on modeling and simulations, can help lower attrition rate in Phase 2, which remains problematic despite our technological achievements and increasing knowledge. Indeed, Phase 2 and 3 attrition are key determinants in research and development productivity and efficiency (81), therefore a decrease in Phase 2 attrition rates will directly impact productivity and efficiency. Additionally, the selection of an optimal dose or dosing regimen as early as possible during the drug development process can lead to considerable savings, in terms of both time and money. This is illustrated by the drug development path taken for nesiritide, a drug indicated for the acute treatment of decompensated congestive heart failure whose NDA was submitted to the FDA in April 1998. From a clinical perspective, the FDA did not agree with the proposed dosing regimen, and sent the company a nonapprovable letter one year after the submission date. Based on some exposure-response analyses conducted by the FDA, the sponsor conducted an additional

study to confirm the selection of the new dosing regimen. Eventually, the product was approved in May 2001, three years after the initial submission. Clearly, if the exposure-response relationship had been well-defined prior to the submission, at early stages in the development process, the optimal dose could have been selected prior to submission, and the ensuing time delays and additional study could have been avoided (115).

Modeling and simulations are excellent tools for making Phase 2 dosing recommendations based on Phase 1 data, because they can make use of prior knowledge. Fortunately, in the field of antimicrobials, there is a considerable body of knowledge pertaining to PK/PD relationships that can be incorporated into models. The first insights into the PK/PD of antimicrobials were described by Eagle in the 1940's and 1950's, who noticed that penicillin efficacy was related to its concentration and time above a threshold concentration (250-253). Research in this subject area was pursued in subsequent decades and continues to this day. Thus, for many classes of antimicrobials, such as fluoroquinolones (154-156, 254-256), beta lactams (151, 257-260) and aminoglycosides (153, 261-263), relationships between PK/PD indices and clinical outcomes are well defined and recognized by the scientific community. In the case of TP-434, when only PK results in healthy volunteers were available, previously established PK-PD relationships for another tetracycline, tigecycline, were used in conjunction with this information to select dosing regimens for further study in a patient population (264-266). This illustrates one of the most appealing features of modeling and simulations, which is its capacity to incorporate information from different sources and make use of all available data.

In the context of drug development, simulations such as those performed for TP-434 dosing regimen selection can answer questions without relying on the conduct of expensive clinical trials. For instance, using the final PK model developed for TP-434, it was possible to conduct many simulations of different dosing regimens in order to determine the probable outcome associated with each of these regimens. This allowed the pharmaceutical

company that was developing TP-434 to be more selective of their dosing regimens and avoid testing regimens that were unlikely to provide the desired clinical outcomes. Rather than test many dosing regimens, of which only maybe one or two would be promising, this allowed the company to test only the most optimal regimens. Similarly, if only one or two regimens were to be tested, this approach ensured that the most promising ones would be tested, thereby avoiding the situation that occurred for nesiritide.

The choice of dosing regimens has important ramifications throughout drug development, as an improper selection can result in an inefficacious exposure or undesirable side effects, which could force companies to conduct additional studies or even worse, halt clinical development. In the world of antimicrobial drug development, another reason for optimizing dosing regimens is the unrelenting development of antimicrobial resistance. Although resistance genes have existed for many millennia (267), unchecked clinical antibiotic use has contributed to increased resistance (149). Thus, one of the ways in which resistance can be reduced is by exerting a better control over the use of such drugs (149). By carefully selecting dosing regimens during the course of drug development, we can therefore ensure that people are not unnecessarily exposed to antibiotics (which is the case when sub-optimal dosing regimens are tested) and that they are being administered the minimally effective dose. In other words, they are not being given more antibiotics than what is required to eradicate the infection. Therefore, the use of modeling and simulations to choose optimal dosing regimens for antibiotics under development also fosters a more rational and controlled use of these drugs, to thwart potential resistance.

In anti-infective research, population modeling and simulations have been used to better understand a drug's PK and/or PD in patient populations (268, 269), including special groups such as burn patients (270, 271), cancer patients (272, 273), cystic fibrosis patients (274, 275), neonates (276, 277), children (278, 279), and pregnant women (280, 281). It has also been used to optimize dosing regimens for special populations (such as

critically ill patients (282-290), cancer patients (291), neonates (292-300), children (301-304), the elderly (305, 306), dialysis patients (307-310), and burn patients (311)), compare drug dosing regimens or potency of anti-viral drugs given as combination therapy (312, 313) and determine PK/PD breakpoints for clinical outcome assessments based on patient data (154, 156, 265, 266, 314-317). Moreover, modeling and simulations were used to guide Phase 3 dose regimen selection during the drug development of doripenem (318) and fusidic acid (319). However, to our knowledge, the results presented in the third article of the thesis are the first report of this approach being used to select Phase 2 dosing regimens based solely on *in vitro* and Phase 1 data. This illustrates an important role for such advanced pharmacokinetic techniques within the drug development context, in that their use can extend to the planning of future studies.

The dose optimization analyses described in articles 3 and 4 are the first published population PK analyses for TP-434. Importantly, they are also the first published accounts of the use modeling and simulations for early-stage dose determination of an antibiotic under development. In other words, in previously published reports where modeling and simulation was performed to refine dosing regimens for an antibiotic (282-311), patient data was always available and included in the analysis, whereas our dose projections were only based on healthy volunteer data. Moreover, although this type of early-phase dose-defining analysis based on results from healthy subjects has been described for other drugs, such as the monoclonal antibody HAE1 (320) and selective factor IXa inhibitor pegnivacogin (321), the research presented within this thesis represents the first of its kind for antibiotics. The impact of our Phase 1 modeling and simulations on the TP-434 dosing regimens selected for Phase 2 investigations was also definitive and significant, whereas in other publications that suggest dosing regimens issuing from compartmental analyses, it is unclear whether or not these recommendations were actually put into place (322-324).

The last article presented within the context of this thesis, also pertaining to TP-434, further highlights one of the strengths of population compartmental analyses within the drug development process. Prior to the conduct of the multiple, ascending dose study in healthy volunteers, only single-dose data was available for TP-434, but once the repeated administration results became available, it was possible to easily incorporate these results into the previously determined population PK model. Thus, the PK model developed for TP-434 could be continuously updated with new information, in order to extract the maximum amount of knowledge from available data. The results obtained from the multiple ascending dose study not only allowed us to learn more about TP-434, but they also served to confirm the PK model that was chosen based on single dose data. This embodies the "learn and confirm" approach to drug development first proposed by Sheiner (136) and advocated by many. In contrast with the view that drug development is a succession of independent steps that ultimately lead to a product's entry on the market, the "learn and confirm" paradigm makes use of advanced pharmacokinetic techniques such as modeling and simulations to integrate data throughout the course of drug development. In the latter, there is always the possibility of learning from studies, even those whose outcomes are unexpected.

The successful application of the learn and confirm paradigm in the development of a novel antibiotic agent was demonstrated in the last two articles of this thesis. Although in this example, advanced pharmacokinetic techniques were employed in the development of a drug belonging to the antibiotic class, the approaches defined by this research can be applied to any type of compound within any therapeutic area. However, some insight into the PK and PD relationship of the investigational drug is necessary to take full advantage of this tool. Nonetheless, these articles serve as an example for other researchers wishing to develop a new drug, and will hopefully encourage others to adopt a model-based, rational approach to dose regimen selection.

The articles presented within the context of this thesis demonstrated the utility of advanced pharmacokinetic techniques such as modeling and simulations, and the creation of innovative models, within the drug development process. The research presented here provides researchers with tools that can be applied not only to drugs with particular PK characteristics (such as iron), innovative biological agents (like pegloticase) or novel antimicrobial agents (such as TP-434), but to drugs in all therapeutic areas. Questions that can arise at different stages of the drug development process (Phase 1 for TP-434 and Phase 3 for pegloticase) can be effectively answered using modeling and simulations, which makes this an invaluable tool. However, to make the most of this approach, data should be collected and analyzed at the earliest stages of the DDP, and this analysis must be reiterated throughout. Although it can be a time-consuming and arduous task, the research presented herein has demonstrated that no matter what the question or phase of drug development, modeling and simulations are well worth the effort.

Chapter 7 - Conclusion

We are reminded time and time again that drug development is a risky, expensive and lengthy process. Nevertheless, although they often defy quantification, the benefits of drug therapy on quality of life and lifespan are undeniable.

In 1993, the Office of Technology Assessment in the U.S. declared that the most important components of pharmaceutical research and development investment were money, time and risk (83). Almost two decades have elapsed since then, but these tenets hold true even today. However, more is being done to improve the drug development process. The research conducted within the context of this thesis have important ramifications on different aspects of the drug development process and provide concrete examples of how advanced pharmacokinetic techniques, such as modeling and simulations, can render the process more efficient.

The first research article demonstrated that compartmental analyses can be used to answer questions pertaining to bioequivalence, and that this approach offers many advantages over the traditional methods normally espoused. It not only forgoes the need to meet specific assumptions about a drug pertaining to its linearity and elimination, but it allows study objectives to be attained without enrolling hundreds of subjects. From a drug development perspective, this translates into significant financial savings and an economy of time. In addition, the research presented in this article makes significant contributions to the relatively unexplored domain of compartmental modeling within bioequivalence assessments. Indeed, few publications exist on this topic and this research explores the use of more elaborate and complex models that have not been previously described within this context. The approach described in this article not only broadens our appreciation of modeling and simulations within the field of generic drug development, but it can also be applied to innovator or biosimilar drug development.

The second research article describes how advanced pharmacokinetic techniques such as population PK/PD modeling improved our understanding of pegloticase. Not only did the research conducted on pegloticase identify covariates that influenced its PK and PD, but it quantified the influence of these covariates. By establishing relationships between patient demographics, laboratory parameters and PK/PD parameters, it was possible to determine that no dosing adjustments were necessary for pegloticase. Thus, the research described in the article contributed directly to product labeling by answering key questions that could otherwise not have been answered from the Phase 3 trials. Due to the sparse sampling collected from patients during these trials, it would not have been possible to perform robust noncompartmental analyses, therefore the approach that was adopted was ideal for meeting study objectives. In other words, the population PK/PD analysis maximized the information that was extracted from the available data, thereby obviating the need to conduct of additional studies or collect many blood samples from patients. Thus, this approach proved to be an economical and efficient tool in the development of a biological therapeutic agent. Furthermore, this tool could be used to gain an improved understanding of pegloticase's immunogenicity, just as it could be used to develop other biological therapeutic agents.

The third and fourth research articles illustrate the use of modeling and simulations in determining optimal antibiotic dosing regimens to be studied in subsequent clinical trials. Although the methods described in these articles can be applied to drugs in diverse therapeutic areas, these articles represent the first account of the use of modeling and simulations to select Phase 2 dosing regimens for an anti-infective drug. The rational dose regimen selection that resulted from the use of modeling and simulations improved the overall drug development process of TP-434 by avoiding the exposure of subjects to dosing regimens that would ultimately prove to be ineffective, and by ensuring that proof-of-concept would likely be established in the Phase 2 study. Consequently, this thwarted the need for re-dosing additional cohorts or conducting another clinical trial, which would both

have been expensive and have added more time to the already lengthy development process. In addition to reducing the cost and time associated with the development of TP-434, the modeling and simulation approach employed in this research provided a flexible and adaptable framework with which the PK TP-434 could be better understood. The PK model developed for TP-434 could be refined as more information became available, thereby demonstrating that the learn and confirm paradigm could be applied to the development of an antibiotic drug. Furthermore, the methods described in these articles could have broader applications, and could be used to develop new drugs in various therapeutic areas.

In conclusion, the projects described in this thesis have shown that advanced pharmacokinetic techniques such as modeling and simulations are able to minimize drug development costs through the use of smaller studies, by avoiding the conduct of additional studies and by ensuring that future studies are designed for a high probability of success. Modeling and simulations, including the development of innovative models, are therefore a tool that can result in a significant economy of time and money, which are two components that contribute to the challenges present during the drug development process. By using such approaches to answer key questions that arise during drug development, the risk involved in the process is decreased, and generic and innovator drugs can be brought to market in a faster and more efficient manner. Ultimately, this will provide clinicians and caregivers with even more therapeutic options to treat or cure diseases, and more importantly, improve the lives of their patients.

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Appendix 1

General Pharmacology of Therapeutic Agents or Classes Studied within this Thesis