PHARMACOMETRIC ANALYSES OF DRUG EXPOSURE AND RESPONSE DATA

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Dedication

To my parents and my husband, Bo Xie
for their endless love and support

Abstract

The current thesis work consists of two projects presenting the pharmacometric analyses in drug exposure and response data. The first project applied population pharmacokinetic modeling approach to estimate the in vivo inhibition constant (Ki) across different CYP2C9 genotypes. In the second project, a model-based meta-analysis was performed to describe the time-course of virologic response in hepatitis C clinical trials across current approved treatments.

The inhibition constant (Ki) of an inhibitor is usually estimated from in vitro experiments. Given the differences between in vivo and in vitro experiments, the in vitro Ki may not truly reflect the inhibitor-enzyme interaction in vivo. A previous study demonstrated that the in vitro Ki of fluconazole varied across different CYP2C9 genotypes, suggesting the inhibitor potency of interacting with CYP2C9 in vivo also depends on enzyme genotypes. The current study presented a population model-based approach to determine the in vivo Ki of fluconazole across three CYP2C9 genotype groups (*1*1, *1*3 and *3*3) with the CYP2C9 substrate, flurbiprofen. An integrated model was developed describing the pharmacokinetic profile of the flurbiprofen and competitive inhibition of fluconazole on flurbiprofen metabolism through CYP2C9. The estimates of in vivo Ki was 14.7 μM for CYP2C9 *1*1, 18.7 μM for CYP2C9*1*3 and 32.9 μM for CYP2C9*3*3. The estimates of in vivo Ki of fluconazole are comparable to those estimates from in vitro studies. The result suggests the presence of CYP2C9*3 is associated with diminished interaction between fluconazole and enzyme. In addition, the contribution of CYP2C9 mediated metabolic clearance to the total flurbiprofen clearance is less significant in CYP2C9*3 group than CYP2C9*1*1 group. The magnitude of overall in vivo drug interaction was also quantitatively evaluated. Patients with CYP2C9*3 is less susceptible to fluconazole inhibition. The present findings showed the potential of metabolism-based drug interaction could be different across individuals featured with various metabolic enzyme polymorphisms. The recommendation on dosing adjustment due to the overall effect of

drug interaction on metabolism, accordingly, should take into consideration genotypes of metabolizing enzyme, elimination pathways of the substrate and inhibitor potency.

In the second project, a model-based meta-analysis was performed to quantitatively assess the time course of longitudinal virologic response across currently approved hepatitis C treatments including peginterferon plus ribavirin (PR), telaprevir plus PR therapy and boceprevir plus PR therapy. A total of 18 studies with 47 treatment arms were enrolled in the current analysis. The information collected in the analytic dataset included numbers of responders whose hepatitis C viral RNA level is undetectable at a given time, treatment strategy and patient population characteristics. The analysis was firstly conducted in NONMEM 7 to obtain maximum likelihood estimates. However, the initial model had several limitations and ran into computational difficulties when trying to incorporate random effects at multiple levels. One of major limitations is that the model did not account for the potential decrease in the response rate which was observed during later phase of treatment in some treatment arms. To overcome these limitations, the meta-analysis was implemented in a Bayesian framework using Markov Chain Monte Carlo algorithm. The response rate model was developed incorporating the component of possibility of response reverse. The analysis found the telaprevir had the fastest response onset, followed by boceprevir and PR therapy alone. The maximum response rates were lower in treatment arms with greater proportion of genotype 1 patients, black patients and prior null-responders. In the model, the SVR rate was compared to the response rate at the end of treatment. Their ratio was associated with the therapy and duration of the treatment. The probability of having a response reverse event increased quite slightly over time in triple therapy with telaprevir or boceprevir, compared to PR therapy alone.

The study demonstrates, for the first time, the model-based meta-analysis of longitudinal hepatitis C virologic response. It allows the indirect comparison between telaprevir and boceprevir on the time-course of response rate.

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CHAPTER 1
Drug-Drug Interactions

1.1 INTRODUCTION

Anticipating a drug-drug interaction (DDI) is an important issue during drug development and clinical practice, due to its potential to alter drug efficacy and safety. The United States Food and Drug Administration (USFDA) and Pharmaceutical Research and Manufacturers of America (PhRMA) have published guidance documents to address the procedure of evaluating DDIs during the development of a new molecular entity (NME) that assess whether the NME would be subject to certain DDIs or would have potential inhibitory or inducing effect on other drug pharmacokinetics.[1,2] These guidelines emphasize the importance of DDI assessment for drug safety and effectiveness and illustrate the integrated approach by means of in vitro and in vivo studies. The drug interaction studies commonly start in vitro. Results from the in vitro studies are subsequently used to predict the potential of drug interactions in vivo and serve as a guide for further in vivo studies. Extrapolating in vitro results to predict the in vivo DDI could offer time and cost savings that minimize the human risk in clinical studies. However, the predictive utility of in vitro approaches can be confounded by uncertainty and variability during in vitro experiments and complexities in clinical practice.[3–5]

1.2 DDI INVOLVING METABOLISM INHIBITION

Drug-drug interactions impact drug pharmacokinetics primarily through metabolism. DDIs involving metabolic inhibition can lead to higher systematic exposure of the inhibited drug and may result in serious adverse events and toxicity, especially in drugs with a narrow therapeutic index.[6–8] Modification of dosage regimen might be suggested in response to the change in drug efficacy or toxicity.[1] Numerous drugs have been

identified as substrates, inhibitors and inducers of cytochrome P450 enzymes (CYP450) that are responsible for drug metabolism. Inhibition in drug metabolism mediated by CYP450 enzymes has been identified with three types of mechanisms (1) reversible inhibition (2) irreversible inhibition and (3) quasi-irreversible inhibition. Among these, reversible inhibition is the most common mechanism for the reported inhibitory DDIs.[3,9] There are four types of reversible inhibition including competitive, noncompetitive, uncompetitive and linear mixed. Competitive inhibition is the most common and simplest type. The inhibitor competes with the substrate for the same binding site at the active site of the enzyme. The metabolic rate (v) with competitive inhibition can be described by Equation 1.1.

$$v = \frac{V_{max}*[S]}{K_m*\left(1+\frac{[I]}{K_i}\right)+[S]} \quad (1.1)$$

where [S] and [I] represent the concentration of substrate and inhibitor at the active site of the enzyme, respectively; V_{max} is the maximum metabolic rate; K_m is the Michaelis-Menten constant of substrate and Ki is the inhibition constant of inhibitor. Competitive inhibition results in an increase in apparent K_m but no change in V_{max}. The inhibitory effect depends on both Ki and [I]. A wide range of substrate and inhibitor concentration levels are required for defining the interaction model that best fits the in vitro data. The in vitro Ki is subsequently determined based on the interaction model.

1.3 PREDICTION OF IN VIVO DRUG INTERACTIONS

According to the USFDA guideline, the potential of in vivo DDI for an NME, that is a reversible inhibitor for a CYP450 enzyme, can be predicted by comparing the in vitro Ki to the estimated in vivo inhibitor concentration.[1,10] The in vivo DDI is assessed to be quite likely if the ratio of in vitro Ki to [I] is greater than 1, possible if the ratio between 0.1 and 1 and is not likely if the ratio is smaller than 0.1.[10–12] Theoretically, [I] should be the inhibitor concentration at the active site of the enzyme. Since that concentration is

quite difficult to measure, the guideline suggests using the estimated mean maximum total (bound and unbound) plasma concentration at steady state of the highest clinical dose of the inhibitor.[1] The ratio has been used to determine the need for further in vivo drug interaction studies. If the ratio is greater than 0.1, a clinical drug interaction study should be conducted in the NME due to its potential risk of a significant pharmacokinetic DDI.[1] This approach also helps to prevent unnecessary in vivo studies in the NME when there is a low risk of in vivo inhibition (e.g., ratio <0.1).

However, there are some limitations and uncertainties when using this method to predict the in vivo DDI.[3,5,13–15] One of the key challenges is which inhibitor concentration should be used to represent [I] in the ratio. Numerous studies have been done for evaluating various estimates of inhibitor concentrations including unbound versus total concentration and systematic plasma versus hepatic inlet concentrations.[12,16–20] The selection of inhibitor concentration affects the predictability of the ratio, but, to date, no common agreement exists on the choice of surrogate inhibitor concentration. A recent study which evaluated the utility of in vitro CYP450 enzyme inhibition data in prediction of in vivo DDI illustrated the unbound hepatic inlet concentration of inhibitors obtained more accurate prediction of in vivo DDI.[20] Using the Ki derived from in vitro studies also causes some inconsistencies, primarily attributed to variation in the in vitro experiments and the biochemical/biophysical differences between the in vivo and in vitro environments.[3,5] More details regarding Ki estimation will be discussed in Section 1.4. In addition, a ratio only based on inhibition potency in vitro and an anticipated inhibitor concentration does not provide sufficient information to definitively evaluate the in vivo DDIs which are also dependent on pharmacokinetics of substrate and inhibitor, and other in vivo factors. Despite these limitations, extrapolation from in vitro data to predict in vivo DDI is plausible in terms of a qualitative assessment. However, the quantitative prediction of in vivo DDI is more complex and challenging.

The inhibitory effect of a DDI on metabolism is directly related to an increase in the systematic exposure of the substrate which can be reflected by the area under the plasma concentration-time curve (AUC). Thus, the magnitude of the inhibitory drug interaction on metabolism can be estimated in clinical studies by comparing AUCs with and without the presence of inhibitor as shown in Equation 1.2. The equation also offers the theoretical basis for the ratio criteria ([I]/Ki) used in prediction of the in vivo DDI of inhibitor (DDI_{inh}).

$$Degree\ of\ DDI_{inh} = \frac{AUC_{inh}}{AUC_{con}} = \frac{CL_{int,con}}{CL_{int,inh}} = 1 + \frac{[I]}{K_i} \quad (1.2)$$

The ratio of AUCs is associated with the ratio of intrinsic metabolic clearance in the absence ($CL_{int,\ con}$) and presence ($CL_{int,\ inh}$) of inhibitor. It should be apparent that this equation only describes the simplest in vivo phenomenon of DDI which requires multiple assumptions. For instance, the substrate is orally administered and exclusively cleared by liver through a single metabolic pathway mediated by a specific CYP450 enzyme which is subject to the inhibition. However, this is usually not true. Drugs may involve parallel elimination pathways including both renal and metabolic clearance involving multiple CYP450 enzymes. The contribution of the metabolic pathway subject to the inhibition to the total drug clearance is an important factor that may influence the overall degree of DDI on drug pharmacokinetics. The USFDA guidelines suggest that an in vivo DDI study is needed for an NME if a metabolizing enzyme contributes greater than 25% of the total clearance of the drug.[1] An analysis of DDI studies involving CYP2D6 showed that quantitative predictions were substantially improved after considering the enzyme contribution as shown in Equation 1.3 where f_m represents the fraction of metabolic pathway of CYP enzyme subject to the inhibition to the total clearance of substrate.[21–23] A significant variation was observed in predicted degree of DDI even with a small change in CYP2D6 contribution when the enzyme was predominately responsible for the substrate elimination (e.g., f_m greater than 0.9).[21]

$$Degree\ of\ DDI_{inh} = \frac{AUC_{inh}}{AUC_{con}} = \frac{1}{\frac{f_m}{1+\frac{[I]}{K_i}}+(1-f_m)} \quad (1.3)$$

Besides substrate disposition in vivo, inhibitor concentration [I] and inhibition constant Ki are still important determinants for predicting the degree of inhibitory metabolism-based drug interaction. The extent and rate of oral absorption of the inhibitor which is associated with inhibitor concentration in vivo could affect the prediction of DDI.[22] The Ki determined from in vitro studies is commonly used for in vivo DDI prediction.

Predicting in vivo DDIs is complicated by large inter-individual variability, which may arise from multiple sources including substrate pharmacokinetics, concentration and potency of inhibitor, and genetic polymorphism of metabolizing enzyme.[9] Uncertainty in selection of inhibitor concentration and variation in inhibitor concentrations resulting from inter-individual variability in inhibitor pharmacokinetics (e.g., oral absorption) could increase the variability in in vivo DDI prediction. As mentioned before, variability in the in vitro experiment (e.g., different microsomal system or assay method) could result in different values of in vitro Ki.[3,5,14] In addition, the affinity of in vitro inhibitor binding to enzyme may not be the same as that in vivo. Although the Ki in vitro is commonly estimated, in vivo Ki should be used to obtain more accurate prediction for in vivo drug interactions involving the enzyme inhibition.[24] Genetic polymorphism of metabolizing enzyme (e.g., CYP2C9, 2C19 and 2D6) is also associated with differential individual response to drug interaction. Varied activity presented by polymorphic variant alleles affect their roles in substrate metabolism and their interaction with the inhibitor. The individual susceptibility to enzyme inhibition is subsequently altered. CYP2C9*3 is one of the mutant alleles that was found to have reduced catalytic activity from both in vitro and in vivo studies.[25,26] Lower oral clearance of flurbiprofen has been observed in individuals with CYP2C9*3 compared to individuals with CYP2C9*1*1(wild type).[27,28] An in vitro study using five probe CYP2C9 substrates showed potential enzyme inhibition was dependent on the CYP2C9 genotype (CYP2C9*1 and CYP2C9*3) and Ki

estimation varied across genotype groups.[29] Individual genotypes for the polymorphic enzyme should be accounted when evaluating the significance of clinical drug interaction.

1.4 INHIBITION CONSTANT KI

The inhibition constant Ki of the inhibitor is the dissociation constant for the inhibitor-enzyme complex and represents the inhibitor potency/affinity of interacting with the enzyme. Ki is usually determined from in vitro experiments using human liver microsomes, hepatocyte, or recombinant enzyme systems. An accurate and reliable estimation of Ki requires an appropriate in vitro experiment that needs multiple levels of substrate and inhibitor concentrations with a proper range. The in vitro Ki has been used to predict the potential of in vivo DDI. However, such extrapolation from in vitro data can bring bias and error to a quantitative prediction.[5,13,14] The estimation of an in vitro Ki can vary from one experiment to another. Multiple factors may contribute to inter-experiment variability, including different in vitro system (e.g., human liver microsomes versus recombinant enzyme system), and different incubation conditions (e.g., pH, temperature, buffer type) and experimental procedures.[3,5,13–15] Another factor that may influence the estimation of in vitro Ki is nonspecific binding of substrate to the microsomes in vitro. Studies have stressed the importance of correction for such binding.[30,31] Furthermore, clinical relevance of the in vitro Ki can be confounded by the discrepancies between in vivo and in vitro systems.[3,5] For example, the inhibitor concentration available at the active site of the enzyme may not be the same in two systems.[24] The nature of two environments is quite different.

Due to these limitations, some studies have proposed to determine the in vivo Ki which is considered a more rational and practical indictor of the inhibitor-enzyme interaction in vivo.[24,32–34] Given competitive or noncompetitive inhibition, the in vivo Ki ($K_{i,\ iv}$) can be directly estimated from formation clearances of metabolite in the presence ($CL_{f,inh}$) and

absence ($CL_{f,con}$) of the inhibitor along with inhibitor concentration in vivo as described in Equation 1.4. The equation assumes that the substrate exhibits linear kinetics and that its concentration is much lower than Km. It also assumes the metabolite formation is only associated with one single CYP enzyme. In studies, plasma concentrations of inhibitor at steady state are used as the value of $[I]$ in Equation 1.4.[24,33]

$$K_{i,iv} = \frac{[I]}{\frac{CL_{f,con}}{CL_{f,inh}} - 1} \quad (1.4)$$

Comparing the estimates of Ki from in vivo and in vitro studies allows evaluation of the in vitro-in vivo correlation of inhibition potency of the inhibitor. The disparity between in vitro and in vivo estimates of Ki reflects the difference in inhibitor-enzyme interactions between in vitro and in vivo systems. Further studies are needed to investigate the potential factors that contribute to this difference. Even though the in vitro Ki is correlated with in vivo Ki, the in vivo Ki is the parameter which directly represents the inhibitor potency given the in vivo environment and provides more accurate information for predicting the magnitude of in vivo drug interactions.

In the view of this approach, the information required before estimating an in vivo Ki are the formation clearances of the metabolite in the presence and absence of the inhibitor and the inhibitor plasma concentration. An appropriate approach in determining this information is necessary to obtain the accurate estimation of the in vivo Ki. Generally, formation clearance of the metabolite is determined based on the amount of the metabolite excreted in the urine and AUCs of the substrate calculated by the trapezoid rule. In these studies, two formation clearances of the metabolite and the inhibitor plasma concentration were estimated first for each individual and then the corresponding in vivo Ki was calculated based on Equation 1.4. The mean of in vivo Kis from all subjects is usually reported as the final estimate of the in vivo Ki. Such sequential approaches involve multiple calculation steps. If one considers inaccuracies in these calculations,

variability in estimation of in vivo Ki could arise from the inter-individual variability in the formation clearance of the metabolite and the inhibitor plasma concentration.

1.5 SCOPE OF CURRENT PROJECT

1.5.1 Rationale

Previous studies have showed that the in vitro Ki was associated with genetic polymorphisms of CYP2C9.[28,29] This suggests the in vivo Ki may also depend on CYP2C9 genotypes. Given the difference between the in vivo and in vitro environment and potential variability in experimental conditions, an in vitro Ki may not accurately describe the inhibitor potency of interacting with the enzyme in vivo. Some studies have proposed a sequential approach to determine the in vivo Ki, where individual in vivo Ki values are calculated from the formation clearance of the metabolite and the inhibitor concentration, followed by taking the average of the individual in vivo Kis. [24,32–34] However, the inter-individual variability in formation clearance of the metabolite and inhibitor plasma concentration affects the estimation of in vivo Ki.

According to USFDA guidance, the potential for an in vivo DDI is evaluated based on in vitro Ki and estimated inhibitor concentration in vivo. This approach serves a good qualitative assessment of in vivo DDI that suggests whether a further clinical study is necessary. In contrast, a quantitative evaluation of in vivo DDI needs a more rigorous and efficient method that enables acknowledgement of multiple clinical factors, such as disposition of the substrate and the inhibitor, inhibitor potency in vivo and genetic polymorphism of enzyme.

1.5.2 Objective

The current project was undertaken to implement a population modeling-based approach to directly determine the in vivo Ki of the inhibitor (fluconazole) with the substrate flurbiprofen across three CYP2C9 genotypes and compare them to in vitro estimates.

Subsequently, the magnitude of DDI was to be evaluated based on the in vivo Ki and flurbiprofen pharmacokinetics.

1.5.3 Approach

A population pharmacokinetic modeling approach was adopted in the current work. An integrated model was developed incorporating the plasma and urine data of CYP2C9 substrate flurbiprofen and its major metabolite 4'-hydroxy flurbiprofen in the presence and absence of the inhibitor fluconazole. This approach allows us to identify and estimate the in vivo Ki of the inhibitor simultaneously with pharmacokinetics of the substrate across three CYP2C9 genotypes. The inter-individual variability arising from substrate pharmacokinetics and unexplained residual variability were also quantitatively described. The population estimates of the in vivo Ki were compared to in vitro estimates previously determined. The degree of drug interaction was further assessed based on estimates of in vivo Ki and the substrate pharmacokinetic parameters. The methods, results and conclusion are presented in Chapter 2.

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CHAPTER 2

Population Model-Based Estimates of Furbiprofen Oral Clearance and In Vivo Inhibition Constant (Ki) of Fluconazole across CYP2C9 Polymorphism

2.1 INTRODUCTION

Given the complexity of certain diseases, patients may receive multiple concomitant medications where a drug-drug interaction needs to be considered in terms of its impact on efficacy and safety. The potential for a drug-drug interaction is usually evaluated with respect to the disposition profile of the target drug (substrate) and mechanism of drug interaction. Genetic polymorphism, especially in metabolizing enzymes (e.g., CYP2C9, CYP2D6 and CYP2C19), has recently emerged as an important factor attributable to the drug interactions.[1–6] The presence of genotype polymorphisms could lead to varied activity of the enzyme.

CYP2C9 is one of the essential P450 enzymes responsible for metabolic oxidation reactions. Among allelic variants coded in CYP2C9, the presence of a *3 allele is associated with significantly lower oral clearance for several CYP2C9 substrates[7–12] and also leads to more frequent adverse events in some substrates with a narrow therapeutic index, such as, warfarin and phenytoin.[13–15] Furthermore, both in vitro and in vivo studies showed the different interacting effect of CYP2C9 inhibitors on the disposition of substrates among different CYP2C9 genotypes. Not only is substrate metabolism affected by CYP2C9 genotypes, the potency (Ki) of the inhibitor is also related to varied activities expressed by CYP2C9 polymorphism.[16] An in vitro study demonstrated that the magnitude of in vitro Ki of fluconazole inhibition on flurbiprofen hydroxylation was larger with the presence of the CYP2C9*3 than in wild type CYP2C9*1*1,[1] suggesting that inhibitor potency is dependent on CYP2C9 genotypes.

The Ki is typically estimated from in vitro rather than in vivo studies. Such in vitro estimates of Ki are usually applied to predicting the in vivo drug interaction. According to United States Food and Drug Administration guidance, [17] the ratio of the estimated maximum plasma concentration of inhibitor to the in vitro Ki is used to predict the potential of competitive inhibition in vivo which determines whether a further in vivo study is needed for a new drug. Such in vitro extrapolation provides a qualitative prediction of the likelihood of an in vivo drug interaction and helps to avoid unnecessary clinical drug interaction studies, such as, a ratio smaller than 0.1 suggests a drug interaction is unlikely.[17,18] There can be issues when the in vitro data are used as a quantitative assessment of an in vivo drug interaction, primarily due to the difference between the in vitro and in vivo environments.[19–24] Similarly, the Ki estimated from in vitro studies may not truly reflect the capability of the inhibitor interacting with the enzyme in vivo.[25] In addition, potential variability could arise when estimating an in vitro Ki, due to differences in experimental design, the in vitro system and data analysis methods.[19,23–25]

In our study, we proposed a population pharmacokinetic modeling approach to identify and determine the in vivo Ki. An integrated pharmacokinetic model of the CYP2C9 substrate flurbiprofen and its metabolite was established along with the interacting effect of the CYP2C9 inhibitor fluconazole. The in vivo Ki of fluconazole was to be assessed for individuals with CYP2C9*1*1, CYP2C9*1*3 and CYP2C9*3*3. Subsequently, the estimated in vivo Ki combined with the pharmacokinetics of flurbiprofen was used to quantitatively evaluate the magnitude of the in vivo drug interaction.

2.2 METHODS

2.2.1 Study Subjects

The institutional review board at the University of Minnesota reviewed and approved the study protocol. One hundred and eighty-nine healthy subjects underwent genotype

screening. From these subjects, twenty-one subjects (11 with CYP2C9*1*1, 8 with *1*3 and 2 with *3*3) were further enrolled in the current drug-drug interaction study. All subjects were non-smokers and were not taking any medicine at the time of enrollment. Their health conditions were determined based on medical history, physical examination, vital signs, and routine biochemical and urinalysis tests.[1]

2.2.2 Assessment of CYP2C9 Genotype

DNA isolation was conducted according to the method previously described. CYP2C9 genotype was subsequently determined by using the isolated DNA samples. The detailed method has been described in Kumar, et al.[1]

2.2.3 Study Design

The study was conducted as a three-way, open-label, randomized, crossover design with a one-week washout period. Subjects were randomly assigned to take one single oral tablet of flurbiprofen at a dose of 50 mg (Mylan Pharmaceutical Inc., Morgantown, WV) alone or following 7 daily tablets of fluconazole at 200 mg or 400 mg (Ivax Pharmaceutical Inc., Miami, FL). Thus, fluconazole was administered at three dosing levels of 0, 200 or 400 mg in the current study.

2.2.4 Sample Collection and Concentration Analysis

Blood samples for measuring flurbiprofen plasma concentration were collected at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after the administration of flurbiprofen. Total voided urine was collected following flurbiprofen administration at intervals of 0 to 12 h and 12 to 24 h. Blood samples of fluconazole were collected before and at 2, 8, 12 and 24 h after the last steady-state dose on the 7^{th} day. Flurbiprofen concentration was quantified in plasma and urine samples. Its primary metabolite, 4'-hydroxyflurbiprofen was analyzed for its concentration in the urine only; its plasma concentration is too low to measure. For the inhibitor fluconazole, the plasma concentration was measured at 0, 2, 8, 12 and 24 h after

the dose on the 7th day. The quantitation method for urine and plasma concentrations have been described previously.[1,26,27]

2.2.5 Population Pharmacokinetic Analysis

The population pharmacokinetic analysis was performed using a nonlinear mixed-effect modeling approach implemented in the NONMEM software (version 7, ICON Development Solutions, Ellicot City, MD, USA). The subroutine ADVAN 6 was used for developing the model. The first-order conditional estimation algorithm with interaction (FOCE-I) was used for parameter estimation in all model runs. The package "Xpose4"[28] was installed through the statistical software R (version 2.14.2)[29] and was used to process NONMEM output and to construct diagnostic plots for evaluating the goodness of model fit. The commands in R were executed through the interface software Rstudio (version 0.97.449, Rstudio Inc., MA, US). A nonparametric bootstrap was performed through Perl-speaks-NONMEM (PsN)[30] and visual predictive checks were performed through the software PDxPop 4.1 (ICON Development Solutions, Ellicott City, MD, USA).

Model Development

Model development was guided based on research objectives, plausible mechanism, prior knowledge, changes in NONMEM maximum likelihood objective function and improvement in various diagnostic plots.

Previous studies showed that a one-compartment model adequately described the pharmacokinetics of flurbiprofen.[1] In the current study, two urine compartments were added to account for the urine data of flurbiprofen and its primary metabolite 4'-hydroxyflurbiprofen as presented in Figure 2.1. Although the metabolite's plasma concentration was too low to quantitate, its urine data still allowed estimation of the formation clearance of the CYP2C9 metabolite. However, the renal clearance of the metabolite itself cannot be estimated. Given the available data, the proposed model permits the analysis of multiple elimination pathways of flurbiprofen, including its renal

clearance, CYP2C9-mediated 4'-hydroxyflubiprofen formation clearance and residual clearance through unknown elimination pathways. Prior studies have established competitive inhibition as the mechanism for fluconazole interacting with flurbiprofen CYP2C9-mediated metabolism.[1,16] The Rowland-Matin equation[31] assuming competitive inhibition was adapted to evaluate the inhibitory effect of fluconazole as a function of inhibition constant (Ki) and fluconazole plasma concentration ([I]) as shown in Equation 2.1.

$$CL_{2C9,inh} = \frac{CL_{2C9,con}}{1+\frac{[I]}{Ki}} \quad (2.1)$$

where $CL_{2C9,inh}$ indicates the inhibited CYP2C9-mediated metabolic clearance, $CL_{2C9,con}$ indicates control CY2C9-mediated metabolic clearance in the absence of inhibitor. It should be noted that $CL_{2C9,con}$ and Ki were estimated separately for each CYP2C9 genetic polymorphism. Since fluconazole concentrations fluctuated little over the dosing interval, the average plasma concentration at steady-state was calculated from the trapezoidal area under concentration-time curve (AUC) of fluconazole plasma data and the dosing interval as shown in Equation 2.2. This single number for each individual was considered as the value of fluconazole concentration $[I]$ in Equation 2.1.

$$\overline{C_{p,ss}} = \frac{AUC_{0-\tau}}{\tau} \qquad \tau = 24\ hours \quad (2.2)$$

The total oral clearance of flurbiprofen can be expressed as the sum of renal clearance (CL_{renal}), CYP2C9-mediated metabolic clearance and residual clearance through unknown elimination pathways ($CL_{residual}$) as in Equation 2.3.

$$CL_{total} = \frac{CL_{2C9,con}}{1+\frac{[I]}{Ki}} + CL_{renal} + CL_{residual} \quad (2.3)$$

where CL_{renal} and $CL_{residual}$ were assumed common across CYP2C9 genotypes. Pharmacokinetic parameters in the model were assumed to follow a log-normal distribution as Equation 2.4.

$$P_i = TVP \times e^{\eta_P} \quad (2.4)$$

$$\eta_P \sim N\ (0, \omega_P^2) \quad (2.5)$$

where P_i is the pharmacokinetic parameter in ith individual, TVP is the typical population mean of the pharmacokinetic parameter and η_P is the inter-individual variability (IIV) which is normally distributed with a mean of zero and variance of ω_P^2 to be estimated as expressed in Equation 2.5.

In the current study, the population means of fluconazole Ki and uninhibited flurbiprofen $CL_{2C9,con}$ were estimated for each of CYP2C9 genotypes. In contrast, the population means of CL_{renal} and $CL_{residual}$ were assumed to be the same across CYP2C9 genotypes and fluconazole concentrations; they were shared among these three CYP2C9 genotype groups. All parameters were assumed to be constant across the three fluconazole dosing arms (0, 200 or 400 mg). The inter-individual variability was assessed for flurbiprofen volume of distribution, first-order absorption rate constant, absorption lag time, metabolic clearance, renal clearance, and residual clearance through unknown elimination pathways.

Residual unexplained variability (RUV) was explored in an additive, proportional and combination error models. Since the concentration data of flurbiprofen and its metabolite were analyzed simultaneously in the pharmacokinetic model, two indicator variables (TYPE1 and TYPE2) were defined to differentiate RUVs which were separately estimated for plasma flurbiprofen, urine flurbiprofen and urine 4'-hydroxyflurbiprofen as shown in Equation 2.6.

$$Y_{obs,ij} = \hat{Y}_{pred,ij} \times (1 + \varepsilon_{1,ij} \times TYPE1 + \varepsilon_{2,ij} \times TYPE2 + \varepsilon_{3,ij} \times (1 - TYPE1) \times (1 - TYPE2)) \quad (2.6)$$

$$\varepsilon_{k,ij} \sim N\ (0, \sigma_k^2),\ \ k = 1,2,3 \quad (2.7)$$

where $Y_{obs,ij}$ and $\hat{Y}_{pred,ij}$ are observed and model-predicted concentration j[th] subject at time point i, $TYPE1$ equals 1 for plasma concentration of flurbiprofen and 0 for others, $TYPE2$ equals 1 for urine concentration of flurbiprofen and 0 for others; $\varepsilon_{1,ij}$, $\varepsilon_{2,ij}$, $\varepsilon_{3,ij}$ represents residual errors for flurbiprofen concentration in plasma and urine and 4'-hydroxyflrubiprofen concentration in urine, respectively. Each of these RUVs is assumed independently, normally distributed with a mean of zero and a variance (σ_k^2) to be estimated as expressed in Equation 2.7.

Model Evaluation

A nonparametric bootstrap was employed to evaluate the reliability and stability of the proposed population model.[32] Bootstrapping is one of the resampling techniques which randomly generate samples with replacement from the original dataset. The simulated dataset has the same number of subjects as the original dataset. Bootstrap is also used to examine the precision of model parameter estimates, especially when the sample size is small. In our study, the numbers of individuals were not balanced among three CYP2C9 genotypes; there were only 2 subjects with the CYP2C9*3*3. It was possible that fewer or no subjects with CYP2C9*3*3 might be drawn during the resampling process. To retain the proportion of subjects in each CYP2C9 genotype as in the original dataset, a stratified bootstrap was used and resampling was stratified based on CYP2C9 genotypes.[30] In this stratified bootstrap, the simulated dataset had approximately the same number of the individuals in each of CYP2C9 genotype as that in the original dataset. Five hundred bootstrap samples were generated and the proposed PK model was fitted in each of these samples to get parameter estimates. The results from successful minimization runs were extracted to compute the summary statistics (e.g., median, 95% confidence internals) for each of the model parameters. The bootstrap results were compared with the original NONMEM results.

Additionally, a visual predictive check (VPC) was carried out to evaluate the performance of the established population pharmacokinetic model. Two hundred datasets were simulated using the pharmacokinetic model and parameter estimates from NONMEM results. The simulated datasets also adopt the same study design as in the original dataset. The median, 5th and 95th percentile of simulated concentration data were calculated at each nominal observation time point after dosing and plotted over these time points. The same percentiles were also constructed in the observed data and the plot was overlaid with those of the simulated data for a visual comparison. VPC plots were evaluated for flurbiprofen plasma concentration with respect to CYP2C9 genotypes. VPC would not be helpful for the evaluation of urine data which were measured only at two time points in the study. An appropriate model is expected to have distributions of predictions generally consistent with observations over time.

2.3 RESULTS

2.3.1 Dataset

The dataset consisted of 630 observed plasma and 126 urine concentrations of flurbiprofen, and 126 observed urine concentration of 4'-hydroxyflurbiprofen from 21 healthy study participants. Among these subjects, 11 persons had CYP2C9*1*1, 8 persons had CYP2C9*1*3 and 2 had CYP2C9*3*3.

2.3.2 Population Pharmacokinetic Model

The pharmacokinetics of flurbiprofen were described in a model consisting of one compartment for flurbiprofen plasma observation and two compartments for flurbiprofen and its metabolite in urine connected to the plasma compartment as shown in Figure 2.1. The parameter estimate and relative standard error from the NONMEM output were summarized along with the bootstrap results in Table 2.1.

The population mean of in vivo Ki was estimated to be 14.7 μM for CYP2C9 *1/*1, 18.7 μM for CYP2C9*1/*3 and 32.9 μM for CYP2C9*3/*3. The CYP2C9-mediated metabolic clearance was 1.09 L/hr for CYP2C9*1/*1, 0.449 L/hr for CYP2C9*1*3 and 0.0481 L/hr for CYP2C9 *3*3. The flurbiprofen renal clearance and residual clearance were estimated at 0.191 L/hr and 0.219 L/hr, respectively.

The inter-individual variability (shrinkage %) was estimated to be 21.1% (12.4), 31.3% (6.09) and 63.2% (7.2) for flurbiprofen metabolic clearance, renal clearance and residual clearance through an unknown elimination pathway, respectively. High inter-individual variability (shrinkage %) was found in the oral absorption rate constant at 94.2% (5.3). The proportional RUV was estimated to be 48.4% for flurbiprofen plasma concentration, 29.6% for flurbiprofen urine concentration and 36.9% for 4'-hydroxyflurbiprofen urine concentration.

2.3.2 Model Evaluation

Diagnostic plots were presented according to the types of concentration data and CYP2C9 genotypes. Observations were plotted versus population predictions and versus individual predictions separately for flurbiprofen plasma concentration, urine concentration and 4'-hydroxy-flurbiprofen urine concentration as shown in Figure 2.2, 2.3, 2.4, respectively. Most of predictions from the model were consistent with the observations. The model had a tendency to under predict at high flurbiprofen plasma concentrations and over predict at high metabolite 4'-hydroxyflubiprofen urine concentrations in subjects with the CYP2C9*1*1genotype.

Conditional weighted residual (CWRES) were plotted against population predicted concentrations in Figure 2.5, 2.6 and 2.7. CWRES of plasma flurbiprofen concentration were generally scattered well and did not show a specific pattern. A large CWRES, approximately 5.8, was seen in one urine flurbiprofen sample in an individual with CYP2C9*1*3, meaning the current model underpredicted this observation by a large

amount. The CWRES of urine flurbiprofen were not evenly scattered across the zero-ordinate in the CYP2C9*3*3 group. It might be due to a small sample size in this subgroup (N=2), sparse sampling in urine flurbiprofen and analytical error in urine assay. For 4'-hydroxy-flurbiprofen urine concentrations, a pattern of negative CWRES trended to become prominent at population predictions that were higher than 10 μg/mL for both CYP2C9*1*1 and CYP2C9*1*3. Different error models were tried, but did not improve the plot. It could be related to model misspecification. CWRES was also evaluated against time in Figure 2.8, 2.9 and 2.10 for each type of concentration. The CWRES plot for plasma flurbiprofen concentrations did not show systemic bias. Urine samples were only collected at 12 hours and 24 hours after dosing. Due to a lack of sampling at other time points, it's difficult to interpret and assess CWRES-time plots and assess model fit in urine data as a function of time. Nevertheless, there appears to be model misspecification of the 4-hydroxyflurbiprofen metabolite data, which may affect the estimation of formation clearance of the metabolite and the Ki. In spite of a slight model misspecification indicated by some of the diagnostic plots, the current model was still capable of adequately describing most of the data and to meet our research objectives.

In addition to diagnostic plots, the model was qualified by visual predictive check (VPC). The VPC plot of flurbiprofen plasma concentrations were inspected for each of CYP2C9 genotypes in Figure 2.11, 2.12, 2.13. Approximately 10.3% of the observed data fell outside 90% of simulated data. The 5^{th}, 50^{th} and 95^{th} percentiles of predictions were comparable to the corresponding percentile of observations. The model generally indicated the overall trend of observed data over time and revealed a good predictive behavior.

2.4 DISCUSSION

The genetic polymorphism of CYP2C9 has been found to be a crucial factor for disposition of multiple CYP2C9 substrates.[11] It is necessary to account for the impact of

genetic polymorphisms when evaluating metabolism-based drug interactions. Not only is the substrate's metabolism affected, but the inhibitor potency in interacting with CYP2C9 is also associated with varied enzyme activity of CYP2C9 genotypes.[16] The inhibition constant (Ki) is usually determined from an in vitro system, e.g., human liver microsomes. A wide range of concentrations of substrate and inhibitor are needed to identify the inhibition model and obtain a reliable estimate of Ki. The in vitro studies can be laborious. Limitations are known due to differences between in vitro and in vivo systems including the distribution of the enzyme, concentrations of inhibitor and substrate at the active site of the enzyme and patient characteristics. It is also recognized that the in vitro study does not account for the pharmacokinetics of substrate and inhibitor that may be important for evaluating the in vivo drug interaction. Inconsistencies and even bias could be introduced when extrapolating the in vitro result to predict the in vivo response. In our study, a population pharmacokinetic modeling approach was employed to estimate the in vivo Ki and evaluate the drug interaction across three CYP2C9 genotypes.

A population pharmacokinetic model was proposed that incorporated the CYP2C9 prototype substrate flurbiprofen. The model described the flurbiprofen concentrations in plasma and urine as well as its primary metabolite, 4'hydroxy-flurbiporfen urine concentration in the presence and absence of fluconazole. The model allowed evaluation of multiple elimination pathways of flurbiprofen. The flurbiprofen total oral clearance consisted of renal clearance, CYP2C9-mediated metabolic clearance and residual clearance through unknown pathways. The inhibitory effect of fluconazole on flurbiprofen CYP2C9-mediated metabolic clearance was described in a competitive inhibition model. The population means of in vivo Ki values for fluconazole interacting with CYP2C9 were estimated separately for each of CYP2C9 polymorphisms. The in vivo Ki values were estimated to be greater in subjects with the presence of *3 allele than in subjects with wild type *1*1. The CYP2C9*3*3 group had the highest value of in vivo Ki, meaning the affinity of fluconazole binding to this genotype was quite weak. In

contrast, fluconazole had a very strong interaction with CYP2C9*1*1 where the estimated in vivo Ki was small. Such differences are attributed to the reduced activity of CYP2C9*3 allele. Of note is that the estimates of in vivo Ki by using the population approach were comparable with those results from in vitro studies, suggesting a good in vitro-in vivo correlation of Ki for fluconazole for these three CYP2C9 genotypes.

In a previous population model, the flurbiprofen clearance was only evaluated based on two elimination pathways which were metabolic clearance through CYP2C9 and non-CYP2C9 clearance.[1] Given the urine data available in this analysis, the current model enabled the differentiation of flurbiprofen renal clearance from CYP2C9 metabolic clearance and the remaining non-CYP2C9 elimination pathways. Renal clearance and residual clearance were assumed to be common among the three groups of CYP2C9 polymorphisms. In contrast, the formation clearance of 4'-hydroxyflurbiprofen was associated with CYP2C9 genotypes and was estimated for each of the groups. As expected, the CYP2C9-mediated metabolic clearance in the absence of inhibitor deceased in the presence of *3 alleles in CYP2C9 genotype. The *1*1 (wild type) group had a much greater formation clearance of 4'-hydroxyflurbiprofen than did *1*3 and *3*3. The estimate of uninhibited CYP2C9 was the smallest in the CYP2C9 *3*3 group compared to *1*1 and *1*3 groups, presumably due to reduced protein function present in CYP2C9*3 allele. This pattern of CL_{2C9} across CYP2C9 genotypes agreed with the findings from the previous study.[1] The population estimates of uninhibited formation clearance of 4'-hydroxyflurbiprofen and the derived total flurbiprofen clearance from the current model were consistent with results from previous noncompartmental analyses,[1] but were smaller than the estimates from earlier population models which only accounted for two elimination pathways for flurbiprofen. [1] It was also notable that the contribution of CYP2C9-mediated metabolism to the total flurbiprofen elimination varied across individuals with different CYP2C9mutations. According to results from the current population analysis, the proportion of CYP2C9 metabolic clearance in total clearance was

quite low in individuals having the CYP2C9*3 allele. Formation clearance of 4'-hydroxyflurbiprofen accounts for approximately 10 % and 52% of the total flurbiprofen clearance in individuals with CYP2C9*3*3 and CYP2C9*1* 3, respectively, which were significantly lower than 73% in those with CYP2C9*1*1. This suggests that the impact of CYP2C9 inhibition was diminished in subjects with CYP2C9*3 due to the small contribution of CYP2C9 metabolism to their flurbiprofen elimination.

The overall influence of the drug interaction between flurbiprofen and fluconazole was therefore evaluated based on substrate disposition and inhibitor interaction, both of which were associated with CYP2C9 polymorphisms. Given the parallel elimination pathways of flurbiprofen, the magnitude of the drug interaction can be evaluated using Equation 2.8.[22,31,33]

$$Degree\ of\ DDI = \frac{AUC_{inh}}{AUC_{con}} = \frac{1}{\frac{f_{m,CYP2C9}}{1+\frac{[I]}{K_i}} + (1 - f_{m,CYP2C9})} \quad (2.8)$$

The degree of drug-drug interaction and the ratio of substrate AUC in the presence (AUC_{inh}) and absence of inhibitor (AUC_{con}) were predicted based on fraction of CYP2C9 mediated metabolism ($f_{m,CYP2C9}$) to the total clearance, inhibitor concentration $[I]$ at the active site of enzyme and inhibitor constant K_i. There has been considerable controversy in what inhibitor plasma concentration should be used as a surrogate for the concentration available at the active site of the enzyme in the hepatocyte which is difficult to measure.[25,34] In our study, the CYP2C9 inhibitor fluconazole had achieved steady-state. The average concentration of fluconazole at steady state in each individual was derived from Equation 2.2 and was used as the value of $[I]$ in Equation 2.3. To predict the overall in vivo drug interaction, the mean of all individual average steady state concentration was calculated at each dose level and used as $[I]$ in Equation 2.8. According to the population estimates from the proposed model, the magnitude of the drug-interaction was derived from Equation 2.8 and the values of relevant parameters were summarized in Table 2.2. The effect of the drug interaction varied across the

CYP2C9 genotype and also changed with inhibitor dosing level. Given the same dose of inhibitor, the inhibitory effect was greater in the CYP2C9*1*1 group than those in the CYP2C9*1*3 and CYP2C9*3*3 groups. Especially in CYP2C9*3*3, the inhibitory effect was quite slight. Within a given genotype group, the effect of fluconazole inhibition on the overall disposition of flurbiprofen was not doubled when the dose of fluconazole was doubled, since not all the elimination pathways of flurbiprofen was subject to the fluconazole inhibition. From the clinical perspective, the present findings showed that the potential of metabolism-based drug interactions could be different across individuals with different metabolic enzyme polymorphisms. The recommendation on dosing adjustment due to the overall effect of a drug interaction involving metabolism by CYP2C9, should take into consideration the presence of particular CYP2C9 polymorphisms and elimination pathways of the substrate and inhibitor potency.

In conclusion, the current study presents a population pharmacokinetic modeling approach to estimate the in vivo Ki and to evaluate the in vivo drug interaction exhibited across three CYP2C9 polymorphisms. The estimates of in vivo Ki were different among genotypes and were comparable to the results from in vitro studies. Such a modeling approach can be used to determine the in vivo Ki for inhibitors interacting with CYP enzymes and to examine the in vitro-in vivo correlation. Incorporation of potential elimination pathways of the substrate refined the evaluation of the net influence of drug interaction. The population pharmacokinetic analysis provides more information regarding the substrate pharmacokinetics and inhibitor potency to characterize the in vivo drug interaction and could help further determination of the dosage modifications needed for the substrate drug. Based on the current findings, the potential drug interaction highly depends on the individual CYP2C9 genotypes. This suggests that CYP2C9 genotypes need to be checked when making decisions on dosage modifications with drugs that interact. One major limitation of the model include a degree of model misspecification, which is probably due to small sample size and could cause the bias in the estimation of

the fm and Ki. In the present work, we only had 2 subjects with CYP2C9*3*3. Future studies are needed with more subjects with each genotype. By using a population pharmacokinetic approach, it is possible to determine the in vivo Ki and the magnitude of the drug interaction in larger numbers of subjects with fewer samples of substrate and fewer levels of inhibitor. The relevant simulation is needed to optimize the study design with an improved sampling technique without introducing bias and decreasing precision of the parameter estimates.

Table 2.1 Final parameter estimates from population analysis in NONOMEM and Bootstrap analysis

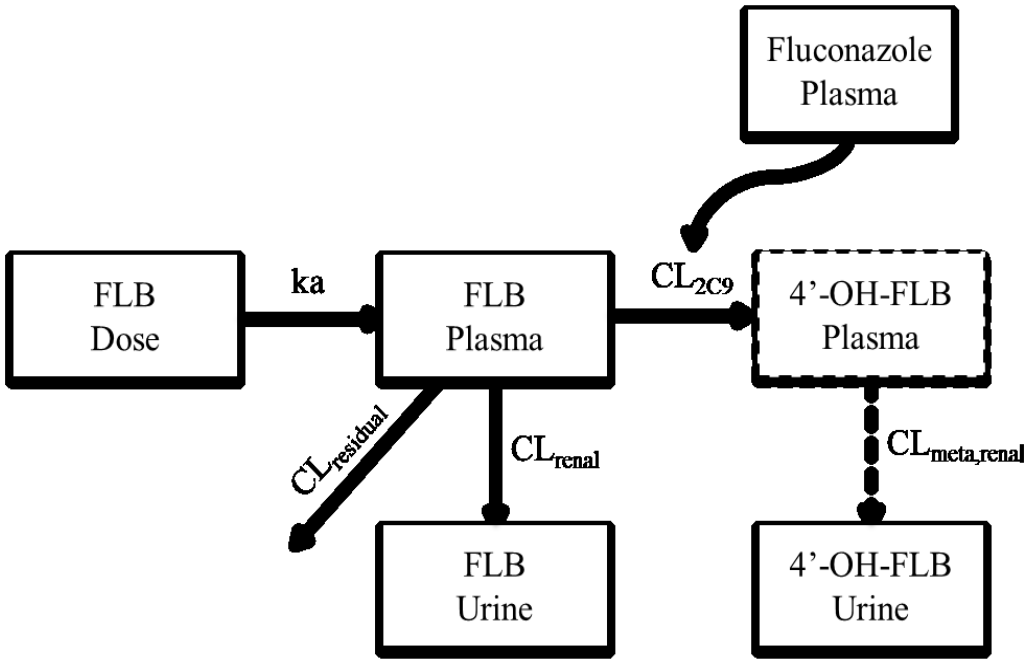
Parameter	Population Analysis (NONMEM)		Bootstrap Analysis	
	Estimate (% RSE)	95% CI	Median	(2.5^{th}, 97.5^{th}) percentile
Ki (μMol)				
CYP2C9*1*1	14.7 (13.7)	(10.8, 18.6)	14.8	(11.3, 19.0)
CYP2C9*1*3	18.7 (16.3)	(12.7, 24.7)	18.8	(14.6, 28.5)
CYP2C9*3*3	32.9 (14.6)	(23.5, 42.3)	32.8	(27.5, 45.8)
CL_{2C9}/F, uninhibited (L/hr)				
CYP2C9*1*1	1.09 (5.39)	(0.975, 1.21)	1.08	(0.987, 1.21)
CYP2C9*1*3	0.449 (20.5)	(0.269, 0.629)	0.456	(0.282, 0.637)
CYP2C9*3*3	0.0481 (9.21)	(0.0394, 0.0568)	0.0483	(0.0404, 0.0562)
CL_{renal}/F (L/hr)	0.191 (8.9)	(0.158, 0.224)	0.191	(0.163, 0.223)
$CL_{residual}$/F (L/hr)	0.219 (14.4)	(0.157, 0.281)	0.219	(0.165, 0.280)
V/F (L/hr)	8.22 (6.25)	(7.21, 9.23)	8.21	(7.35, 9.18)
Ka (hr^{-1})	2.05 (22.4)	(1.15, 2.95)	2.05	(1.37, 3.44)
T_{lag} (hr)	0.2 (4.9)	(0.181, 0.219)	0.2	(0.179, 0.217)
Variance of Interindividual Variability (IIV)				
ω^2_{CL2C9}	0.0447 (75.8) CV%=21.2	(0, 0.111)	0.0328	(0.0026, 0.0991)
$\omega^2_{CLrenal}$	0.0981(41.9), CV%=31.1	(0.0175, 0.179)	0.0912	(0.0373, 0.191)
$\omega^2_{CLresidual}$	0.399 (51.4), CV%=63.2	(0, 0.801)	0.365	(0.112, 0.793)
ω^2_{V}	0.0443 (44.2), CV%=21.0	(0.00588, 0.0827)	0.0411	(0.0125, 0.0875)
ω^2_{Ka}	0.887 (28.5), CV%=94.2	(0.391, 1.38)	0.86	(0.452, 1.41)
ω^2_{Tlag}	0.0197 (50.8), CV%=14.0	(0.0001, 0.0393)	0.0181	(0.00602, 0.0474)
Variance of Residual Unexplained Variability (RUV)				
$\sigma^2_{plasma\ flurbiprofen}$	0.234 (9.15), CV%=48.4	(0.192, 0.276)	0.235	(0.197, 0.278)
$\sigma^2_{urine\ flurbiprofen}$	0.0875 (27.2), CV%=29.6	(0.0409, 0.134)	0.085	(0.0483, 0.134)
$\sigma^2_{urine\ 4'\text{-}hydroxyflurbiprofen}$	0.136 (16.6), CV%=36.9	(0.0917, 0.180)	0.131	(0.0954, 0.173)

Table 2.2 Predicted degree of drug interaction according to Equation 2.8

		CYP2C9*1*1	CYP2C9*1*3	CYP2C9*3*3
	Ki (μMol)	14.7	18.7	32.9
	$f_{m,CYP2C9}$	0.73	0.52	0.11
Degree of DDI	Fluconazole (Dose=200mg) [I]=33 μMol	2.02	1.5	1.06
	Fluconazole (Dose=400mg) [I]=69 μMol	2.51	1.69	1.08

Note: DDI: drug-drug interaction; Ki, inhibition constant; [I] inhibitor (Fluconazole) plasma concentration

Figure 2.1 Population pharmacokinetic model of flurbiprofen and its metabolite, 4'-hydroxy flurbiprofen



Note: FLB, flurbiprofen; ka: first-order absorption rate constant; 4'-OH-FLB, 4'-hydroxy flurbiprofen;CL_{2C9}, CYP2C9 mediated formation clearance of 4'-hydroxy flurbiprofen; CL_{renal}, flurbiprofen renal clearance; $CL_{residual}$, flurbiprofen clearance through residual elimination pathway; $CL_{meta,renal}$, metabolite renal clearance. 4'-hydroxy flurbiprofen plasma data was not available in the study (dash box) and its renal clearance was not estimated (dash arrow).

Figure 2.2 Observations versus Predictions of flurbiprofen (FLB) plasma concentration across CYP2C9 genotypes.

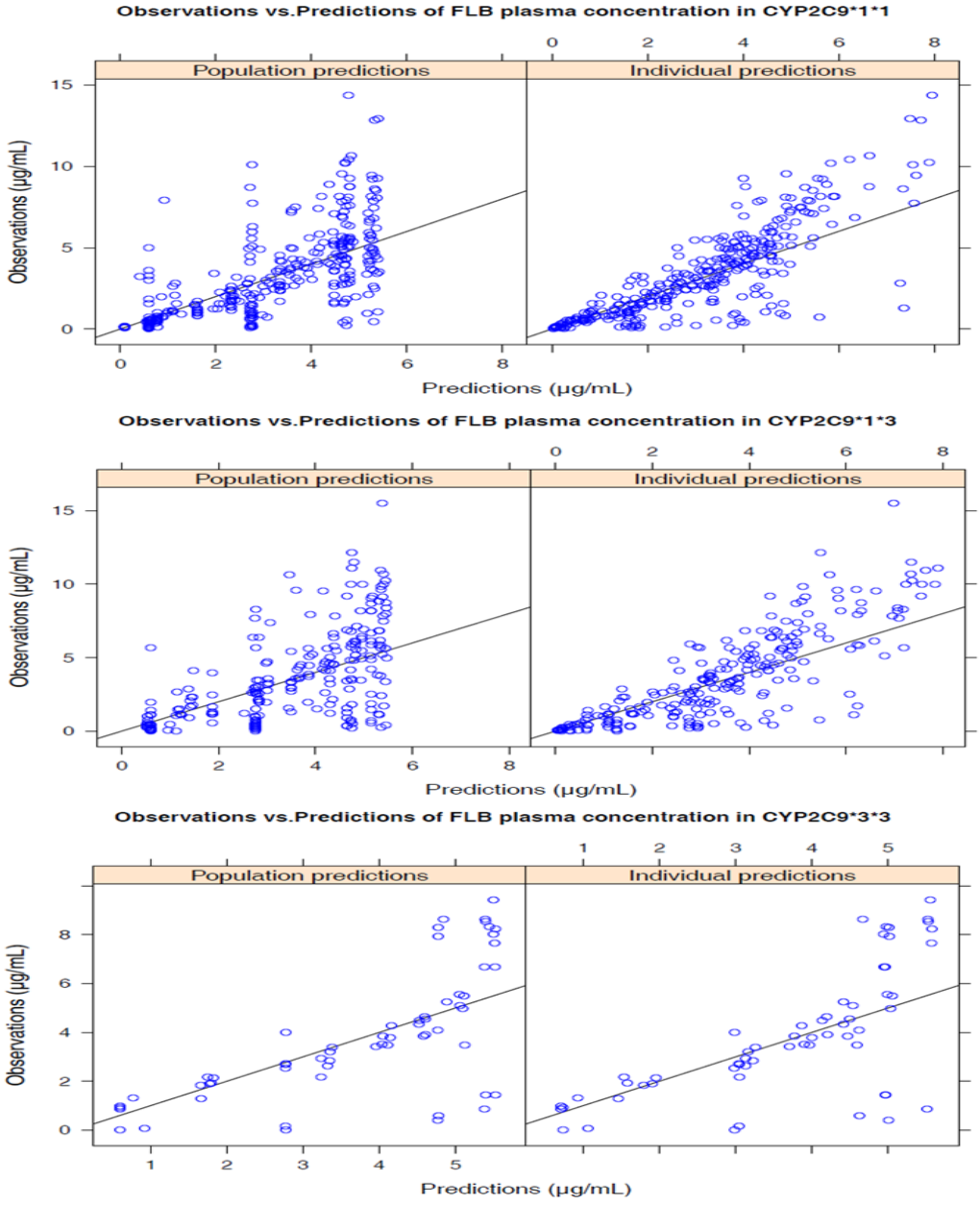


Figure 2.3 Observations vs. Predictions of flurbiprofen (FLB) urine concentration across CYP2C9 genotypes

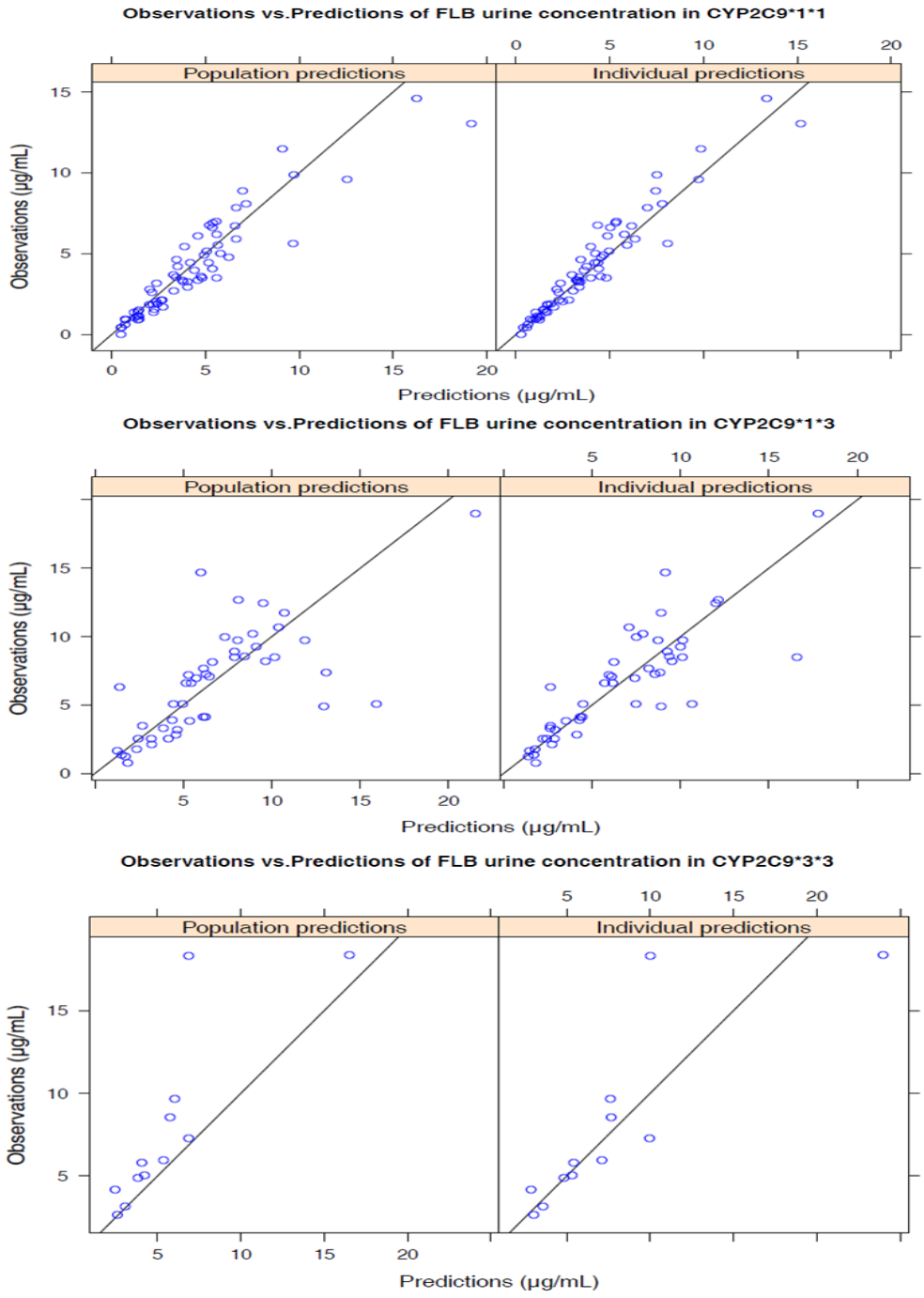


Figure 2.4 Observations vs. Predictions of 4'-hydroxyflurbiprofen (4'-OH-FLB) urine concentrations across CYP2C9 genotypes

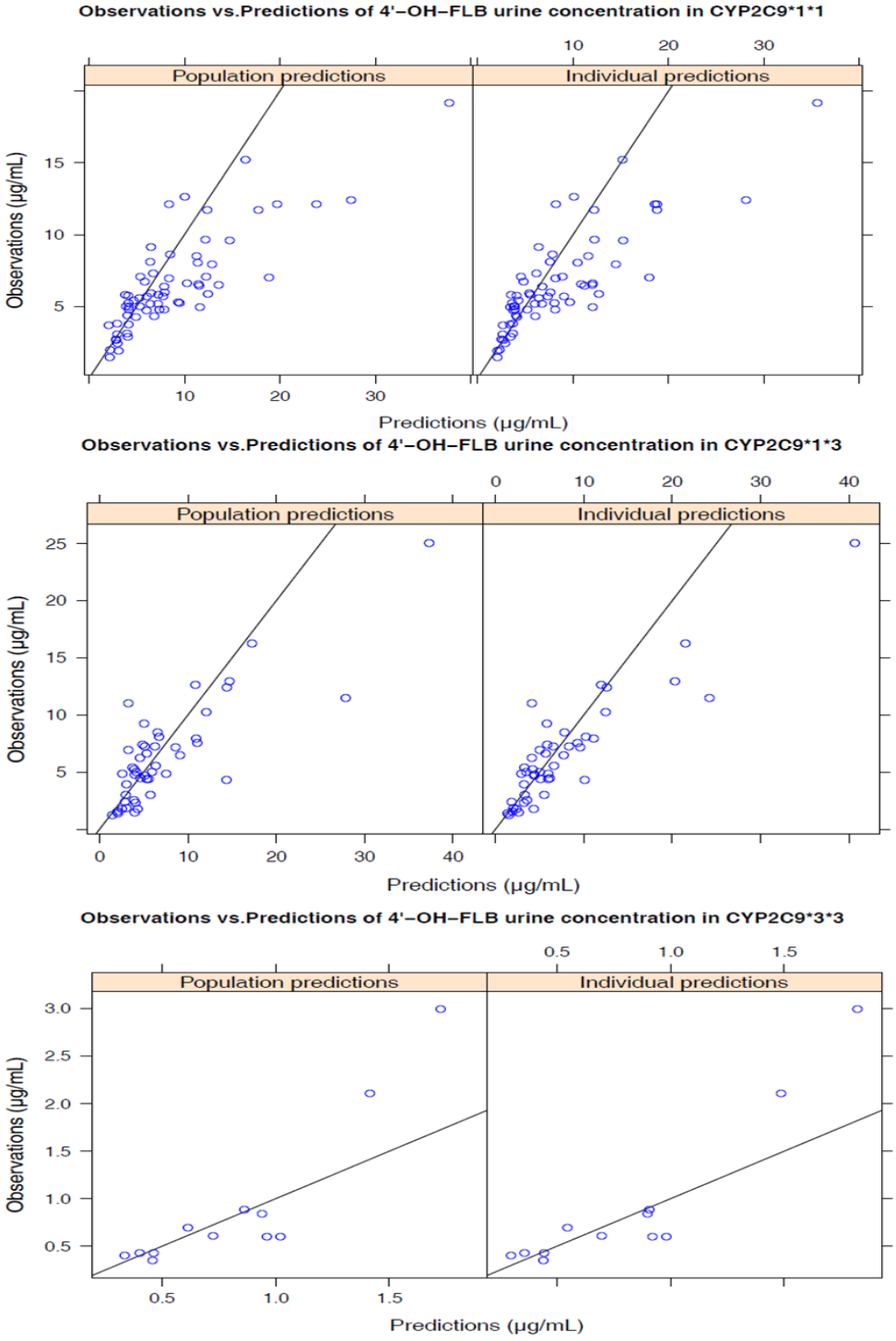


Figure 2.5 CWRES vs. Population Predictions (PRED) of FLB plasma concentrations across CYP2C9 genotypes

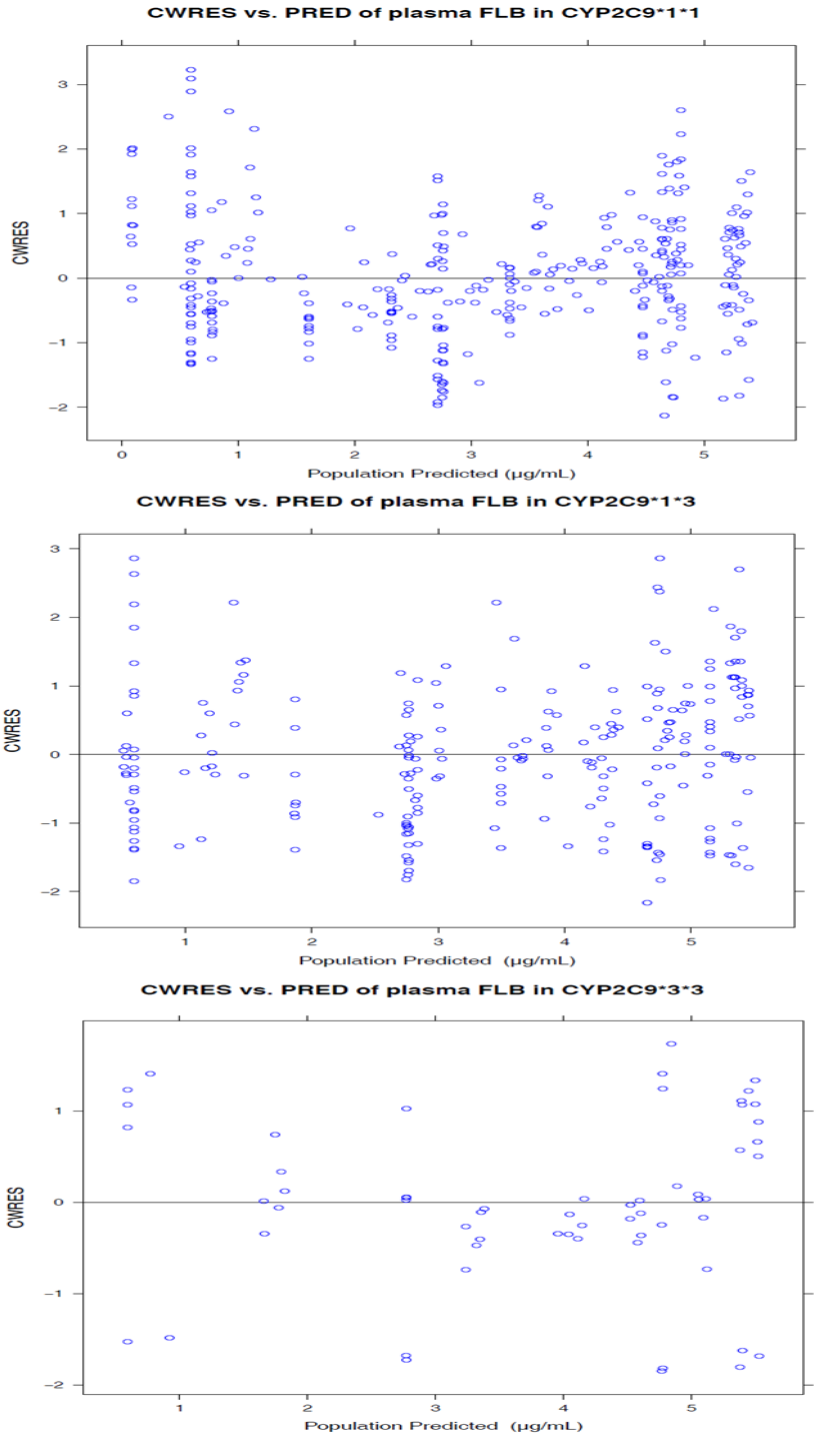


Figure 2.6 CWRES vs. Population Predictions (PRED) of flurbiprofen urine concentrations across CYP2C9 genotypes

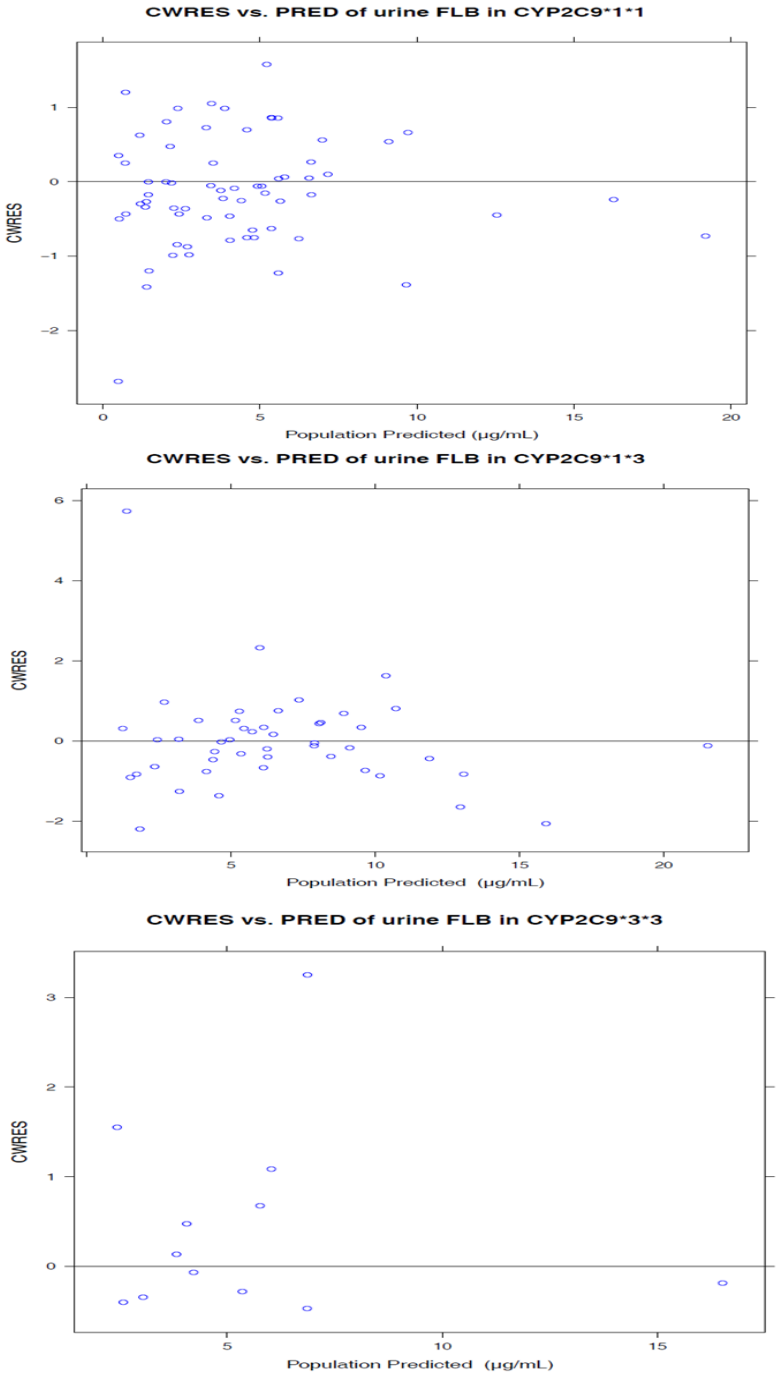


Figure 2.7 CWRES vs. Population Predictions (PRED) of 4'-hydroxyflurbirpofen (4'-OH-FLB) urine concentrations across CYP2C9 genotypes

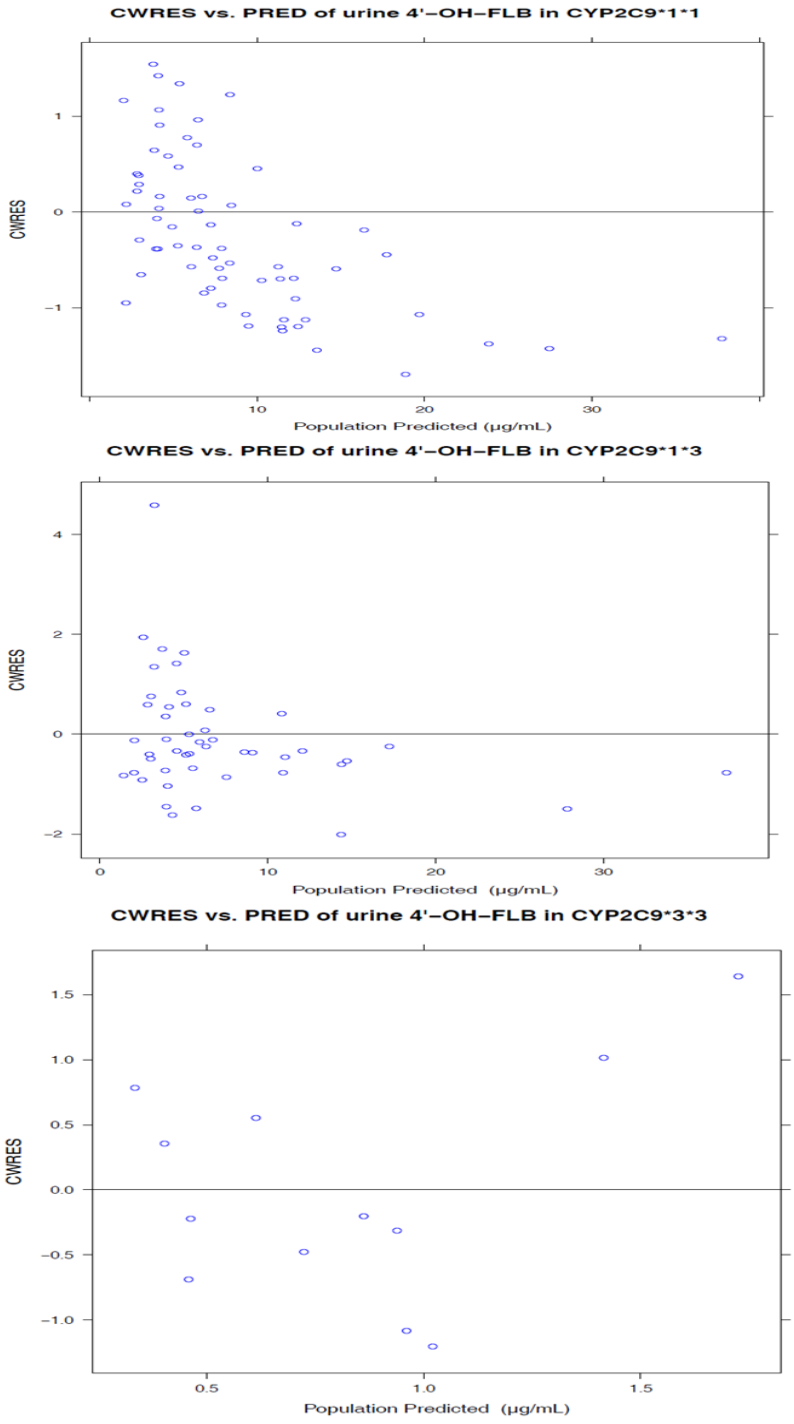


Figure 2.8 CWRES vs.Time of flurbiprofen (FLB) plasma concentration across CYP2C9 genotypes

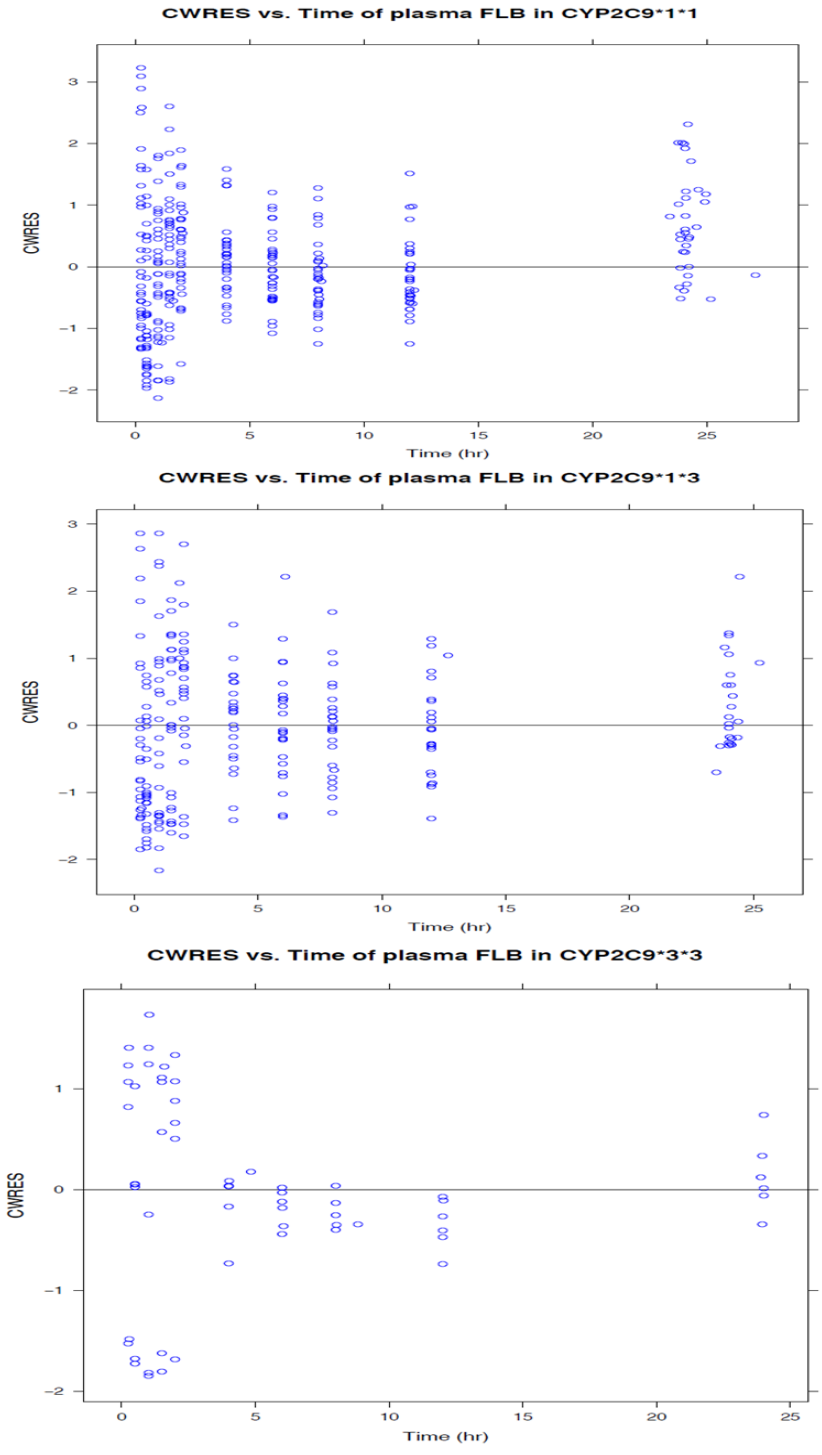


Figure 2.9 CWRES vs.Time of flurbiprofen (FLB) urine concentration across CYP2C9 genotypes

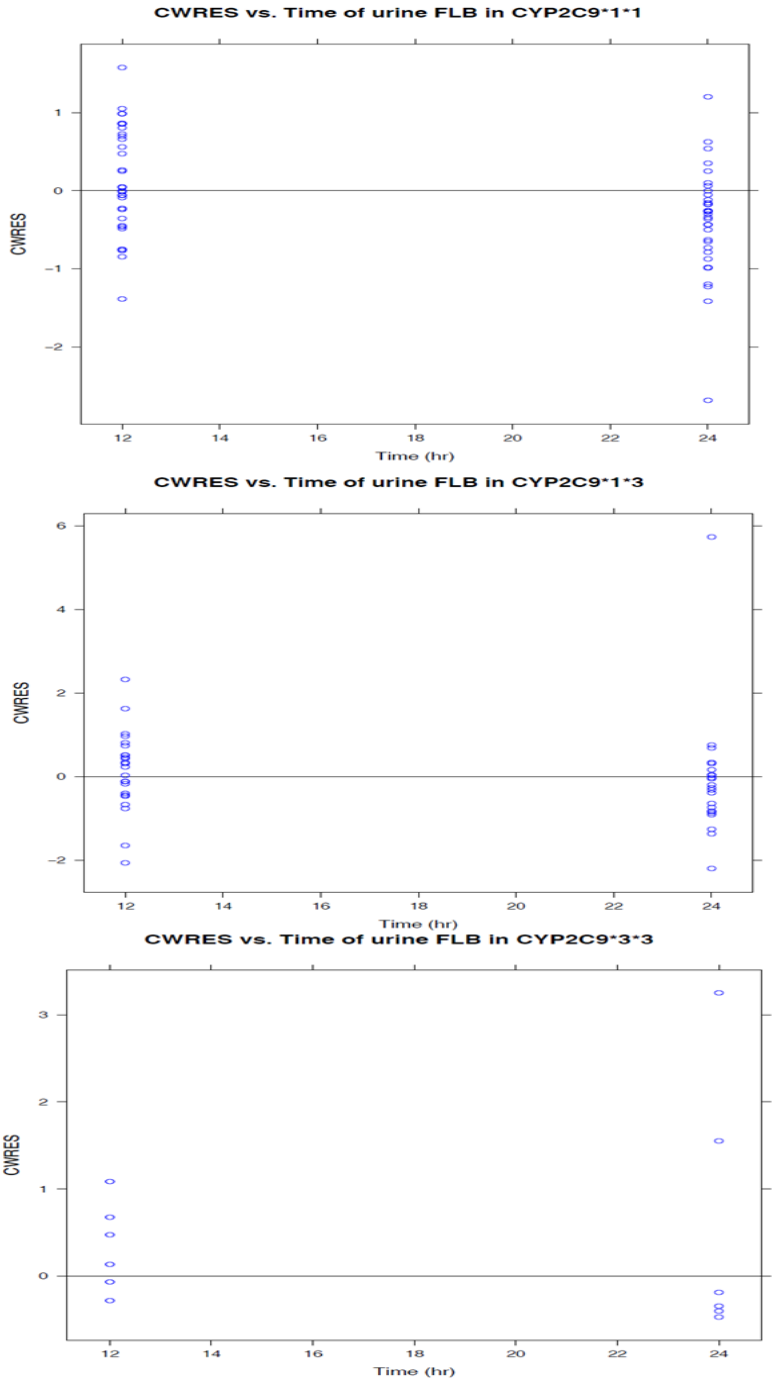


Figure 2.10 CWRES vs.Time of4'-hydroxyflurbiprofen (4'-OH-FLB) urine concentration across CYP2C9 genotypes

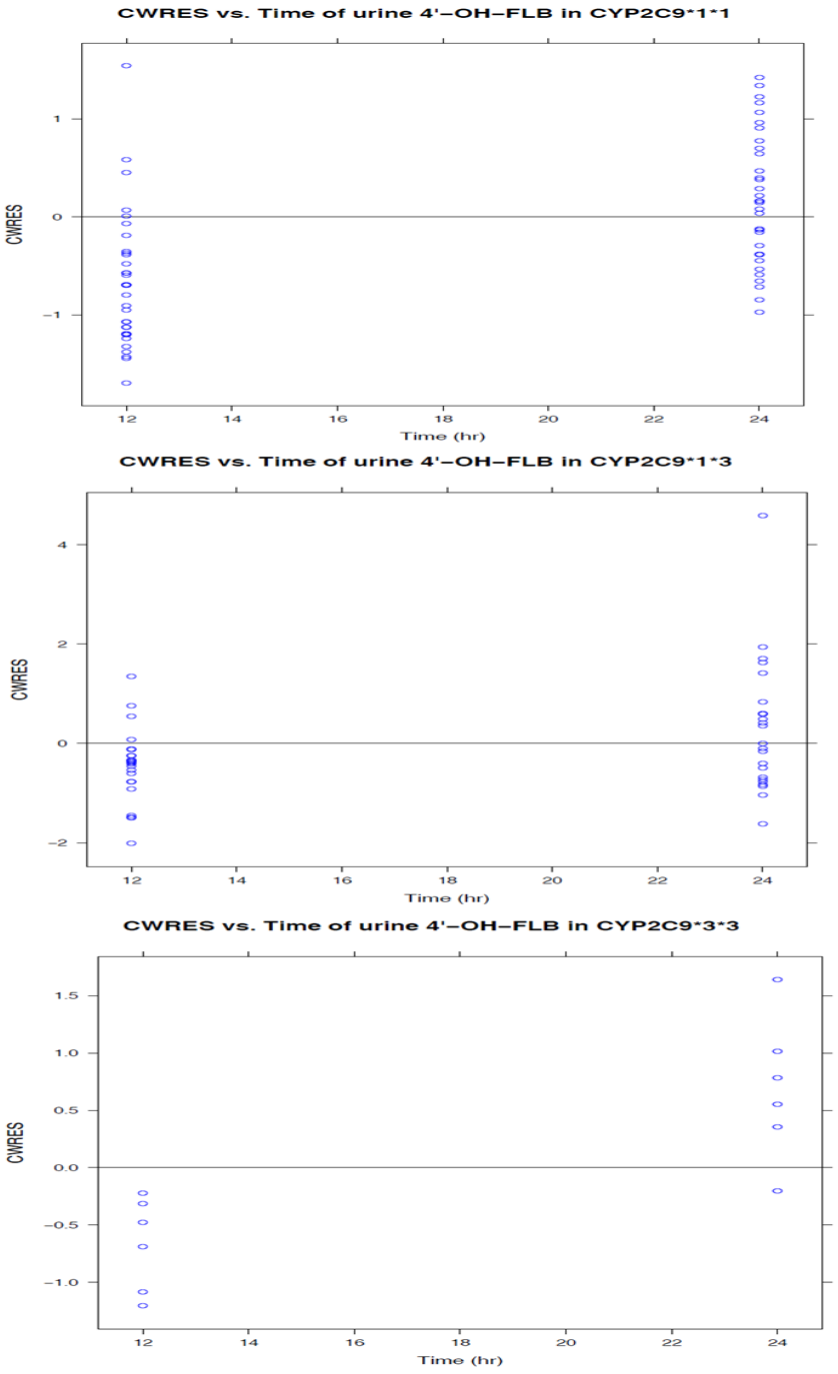


Figure 2.11 Visual predictive check (VPC) of flurbiprofen (FLB) plasma concentration in CYP2C9*1*1

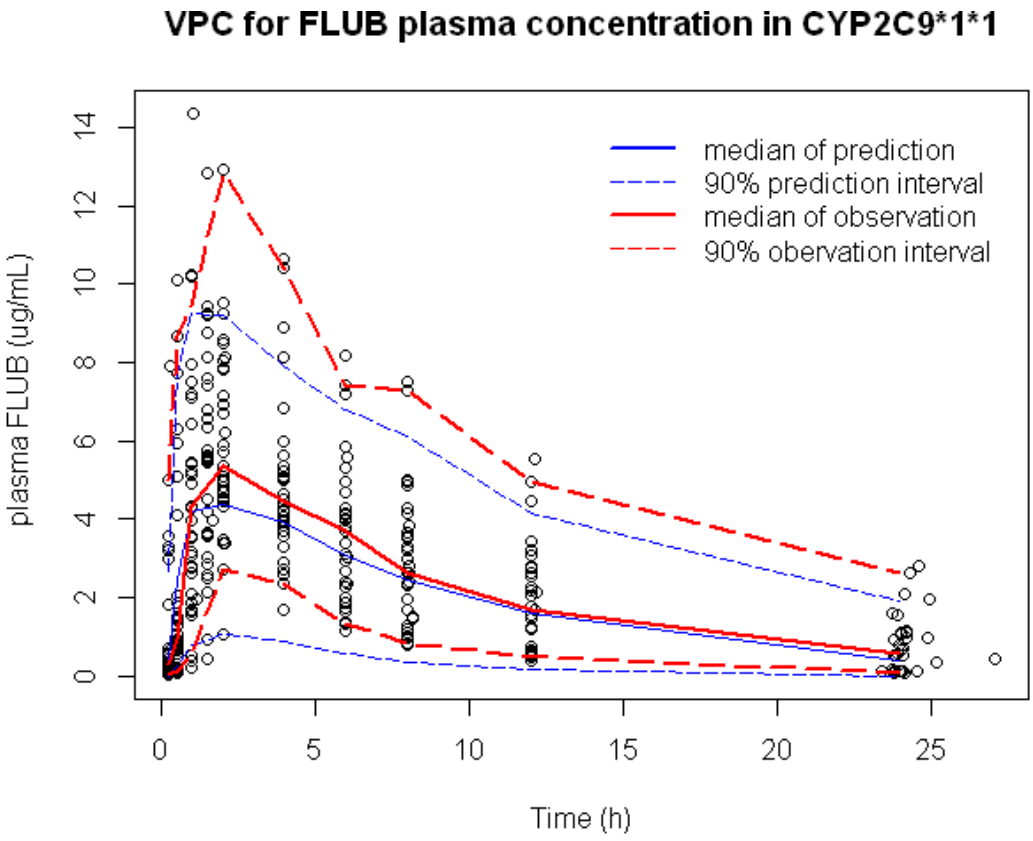


Figure 2.12 Visual predictive check (VPC) of flurbiprofen (FLB) plasma concentration in CYP2C9*1*3

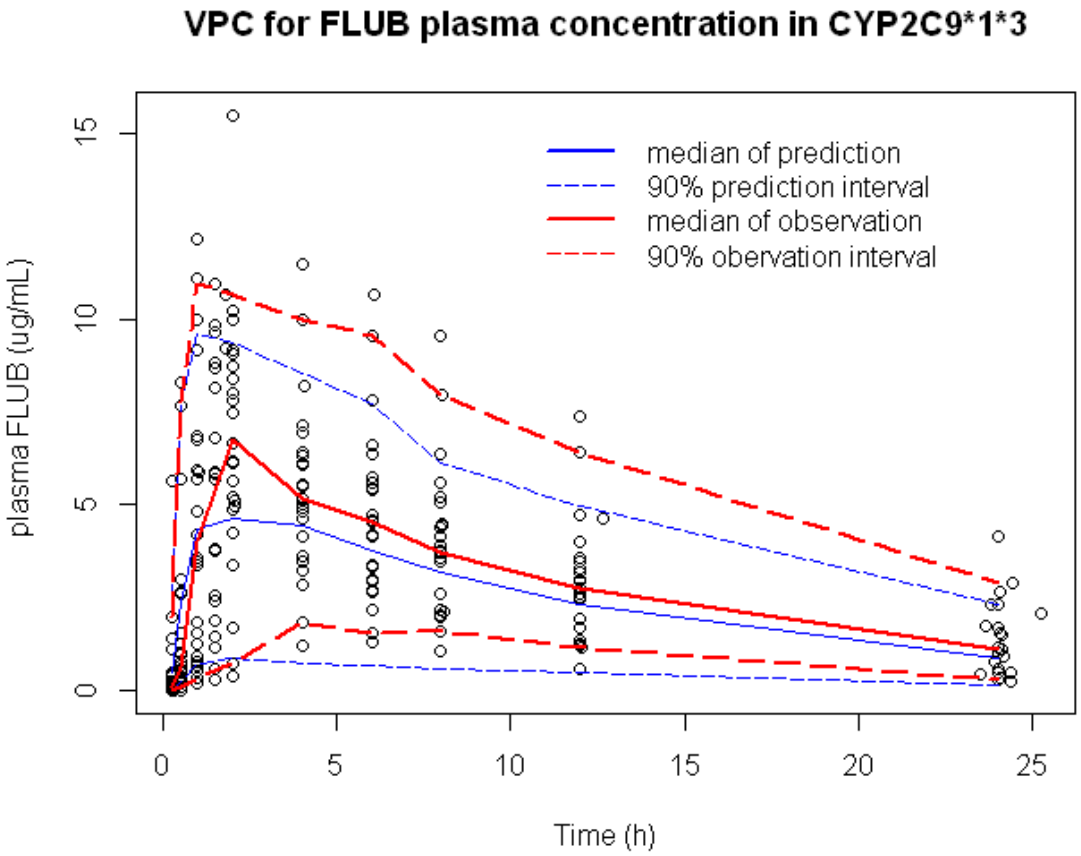
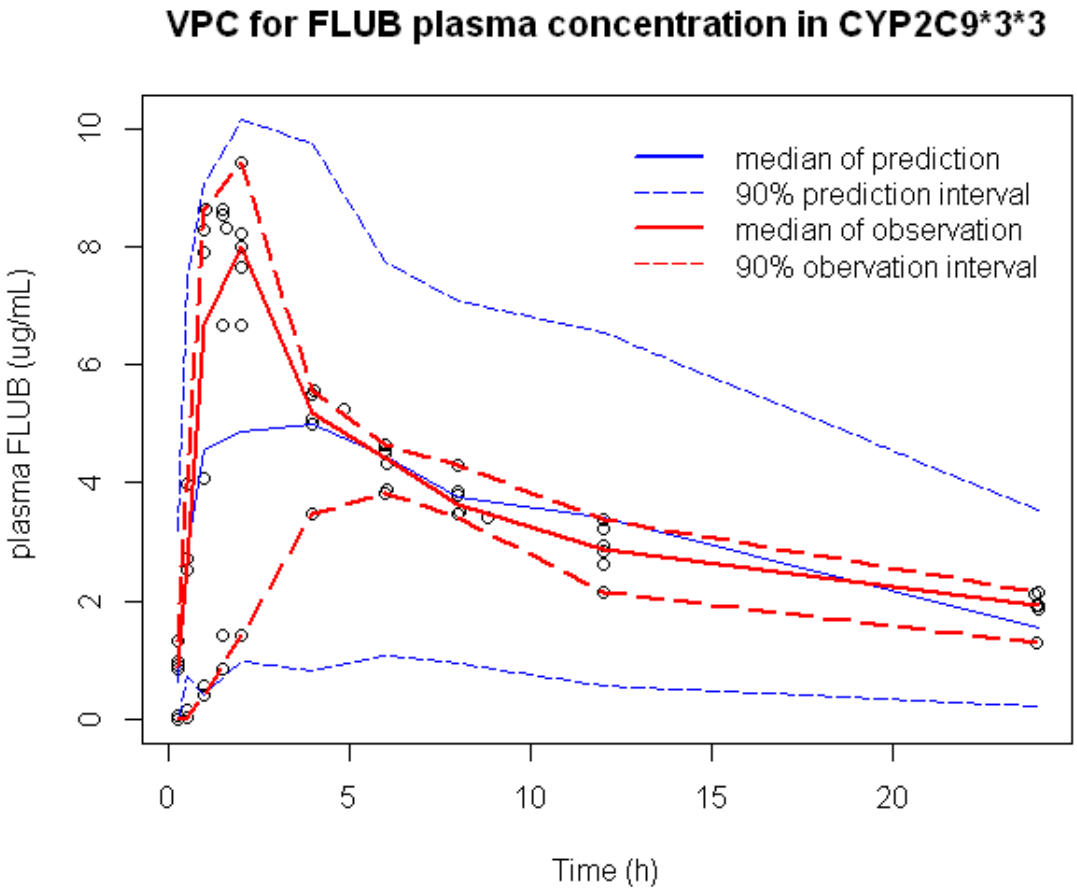


Figure 2.13 Visual predictive check (VPC) of flurbiprofen (FLB) plasma concentration in CYP2C9*3*3



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CHAPTER 3
Hepatitis C and Current Treatments

3.1 INTRODUCTION

3.1.1 Hepatitis C

Hepatitis C is a contagious liver disease caused by infection with the hepatitis C virus (HCV). Currently, it has been estimated that nearly 130-170 million people worldwide, approximately 3% of population, are chronically infected with HCV.[1–3] HCV is mainly transmitted through exposure to contaminated blood or needles. About 3 to 4 million people are newly infected by HCV every year.[1] Although not everyone carrying HCV develops chronic hepatitis, there is approximately a 60%-70% chance that a HCV infection will become chronic, leading to a high risk of progression to severe liver disease including cirrhosis, hepatic dysfunction and hepatocellular carcinoma.[1,4–6] In the United States, hepatitis C is the most common cause of liver transplantation and a major cause of death from liver disease.[7]

3.1.2 Hepatitis C Virus

HCV is a virus primarily infecting the hepatocytes and causing inflammation in the liver. It is a single positive strained RNA virus. HCV is reproduced through RNA-dependent-RNA replication. With better understanding of the viral life cycle, more efforts have been focused on developing novel agents which directly interfere with certain steps during the viral replication process.[8]

HCV is characterized by a broad variety of genotypes and subtypes. This heterogeneity makes HCV treatment and management challenging due to their different responsiveness to therapy.[9,10] At least six major genotypes have been identified and their distribution varies geographically. Genotype 1, genotype 2 and genotype 3 are commonly observed worldwide. Subtype 1a is predominating in North America and Northern Europe, and

subtype 1b is prevalent in Southern and Eastern Europe and Japan. [9,10] Genotype 3 is more common in Southeast Asia. Genotype 4 predominates in the Middle East, especially in the Egypt, and is also found in Central Africa.[9,10] Genotype 5 is primarily found in South Africa and genotype 6 distributes differently across countries in Asia. [9,10]

3.1.3 Current HCV Antiviral Treatments

Unlike hepatitis A or B virus, there is no vaccine available for preventing HCV infection. However, HCV is curable through the use of antiviral treatment. The primary goal of the treatment is to eradicate the HCV in the long term. The success of HCV therapy is established by achieving sustained virologic response (SVR, more details in Section 3.2). Monotherapy with interferon was initially used for treating HCV by its inhibitory effect on viral replication. Pegylated interferon was then formed by adding the polyethylene glycol to interferon to prolong the elimination half-life and reduce the clearance. Meanwhile, ribavirin was found to have a synergistic effect with interferon-based therapy by enhancing the viral elimination and decreasing virologic breakthrough and relapse with multiple mechanisms of action.[11,12] Until recently, the combination of peginterferon alpha (pegINF) and ribavirin (RBV) has been used as standard of care for chronic hepatitis among genotype 1 to 6.[13] The dual therapy of pegINF and RBV (PR) is generally administered for 48 weeks in patients with HCV genotype 1, 4, 5 or 6 infection and given for 24 weeks in patients with HCV genotype 2 or 3 infection.[13] Studies have shown SVR rates were 40-50% in patients with HCV genotype 1 infection and 70-80% in patients with genotype 2 or 3 infection.[14–18]

With the increasing knowledge on molecular pathways of the viral life cycle, more efforts have been focused on developing agents that directly act on certain targets during viral replication.[8] These direct acting agents (DAAs) are classified based on their action. One target is a nonstructural 3/4A (NS3/4A) serine protease essential for processing viral polyprotein to be mature and ready for further viral replication. Two protease inhibitors (PIs), telaprevir (Inciveck™, Vertex Pharmaceutical) and boceprevir (Victrelis™, Merck),

were approved by the US Food and Drug Administration (USFDA) and European Medicines Agency (EMA) in 2011 as a treatment for chronic HCV genotype 1 infection in adult patients. Both of them should be administered along with pegIFN and RBV, which enhance the suppression of HCV and reduce the development of viral resistance-associated mutation. With the addition of the PI, SVR rate is improved up to 68%-75% in treatment naïve patients and to 41%-52% in patients who failed to achieve SVR in previous PR treatment.[19–22] Furthermore, the duration of PI-based therapy could be adapted based on the viral response measured during the early stage of treatment by using a response-guided approach.[19–22] However, current PI-based therapy is accompanied by viral resistant mutation and more side effects. Findings from major Phase 2 and Phase 3 clinical trials of telaprevir and boceprevir are summarized in Table 3.1 and Table 3.2, and will be discussed in Section 3.4 and Section 3.5.

3.1.4 Scope of Current Research

Rationale

To better understand the therapeutic effect of antiviral treatment and minimize the potential risk, it has been recommended that patient HCV RNA levels be monitored during treatment. More data have emerged regarding the virologic response evaluated at various time points, typically at treatment weeks 4, 12, 24 and at the end of treatment as well as 24 weeks after treatment completion. Virologic responses assessed early in the treatment have been demonstrated to have a strong predictive value for attaining SVR during the follow-up period. Most clinical trials reported virologic response as the number of responders whose HCV RNA is undetectable at a given time. However, the evaluation of clinical efficacy of HCV antiviral treatment is generally based on the primary endpoint, SVR. Several published meta-analyses or systematic reviews compared the treatment effectiveness only according to the SVR data, while virologic responses at other time points were not included.[23–28] Such one-endpoint analysis might

be affected by the variability in treatment duration. In addition, it did not account for the information of virologic responses developed during treatment.

Objectives

The objectives of the current work were:

(1) to develop a statistical model describing the time course of longitudinal virologic response during treatment and follow-up periods across three HCV antiviral treatments: PR, telaprevir plus PR, and boceprevir plus PR.
(2) to investigate the potential influence of patient population characteristics (e.g., race and advanced fibrosis) and viral factors (e.g., HCV genotype and baseline viral level) on the response-time profile
(3) to compare response parameters across different antiviral therapeutic regimens

Approach

A longitudinal model-based meta-analysis was applied to provide a framework for the quantitative assessment of response data across different HCV antiviral treatments. The initial analysis was conducted in NONMEM 7 by using a maximum likelihood algorithm. However, the initial model had some limitations and ran into computational difficulties when trying to incorporate variability at multiple levels. Later, a Bayesian analysis was used with a Markov Chain Monte Carlo algorithm. A hierarchical nonlinear mixed-effect model was developed characterizing the longitudinal HCV response data with acknowledgement of variability at both arm and study levels.

3.2 HCV VIROGLOGIC RESPONSE IN CLINICAL TRIALS

Sustained virologic response (SVR) is the primary clinical endpoint indicating the success of HCV therapy. The endpoint has a strong correlation with enduring clearance of virus and a reduced risk of progression to severe liver disease with improved outcomes in patients.[29–31] SVR is defined as undetectable HCV RNA level 24 weeks after completion of therapy. Detectability of HCV RNA for defining SVR should be

determined through a sensitive assay with lower limit of detection of 50 IU/mL or less. Patient HCV RNA level has been recommended to be monitored at multiple time points along the course of therapy.[32,33] Recently, more data have emerged regarding assessments of treatment effectiveness during treatment and at the end of treatment shown in Figure 3.1 and Table 3.3. These endpoints provide more information about how patients typically respond to treatment and may also aid clinicians in modifying and optimizing the treatment strategy based on patient viral kinetics.[34] Generally, a more sensitive assay, with a lower limit of detection in the range of 10-15 IU/mL, is used to determine if HCV RNA is detectable when evaluating the virologic response during treatment and making decisions on response-guided therapy and treatment discontinuation. Furthermore, clinical studies have found the undetectable HCV RNA levels during treatment, e.g., at week 4 and 12, were significantly associated with the SVR during the follow-up period. Thus, SVR can be predicted given the knowledge of early response. In our study, we aimed to understand the evolvement of virologic response during treatment and to further relate it to the primary clinical outcome, SVR.

Virologic breakthrough occurs with reappearance of detectable HCV RNA while on treatment. The incidence of breakthrough could rise with reduced doses of ribavirin or peginterferon alpha in PI-based therapy, or patient non-adherence to the treatment. The emergence of HCV mutations with resistance or a reduced sensitivity to PIs are major contributing factors of virologic breakthrough in PI-based therapy.

Null response and *partial response* are usually used to describe the treatment-experienced patients who failed the previous PR therapy. Retreatment with the addition of telaprevir or boceprevir substantially improves the SVR rates in these patients.[20,22] In addition, the improvement is more significant in partial responders than null responders.[20,22]

Virologic relapse occurs when the HCV RNA level reappears as detectable during the follow-up period even with an undetectable level at the end of treatment. Among those

who failed to achieve SVR in previous PR therapy, patients with virologic relapse benefited the most from addition of a protease inhibitor (telaprevir or boceprevir) to retreatment, compared to prior null and partial responders.[20,22]

3.3 PREDICTORS FOR SVR

Although chronic hepatitis C can be cured by antiviral therapy, high medication cost, complicated treatment regimen and frequent adverse events make the treatment burden heavy in patients. The emergence of resistant mutations to DAA is another issue for newly approved triple therapy of protease inhibitors (telaprevir or boceprevir) plus PR.[35] It is important to recognize factors which are predictive of the treatment outcomes. These factors may guide the choice of initial treatment for a patient; they also provide information concerning adjustment of the treatment regimen for patients to maximize the therapeutic benefit and minimize the cost and risk.

The predictors for SVR can be categorized into several classes based on their sources: baseline patient (host) characteristics, baseline viral features, antiviral intervention and on-treatment virologic response. The first three classes of factors are usually determined before initiation of the treatment. These factors can help to select a more appropriate regimen for patients and identify patients who are more likely to respond to treatment. The virologic benchmarks assessed during the course of treatment can help to adapt the regimen with respect to the actual patient viral kinetics, which avoids unnecessary prolonged treatment and minimizes the potential risk while improving the clinical outcome.

In this section, several factors are presented that are potentially associated with SVR in HCV antiviral treatment. Some predictors for PR therapy could remain important for new PI triple therapy, whereas some might not be significant.

3.3.1 Viral Factors

Genotype

HCV genotype is one of the most important baseline predictors of the response to HCV antiviral treatment, especially in PR therapy. Genotype 1 has less responsiveness to PR therapy than does genotype 2 or 3. It usually requires a higher dose of ribavirin during a longer term of treatment than genotype 2 or 3.[13] Patients with chronic HCV genotype 2 or 3 infection usually receive a lower dose of ribavirin at 800 mg per day in PR therapy for 24 weeks, while patients with chronic HCV genotype 1are usually given a weight-based ribavirin dose of 1000 to 1400 mg per day in PR therapy for 48 weeks. PR therapy leads to a higher SVR rate (up to 80%) in patients with HCV genotype 2 or 3 infection than in those with genotype 1 (40%-50%).[13,14,36] Protease inhibitors, telaprevir and boceprevir, are approved by the FDA only for treatment of chronic HCV genotype 1 infection. One telaprevir study enrolled a small number of treatment-naïve patients with HCV genotype 2 and 3 infections. The telaprevir showed antiviral activity in patients with genotype 2 HCV but limited effect against genotype 3.[37] However, it appears less effective in these two genotypes compared to that in genotype 1.

Baseline Viral Load

Baseline viral load is another factor predicting the response in PR therapy. Patients with higher viral RNA levels (greater than 800,000 IU per ml or 200,000 copies per ml) are less likely to achieve SVR, possibly due to rapid viral replication and reproduction.[14,16,36] The predictive value of baseline viral load for SVR rate is also observed in PI triple therapy among patients with chronic HCV genotype 1 infection. In the SPRINT-2 study, boceprevir-based therapy led to SVR rates of 61% and 63% in patients with baseline viral levels more than 800,000 IU per ml, while SVR rates were 76% and 85% in those with viral loads less than 800,000 IU per ml.[21] The ADVANCE trial showed SVR rate of telaprevir arms were 78% in patients with baseline viral loads less than 800,000 IU per ml and 74% in baseline viral higher than 800,000 IU per ml.[19] Both phase 3 trials showed

that lower baseline viral load was associated with slightly higher SVR rate. However, the predictive effect of baseline viral load was not as significant as in PR therapy.

Virologic Responses During Treatment

The patient HCV RNA level is recommended to be monitored during treatment. A virologic response is defined as an undetectable HCV RNA level is achieved. Achieving a rapid virologic response at week 4 of treatment (RVR) is strongly associated with the success of SVR in PR therapy, even in patients with unfavorable baseline factors. It has been demonstrated as the most important predictor of SVR.[38–40] Although the predictive value of an early virologic response at week 12 (EVR) is less significant than RVR, failure to obtain the EVR strongly suggests little chance of having the SVR in PR therapy.[13,41,42] Accordingly, a negative EVR sometimes is used as one of the study futility rules to discontinue treatment in patients who are unlikely to achieve the SVR due to inadequate response to the treatment.[13]

Early on-treatment viral kinetics remains important in the context of PI triple therapy. Phase 3 studies of telaprevir confirmed the predictability of virologic response at week 4 and week 12 for SVR among treatment-naïve and previously treated patients[19,20], and suggested a therapy strategy guided by response.[19,43] Virologic response at treatment week 8 of boceprevir treatment was found to be significantly associated with SVR rate.[21,22] A response-guided strategy is also applied to guide the further boceprevir therapy among both treatment-naïve and treatment-experienced patients.[21,22,44] In addition, virologic response during lead-in PR therapy was also demonstrated as a strong predictor of SVR for the subsequent boceprevir therapy. [21,22] In the SPRINT 2 study, 81% and 79% of treatment naïve patients who had a lead-in response achieved the SVR in response guided boceprevir therapy and 48-week boceprevir-base therapy, respectively. The strong predictive effect of lead-in PR response on SVR even overcame the negative impact of undesirable baseline host (e.g., IL28B TT or CT genotype) or viral factors (e.g., high baseline viral level).[21] In the RESPONSE 2 trial among previously treated patients, the

odds ratio of the SVR rate in patients with lead-in response to those without the lead-in response was 5.2 ($p<0.001$). [22]

Monitoring viral kinetics during treatment also helps to determine treatment discontinuation in patients who are unlikely to achieve the SVR. It is important to recognize PI treatment futility to avoid unnecessary prolonged therapy and to reduce the potential risk of adverse events and further development of viral resistibility which may comprise future therapy. It is recommended that telaprevir treatment should be discontinued in patients whose HCV RNA levels are greater than 1000 IU/ml at week 4 or in patients who fail to have a 2 log drop in HCV RNA level by week 12 or in those who have detectable HCV RNA at week 24 of therapy.[45] Boceprevir treatment should be discontinued in patients with HCV RNA levels greater than 100 IU/ml at week 12 or in those who have detectable HCV RNA at week 24.[44]

3.3.2 Baseline Patient Characteristics

Race/Ethnicity

Clinical trials conducted in western countries reported that dual therapy of PR led to the SVR rates of 40%-50% and70%- 80% in patients with HCV genotype1 infection and in patients with genotype 2 or 3 infection respectively.[14–18] Remarkably higher SVR rates were reported from PR therapy trials in Asia as approximately 70% in genotype 1 and 90% in genotype 2 or 3 infection.[46] Furthermore, African Americans are less likely to achieve SVR during the PR therapy than non-African Americans. The SVR rates in African Americans were 19% -28% compared to 39%–52% in non-African-Americans.[47–51] Hispanic patients also have less chance of achieving SVR in PR therapy than non-Hispanic white patients.[52]

The addition of a PI (telaprevir or boceprevir) to PR therapy significantly improves the SVR rate in black patients; however, the SVR rate is still lower than that in nonblack

patients.[19,21] Telaprevir-based therapy led to 62% of SVR rate among black patients and 75% in nonblack patients, compared to 25% and 46% in PR alone. In the black cohort, SVR rates were 53% in 48-week boceprevir-based therapy, 42% in response-guided boceprevir therapy and 23% in PR therapy; whereas in nonblack cohort, SVR rates were 68%, 67% and 40% respectivelly.[21] It is still unclear how race/ethnicity affects the clinical outcome of HCV treatment. Race has been found to be correlated with the frequency of IL28B genotypes, suggesting that a lack of the favorable IL28B CC genotype in African Americans might be responsible for poor response in this subgroup.[53]

Liver Fibrosis

The progression to advanced fibrosis, e.g., bridging fibrosis or cirrhosis, is linked to failure in response to HCV treatment. Given the small number of patients with cirrhosis in most studies, the effect of advanced fibrosis on clinical outcome is not always found to be significant in the analysis. Among patients with HCV genotype 1 or 4 treated by PR therapy, the SVR rate was gradually diminished as 60% in patients without advanced fibrosis to 51% in patients with bridging fibrosis followed by 33% in those with cirrhosis.[54]

The combination of a PI (telaprevir or boceprevir) with PR therapy improved the SVR rate in patients with mild or advanced fibrosis. However, patients with advanced fibrosis, especially with cirrhosis, are still at a great risk of failure in response to PI triple therapy. Boceprevir-based treatment led to a SVR rate of 67% in patients with mild fibrosis (metavir score 0-2) while a SVR rate of 52% in patients with advanced fibrosis (metavir score 3 or 4), compared with PR therapy which led to a 38% SVR rates in both subgroups.[21] Sixty-two percent of patients with bridging fibrosis achieved SVR in telaprevir-based therapy, compared to 33% in PR therapy.[19] The likelihood of SVR in patients with minimal or portal fibrosis was dramatically increased by telaprevir and was higher than that in patients with advanced cirrhosis.[19] Still, the effect of advanced fibrosis

should be interpreted with caution in these studies due to the enrolment of a limited number of patients with severe fibrosis enrolled.

Polymorphism of IL28B

A genetic polymorphism identified near the human gene interleukin 28B (IL28B) encoding interferon lambda 3 gene is associated with the elimination of HCV and is a strong predictor of SVR in PR therapy. [53,55,56] The genome wide association study (GWAS) showed patients with the CC genotype achieved a higher SVR rate than those with TT genotype in PR therapy.[56] IL28B polymorphism was also associated with early on-treatment virologic response in PR therapy.[57] Varied distribution of genetic polymorphism of IL28B among races/ethnicity could partially explain the difference in treatment outcome in these groups of patients. [53] The C allele leading to greater HCV clearance has the highest prevalence in Asia, followed by Europe. Low frequency of the C allele and high frequency of the T allele in African American patients could contribute to the poor response to PR therapy.[53]

However, the predictive effect of IL28B polymorphism in PI triple therapy is not found to be as strong as that in PR therapy, but is still associated with response to the therapy. Knowing the patient IL28B genotype could help to predict the likelihood of achieving SVR and the possibility of shortening the PI-based treatment. From retrospective analysis of Phase 3 studies of boceprevir and telaprevir, the improvement in SVR rate by adding a PI to PR therapy was more substantial in treatment-naïve patients with the unfavorable T allele (e.g., CT or TT genotype) than was that in patients with CC genotype.[58,59] But the CC genotype was still associated with a higher SVR rate in PI-based therapy and early virologic response which might lead to a shorter term treatment. [58–60] Furthermore, IL28B genotype appeared to be associated with the responsiveness to lead-in PR therapy at week 4 of boceprevir treatment. However, the predictive effect of IL28B for SVR was diminished when considering the response at the end of lead-in PR therapy in boceprevir. The REALIZE trial of telaprevir among the treatment-experienced patients showed that

the SVR rates were comparable among groups of CC, CT and TT genotypes given one type of prior response to PR therapy. The predictive effect of IL28B genotype for SVR rate was not as strong as prior PR response in telaprevir retreatment.[61]

Response to Prior Interferon-based Therapy

Previous response to interferon therapy is a crucial determinant for predicting patient response to retreatment. It has been demonstrated that prior patient response to PR therapy is strongly associated with SVR rate of retreatment by adding a PI to PR therapy. PI-based treatment is given to retreat three major types of patients who failed previous PR therapy, which are null responders, partial responders and relapsers (definitions seen in Section 3.2.1). The addition of PI to PR therapy significantly improved the likelihood of SVR by 41%-52% in patients who did not response well to previous PR therapy.[20,22] However, the incremental benefit in SVR rate varies with respect to patient prior interferon response. Prior relapsers are most likely to achieve the SVR in retreatment of PI triple therapy, followed by prior partial responders. Null responders have the lowest SVR rate as compared to prior relapsers and partial responders. In the REALIZE trial, the SVR rate in two telaprevir arms were 83% and 88% in prior relapsers, 59% and 54% in partial responders and 29% and 33% in null responders .[20] For boceprevir, SVR rates were higher in prior relapsers (75% and 69% in two boceprevir arms) than in prior partial responders (52 % and 40% in boceprevir arms).[22]

3.4 CLINICAL TRIALS OF TELAPREVIR

Telaprevir is an inhibitor with specific binding to HCV nonstructural (NS) 3/4A serine protease which an essential factor catalyzing the HCV polyprotein cleavage during the polyprotein processing. The viral replication is thus inhibited by telaprevir. The drug is produced by Vertex Pharmaceuticals (Cambridge, Massachusetts, United States).[45] It was approved by the USFDA for treating chronic hepatitis C genotype 1 infection in adult patients who are treatment-naïve or have failed to respond to previous therapy of

interferon and ribavirin, including null responders, partial responders and relapsers. The labeled dose is 750 mg orally taken three times a day (every 7 to 9 hours) with food.[45] Telaprevir must be administered in combination with peginterferon alpha and ribavirin.[45]

The clinical efficacy and tolerability of telaprevir-based triple therapy has been established in several clinical trials including three Phase 2 studies (PROVE 1[62], PROVE 2[63], and PROVE 3[64]) and three Phase 3 studies (ADVANCE[19], ILLUMINATE[43] and REALIZE[20]). Among these studies, four (PROVE 1, PROVE2, ADVANCE and ILLUMINATE) were conducted in treatment-naïve patients and two (PROVE 3 and REALIZE) in patients previously treated but failing interferon-based therapy. In all studies, telaprevir was administered with peginterferon alpha-2a and ribavirin. Peginterferon alpha-2a was given at 180 μg per week by subcutaneous injection and ribavirin was orally taken at weight-based doses of 1000 mg per day in patients with body weight less than 75 kg or at 1200 mg per day in those with body weight greater than or equal to 75 kg. The assays used for quantifying the plasma HCV RNA levels during the trials had lower limit of quantification of 25 IU/mL and a lower limit of detection of 10 IU/mL.

3.4.1 Therapeutic Efficacy

In the Phase 2 studies, telaprevir was given in combination with PegINF and RBV for 12 weeks, followed by PR therapy alone for addition 12 weeks or 36 weeks, which made the whole treatment for 24 weeks (T12PR24) or 48 weeks (T12PR48). Studies showed the addition of telaprevir significantly improved the SVR rate up to 69% among treatment naïve patients, compared to less than 50% in the 48-week PR therapy alone.[62,63] Telaprevir-based therapy also yielded a substantial increase in SVR rate in treatment-experienced patients who failed to achieve the SVR in previous interferon-based therapy. The SVR rate was improved up to 53% among previously treated patients compared to 14% in 48-week PR therapy (PR48) alone.[64] Virologic relapse occurred less frequently in telaprevir-based therapy given for at least 24 weeks in total than that in 48-week PR

therapy. The treatment with only 12-week triple therapy of telaprevir plus PR led to a high virologic relapse rate. It suggests the extended PR therapy after telaprevir period helped to enhance the viral elimination and prevent future viral relapse when the whole treatment was completed.[62,63] Virologic breakthrough was seen more commonly in patients treated with telaprevir-based therapy. It was attributed to the variants with resistance to telaprevir. Telaprevir treatment without RBV was also evaluated and resulted in the diminished SVR rates and increased viral breakthrough. [63,64]

The Phase 3 studies further evaluated and confirmed the efficacy and safety of telaprevir in combination with PegINF and RBV. A response-guided therapy (RGT) was introduced to guide the telaprevir-based therapy. In the ADVANCE trial[19], telaprevir was administered in conjunction with PegINF and RBV for 8 weeks (T8PR) or 12 weeks (T12PR), and the subsequent PR alone therapy was determined based on patient HCV RNA levels measured at week 4 and week12. If patients achieved the eRVR (undetectable HCV RNA at both weeks 4 and 12), the PR therapy after the telaprevir period was given up to week 24. Otherwise, patients received a longer PR therapy until week 48. The study showed the superiority of telaprevir-based treatment with higher SVR rate over PR therapy alone. The SVR rates were 75% in the T12PR and 69% in the T8PR, significantly higher than 44% in the 48-week PR therapy (PR48). [19] Based on response-guided therapy, 57% and 58% of patients in the T8PR and T12PR groups achieved the eRVR, respectively, and 83% and 89% obtained the SVR, respectively. [19] It suggested the eRVR was a strong predictor for the SVR. The ILLUMINATE study further presented the robustness of eRVR-guided telaprevir treatment. After receiving 12-week telaprevir triple therapy, patients who had the eRVR were randomly assigned to receive the PR therapy for either 12 weeks or 36 weeks, leading to the whole treatment for 24 weeks (T12PR24) or 48 weeks (T12PR48). [43] A total of 65% of patients achieved the eRVR and received the RGT which led to SVR rates of 92% in the T12PR24 and 88% in the T12PR48. [43] In contrast, The SVR rate was only 64% in patients who did not

achieve the eRVR during the telaprevir period and received the PR therapy up to week 48. [43]The study suggested the telaprevir-based therapy could be shortened by using the RGT treatment without diminishing the therapeutic effect in treatment-naïve patients. [43]

The REALIZE study evaluated the telaprevir-based retreatment among patients who previously failed to achieve the SVR during the PR therapy. The study consisted of three treatment arms, one received telparevir triple therapy for first 12 weeks and PR therapy alone for additional 36 weeks (T12PR48), one received a 4-week lead in period of PR therapy followed by 12-week telaprevir triple therapy and 36-week PR therapy alone (Lead-in T12PR48), and the control arm received 48-week PR therapy (PR48). [20] The addition of telaprevir to PR therapy significantly improved the SVR rate in all these previous treated patients (64% in T12PR48 and 66% in Lead-in T12PR48 versus 17% in PR48). [20] Similar to the PROVE 3 study, the prior response to interferon-based therapy was strongly associated with the outcome of the telaprevir-based retreatment. The SVR rates for relapsers were 83% in the T12PR48 and 88% in the Lead-in T12PR48, were 59% and 54% for partial responders, respectively, and were 29% and 33% for null responders.[20] Relapsers retreated with telaprevir achieved the SVR rate as high as treatment naïve patients. Although there is no study directly evaluating the RGT telaprevir therapy in relapsers, the current label approved by FDA recommends the RGT guided by eRVR could be used in relapsers.[45] As in the Phase 2 studies, virologic relapse was less common in telaprevir combination therapy than in PR therapy, irrespective of treatment-naïve or treatment-experience patients.[19,20] Variants with resistance to telaprevir were observed in the telaprevir treatment.[19,20]

3.4.2 Tolerability

Since telaprevir is used in combination with pegINF and RBV, interferon-related adverse events are consistently observed in such combination therapy, such as fatigue and influenza-like symptoms. However, more adverse events were noted and attributed to telaprevir administration than PR therapy alone, including rash (56% in telaprevir versus

34% in PR), pruritus (47% versus 28%), nausea (39% versus 28%), anemia (36% versus 17%) and diarrhea (26% versus 17%).[65] Among these, rash, anemia and pruritus were the most common serious adverse events occurring during the telaprevir combination therapy with pegINF and RBV. In the Phase 2 and 3 clinical trials, treatment discontinuation due to adverse events occurred more frequently in telaprevir –based therapy versus that in PR therapy , 21% versus 11% in the PROVE 1 study[62], 12% versus 7% in the PROVE 2 study[63], 15% versus 4% in the PROVE 3 study[64], 10% versus 7% in the ADVANCE study[19], and 13% versus 3% in the REALIZE study. [20]

3.5 CLINICAL TRIALS OF BOCEPREVIR

Boceprevir is another DAA which directly targets the hepatitis C viral life cycle. Like telaprevir, it inhibits HCV polyprotein processing by specifically binding to the nonstructural protein NS3/4A serine protease. The drug is produced by Merck (New Jersey, United States). It is approved by the USFDA as a treatment for chronic hepatitis C genotype 1 infection in adult patients who are previously untreated before or who have failed previous treatment of interferon and ribavirin, including prior null responders, partial responders and relapsers. Boceprevir must be used in combination with peginterferon alpha and ribavirin. It is recommended to orally take 800 mg (four 200 mg capsules) three times a day (every 7 to 9 hours) with food.[44]

The clinical efficacy and tolerability of boceprevir-based therapy have been established in several clinical trials among treatment-naïve patients in a Phase 2 study (SPRINT-1)[66] and a Phase 3 study (SPRINT-2)[21] and among previously treated patients in a Phase 3 study (RESPOND-2).[22] In all clinical trials, boceprevir was administered with peginterferon alpha-2b and ribavirin. Peginterferon alfa-2b was subcutaneously injected at a dose of 1.5 μg/kg, and ribavirin was orally taken with a total dose of 600 mg to 1400 mg per day. The assays used for quantifying the plasma HCV RNA levels during the

trials had lower limit of quantification of 25 IU/mL and a lower limit of detection of 9.3 IU/mL.

3.5.1 Therapeutic Efficacy

The Phase 2 study SPRINT-1 consisted of two parts to evaluate the efficacy and safety of boceprevir-based therapy. The first part of the study included the boceprevir therapy with and without a 4-week lead-in PR therapy for 28 weeks (PR4/PRB24, PRB28) or 48 weeks (PR4/PRB44, PRB48). The second part of the study compared the efficacy of 48-week boceprevir-therapy with a standard dose (800-1400 mg per day) of ribavirin and a lower dose (400-1000 mg per day) of ribavirin. [66] All treatments containing boceprevir significantly improved the likelihood of achieving the SVR, compared to the 48-week PR therapy (PR48) alone. The SVR rates were 54% in PRB28, 56% in PR4/PRB24, 67% in PRB48, and 75% in PR4/PRB48, versus 38% in PR48. [66] Interestingly, the regimen including a lead-in PR period before the addition of boceprevir appeared to have the higher SVR rate and the lower rates of relapse and virologic breakthrough. The responsiveness to the lead-in PR therapy was introduced as having a drop in HCV RNA by at least 1.5-$\log_{10}$ at week 4, which was shown as a strong predictive factor for the SVR. Patients responding to the lead-in PR were more likely to achieve the SVR. [66] The second part of the study showed the reduced dose of RBV in boceprevir combination therapy was associated with a lower rate of SVR and higher rate of relapse compared to the therapy with standard dose of ribavirin, suggesting the crucial role of RBV in the boceprevir therapy.

In the Phase 3 studies, the boceprevir-based therapy with lead-in PR period was further investigated given with response-guided therapy (RGT) strategy and fixed term. In the SRPINT-2 study, treatment-naïve patients were randomized to one of three regimens. The control group received PR therapy alone for 48 weeks (PR48). Following the 4-week PR therapy period, one of boceprevir arms was given with boceprevir combined with PR therapy up to week 48 (PR4/PRB48). [21] The other boceprevir arm was adopted with RGT

strategy that patients who had the undetectable HCV RNA at week 8 through week 24 (i.e., early responders) received boceprevir plus PR therapy up to week 28; and patients whose HCV RNA level was detectable at week 8 or any subsequent visit but was still undetectable at week 24 (i.e., slow responders) continued with PR therapy until week 48 after boceprevir triple therapy stopped at week 28 (RGT-PRB). [21] Overall, 57% of the patients in both boceprevir arms and 17% in PR48 arm had the undetectable HCV RNA at week 8 during treatment. Among these patients, the SVR rates were 88% in the RGT-PRB, 90% in PR4/PRB48 and 85% in PR48. In contrast, among patients who did not show the early response at week 8, the SVR rates were 36%, 40%, and 30%, respectively. [21] This result suggests undetectable HCV RNA at week 8 is a strong predictive factor for a high SVR rate. Response to the lead-in PR therapy (defined as having at least 1-$\log_{10}$ drop in HCV RNA level at week 4) was also associated with higher SVR rate in both boceprevir treatment arms. [21] Theoretically, the lead-in PR therapy aid to decrease the HCV RNA level before the patient is exposed to boceprevir and to lower the risk of viral resistance. In addition, a lead-in regimen allows the assessment of the patient responsiveness, compliance and tolerability to PR which is the backbone of boceprevir triple therapy before the boceprevir is initiated. [21]

The boceprevir treatment was also evaluated in previously treated patients who failed to respond to PR therapy in the RESPOND-2 trial. In this study, the boceprevir treatment was guided by the viral responses at week 8 and week 12. After 4-week lead-in period, patients with undetectable HCV RNA levels at both weeks 8 and 12 completed the boceprevir triple therapy at week 36, and patients with detectable HCV RNA levels at week 8 but undetectable HCV at week 12 continued to receive PR therapy for additional 12 weeks up to week 48 after boceprevir triple therapy stopped at week 36. [22] The RESPOND-2 trial showed boceprevir combined with PR therapy significantly improved the SVR rate in prior partial responders and relapsers. The SVR rates were 75%, 69% and 29% in prior relapsers with PR4/BPR48, response-guided boceprevir treatment and PR48,

respectively, and 52%, 40% and 7% in partial responders in the corresponding treatment groups. [22] Like treatment-naïve patients, the study also showed the association of undetectable HCV RNA at week 8 with the success of SVR, suggesting the boceprevir therapy could be shortened based on virologic response at week 8 without jeopardizing the clinical outcome for treatment-experienced patients. It is noted that null responders were not included in the RESPOND-2 trial. An ongoing, open-label, single-arm trial recently evaluated the boceprevir therapy in prior null responders to interferon and ribavirin therapy (PROVIDE). [67] In this study, null responders received 4-week PR lead-in treatment followed by the triple therapy of boceprevir plus PR up to week 48. The SVR rate was significantly higher in boceprevir-based therapy than that in the PR48 arm as 38% versus 14%. [67]

3.5.2 Tolerability

Boceprevir-based therapy is associated with a higher rate of serious adverse events and more treatment discontinuation due to adverse events, although there were no new side effects reported from boceprevir. The most notable side effects in boceprevir treatment include anemia and dysgeusia compared to PR therapy. In the SPRINT-1 study, rates of dysgeusia and anemia were higher in groups receiving boceprevir treatment than that in PR group (27% vs. 9% for dysgeusia and 55% vs. 34% for anemia).[66] In the SPRINT-2 study, dysgeusia occurred more than twice frequently in boceprevir arms than in PR arm. Rates of anemia (hemoglobin<10 g/dL) were 49% for both fixed-duration and response-guided boceprevir treatment, compared to 29% in PR48.[21] In the RESPOND-2 trial among treatment-experienced patients, 45% of patients with boceprevir treatment and 20% patients with PR therapy reported anemia as adverse events and 20% of patients. [22]

Table 3.1 Findings from Phase 2 and Phase 3 studies of telaprevir and boceprevir in treatment-naïve patients with chronic hepatitis C genotype 1 infection

Trial	Intervention	Regimen group (N)	SVR (%)	P value	Relapse (%)
PROVE-1[59]	Telaprevir (PO): 1250 mg loading dose on 1st day, followed by 750mg every 8 hours; Peginterferon alfa-2a (SC): 180 μg once per week; Ribavirin (PO): 1000 mg (BW<75kg) or 1200 mg (BW≥75kg) per day	T12PR12 (N=17)	35	_	33
		T12PR24 (N=79)	61	0.02	2
		T12PR48 (N=79)	67	0.002	6
		PR48 (control, N=75)	41	_	23
PROVE-2[60]	Telaprevir (PO): 1250 mg loading dose on 1st day, followed by 750mg every 8 hours; Peginterferon alfa-2a (SC): 180 μg once per week; Ribavirin (PO): 1000 mg (BW<75kg) or 1200 mg (BW≥75kg) per day	T12PR12 (N=82)	60	0.12	30
		T12PR24 (N=81)	69	0.004	14
		T12P12 (N=78)	36	0.2	48
		PR48 (control, N=82)	46	_	22
ADVANCE[19]	Telaprevir (PO):750 mg every 8 hours; Peginterferon alfa-2a (SC): 180 μg once per week; Ribavirin (PO): 1000 mg (BW<75kg) or 1200 mg (BW≥75kg) per day	T8PR (N=363)	69	<0.001	9
		T12PR (N=364)	75	<0.002	9
		PR48(control, N=361)	44	_	28
ILLUMINATE[45]	Telaprevir (PO):750 mg every 8 hours; Peginterferon alfa-2a (SC): 180 μg once per week; Ribavirin (PO): 1000 mg (BW<75kg) or 1200 mg (BW≥75kg) per day	With eRVR, T12PR24 (N=162)	92	_	6
		With eRVR, T12PR48 (N=160)	88	_	3
		Without eRVR, T12PR48 (N=118)	64	_	11
SPRINT-1[64]	Boceprevir (PO): 800 mg three times per day; Peginterferon alfa-2b (SC): 1.5 μg/kg once per week; Ribavirin (PO): 800-1400 mg per day based on body weight as standard dose (SD) or 400-1000 mg per day base on body weight as low dose (LD)	PRB28 (N=107)	54	0.013	30
		PRB48 (N=103)	67	<0.0001	7
		PR4/PRB24 (N=103)	56	0.005	24
		PR4/PRB44 (N=103)	75	<0.0001	3
		PR48 (control, N=104))	38	_	24
		LD-PRB48 (N=59)	36	_	22
		SD-PRB48 (N=16)	50	_	11
SPRINT-2[21]	Boceprevir (PO): 800 mg three times per day; Peginterferon alfa-2b (SC): 1.5 μg/kg onceper week; Ribavirin (PO): 600-1400 mg per day based on body weight	PR4/PRB44 (N=366)	76	<0.001	9
		PR4/PRB (N=368)	71	<0.001	9
		PR48 (control, N=363)	53	_	22

Note: PO, orally; SC, subcutaneously; BW, body weight; eRVR: extended rapid virologic response; PR: peginterferon alpha and ribavirin; PR48: peginterferon alpha and ribavirin were given for 48 weeks; T12PR12, triple therapy of telaprevir combined with PR was given for 12 weeks; T12PR24, triple therapy of

telaprevir and PR was given for 12 weeks, followed by PR therapy alone for another 12 weeks; T12PR48, triple therapy of telaprevir and PR was given for 12 weeks, followed by PR therapy alone for another 36 weeks; T12P12, telaprevir was administered with only peginterferon alpha for 12 weeks; T8PR, telaprevir combined with PR was given for 8 weeks and the following term of PR-alone therapy was determined by patient viral response (eRVR); T12PR, telaprevir combined with PR was given for 12 weeks and the following PR-alone therapy the term of which was determined by patient viral responses at treatment weeks 4 and 12 (eRVR); PRB28, triple therapy of boceprevir and PR was given for 28 weeks; PRB48, triple therapy of boceprevir and PR was given for 48 weeks; PR4/PRB24, triple therapy of boceprevir and PR was given for 24 weeks after 4-week lead-in PR therapy; PR4/PRB44, triple therapy of boceprevir and PR was given for 44 weeks after 4-week lead-in PR therapy; LD-PRB48, boceprevir was administered together with peginterferon alpha and low-dose ribavirin for 48 weeks; SD-PRB48, boceprevir was administered together with peginterferon alpha and standard-dose ribavirin for 48 weeks. PR4/PRB, 4-week lead-in PR therapy was given, followed by triple therapy of boceprevir plus PR the duration of which was guided by patient viral response at treatment weeks 8 and 12.

Table 3.2 Findings from Phase 2 and Phase 3 studies of telaprevir and boceprevir in previously treated patients with chronic HCV genotype 1 infection

Trial	Intervention	Previous virologic response	Regimen group (N)	SVR (%)	P value	Relapse (%)
REALIZE[20]	Telaprevir(PO): 750 mg every 8 hours; Peginterferon alfa-2a (SC): 180 μg once per week; Ribavirin (PO): 1000 mg (BW<75kg) or 1200 mg (BW≥75kg) per day	total	T12PR48 (N=266)	64	<0.001	12
			Lead-in T12PR48 (N=264)	66	<0.001	12
			PR48 (control, N=132)	22	_	65
		null response	T12PR48 (N=72)	29	<0.001	27
			Lead-in T12PR48 (N=75)	33	<0.001	25
			PR48 (control, N=37)	5	_	60
		partial response	T12PR48 (N=49)	59	<0.001	21
			Lead-in T12PR48 (N=48)	54	<0.001	25
			PR48 (control, N=27)	15	_	0
		relapse	T12PR48 (N=145)	83	<0.001	7
			Lead-in T12PR48 (N=141)	88	<0.001	7
			PR48 (control, N=68)	24	_	65
RESPOND-2[22]	Boceprevir(PO): 800 mg three times per day; Peginterferon alfa-2b (SC): 1.5 μg/kg once per week; Ribavirin (PO): 600-1400 mg per day based on body weight	total	PR4/PRB44(N=161)	66	<0.001	12
			PR4/PRB (N=162)	59	<0.001	15
			PR48(control, N=80)	21	_	32
		partial responder	PR4/PRB44 (N=58)	52	_	_
			PR4/PRB (N=57)	40	_	_
			PR48 (control, N=29)	7	_	_
		relapse	PR4/PRB44 (N=103)	75	_	_
			PR4/PRB (N=105)	69	_	_
			PR48 (control, N=51)	29	_	_

Table 3.2 Continued

Trial	Intervention	Previous virologic response	Regimen group (N)	SVR (%)	P value	Relapse (%)
PROVE-3[61]	Telaprevir(PO): 1125 mg loading dose on 1st day, followed by 750 mg every 8 hours; Peginterferon alfa-2a (SC): 180 μg once per week; Ribavirin (PO): 1000 mg (BW<75kg) or 1200 mg (BW≥75kg) per day	total	T12PR24(N=115)	51	<0.001	30
			T24PR48(N=113)	53	<0.001	13
			T12P24(N=111)	24	0.02	53
			PR48(control,N=114)	14	_	53
		null response	T12PR24(N=66)	39	_	37
			T24PR48(N=64)	38	_	4
			T12P24(N=62)	11	_	68
			PR48(control,N=68)	9	_	40
		breakthrough	T12PR24(N=7)	57	_	0
			T24PR48(N=8)	62	_	20
			T12P24(N=11)	36	_	20
			PR48(control,N=5)	40	_	0
		relapse	T12PR24(N=42)	69	_	18
			T24PR48(N=41)	76	_	0
			T12P24(N=38)	42	_	46
			PR48(control,N=41)	20	_	62

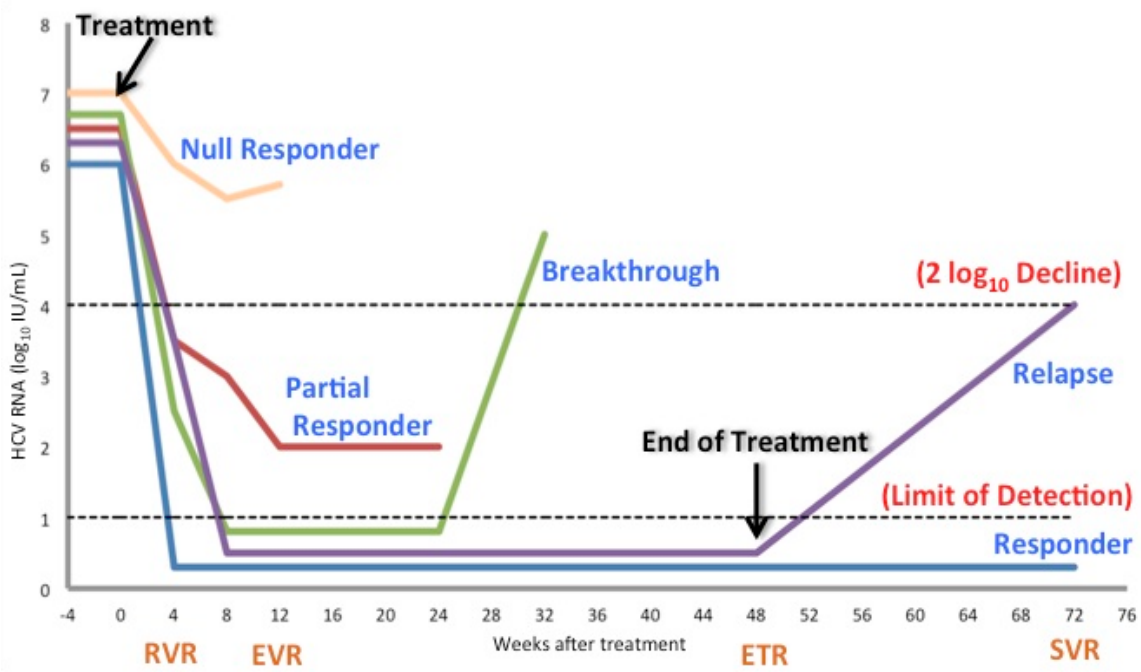
Note: PO, orally; SC, subcutaneously; BW, body weight; eRVR: extended rapid virologic response; PR: peginterferon alpha and ribavirin; PR48: peginterferon alpha and ribavirin were given for 48 weeks; T12PR24, triple therapy of telaprevir and PR was given for 12 weeks, followed by PR therapy alone for another 12 weeks; T12PR48, triple therapy of telaprevir and PR was given for 12 weeks, followed by PR therapy alone for another 36 weeks; T12P24, telaprevir was administered with only peginterferon alpha for 24 weeks; Lead-in T12PR48, a 4-week lead-in PR therapy was given, followed by 12-week telaprevir triple therapy and then PR therapy through treatment week 48; PR4/PRB44, triple therapy of boceprevir and PR was given for 44 weeks after 4-week lead-in PR therapy; PR4/PRB, 4-week lead-in PR therapy was given, followed by triple therapy of boceprevir plus PR the duration of which was guided by patient viral response at treatment weeks 8 and 24.

Table 3.3 Definition of virologic response

Virologic response	Definition
RVR (rapid virologic response)	Undetectable HCV RNA at TW 4
EVR (early virologic response)	Complete (cEVR): Undetectable HCV RNA at TW12; Partial(pEVR): At least 2-$\log_{10}$ drop in HCV RNA from baseline at TW12
eRVR(extended rapid virologic response)	Undetectable HCV RNA at both TW 4 and TW12 (telaprevir)
ETR(end-of-treatment response)	Undetectable HCV RNA at the end of treatment
SVR (sustained virologic response)	Undetectable HCV RNA 24 weeks after treatment completion
Null response	Less than 2-$\log_{10}$ drop in HCV RNA from baseline at TW12
Partial response	Detectable HCV RNA although with 2-$\log_{10}$ drop in HCV RNA from baseline at TW12
Virologic breakthrough	Reappearance of detectable HCV RNA while on therapy
Virologic relapse	Reappearance of detectable HCV RNA after treatment completion

Note: TW, treatment week; HCV RNA viral load should be assessed by using a sensitive assay with at least 50 IU/mL in lower limit of detection. Generally, telaprevir and boceprevir-based therapies use a lower limit of detection of 10-15 IU/mL to determine the "undetectable" HCV RNA during treatment when considering the response-guided therapy and treatment discontinuation.

Figure 3.1 Virologic response based on the course of treatment



Note: RVR, rapid virologic response; EVR, early virologic response; ETR, end-of-treatment response; SVR, sustained virologic response. Modified the figure from http://www.hepatitis.va.gov/provider/guidelines/2012HCV-definitions-of-response.asp

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CHAPTER 4
Systematic Review of Hepatitis C Clinical Trials

4.1 LITERATURE SEARCH

A broad systematic literature search was carried out in January 2012 in public databases including PubMed and clinicaltrials.gov. We also reviewed the Summary Basis of Approval available from the website of United States Food and Drug Administration and the equivalent approval documents from the European Medicines Agency for approved agents. The literature search was focused on telaprevir, boceprevir, and peginterferon plus ribavirin (PR). The key search terms included, but were not limited to: "chronic hepatitis C", "sustained virologic response", "hepatitis C virus", "peginterferon alfa","telaprevir" and "boceprevir". Various combinations of search terms were applied according to disease and intervention of interest, such as "telaprevir + chronic hepatitis C". We restricted the search to English-language reports of human clinical trials.
A flow diagram of systematic review was constructed according to the template from PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[19] in Figure 4.1. The initial search resulted in 101 records. Further inspection was given to the title and abstract of the literature, followed by a full-paper examination. Information of HCV clinical studies was reviewed, including study design, patient population, intervention, clinical outcomes, analysis and results. Treatments of interest included dual therapy of PR, and telaprevir and boceprevir combined with PR therapy. The virologic response/ treatment outcomes of interest for evaluating the HCV response included the number and percent of patients with undetectable HCV RNA measured at protocol-determined time points and SVR rate (24 weeks after the end of treatment).

Studies that met the following criteria were included for meta-analysis. (1) Studies should be human trials enrolling adult patients aged at least 18 years; (2) Patients were

diagnosed with chronic hepatitis C virus infection; (3) Study design can be randomized or non-randomized, open-label or blinded; (4) Studies should report at least one clinical outcome of HCV response; (5) Studies should evaluate and report clinical outcomes at protocol-determined time points; (6) Treatments should be administered for a standard or fixed term which have been specified in the protocol; (7) Patients should be given a standard dose of ribavrin and peginterferon alpha in PR or PI-based therapy; (8) The sample size of a treatment arm or study should be greater than 15.

Studies were excluded if trials were conducted in animal or in vitro or in patients who had undergone liver transplantation, or had other severe disease besides HCV, such as HIV and HBV, or had decompensated liver disease. Trials or treatment arms were excluded if the course of treatment was determined based on patients' early on-treatment virologic responses by using response-guided therapy. Treatment regimens without standard dose of medication (e.g., ribavirin or peginterferon alpha) were not considered in this meta-analysis. Other reasons for literature exclusion included pharmacokinetic studies, reviews, case studies, editorials, letters and meta-analyses.

4.2 DATASET CONSTRUCTION

Data pertaining to baseline characteristics of patient population, clinical outcomes and treatment strategy were extracted from published manuscripts. The information was usually summarized and reported for each of the treatment arms that were administered with different therapeutic regimens. Where possible, data were extracted for the intent-to-treat patients, generally defined as patients who underwent the randomization and received at least one dose of study medication. The numbers of patients were extracted according to patient demographics, such as race/ethnicity, and clinical characteristics, including prior treatment experience and advanced fibrosis. Data were also extracted on patient viral information, for example, baseline viral load and HCV genotype. Clinical outcomes were recorded as number of responders with undetectable HCV RNA level.

The sample size in each arm of the trial was retained in the dataset. Due to high variability in treatment strategies, information was abstracted on medication, start and end times of drug administration and the duration of the whole therapy. Data extraction was conducted by one researcher and independently reviewed by another researcher. All the data were synthesized to a dataset ready for the model-based meta-analysis.

4.3 DATASET FOR META-ANALYSIS

After screening the literature identified from the database, a total of 24 studies were eligible for further assessment. Of these 24 studies, six studies were excluded due to small sample size and use of response-guided therapy (RGT) without fixed course of treatment. Studies that were included in the analysis may have specific arms excluded if they met the exclusion criteria. The final dataset available for model-based meta-analysis consisted of 18 HCV clinical trials with 47 treatment arms in total. The median (range) number of patients in each study is 407 (32-2054), and the median (range) number of patients per treatment arm is 114 (16-1035). Sustained virologic response at 24 weeks after completion of treatment was reported in all trials while virologic response assessed at other time points were available to different extents across studies.

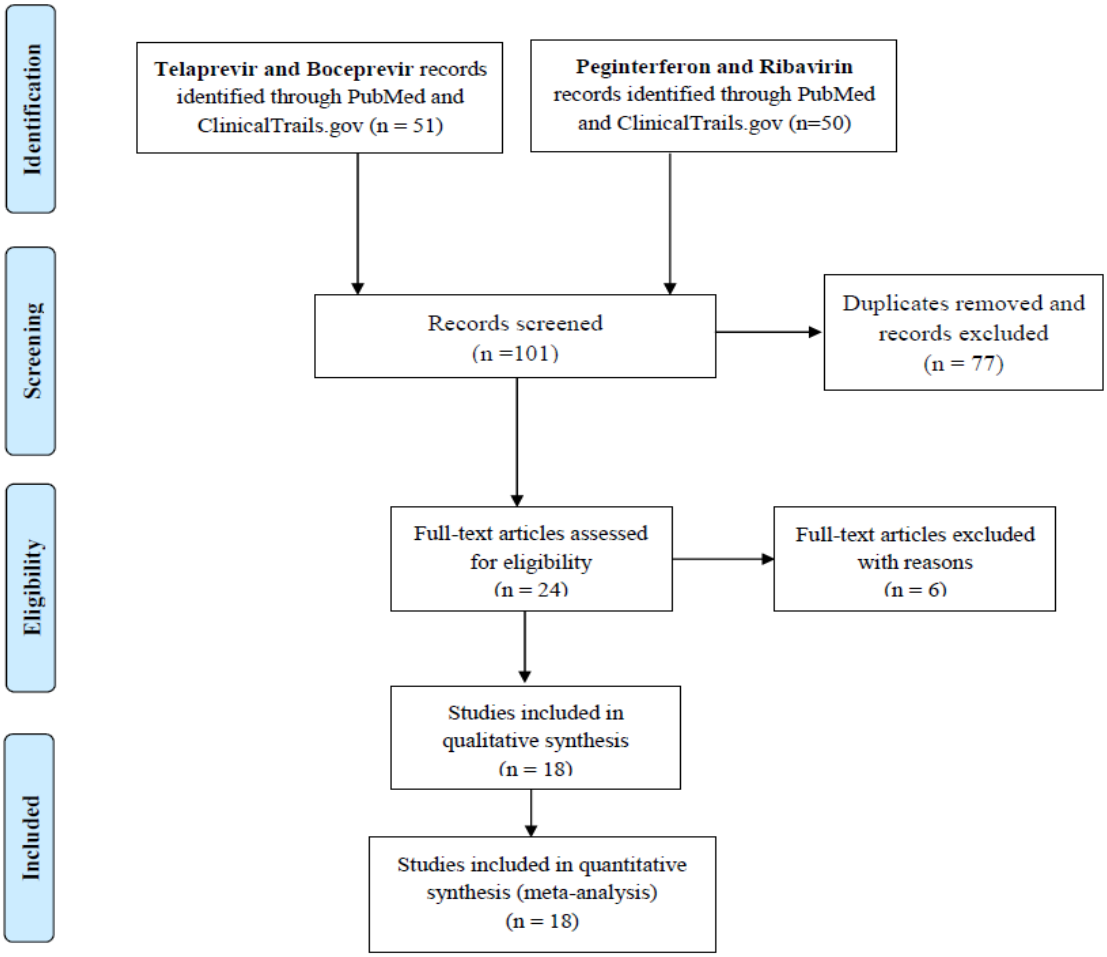
Among arms enrolled in the dataset, 27 arms received the dual therapy of peginterferon alpha and ribavirin, and 20 arms received the triple therapy of a protease inhibitor combined with PR (12 with telaprevir and 8 with boceprevir). In one PR therapy, peginterferon alfa-2a was subcutaneously injected at a dose of 180 μg once per week and ribavirin was orally taken at a weight-based dose of 1000 mg for patients with body weight less than 75 kg or 1200 mg for patients with body weight greater than or equal to 75 kg. For HCV genotype 2 or 3 infection, ribavirin was administered with a lower dose level at 800 mg. In another PR therapy, peginterferon alfa-2b was subcutaneously injected at a dose of 1.5 μg per kg per week and ribavirin is orally taken at weight-based dose of 800 to 1400 mg per day. Telaprevir was administered with an oral dose of 750

mg three times a day (every 7-9 hours) or a loading dose the first day followed by 750 mg every eight hours. [3-5] Telaprevir was used in combination with peginterferon alfa-2a and ribavirin. Boceprevir was administered at an oral dose of 800 mg three times a day (every 7-9 hours) together with peginterferon alfa-2b and ribavirin. Arms receiving a PI-based treatment but in the absence of ribavirin or with a low-dose ribavirin were not included in current analysis. PR therapy was generally given for 48 weeks in patients with genotype 1 and for 24 weeks in patients with genotype 2 or 3.The duration of the whole PI-based therapy varied from 12 weeks to 48 weeks. Any study or arm receiving response-guided therapy had been excluded. The current meta-analysis is only focused on treatments with fixed course which was previously determined by the protocol.

Twelve studies were conducted in treatment naïve patients who had not received any HCV treatment before, and six studies were in patients who had been previously treated by interferon-based therapy but failed to achieve the SVR. Most studies (n=12) only enrolled patients with chronic HCV genotype 1 infection. Two studies were focused on HCV genotype 2 or 3 infection and four studies enrolled patients infected with various HCV genotypes. Telaprevir and boceprevir are approved only for HCV genotype 1 infection, while, PR therapy is used for treating both genotype 1 and other genotypes of HCV infection. To investigate the effect of treatment term, a covariate called “short term” was created as a binary data item with only two values of 1 and 0. For patients with genotype 1 HCV infection, treatments given for less than 48 weeks (standard term) were considered as a short-term therapy with 1as the value of this covariate; otherwise, treatments with 48 weeks were assigned with 0. For non-genotype 1 patients (genotype 2 or 3), 24-week PR therapy is the standard course for the treatment, while any treatment shorter than 24 were assigned with 1 as the value of the covariate “short term”. Baseline characteristics that potentially influence the treatment outcome were incorporated in the current dataset with respect to HCV genotype, race/ethnicity, prior HCV treatment experience (e.g., null responders, relapsers), fibrosis stage (bridging fibrosis or cirrhosis),

and baseline HCV RNA level. The effect of IL28B genotype was investigated separately in retrospective analyses of Phase 3 studies of telaprevir and boceprevir.[20-23] However, the information regarding IL28B genotype was not available in most enrolled studies. The current dataset did not incorporate the information of the patient IL28B genotypes, although IL28B may have significant effect on SVR and the time course of response. The covariates incorporated in the data set were summarized based on treatment arms in Table 4.2. The median (mean) proportion of black patients across all arms was 7.79% (11.3%), of patients with HCV genotype 1 infection was 100% (81.1%), of patients with bridging fibrosis or cirrhosis was 20.2% (20.8%), and patients with high baseline HCV RNA level was 82.9% (77.6%). Several studies were lacking of information regarding some of baseline population characteristics, such as black race [13,17,18] and advanced fibrosis[15]. During the analysis, these missing values were replaced with the corresponding median value. The study and relevant information in the analytic dataset were summarized in Table 4.2.

Figure 4.1 Flow chart of systematic review



Note: Among 6 full-text articles excluded with reasons, five studies were excluded because of using only response-guided therapy and one study was excluded due to sample size smaller than 15.

Table 4.1 Summary information of baseline characteristics in arms included in the analysis

Baseline Characteristics	Mean	Median	Range
Number of subjects	208	114	(16, 1035)
Patients with HCV genotype 1 infection (%)	81.1	100	(0, 100)
Prior null responder (%)	10.1	0	(0, 100)
Prior relapsers (%)	11.3	0	(0, 84.4)
Black patients (%)	11.3	7.79	(0, 100)
Patients with bridging fibrosis or cirrhosis (%)	20.8	20.2	(0, 49.6)
Patients with high baseline HCV RNA level (%)	77.6	82.9	(20.6, 100)

Table 4.2 Summary of studies in dataset for meta-analysis

Study	Treatment Regimen	Treatment duration (weeks)	Short term (1: yes,0: no)	Patients (number)	Treatment-naïve patients (1: yes, 0: no)	HCV genotype 1 infection (%)	Prior null responders (%)	Prior relapsers (%)	Bridging fibrosis or cirrhosis (%)	Black (%)	High baseline HCV RNA (%)	Comments
Jacobson et al[1]	PR48	48	0	361	1	100	0	0	20.2	7.76	77.3	Two telaprevir arms using RGT were excluded
Zeuzem et al[2]	T12PR48	48	0	266	0	100	27.1	54.5	49.6	4.14	89.5	
	Lead-in T12PR48	48	0	264	0	100	28.4	53.4	47.3	3.03	88.6	
	PR48	48	0	132	0	100	28	51.5	44.7	8.33	86.4	
McHutchison et al[3]	T12PR24	24	1	115	0	100	57.4	36.5	39.1	7.83	92.2	T12P24 arm without ribavirin was excluded
	T24PR48	48	0	113	0	100	56.6	36.3	46.9	9.73	92	
	PR48	48	0	114	0	100	59.6	36	38.6	8.77	91.2	
McHutchison et al[4]	T12PR24	24	0	79	1	100	0	0	17.7	8.86	83.5	
	T12PR48	48	0	79	1	100	0	0	17.7	10.1	86.1	
	T12PR12	12	1	17	1	100	0	0	23.5	17.6	88.2	
	PR48	48	0	75	1	100	0	0	25.3	12	92	
Hezode et al[5]	T12PR24	24	0	81	1	100	0	0	11.1	1.23	90.1	T12P12 arm without ribavirin was excluded
	T12PR12	12	1	82	1	100	0	0	7.32	2.44	81.7	
	PR48	48	0	82	1	100	0	0	9.76	2.44	82.9	
Muir et al[6]	T12PR24	24	1	24	0	100	100	0	45.8	8.33	95.8	Arms receiving RGT were excluded.
	T12PR48	48	0	27	0	100	100	0	40.7	11.1	100	
Poordad et al[7]	PR48	48	0	311	1	100	0	0	7.4	0	83	Arms receiving RGT were excluded
	PR4/PRB44	48	0	311	1	100	0	0	11.6	0	84.2	
	PR48	48	0	52	1	100	0	0	1.92	100	96.2	
	PR4/PRB44	48	0	55	1	100	0	0	10.9	100	92.7	
Bacon et al[8]	PR48	48	0	80	0	100	0	63.8	18.8	15	81.3	RGT arm was excluded
	PR4/PRB44	48	0	161	0	100	0	64	19.3	11.8	87.6	
Kwo et al[9]	PR48	48	0	104	1	100	0	0	7.69	15.4	90.4	Boceprevir arm with low-dose ribavirin was excluded
	PR4/PRB24	28	1	103	1	100	0	0	6.8	14.5	87.4	
	PR4/PRB44	48	0	103	1	100	0	0	5.83	14.6	90.3	
	PRB28	28	1	107	1	100	0	0	6.54	16.8	91.6	
	PRB48	48	0	103	1	100	0	0	8.73	13.6	91.3	
	PRB48	48	0	16	1	100	0	0	0	25	81.3	

Table 4.2 Continued

Study	Treatment Regimen	Treatment duration (weeks)	Short term (1: yes,0: no)	Patients (number)	Treatment-naïve patients (1: yes, 0: no)	HCV genotype 1 infection (%)	Prior null responders (%)	Prior relapsers (%)	Bridging fibrosis or cirrhosis (%)	Black (%)	High baseline HCV RNA (%)	Comments
Shiffman et al[10]	PR16	16	1	372	1	0	0	0	27.7	3.2	78	
	PR16	16	1	358	1	0	0	0	22.6	2.8	60.3	
	PR24	24	0	369	1	0	0	0	20.3	0.813	62.9	
	PR24	24	0	356	1	0	0	0	25.3	5.05	75	
Fried et al[11]	PR48	48	0	453	1	65.8	0	0	12.4	6	64.7	Arm with interferon or low-dose peginterferon was excluded
Rodriguez-Torres et al[12]	PR48	48	0	569	1	100	0	0	11.4	0	75.6	
Manns et al[13]	PR48	48	0	511	1	68.1	0	0	26.6	NA	68.7	Arm with interferon or low-dose peginterferon was excluded
Hadziyanniset al[14]	PR24	24	0	96	1	0	0	0	20.8	0	64.6	
	PR24	24	0	118	1	100	0	0	22.9	0	39.8	
	PR24	24	0	144	1	0	0	0	27.1	0	67.4	Arms with interferon or low-dose ribavrin were excluded
	PR48	48	0	99	1	0	0	0	20.2	0	66.7	
	PR48	48	0	271	1	100	0	0	28.8	0	68.6	
	PR48	48	0	153	1	0	0	0	21.6	0	68.6	
Kumada et al[15]	T12PR24	24	0	126	1	100	0	0	NA	0	20.6	
	PR48	48	0	63	1	100	0	0	NA	0	28.6	
McHutchison et al[16]	PR48	48	0	1019	1	100	0	0	10.9	18	82	Arm with low dose peginterferon was excluded
	PR48	48	0	1035	1	100	0	0	10.6	19.3	82.3	
Husa et al[17]	PR48	48	0	203	0	100	43.3	51.7	9.85	NA	39.4	
Herrine et al[18]	PR48	48	0	32	0	78.1	0	84.4	28.1	NA	56.3	

Note: RGT, response-guided therapy; PR: peginterferon alpha and ribavirin; PR48: peginterferon alpha and ribavirin were given for 48 weeks; T12PR12, triple therapy of telaprevir combined with PR was given for 12 weeks; T12PR24, triple therapy of telaprevir and PR was given for 12 weeks, followed by PR therapy alone for another 12 weeks; T12PR48, triple therapy of telaprevir and PR was given for 12 weeks, followed by PR therapy alone for another 36 weeks; T12P12, telaprevir was administered with only peginterferon alpha for 12 weeks; T12P24, telaprevir was administered with peginterferon alpha for 12 weeks, followed by 12-week peginterferon alone; PRB28, triple therapy of boceprevir and PR was given for 28 weeks; PRB48, triple therapy of boceprevir and PR was given for 48 weeks; PR4/PRB24, triple therapy of boceprevir and PR was given for 24 weeks after 4-week lead-in PR therapy; PR4/PRB44, triple therapy of boceprevir and PR was given for 44 weeks after 4-week lead-in PR therapy; Short term: For patients with genotype-1 infection, PR therapy, telaprevir and boceprevir therapy

were considered as a short-term therapy if treatment was given less than 48 weeks, while, for patients with genotype 2 or 3 infection, PR therapy was considered to be short if less than 24 weeks.

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CHAPTER 5

Initial Model-Based Meta-Analysis of Virologic Response in Hepatitis C Clinical Trials Using NONMEM

5.1 INTRODUCTION

Hepatitis C is an infectious liver disease which is associated with a high risk of cirrhosis, hepatic dysfunction and hepatocellular carcinoma.[1–4] Recently, it has been estimated that approximately 170 million people worldwide and 3 to 4 million people in the United States are chronically infected with hepatitis C.[1,5–7] Although there is no vaccine available to prevent the hepatitis C disease, it can be cured by antiviral treatments. The success of treatment is presented by achievement of sustained virologic response (SVR) at week 24 after completion of treatment. The combination therapy of pegylated interferon and ribavirin (PR) has been used for decades as a standard of care for chronic hepatitis C. It lead to a 70%-80% SVR rate in patients with chronic HCV genotype 2 or 3 infection, but only 40%-50% in those with genotype 1.[8–13] With the increasing knowledge in viral replication, more research interest and efforts have been focused on developing drugs which can target directly certain steps of viral life cycle.[14] In 2011, United States Food and Drug Administration (USFDA) approved the first two direct-acting antiviral agents, telaprevir and boceprevir. [15,16] These two drugs are the inhibitors specifically for NS3/4A protease which is needed for translating and processing the viral polyproteins. Both of these two protease inhibitors (PI) are required to be administered in combination with peginterferon and ribavirin for treating chronic hepatitis C genotype 1 infection in adult patients. These new "triple therapies" dramatically improved the SVR rate to 68-75% in patients chronically infected with HCV genotype 1.[17–20]

To better understand the therapeutic effect of antiviral treatment and prevent the potential risk, it has been recommended that patient HCV RNA level be monitored along the

therapy at various time points, such as, treatment week 4, 12, 24 and at the end of treatment. Virologic response is defined and reported in clinical trials as the number or percentage of patients with undetectable HCV RNA at a given time point. The efficacy of HCV antiviral treatment is frequently evaluated based on the primary efficacy endpoint, the SVR rate evaluated at 24 weeks after completion of treatment. Recently, meta-analyses and systematic review have been performed to provide an indirect comparison in therapeutic effectiveness across current antiviral treatments.[21–26] However, these analyses only included primary efficacy endpoint, the SVR rate. Virologic response evaluated at other time points was not taken into consideration. A one-endpoint analysis could be affected by inconsistence in treatment duration. The response from 24-week treatment could be different from that 48-week treatment. In addition, the risk of bias may arise from potential heterogeneity among studies, including differences in study design and study population. Moreover, the development of virologic response during the treatment period could be different among different treatments.

In the current study, a model-based meta-analysis (MBMA) was employed to integrate the longitudinal information of virologic response from multiple clinical trials. It permits us to quantitatively describe the time course of virologic response and to investigate the comparative effect of current HCV antiviral treatments on response-time profiles. Given the nature of meta-analysis, potential variability arises when pooling the data from different sources. Both inter-study variability and inter-arm variability should be considered. A previous study by Ahn and French presented how multiple random effects in meta-analysis were handled using NONMEM software for nonlinear mixed effects modeling.[27] In this chapter, we explored the MBMA of longitudinal HCV virologic response in NONMEM using a maximum likelihood estimation algorithm.

The objectives of the current study were to develop a mathematical model describing the time-course of longitudinal virologic response and compare the response-time profile between PI-based therapy and PR therapy. The study also explored multiple ways to

incorporate and estimate the inter-study variability and inter-arm variability. Potential covariates (e.g., HCV genotype) were evaluated for their effects on parameters of time-course of response.

5.2 METHODS

5.2.1 Literature Review and Dataset Construction

The literature search and systematic review of published HCV clinical trials were described in Chapter 4. Information has been abstracted from literature including longitudinal virologic response rates, baseline patient characteristics and treatment regimen. The dataset developed for model-based meta-analysis was summarized in Chapter 4. Additional details regarding the study selection and dataset construction were provided in Chapter 4.

5.2.2 Model Development

The longitudinal model-based meta-analysis was implemented by means of a nonlinear mixed-effects approach using NONMEM version 7 (ICON Development Solutions, Ellicott City, MD, USA). NONMEM was initiated to run through an R package "metrumrg" (Metrum Research Group, Tariffville, CT, USA) installed in the statistical software R (version 3.0). [28] Data management, graphic exploration, post-processing of NONMEM output and simulations were performed using R with the interface software RStudio (version 0.97.449, RStudio.Inc, Boston, MA, USA). Model development and selection were guided by research interest, exploratory data analysis, change in objective function value (OFV) from importance sampling, and improvement in the diagnostic plots. A visual predictive check was conducted based on treatments to assess whether predictions from final model were consistent with observations.

The response data collected in the meta-dataset was the number of patients who responded to the treatment with HCV RNA level below detection limit at a given time.

The number of responder (Y_{ijs}) at time *s* in arm *j* in study *i* is considered to follow a binomial distribution that depends on the sample size in arm *j* in study *i* and the response rate at time *s* in the arm *j* in study *i* ($P_{resp,ijs}$) as described in Equation 5.1.

$$Y_{ijs} \sim Binomial\ (P_{resp,ijs}, N_{ij}) \quad (5.1)$$

The observed response rate was calculated using number of responders divided by sample size in the corresponding arm. Data visualization was achieved by graphic exploration. Figure 5.1 shows how the observed response rate changed over time according to dual therapy of PR and triple therapy of a PI (telaprevir or boceprevir) plus PR. According to the graph, the observed response rate increased dramatically in the beginning of the treatment. Most of arms seemed to reach an asymptote later during the treatment period. An asymptotic structural model was chosen to describe the time course of response rates as expressed in Equation 5.2.

$$P_{resp,ijs} = P_{max,ij} \times \left[1 - e^{\left(-k_{ij} \times (t_{ijs} - tlag_{ij})\right)}\right] \quad (5.2)$$

where $P_{max,ij}$, k_{ij} and $tlag_{ij}$ denote the maximal response rate, response onset speed and delayed time of response onset in the j[th] treatment arm of the i[th] study, respectively. The model assumed response start to onset until tlag that the probability of response was 0 when time < tlag.

After the treatment was completed, the only virologic response examined was the SVR rate measured at week 24 of the follow-up period. It was evaluated relative to the predicted response rate at the end of treatment ($P_{\mathrm{ETR,ij}}$) as in Equation 5.3.

$$P_{resp,ij} = P_{ETR,ij} * RR_{ij} = P_{max,ij} \times \left(1 - e^{\left(-k_{ij} * (EOT_{ij} - tlag_{ij})\right)}\right) \times RR_{ij} \quad (5.3)$$

Where EOT_{ij} and RR_{ij} denote the end of treatment time and response ratio of SVR rate to ETR rate in the j[th] treatment arm of the i[th] study, respectively. An effective HCV antiviral treatment is expected to have virus suppressed for a long term that the response rate after

completion of treatment does not decrease much. The RR is expected to be close to 1. The information regarding when the treatment was completed was collected from studies and retained in the dataset as a covariate called "EOT" (end of treatment).

Random effects were accommodated by incorporating the inter-study variability and inter-arm variability for each of parameters. The inter-arm variability was weighted by the sample size (N_{ij}) in the j[th] treatment arm of the i[th] study.

$$log(k_{ij}) = TVlogk + \eta_i^{study,k} + \eta_{ij}^{arm,k} \quad (5.4)$$

$$\eta_i^{study,k} \sim N\left(0, \omega_{study,k}^2\right), \quad \eta_{ij}^{arm,k} \sim N\left(0, \frac{\omega_{arm,k}^2}{N_{ij}}\right) \quad (5.5)$$

where the log-scaled k_{ij} is described in Equation 5.4 incorporating the population mean $TVlogk$ and inter-study random effect $\eta_i^{study,k}$ and inter-arm random effect $\eta_{ij}^{arm,\mathrm{k}}$. Random effects at study and arm levels are normally distributed with a mean of zero and variances to be estimated of $\omega_{study,\mathrm{k}}^2$ and sample-size weighted $\frac{\omega_{arm,k}^2}{N_{ij}}$, respectively, as shown in Equation 5.5.

$$logit(P_{max,ij}) = TVlogitP_{max} + \eta_i^{study,\,P_{max}} + \eta_{ij}^{arm,P_{max}} \quad (5.6)$$

$$\eta_i^{study,P_{max}} \sim N\left(0, \omega_{study,P_{max}}^2\right), \quad \eta_{ij}^{arm,P_{max}} \sim N\left(0, \frac{\omega_{arm,P_{max}}^2}{N_{ij}}\right) \quad (5.7)$$

where $P_{max,ij}$ is the maximum response rate with values between 0 and 1. The logit model of $P_{max,ij}$ in Equation 5.6 consists of the population mean $TVlogitP_{max}$ and inter-study random effect $\eta_i^{study,P_{max}}$ and inter-arm random effect $\eta_{ij}^{arm,P_{max}}$. Both of the random effects are normally distributed with a mean of zero and variances to be estimated of $\omega_{study,P_{max}}^2$ and $\frac{\omega_{arm,P_{max}}^2}{\mathrm{N}_{ij}}$, respectively, as shown in Equation 5.7.

$$log(RR_{ij}) = TVlogRR + \eta_i^{study,RR} + \eta_{ij}^{arm,RR} \quad (5.8)$$

$$\eta_i^{study,RR} \sim N\left(0, \omega_{study,RR}^2\right),\ \eta_{ij}^{arm,RR} \sim N\left(0, \frac{\omega_{arm,RR}^2}{N_{ij}}\right) \quad (5.9)$$

where the log-scaled RR_{ij} is modeled including the population mean $TVlogRR$ and inter-study variability $\eta_i^{study,RR}$ and inter-arm variability $\eta_{ij}^{arm,\mathrm{RR}}$ in Equation 5.8. Both of random effects are normally distributed with a mean of zero and variances to be estimated of $\omega_{study,\mathrm{RR}}^2$ and $\frac{\omega_{arm,\mathrm{RR}}^2}{N_{ij}}$, respectively in Equation 5.9.

Figure 5.1 presents the observed response rate according to PR therapy and PI-based therapy. It also reveals the triple therapy led to a significantly quicker response onset than did the PR therapy alone. Thus, treatment effect was evaluated on speed of response onset (k). Furthermore, exploratory analysis of the data found the treatment arms with HCV non-genotype 1 infection tended to higher response rate than those with genotype 1 infection (as shown in Figure 5.1). HCV genotype is also of interest for its effect on the magnitude of maximal response rate.

5.2.3 SAEM Estimation

The nonlinear-mixed effect model was developed in NONMEM 7 using stochastic approximation expectation maximization (SAEM) estimation method.[29,30] SAEM is one of the maximum likelihood estimation methods using expectation-maximization algorithms. The expectation of the log-likelihood can be achieved by stochastic approximation through Markov Chain Monte Carlo (MCMC).[30,31] Estimates of parameters are updated in the maximization step until a stable and improved objective function is achieved. The process is iterative until convergence is reached. Since SAEM involves a highly stochastic process, the objective function monitored and computed from SAEM cannot be used for model comparison and hypothesis testing. An importance sampling is usually performed on the final run of SAEM and provides an appropriate

objective function and standard error for model selection and evaluating the parameter precision.[29]

The execution of SAEM estimation in NONMEM 7 allows the user to define the convergence criteria. In this study, the convergence was examined according to the fixed parameters, random parameters and objective functions as CTYPE=3. This study uses 10,000 iterations in the burn-in period and 1,000 as the number of iterations to run after burn-in. Default settings were applied to the rest options regarding the implementation of SAEM.

5.3 RESULTS

5.3.1 Systematic Review Result and Analytic Dataset

The procedure of systematic review of literature of HCV antiviral treatment was described in Chapter 4. The model-based meta-analysis was conducted on longitudinal response data, arising from 18 studies and 47 arms in total. Twenty-seven arms received the dual therapy of peginterferon alpha and ribavirin, and twenty arms received the triple therapy of a protease inhibitor combined with PR (12 with telaprevir and 8 with boceprevir). The median (range) number of patients in each study is 407 (32-2054), and the median (range) number of patients per treatment arm is 114 (16-1035). Only arms with approved dosage of medication were enrolled. Studies or arms within the included study were excluded if they received response-guided therapy. The course of treatment and time points of response assessment had been determined in the study protocol. Information regarding baseline population characteristics and treatment strategies were also collected in the dataset. Among studies enrolled in the dataset, twelve studies were conducted in patients with HCV genotype 1 infection only, two studies in patients with HCV genotype 2 or 3 and four studies in patients with HCV various genotypes. PI-based triple therapy was administered only in patients with HCV genotype 1 infection, while PR therapy was given to those with genotype 1 or non-genotype 1infection. Further

information regarding the final dataset for meta-analysis was described and summarized in Chapter 4.

5.3.2 Longitudinal HCV Response Meta-Analysis

According to the exploratory graph (Figure 5.1), an asymptotic model was selected to describe the time course of HCV virologic response. Different models were attempted to explore the inter-arm and inter-study variability. Unfortunately, the covariance step always failed in the importance sampling step, although convergence was achieved during SAEM process. When both inter-study variability and inter-arm variability were added to all model parameters of interest (e.g., P_{max}, k and RR), the inter-study variability was significantly smaller in parameter RR than its inter-arm variability. The estimates of variance of inter-study and inter-arm variability in RR were 1.47E-17 and 3.7, respectively. However, the inter-study variability in P_{max} and k were comparable to their corresponding inter-arm variability. Thus, inter-study variability was retained for P_{max} and k, and inter-arm variability adjusted by sample size was estimated for each of these three model parameters. The resulting OFV in the reduced model was smaller than the previous model. The exploratory graph also revealed the significant difference in response-time profile between PI-based therapy and PR therapy and variation among treatment arms with different HCV genotypes. These covariates were explored for their potential effects on model parameters.

The proportion of patients with HCV genotype1 infection in a treatment arm was incorporated in the data set. The analysis found a negative effect of HCV genotype1 on the maximum response rate (P_{max}). Arms with more genotype 1 infected patients tend to reach lower Pmax during treatment. After accounting for the effect of HCV genotype, inter-study variability in P_{max} decreased dramatically and was estimated to be much smaller than the inter-arm variability. The variances of inter-study and inter-arm variability on P_{max} were 6.25E-17 and 43.3, respectively. The insignificant inter-study variability on P_{max} was then dropped from the model. The fixed effect of genotype 1 and

random effect at arm level on P_{max} were still included in the model. The analysis also found the effect of treatment on P_{max}. After adjusting the genotype, PI therapy has higher P_{max} than PR therapy. For the treatment arm with HCV genotype 1 patients only, the maximum response rate is estimated to be 0.594 in PR therapy and 0.758 in PI-based therapy. For the treatment arm only including patients with non-genotype 1 infection, the maximum response rate is estimated to be 0.873 in PR therapy.

The analysis showed that PI-based therapy had more rapid response increase than for PR therapy. The rate constant of response onset was estimated to be 0.401week $^{-1}$ and 0.242 week^{-1} for PI therapy and PR therapy, respectively. The time needed to achieve the half of maximum response rate was derived from the rate constant of response onset. PI therapy needs 1.7 weeks to reach half the maximal effect, while the PR therapy needs almost 3 weeks. The SVR rate during the follow-up period was compared to the response rate at the end of treatment. Their ratio was estimated to be 0.76. Incorporating the treatment effect on P_{max} and k resulted in decreased inter-arm variability in these two parameters. For some treatment arms, the protease inhibitor was initiated after 4-week PR therapy. Such delayed administration resulted in a longer lag time estimated at 3.87 week. Arms without the lead-in period had a lag time of 1.67 week. The objective function value was 43595 in the final model, compared to 43634 in the base model. The NONMEM estimates of parameters in final model were summarized in Table 5.1. The NONMEM code for final model was included in the Appendix.

The individual predictions from the final model showed good agreement with observations in each treatment arm (Figure 5.2). Prediction was performed from the final model for treatment arms with genotype 1 patients only and treated for 48 weeks. The plot (Figure 5.3) presents the median and 90% prediction intervals of model prediction compared to the observation. The model appeared to predict the central tendency of response rate over time. The 90% prediction interval included most of observations. But the prediction interval was wide, which was mainly due to inter-study and inter-arm

variability in model parameters. The median predicted SVR rate (90% prediction interval) was 0.56 (0.36, 0.79) in PI therapy and 0.43 (0.24, 0.65) in PR therapy for treatment arms with genotype1 patients and 48-week therapy.

5.4 DISCUSSION

Our study proposed a mathematical model to describe the time course of virologic response across different HCV antiviral treatment regimens. Several meta-analyses and systematic reviews have been published to compare the effectiveness among current approved treatments including PR therapy and PI combination therapy with telaprevir or boceprevir. These analyses are only focused on one single endpoint, the SVR rate evaluated during the follow-up period. However, virologic response can vary as the treatment proceeds. By incorporating longitudinal information, the current model-based meta-analysis enables the response data measured at different time points from various studies to be combined and as a result to characterize the time course of virologic response. The analysis increases our knowledge in how response evolves along the treatment by capturing the speed of response onset and maximum response rate. In addition, indirect comparisons were made among treatments for their differing impacts on the response-time profile.

The present study showed that triple therapy with a protease inhibitor combined with PR therapy led to a quicker response onset than PR. Previous Phase 3 studies also illustrated that telaprevir and boceprevir-based treatment are associated with higher virologic response during the early stage of treatment compared to the PR therapy.[17–20] Studies on HCV viral dynamics indicated telaprevir exhibited a more rapid second phase of HCV viral decline than PR therapy, which might be due to treatment effectiveness on infected cell death.[32–34] The proportion of patients with HCV genotype 1 infection was found to have a negative effect on the maximum response rate during treatment. Arms with more genotype 1 patients tend to reach a lower magnitude of maximum response rate. This

finding agrees with previous clinical studies that response to treatment depends on HCV genotype. Patients with genotype 2 or 3 have better response to PR treatment than those with genotype 1 and are eligible to receive a lower dose of ribavirin for a shorter term without sacrificing the treatment efficacy. Since PI-based treatment was approved only for treating HCV genotype 1 infection, our current study did not include PI studies in patients with HCV non-genotype 1 infection. Compared to HCV genotype 1 infection, protease inhibitors appeared to be less effective for genotype 2, 3 and 4. [35,36] After the treatment is completed, the rate of virologic response decreases slightly. The SVR rates predicted from the final model were compared to those observed in clinical trials which demonstrated greater SVR rate in PI therapy with telaprevir or boceprevir than PR therapy.[17–20] The model prediction plot shows that response rate observed in the beginning of the treatment (at week 4) are not described well by the current model, especially for PR therapy (Figure 5.3). It suggests the lag time might be different between PR therapy and PI therapy and should be estimated separately for each treatment. In addition, large variation was observed in the prediction plot which rises from inter-arm and inter-study variability in model parameters. The potential variability could be explained by incorporating covariate effect. The current study did not perform a comprehensive covariate exploration. Without achieving the precision of the parameter estimates, it's hard to evaluate the accuracy of the estimation.

Several limitations were presented in our current study. We encountered computational issues with NONMEM when we were trying to account for both inter-study and inter-arm variability on model parameters (e.g., P_{max}, k and RR). Despite the estimation of model parameters and variance of random effects were obtained, the covariance step during the importance sampling always failed and as a result we were unable to access the precision of the estimates. It might be due to the complexity of the model that the current data set does not provide sufficient information to estimate the parameters. It might also relate to the computation challenges in handling with nonlinear mixed effects model when using

maximum likelihood algorithm in NONMEM. Model misspecification could also contribute to the problem. The current asymptotic model assumed the response rate was maintained after it reached the maximum level. However, some treatment arms showed a reduction in observed response rate at later time points during treatment. It can be attributed to inadequate virologic response, treatment discontinuation or patient non-compliance to the treatment, where the patient may not be eligible to be considered as a responder. The current model did not account for the effect of potential response reverse events, which could raise the risk of inaccuracy and bias in the model estimation and prediction.

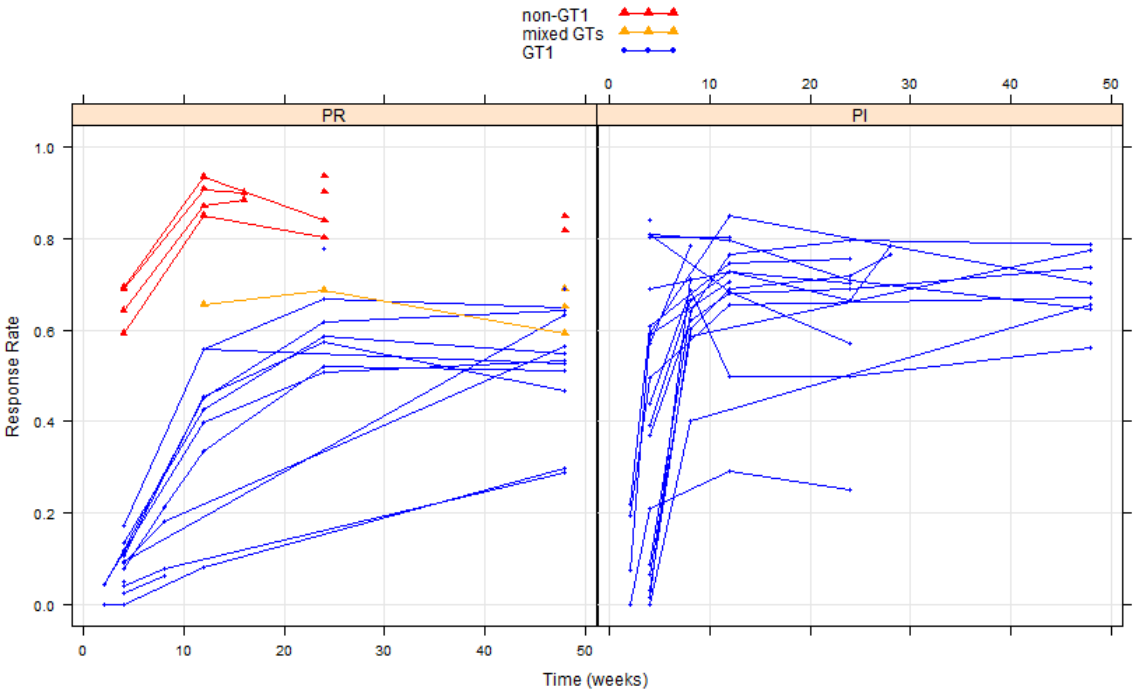
During the model development, we found inter-study variability was insignificant compared to the inter-arm variability in RR. After accounting for the effect of HCV genotype, inter-study variability in P_{max} also became very small. To avoid the potential of over-parameterization and simply the model, the inter-study variability was dropped from these two parameters in the final model. However, it should be acknowledged that ignoring the inter-study variability can inflate the inter-arm variability and may cause the bias. Given the nature of meta-analysis, it's important to investigate potential sources of heterogeneity.

The current study was conducted as an initial analysis to characterize and compare the response-time profile between PI-based therapy and PR therapy. However, the analysis did not compare the response-time profile between telaprevir and boceprevir which have not been compared head-to-head. In addition, the present work only evaluated the effects of treatment and HCV genotypes. It's necessary to conduct further studies to have an indirect comparison between telaprevir and boceprevir, and a comprehensive exploration of potential covariate effects on the time-course of response rate. Considering these limitations in the current NONMEM analysis, we continued our model-based meta-analysis work and proposed a more rational model in the Bayesian framework.

Table 5.1 Population estimates of model parameters

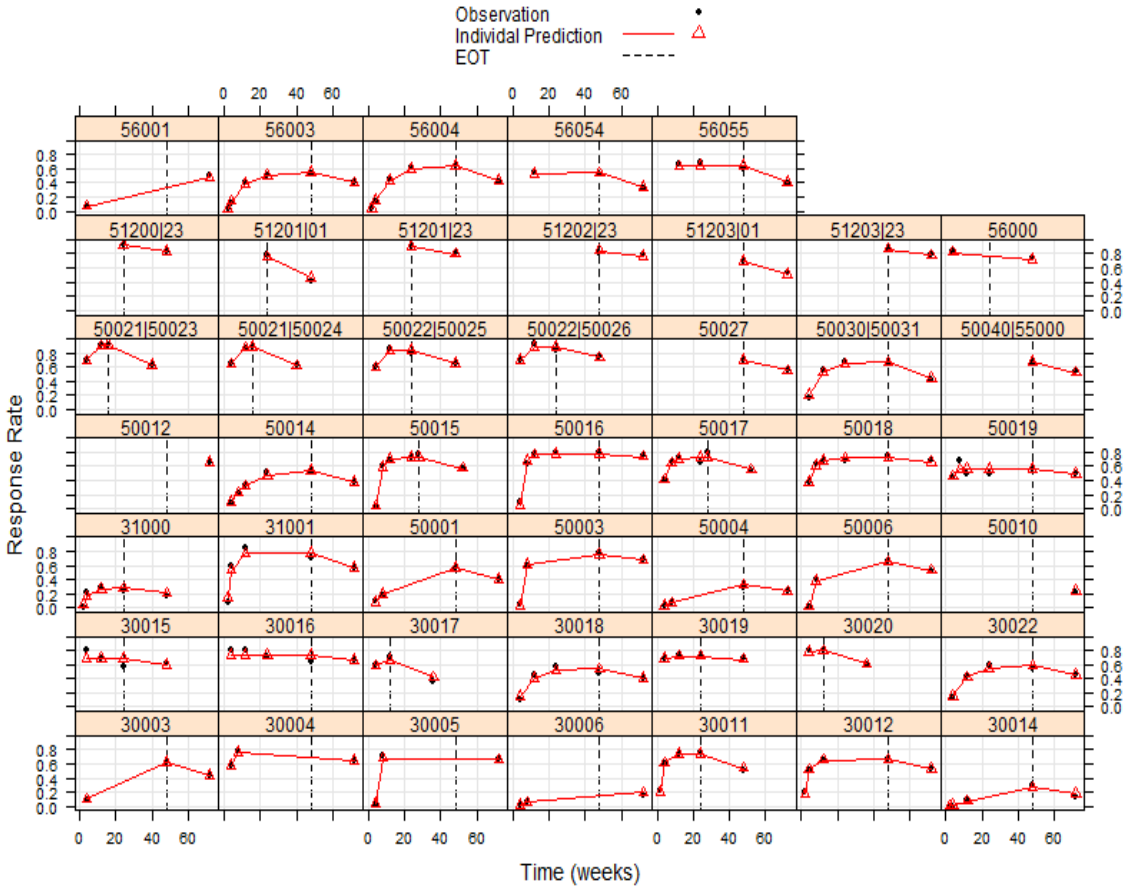
Parameter	Description	Population Estimate
k_{PR}	Speed of response onset in PR therapy	0.242
k_{PI}	Speed of response onset in PR therapy	0.401
$P_{max, GT1, PR}$	Maximum response rate in arms with genotype-1 patients only in PR therapy	0.594
$P_{max, GT1,PI}$	Maximum response rate in arms with -genotype-1 patients only in PI therapy	0.758
$P_{max, non-GT1, PR}$	Maximum response rate in arms with all non-genotype-1 patients in PR therapy	0.873
tlag	Lag time in response onset without lead-in period	1.67
$tlag_{lead-in}$	Lag time in response onset with lead-in period	3.90
RR	Ratio of SVR rate to ETR rate	0.739
$\omega^2_{study,Pmax}$	Between-study variability (BSTV) on logit-scale Pmax	4.53e-16
$\omega^2_{arm,k}$	Between-arm variability (BAV) on log-scale k	1.62
$\omega^2_{arm,Pmax}$	BAV on logit-scale Pmax	0.25
$\omega^2_{arm,RR}$	BAV on log-scale RR	0.0158

Figure 5.1 Observed response rate over time in a treatment arm during treatment



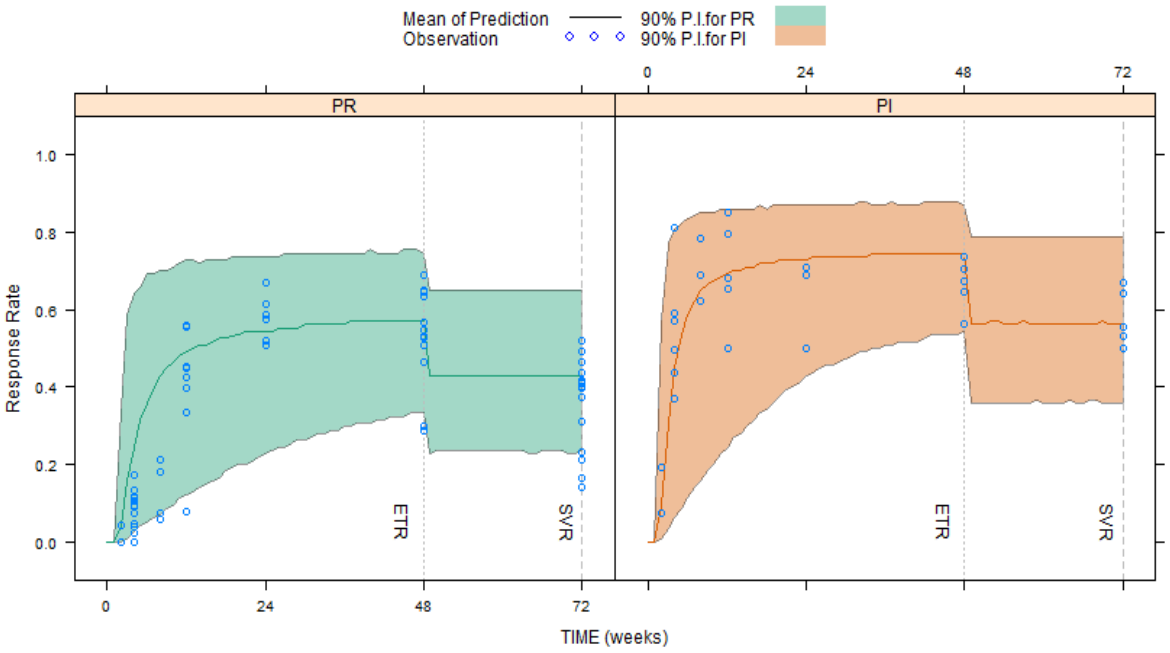
Note: The graph only showed the virologic response during treatment. There is no SVR rate included. PR: peginterferon plus ribavirin; PI: therapy of a protease inhibitor (telaprevir or boceprevir) combined with PR; GT1: arms with HCV genotype 1 patients only; Non-GT1: patients in the arm are all non-genotype 1 HCV infection; Mixed GT1: arms including patients with various HCV genotype infection.

Figure 5.2 Individual Prediction versus Observation in each arm



Note: EOT: end of treatment.

Figure 5.3 Model-based prediction and observation versus time



Note: prediction was performed for 48-week treatment in HCV genotype-1 patients. PI: prediction interval; PR: peginterferon plus ribavirin; PI: therapy of a protease inhibitor (telaprevir or boceprevir) plus PR.

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CHAPTER 6

Model-Based Meta-Analysis of Hepatitis C Virologic Response Using a Bayesian Approach

6.1 INTRODUCTION

In Chapter 5, the model-based meta-analysis was conducted to describe aggregate longitudinal HCV response data using nonlinear-mixed effect modeling in NONMEM 7. A simple asymptotic model was developed to characterize the time course of response rate during treatment in terms of speed of response onset and maximum response rate. However, there were several limitations in this model. First, the asymptotic model assumed the response rate was maintained after it achieved the highest magnitude. This ideal situation does not truly reflect the real HCV clinical management. In fact, HCV antiviral treatment could be discontinued due to a severe adverse effect or inadequate response. Treatment futility rules are usually specified for HCV treatment to avoid the unnecessary prolonged therapy in patients who are unlikely to response in order to reduce the risk of potential side effects and viral resistance.[1–3] Given the complexity of treatment regimen and increased adverse effects, it becomes challenging to keep patients adherent to therapy, which is associated with virologic response both during and after the treatment.[4,5] Non-adherence to the treatment regimen could lead to virologic failure and subsequent discontinuation in treatment. The plot of observed response rate over time showed that some treatment arms had a reduced response rate in later phase of treatment (Figure 6.1). The asymptotic model does not account for this decline. In this chapter, we propose an alternative model which allows capturing the response reverse during treatment.

Another concern is the heterogeneous variability in the meta-analysis. During the model development process, multiple random effects were explored at the study and arm levels.

However, we developed computational challenges when incorporating both inter-study and inter-arm variability in NONMEM. The model containing inter-arm variability alone seemed to run well. However, disregarding the inter-study variability ignores the data structure and within-study randomization, which can lead to bias in parameter estimates.[6] In this chapter, we explore new modeling techniques to estimate and evaluate the nonlinear mixed effects model that includes both study- and arm-level effects.

Both telaprevir and boceprevir are required to be administered in conjunction with peginterferon alpha and ribavirin (PR). However, treatment can be different in the schedule of administration, such as, when to commence the triple therapy and how long it lasts. In some treatment arms, triple therapy was not initiated at the start of treatment. Instead, PR was given alone for 4 weeks before the protease inhibitor was added. The effect of this 4-week lead-in period was accounted for as a lag time of response onset in the Chapter 5 model, assuming that the response probability is 0 from the start of treatment until the lag time. The results showed the 4-week lead-in period was associated with a longer delay in response. As a matter of fact, arms had developed the response to PR treatment even before protease inhibitor was initiated, although the magnitude is relatively small. Besides, boceprevir clinical trials showed that responsiveness of lead-in PR therapy was associated with the outcome of the subsequent triple therapy.[7,8] The model developed in this chapter accommodates the virologic response developed during the lead-in period.

In this chapter, a Bayesian approach was applied to the model-based meta-analysis using the same longitudinal response data. A new model was proposed that takes into account the potential response reverse during treatment. The hierarchical nonlinear mixed-effect model was developed considering covariate effects and multiple levels of random effects, such as inter-study variability and inter-arm variability. The software JAGS was used to implement the analysis in the Bayesian framework using Marko chain Monte Carlo (MCMC) methodology.

6.2 METHODS

6.2.1 Systematic Review and Dataset Construction

The systematic review of published literature and clinical trials of HCV antiviral treatment has been described in the Chapter 4. Information and data related to the current research was abstracted from the selected literatures and synthesized to the data set ready for the model-based meta-analysis. Chapter 4 provided the detailed description regarding data set construction and the final analytic dataset.

6.2.2 Bayesian Analysis

Bayesian analysis was adopted in the current model-based meta-analysis. A full nonlinear Bayesian hierarchical model was developed in the software JAGS. [9] Like other Bayesian software, such as OpenBUGS and WinBUGS, JAGS generates Bayesian inference by means of Monte Carlo Markov Chain algorithm (MCMC).[9] The statistical software, R (version 3.0.0)[10], was used as a working platform where JAGS was directly implemented and managed through R packages "rjags" and "r2jags". The MCMC simulation output from JAGS was processed by R package "coda". RStudio (version 0.97.449, RStudio.Inc., Boston, Massachusetts, United States) was used as a user interface to R.

In the current project, multiple Markov chains were run with different settings of initial values. The convergence of MCMC chains were visually inspected based on trace plots of multiple chains for every monitored parameter, where a well-mixed "fuzzy caterpillar" pattern suggests a stationary distribution has been reached. The Gelman-Rubin statistic was also used to assess the convergence of MCMC chains.[11] The statistics was calculated by examining within-chain variance and between-chain variance. When MCMC chains converge, the estimated Gelman-Rubin statistics should be nearly 1 (generally less than 1.2) for every parameter.[12] If the statistic is greater than 1.2, it means MCMC chains need to run longer to reach the stationary distribution .[13] Pooled samples from the converged chains were used to generate the posterior distribution of each model parameter. The

median of the posterior distribution is considered as the Bayes estimates of the parameter. 95% Bayesian credible intervals also are presented using the 2.5th and 97.5th percentiles of the posterior distribution. Deviance information criteria (DIC) was used to direct the model selection, which takes into consideration both model fit and model complexity.[14]

6.2.3 Model Development

Base model

The number of responders (Y_{ijs}) at time *t* in arm *j* in study *i* is considered to follow a binomial distribution that depends on the sample size in arm *j* in study *i* and the response rate at time *s* in the arm *j* in study *i* ($P_{resp,ijs}$) as described in Equation 6.1.

$$Y_{ijs} \sim Binomial\ (P_{resp,ijs}, N_{ij}) \quad (6.1)$$

A response reverse model was introduced to address the potential of decline in response rate. It should be noted that "response reverse" is used here to indicate any circumstance where patients may not be eligible to be considered as a responder. It could be due to treatment discontinuation, patient non-adherence to the medication and inadequate response. These events increased the number of patients who were not classified as responders and therefore decreased the response rate. The response rate during treatment can be described as Equation 6.2.

$$P_{resp} = P(resp|no\ reverse) \times P(no\ reverse) + P(resp|reverse) \times P(reverse) \quad (6.2)$$

where $P(resp|no\ reverse)$ refers to the probability of a patient having a response without a reverse event, whereas $P(resp|reverse)$ means the probability of a patient responding to the treatment given a reverse event. $P(no\ reverse)$ is the probability of not having a response reverse event, and $P(reverse)$ is the probability of having a response reverse event. Since the patient cannot be considered as a responder in any situation where a reverse event happens, $P(resp|reverse)$ becomes zero. The second additive part in the equation was removed.

Therefore, the response rate during treatment phase can be explained by a product of two components as shown in Equation 6.3, one is the probability of having a response given the absence of a reverse event ($P(resp|no\ reverse)$), and the other is probability of not having reverse event ($P(no\ reverse)$). These two factors are both essential to determine the pattern of longitudinal response rate. Each of them was specified as a function of time and model parameters.

$$P_{resp} = P(resp|no\ reverse) \times P(no\ reverse) \tag{6.3}$$

Without any occasion hampering the response to the treatment, the time-course of the response rate is assumed to gradually reach a maximum response during treatment as an asymptotic model describe in Equation 6.4.

$$P_{resp|no\ rev,ijs} = P_{max,ij} \times \left[1 - e^{\left(-k_{ij} \times (t_{ijs} - tlag_{ij})\right)}\right] \tag{6.4}$$

where $P_{resp|no\ rev,ijs}$ is the response rate given no reverse at time *s* in arm *j* in study *i*, $P_{max,ij}$ is the maximal response that arm *j* in study *i* can achieve, k_{ij} is the first-order rate constant of response increase and represents the speed of response onset after treatment commences, and $tlag_{ij}$ is the lag-time for delayed initiation of response. The assumption for this model was the probability of response was 0 until $tlag_{ij}$.

The probability that no reverse event ($P_{no\ rev,ijs}$) happened at time *s* in treatment arm *j* in study *i* can be expressed as 1 minus the probability of treatment reverse ($P_{rev,ijs}$) as shown in Equation 6.5. Since the value of a probability falls only between 0 and 1, logit transformation was applied to constrain the probability of response reverse event within the range. Furthermore, the logit regression was conducted to evaluate the time effect.

$$P_{no\ rev,ijs} = 1 - P_{rev,ijs} \tag{6.5}$$

$$logit\left(P_{rev,ijs}\right) = log\left(\frac{P_{rev,ijs}}{1-P_{rev,ijs}}\right) = \beta_{0.ij} + \beta_{1,ij} \times (t_{ijs} - 2) \tag{6.6}$$

Where $P_{rev,ijs}$ denotes the probability describing a response reverse event at time *s* in arm *j* in study *i*, $\beta_{0.ij}$ and $\beta_{1,ij}$ are arm-level regression coefficients of time (t_{ijs}). Among the studies included, the response was evaluated at as early as week 2 of therapy. Time was centered on week 2 at the earliest evaluation. Therefore, the formula of on-treatment response rate is updated to Equation 6.7.

$$P_{resp,ijs} = P_{resp|no\ rev,ijs} \times P_{no\ rev,ijs}$$

$$= P_{max,ij} \times \left[1 - e^{\left(-k_{ij} \times (t_{ijs} - tlag_{ij})\right)}\right] \times \left[1 - \frac{1}{1+e^{-(\beta_{0.ij} + \beta_{1,ij} \times (t_{ijs}-2))}}\right] \quad (6.7)$$

In some arms, the therapy was initiated with PR alone for 4 weeks followed by addition of a protease inhibitor, such as, boceprevir. The analysis of the response to the subsequent triple therapy should be adjusted for the response gained by the prior lead-in PR treatment. The response rate during the lead-in period was assumed to have the same response-time course in PR treatment. It can be modeled using Equation 6.7. The response after adding the protease inhibitor was associated with the response yielded at the end of lead-in period. The response rate at week 4 of PR could be derived as shown in Equation 6.8. Then, the response rate during the triple therapy was modeled as Equation 6.9.

$$P^{PR}_{resp\ at\ 4,ij} = P^{PR}_{resp|no\ rev\ at\ 4,ij} \times P^{PR}_{no\ rev\ at\ 4,ij}$$

$$= P^{PR}_{max,ij} \times \left[1 - e^{\left(-k^{PR}_{ij} \times (4 - tlag_{ij})\right)}\right] \times \left[1 - \frac{1}{1+e^{-(\beta^{PR}_{0,ij} + \beta^{PR}_{1,ij} \times (4-2))}}\right] \quad (6.8)$$

$$P^{TRI}_{resp,ijs} = P^{PR}_{resp\ at\ 4,ij} + \left(P^{TRI}_{max,ij} - P^{PR}_{resp\ at\ 4,ij}\right) \times \left[1 - e^{\left(-k^{TRI}_{ij} \times (t_{ijs} - 4)\right)}\right] \times P^{TRI}_{no\ rev,ijs} \quad (6.9)$$

Where $P^{PR}_{resp\ at\ 4,ij}$ represents the response rate at week 4 of lead-in PR therapy, $P^{TRI}_{\mathrm{resp,ijs}}$ represents the response rate during the triple therapy period, $P^{PR}_{max,ij}$and $P^{TRI}_{max,ij}$ denotes the maximal response rates to lead-in therapy (PR) and triple therapy, respectively.

k_{ij}^{PR} and k_{ij}^{TRI} are the speed of response onset given lead-in therapy (PR) and triple therapy, respectively. $\beta_{0,ij}^{PR}$ and $\beta_{1,ij}^{PR}$ are coefficients in logistic regression of probability of response reverse event on time in PR therapy.

The SVR rate in arm *j* in study *i* was modeled relative to response rate predicted at the end of treatment (EOT) as shown in Equation 6.10.

$$P_{SVR,ij} = P_{resp\ at\ EOT,ij} \times RR_{ij} \quad (6.10)$$

where $P_{SVR,ij}$ represents the SVR rate in arm *j* in study *i*, $P_{resp\ at\ EOT,i}$ is the predicted response rate at EOT (ETR) and RR_{ij} denotes relative response, which is the ratio of SVR rate to predicted ETR rate.

To account for inter-study and inter-arm variability, multiple levels of random effects were evaluated on model parameters, especially on speed of response onset (k) and maximum response rate (Pmax) given no reverse event as well as relative response rate (RR) of SVR to ETR. The variance-covariance matrix was modeled separately for inter-study variability and inter-arm variability and inter-arm random effects were assumed to be independent of the inter-study random effects.

Covariate model

Heterogeneous variability was primarily attributed to differences in study population and in treatment regimens across arms and studies. Potential association of covariates with parameters was explored based on initial exploratory analysis, prior knowledge from HCV clinical trials, and physiological and pharmacological plausibility. Covariates of interest included race/ethnicity, baseline viral load, HCV genotype, advanced fibrosis (bridging fibrosis or cirrhosis) and prior treatment experience. The treatment regimen was also evaluated in terms of medication composition and duration of therapy. Model parameters were assumed log-normally distributed, except the maximum response rate. The potential effect of a covariate was investigated on the log-scale of the parameters in a

linear way as Equation 6.11. Since the value of maximum response rate should fall between 0 and 1, logistic regression was used to explore the covariate effect as shown in Equation 6.12.

$$log(Para) = \theta_1 + \theta_2 \times COV \quad (6.11)$$

$$logit(Pmax) = \theta_3 + \theta_4 \times COV \quad (6.12)$$

where $\log(Para)$ represents the log scale of parameter, $\text{logit}(Pmax)$ is the logit-transformed maximal response rate, $\theta_1, \theta_2, \theta_3, \theta_4$ are regression coefficients of covariate (COV).The selection of covariate into the final model was guided by exploratory data analysis, prior knowledge from clinical studies, change of DIC and whether the 95% posterior credible intervals of covariate coefficients covers zero or not. In addition, convergence of the model with the selected covariates was evaluated to ensure the reliable and stable parameter estimation.

Prior distribution

Uninformative or poor informative prior distributions were assigned to most model parameters and random effects. Normal prior distributions were specified to parameters with large variance which led to low precision (inverse of variance). Inter-study and Inter-arm variance-covariance matrices were given with a Wishart prior distribution.

6.2.4 Model Evaluation

The goodness of model fit was evaluated by comparing the observed data and model prediction for each treatment arm. A posterior predictive check was conducted to assess the predictive performance of the final model. The model parameters (including fixed and random effects) were sampled from their resulting posterior distributions. The response rate was simulated at each time point from the final model structure and model parameters. The median and 90% prediction intervals of simulated response rates were calculated and plotted overlaid with observed data to assess whether observations were

consistent with the range of model predictions. The predictability of the final model was assessed with respect to various treatment strategies and patient characteristics.

6.3 RESULTS

6.3.1 Systematic Review Result and Analytic Dataset

The model-based meta-analysis was conducted on longitudinal response data, arising from 18 studies and 47 arms in total. Twenty-seven arms received the dual therapy of peginterferon alpha and ribavirin, and twenty arms received the triple therapy of a protease inhibitor combined with PR (12 with telaprevir and 8 with boceprevir). The median (range) number of patients in each study is 407 (32-2054), and the median (range) number of patients per treatment arm is 114 (16-1035). Only arms with approved dosage of medication were enrolled. Studies or arms within the included study were excluded if they received response-guided therapy. The course of treatment and time points of response assessment had been determined in the study protocol. Information regarding baseline population characteristics and treatment strategies were also collected in the dataset. Most studies (n=12) only enrolled patients with chronic HCV genotype 1 infection. Two studies were focused on HCV genotype 2 or 3 infection and four studies enrolled patients infected with various HCV genotypes. Treatment-naïve patients were given HCV antiviral therapy in 12 studies and patients who had been previously treated but failed PR therapy were retreated in the remaining 6 studies. Further information regarding the final dataset for meta-analysis were described and summarized in Chapter 4.

6.3.2 Longitudinal Model-Based Meta-Analysis

A Bayesian nonlinear mixed effects model was developed to describe the response data over time during treatment and follow-up periods. Both inter-study and inter-arm variability were incorporated into the model. Several covariates were found to be significant for response parameters. For the final model, uninformative priors (with wide variance) were assigned to most model parameters as presented in Table 6.1. Due to

model identifiability, an informative prior was assigned to the maximum response rate (Pmax). Each of three MCMC chains started to run at different sets of initial values for 400,000 iterations with 5,000 samples discarded during the burn-in period in each chain. The thinning factor was set as 10 so that only one in every ten samples was saved. The posterior distribution for each model parameter was obtained from a total of 105,000 samples saved from post-burn-in MCMC chains. The posterior median and 95% credible intervals of model parameters and relevant convergence statistics were summarized in Table 6.2. Trace plots were constructed for each model parameter to assess the convergence of MCMC chains. JAGS code for the final model and trace plots of model parameters are included in Appendix.

Structural model

The potential decrease in response rate during the later phase of treatment was taken into consideration in the current analysis by incorporating a component which described the response reverse event, such as, patient non-adherence (i.e., drop out) and treatment discontinuation due to severe side effects or inadequate response (i.e., virologic breakthrough). Accordingly, the model for response rate during treatment period consisted of two parts, one of which was used to demonstrate the response rate-time profile given the absence of reverse event and the other part was associated with probability of having a response reverse event. The first part was well described with an asymptotic model characterized with speed of response onset (k) and the maximum response rate (Pmax) if no reverse event occurred. In the second part, a logistic regression was used to describe the probability of having a response reverse event as a function of time. The intercept ($\beta_{0,ij}$) in the logistic model was fixed to -5, since there was lack of sufficient data at treatment week 2 to support estimation of the parameter. In this way, the model assumed response reverse was not likely to occur at treatment week 2. The probability of having response reverse at week 2 was 0.0067. After completion of the

treatment, the SVR rate was modeled relative to the predicted response rate at the end of treatment (ETR). The ratio was defined as relative response (RR) here.

The heterogeneity that arises from meta-analysis was quantitatively analyzed by incorporating covariate effects, and inter-study and inter-arm variability on response model parameters. Meanwhile, inter-arm variability was weighted by sample size in the corresponding arm. The structural of final model was shown in Figure 6.2.

Effects of treatment strategies

Three approved HCV antiviral therapies were evaluated in the current meta-analysis, including PR therapy, telaprevir plus PR therapy and boceprevir plus PR therapy. Plots revealed these therapies led to different time courses of response rate during treatment in Figure 6.1. The PI-based therapy is associated with a more rapid response onset than PR therapy alone. The posterior median of speed of response onset (k) was 3.9 $week^{-1}$ with a 95% credible interval (95% C.I.) of 1.92 to 8.71for telaprevir, 0.617 $week^{-1}$ (95% C.I.: 0.312, 1.322) for boceprevir, and 0.0748 $week^{-1}$ (95% C.I.: 0.0389, 0.156) for PR therapy. The half-time of response increase ($T_{1/2}$) which represents the time needed to achieve 50% of the maximal response rate was then calculated from the estimates of k. $T_{1/2}$ was 0.18 weeks in telaprevir and 1.12 weeks in boceprevir, compared to 9.27 weeks in PR therapy. There was no statistically significant difference found in the maximum response rate (P_{max}) among the three treatments.

For the logistic response reverse model, the coefficient of probability of reverse event on time (β_1) was estimated to be 0.07 $week^{-1}$ (95% C.I.: 0.056, 0.081) in PR therapy and was much smaller (0.000111 $week^{-1}$) in triple therapy, suggesting the probability of having a reverse event changed only slightly along PI-based therapy. For the PR therapy, the estimated probability of response reverse (95% C.I.) were 0.013 (0.012, 0.015) at treatment week 12, 0.031 (0.022, 0.039) at week 24 and 0.15 (0.081, 0.22) at week 48.

For PI therapy, the estimated probability of response reverse (95% C.I.) was 0.0067 (0.00669, 0.0163) at week 24 and 0.0067(0.00669, 0.043) at week 48.

After treatment completion, the SVR rate during the follow-up period was comparable to ETR rate at the end of treatment in PI-based therapy. The relative response (95% C.I.) of SVR rate to ETR rate was estimated to be 0.89 (0.78, 1.00), 0.93(0.81, 1.07) and 0.73 (0.66, 0.79) for telaprevr, boceprevir and PR therapies with the standard term, respectively. Treatment duration was found to have a significant effect on the RR. Given the same medication composition, RR was decreased by 17% (10%, 25%) if the treatment was administered for a shorter term.

Effects of baseline patient characteristics

Although the maximum response rate did not show a significant difference across treatments, baseline characteristics were explored for their potential effects in this model parameter. Arms with a greater proportion of patients with the HCV genotype 1infection, black patients and null responders tend to have a lower maximum response rate. Table 6.3 shows the estimated maximum response rate for the patient populations containing combinations of these characteristics.

Inter-study and inter-arm variability

After adjusting the inter-arm variability by the median value of sample size in a treatment arm (n=114), the standard deviations of inter-arm variability was estimated to be 0.0479, 0.322, 0.0486 for speed of response onset, maximum response rate and relative response. The standard deviation of inter-study variability for these parameters was estimated to be 1.277, 0.404 and 0.133, respectively.

Model evaluation and prediction

Figure 6.3 shows individual predictions of response rate compared with observations. The observed data in each treatment arm fall mostly within 90% Bayesian predictive

intervals. No systematic overprediction or underprediction was found. For the posterior predictive check, the simulations were performed based on model structure and posterior distributions of model parameters given different treatments and different patient populations. The plots show that the 90% prediction intervals include most of observations (Figure 6.4 to Figure 6.6). These plots were constructed for patients with genotype 1 infection and used to compare the time course of the response rate among PR, telaprevir and boceprevir therapy. For standard-term PR therapy (48 weeks), the median of predicted SVR rate (90% prediction interval) was 38.8% (12.5%, 55.9%) in the treatment arm with HCV genotype 1 patients only, no prior null responders and 5.05% black patients. For 48-week telaprevir therapy, the median predicted SVR rate (90% prediction interval) was 64.1% (46.2%, 83.2%) in the treatment arm with HCV genotype 1 patients only, no prior null responders and 14.6% black patients. For 48-week boceprevir therapy with a 4-week lead-in PR period, the median of predicted SVR rate (90% prediction interval) was 65.9% (48.9%, 84.25) in treatment arms with HCV genotype 1 patients only, no prior null responders and 14.6% black patients. Predictions of response rate at other time points (e.g., treatment week 4, 12 and the end of treatment) were compared among treatments and are summarized in Table 6.4.

6.4 DISCUSSION

In the current work, a model-based meta-analysis was performed to quantitatively describe the aggregate longitudinal response data by means of Bayesian methodology. The proposed model presents an approach to accomplish indirect treatment comparisons in the time course of clinical responses. To date, several meta-analyses have been published to compare the effectiveness across approved HCV antiviral treatments including PR therapy, telaprevir and boceprevir.[15–20] However, one major limitation of these analyses is that only the primary clinical outcome, SVR, was evaluated and compared as an efficacy endpoint across treatments. These SVR-based analyses did not find a significant difference between telaprevir and boceprevir therapy. In addition, such

one-endpoint analyses did not capture the variation in treatment effect/response over the time, especially during treatment. In fact, monitoring patient response along the treatment course is important for HCV management. Furthermore, it may not be appropriate to generalize the covariate effect found on one endpoint to other responses assessed at different time points. To address these limits, the current study integrated the longitudinal information of response data, allowing us to enroll more studies with different treatment regimens and different response assessment schedules. The current analysis enables the indirect treatment comparison based on the time course of response featured with the speed of response onset and maximum response. Baseline patient population characteristics were also acknowledged for their potential effects on the response-time profile, which provided additional information to better understand the variability in treatment outcomes.

A simple asymptotic model was presented in Chapter 5 to describe the time course of response rate during treatment. The major drawback of this model was not accounting for the potential reverse in response rate during the later phase of treatment. Such a decrease in response rate could be attributable to treatment discontinuation due to inadequate response or adverse effects and patient non-adherence to the treatment. In these events, a patient might not be eligible to be considered as a responder, which usually occurs in a HCV antiviral therapy. A model ignoring these factors might not be practical for clinical management and may lead to a biased estimate and prediction. Appropriately considering these events is important during the model development. The current study adopted a latent logistic model to describe the probability of having a response reverse event as a function of time. Accordingly, the response rate was driven together by two components, which are the probability of response without any reverse event and the probability of no reverse event. In the response reverse model, the chance of having a reverse event increased as treatment proceeded. It was estimated that PI-based therapy had much slower increase in probability of reverse event over time. Several clinical trials showed

PI-based therapies were associated with more adverse events than PR therapy. Virologic breakthrough was also observed with the presence of resistant variants to the PI. The occurrence of these events is generally summarized at the end of study. However, the detailed information is missing regarding when and how many patients have experienced what kind of response-reverse events. Lack of such information makes it challenging to differentiate the independent effects of these events and affect the interpretation of the current response reverse model. Further analysis is needed with more data regarding the response reverse events available.

The response rate without any reverse event was well described using an asymptotic model, where treatment effect increased and reached a maximum over time. Response rates grew more rapidly in arms with PI-based therapy than did those in PR therapy. Phase 3 studies illustrated addition of telaprevir or boceprevir was associated with a higher rate of early responses at treatment week 4 or 12. [7,8,21,22] Furthermore, the current meta-analysis found that the response increased more rapidly with telaprevir therapy than boceprevir therapy. In addition, the response developed during the 4-week PR lead-in period was also accounted in the model. The subsequent response after boceprevir initiated from week 4 was developed based on the lead-in PR response. However, the previous model in Chapter 5 only estimated the different lag times for treatments with and without a lead-in period, which also assumed the response rate was 0 until the lag time. That model led to under-prediction in the response during the lead-in period and also affected prediction of the response after boceprevir was added.

For the maximum treatment response/ efficacy, HCV genotype, race and previous response to PR therapy were illustrated as significant factors. Maximum treatment effect was lower in arms including more patients with HCV genotype 1 infection, black race and prior null response. However, the current analysis did not find a significant difference in the maximum response rates between PI-based therapy and PR therapy. It should be noted that the maximum response rate represents the magnitude of treatment

efficacy while on treatment, which is unlike the SVR rate which is assessed after completion of treatment.

The SVR was the only response evaluation performed after completion of treatment; the relative response was introduced in our analysis as a ratio to describe how SVR rate during the follow-up period differed from response rate at the end of treatment (ETR). The estimates of the ratio were closer to 1 in PI-based therapy, suggesting the SVR rate is more comparable to the ETR rate than PR therapy. It may be related to less frequent virologic relapse in patients given triple treatment. In addition, the current study showed boceprevir had a slightly higher RR than telaprevir. Higher SVR rates and lower relapse rates have been observed in several clinical trials of telaprevir or boceprevir therapy combined with PR therapy, which confirms the superiority of triple therapy to PR therapy.[7,8,21,22] Extending the course of therapy could enhance the suppression of HCV and prevent virologic relapse. PR therapy is generally given for the standard 48 weeks to treat HCV genotype 1 infection. The combination of telaprevir or boceprevir with PR therapy is usually followed by PR therapy alone for several weeks. The whole PI-based treatment could vary from 24 weeks to 48 weeks. According to approved prescribing information, the treatment duration of telaprevir or boceprevir-based therapy can be determined based on patient early response.[2,3] In the current analysis, we only focused on treatment regimens with a fixed course which have been defined in the protocol. Because treatment is generally given for 48 weeks in patients with genotype 1 infection, any therapy with less than 48 weeks were considered as short term treatment, whereas for patients with non-genotype 1(e.g., 2 or 3), 24 weeks are standard duration for the treatment and any therapies less than 24 weeks were defined as short term. Our analysis found treatments with truncated periods were associated with a smaller RR, suggesting that the response rate at week 24 of follow-up period (SVR rate) dropped markedly from the response rate assessed at the end of treatment (ETR). It suggested the longer treatment could improve the SVR rate. However, it should also be kept in mind that the

longer treatment may raise the risk of adverse effects and patient non-compliance. An appropriate design in treatment regimen should take into consideration both benefit and risk. Other covariates (e.g., baseline HCV RNA, relapse, advanced fibrosis) were also explored for their impact on model parameters. However, they were not found to be significant. It might be due to a confounding effect among some covariates. Studies in PI-based therapy had more patients with high baseline HCV RNA. The cut-off value to define high or low baseline HCV RNA level was not consistent among studies. Small numbers of patients with bridging fibrosis or cirrhosis in studies also affected the analysis results. Given these limitations in the available data, the current analysis cannot estimate the effects of all these covariates. However, some clinical studies demonstrated these covariates were associated with primary clinical outcome, SVR. These covariates are still important for further study due to their potential clinical significance.

In the meta-analysis framework, heterogeneity arises when combining information from different trials with various treatment, patient population and study design. In the current study, it is addressed by incorporating covariate effects, and inter-study and inter-arm variability. After accounting for the impact of the covariate on model parameters, high variability was still observed in the model. For example, inter-study variability was relatively high in model parameter k which represents the speed of response onset. It was primarily attributable to inconsistencies in study design and difference in patient population among enrolled trials. Not all studies assessed/reported the response early in the treatment. Underlying inconsistencies among patient populations also raise the between-study variability. Further work is needed to further explore the inter-study variability in this parameter.

The current model-based meta-analysis presents a quantitative evaluation and indirect comparison in the time profile of HCV response across PR and novel triple therapy with direct acting agents. Protease inhibitors were found to have a favorable efficacy with more rapid response and a higher SVR rate. The impact of patient population

characteristics was also taken into consideration. The analysis could be useful to predict the response at certain time points and in particular patient populations that have not been studied. It might also be helpful to apply the current model to compare a new PI drug with approved treatments.

Table 6.1 Prior distribution

Fixed effect			
$\mu_k \sim N(-2, 10^6)$	$\theta_{1,k} \sim N(0, 10^6)$	$\theta_{2,k} \sim N(0, 10^6)$	
$\mu_{P_{max}} \sim N(3, 0.01)$	$\theta_{1,Pmax} \sim N(0, 10^6)$	$\theta_{2,Pmax} \sim N(0, 10^6)$	$\theta_{3,Pmax} \sim N(0, 10^6)$
$\mu_{RR} \sim N(-0.2, 10^6)$	$\theta_{1,RR} \sim N(0, 10^6)$	$\theta_{2,RR} \sim N(0, 10^6)$	$\theta_{3,RR} \sim N(0, 10^6)$
$\mu_{\beta_1} \sim N(-2, 10^2)$	$\theta_{\beta_1} \sim N(0, 10^6)$	$\mu_{tlag} \sim Unif(0, 2)$	$\theta_{tlag} \sim Unif(0, 2)$
Random effect			
Inter-arm variability for parameters *k, P_{max}, RR*			
$\Omega_{arm}^{-1} \sim Wishart\ (R[1:3,1:3], 3)$			
Inter-study variability for parameters *k, P_{max}, RR, β_1*			
$\Omega_{study}^{-1} \sim Wishart\ (R[1:3,1:3], 3)$			
$\omega_{\beta_1}^{study} \sim Unif(0,10)$			

Table 6.2 Summary of Bayes estimate and 95% credible interval of model parameters and convergence statistics

Parameter	Description	Posterior Median	Posterior 95% credible interval	Rhat	Effective size
μ_k	Log-scaled speed of response onset (log (k)) in PR therapy	-2.593	(-3.280, -1.955)	1.005	470
$\theta_{1,k}$	Telaprevir therapy effect on log (k)	3.954	(3.599, 4.324)	1.001	10000
$\theta_{2,k}$	Boceprevir therapy effect on log (k)	2.108	(1.864, 2.370)	1.001	30000
μ_{Pmax}	Logit-transfromed maximum response rate (logit (Pmax)) in non-genotype 1, non-black and non-null response patients	3.022	(2.834, 3.215)	1.001	6300
$\theta_{1,Pmax}$	HCV genotype 1 effect on logit(P_{max})	-1.838	(-2.232, -1.478)	1.002	1700
$\theta_{2,Pmax}$	Black race effect on logit(P_{max})	-1.309	(-1.988, -0.649)	1.001	12000
$\theta_{3,Pmax}$	Prior null responder effect on logit (P_{max})	-1.366	(-2.065, -0.667)	1.001	59000
μ_{RR}	Log-scaled relative resposne (log(RR)) of SVR to ETR in standard-term PR therapy	-0.290	(-0.414, -0.232)	1.003	920
$\theta_{1,RR}$	Telaprevir therapy effect on log (RR)	0.200	(-0.060, 0.338)	1.001	19000
$\theta_{2,RR}$	Boceprevir therapy effect on log(RR)	0.250	(0.109, 0.391)	1.001	15000
$\theta_{3,RR}$	Short-term treatment effect on log (RR)	-0.191	(-0.278, -0.106)	1.001	35000
$\mu_{\beta 1}$	Log-scaled slope of response reverse (log(β_1)) in PR therapy	-2.654	(-2.886, -2.505)	1.002	2600
$\theta_{\beta 1}$	Triple therapy with protease inhibitor effect on log(β_1)	-6.444	(-22.262, -0.536)	1.001	54000
tlag	Lag time (tlag) in response onset in PR therapy	1.358	(1.186, 1.497)	1.001	7400
θ_{tlag}	Lag time in triple therapy with protease inhibitor	1.546	(1.302, 1.711)	1.001	59000
$\omega_{study,k}$	Between-study variability (BSTV) on log(k)	1.230	(0.854, 1.899)	1.002	3800
$\omega_{study,Pmax}$	BSTV on logit(P_{max})	0.401	(0.206, 0.727)	1.001	11000

$\omega_{study,RR}$	BSTV on log (RR)	0.132	(0.091, 0.207)	1.001	24000
$\omega_{study,\beta1}$	BSTV on log ($\beta1$)	0.197	(0.022, 0.456)	1.003	3100
$\omega_{arm,k}$	Between-arm variability (BAV) on log (k)	0.511	(0.166, 1.284)	1.001	16000
$\omega_{arm,Pmax}$	BAV on logit (P_{max})	3.435	(2.326, 4.979)	1.001	24000
$\omega_{arm,RR}$	BAV on log (RR)	0.519	(0.219, 0.953)	1.002	3100

Table 6.3 Posterior estimation of maximum response rate (P_{max}) in arms with certain population characteristics

Population characteristics			Posterior median (95% Bayesian credible interval)
Genotype 1	Black	Prior null responder	
+	+	+	0.184 (0.076, 0.372)
+	+	-	0.468 (0.313, 0.626)
+	-	+	0.456 (0.280, 0.628)
+	-	-	0.766 (0.698, 0.816)
-	-	-	0.954 (0.944, 0.961)
-	+	-	0.846 (0.733, 0.916)

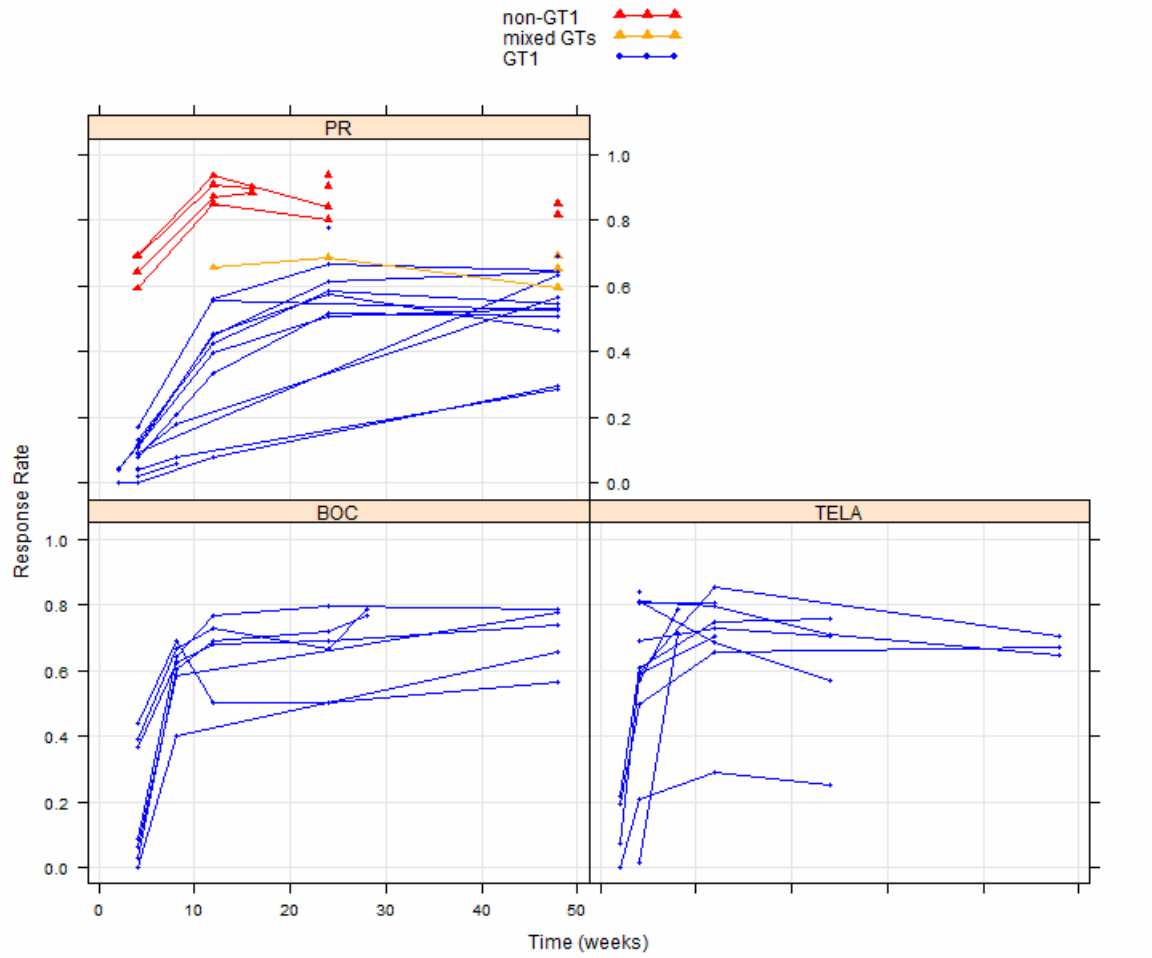
Note: + means all patients in the arm have the corresponding characteristics (covariate value is 100%); - means none of patients in the arm has the corresponding characteristics (covariate value is 0).

Table 6.4 Model-based prediction of response rate in arms with certain population characteristics (90% prediction interval)

TRT	Population characteristics			Response rate			
	GT 1	Black[a] (%)	Null-RESP	TW 4	TW12	ETR	SVR
PR	+	5.06	-	0.132 (0.0185, 0.490)	0.392 (0.0717, 0.685)	0.536 (0.189, 0.704)	0.388 (0.125, 0.559)
TELA	+	8.08	-	0.709 (0.456, 0.834)	0.738 (0.521, 0.864)	0.738 (0.524, 0.870)	0.641 (0.462, 0.832)
BOCE	+	14.6	-	0.127 (0.0182, 0.469)	0.658 (0.341, 0.800)	0.718 (0.495, 0.846)	0.659 (0.471, 0.839)

Note: TRT: treatment; PR: dual therapy of peginterferon alpha and ribavirin; TELA: telaprevir plus PR therapy; BOCE: boceprevir plus PR therapy; GT1: HCV genotype 1 infection; Null-RESP: null-responders; TW: treatment week; ETR: response rate at the end of treatment; SVR: sustained virologic response; Black[a]: the median value according to each therapy in original dataset; + means all patients in the arm have the corresponding characteristics (covariate value is 100%); - means none of patients in the arm has the corresponding characteristics (covariate value is 0).

Figure 6.1 Observed response rate over time during treatment period according to therapies



Note: PR, therapy of peginterferon alpha and ribavirin; BOC:therapy of boceprevir plus PR; TELA: therapy of telaprevir plus PR; non-GT1: none-genotype 1; GT1: HCV genotype 1; mixed GTs: mixed HCV genotypes.

Figure 6.2 Mathematical structure of model

BASE Model

$$Y_{ijs} \sim Binomial\ (P_{resp,ijs}, N_{ij})$$

Response rate during treatment period

Without 4-week lead-in period

$$P_{resp,ijs} = P_{resp|no\ rev,ijs} \times P_{no\ rev,ijs}$$

$$= P_{max,ij} \times \left[1 - e^{\left(-k_{ij} \times (t_{ijs} - tlag_{ij})\right)}\right] \times \left[1 - \frac{1}{1 + e^{-(-5 + \beta_{1,ij} \times (t_{ijs} - 2))}}\right]$$

With 4-week lead-in period

$$P^{PR}_{resp\ at\ 4,ij} = P^{PR}_{resp|no\ rev\ at\ 4,ij} \times P^{PR}_{no\ rev\ at\ 4,ij}$$

$$= P^{PR}_{max,ij} \times \left[1 - e^{\left(-k^{PR}_{ij} \times (4 - tlag_{ij})\right)}\right] \times \left[1 - \frac{1}{1 + e^{-(-5 + \beta^{PR}_{1,ij} \times (4-2))}}\right]$$

$$P^{TRI}_{resp,ijs} = P^{PR}_{resp\ at\ 4\ ,ij} + \left(P^{TRI}_{max,ij} - P^{PR}_{resp\ at\ 4\ ,ij}\right) \times \left[1 - e^{\left(-k^{TRI}_{ij} \times (t_{ijs} - 4)\right)}\right] \times P^{TRI}_{no\ rev,ijs}$$

Response rate during the follow-up period

$$P_{resp,ij} = P_{resp\ at\ EOT,ij} \times RR_{ij}$$

Covariate and Random Effect Model

$$log(k_{ij}) = \mu_k + \theta_{1,k} \times TELA_{ij} + \theta_{2.k} \times BOCE_{ij} + \eta_i^{study,k} + \eta_{ij}^{arm,k}$$

$$\eta_i^{study,k} \sim N\left(0, \omega_{study,k}^2\right), \quad \eta_{ij}^{arm,k} \sim N\left(0, \frac{\omega_{arm,k}^2}{N_{ij}}\right)$$

$$logit(P_{max,ij}) = \mu_{pmax} + \theta_{1,Pmax} \times GTONE_{ij} + \theta_{2,Pmax} \times BLK_{ij} + \theta_{3,Pmax} \times NRESP_{ij} + \eta_i^{study,\,P_{max}} + \eta_{ij}^{arm,\,P_{max}}$$

$$\eta_i^{study,P_{max}} \sim N\left(0, \omega_{study,P_{max}}^2\right), \qquad \eta_{ij}^{arm,P_{max}} \sim N\left(0, \frac{\omega_{arm,P_{max}}^2}{N_{ij}}\right)$$

$$log(RR_{ij}) = \mu_{RR} + \theta_{1,RR} \times TELA_{ij} + \theta_{2.RR} \times BOCE_{ij} + \theta_{3,RR} \times STERM_{ij} + \eta_i^{study,RR} + \eta_{ij}^{arm,RR}$$

$$\eta_i^{study,RR} \sim N\left(0, \omega_{study,RR}^2\right), \qquad \eta_{ij}^{arm,RR} \sim N\left(0, \frac{\omega_{arm,RR}^2}{N_{ij}}\right)$$

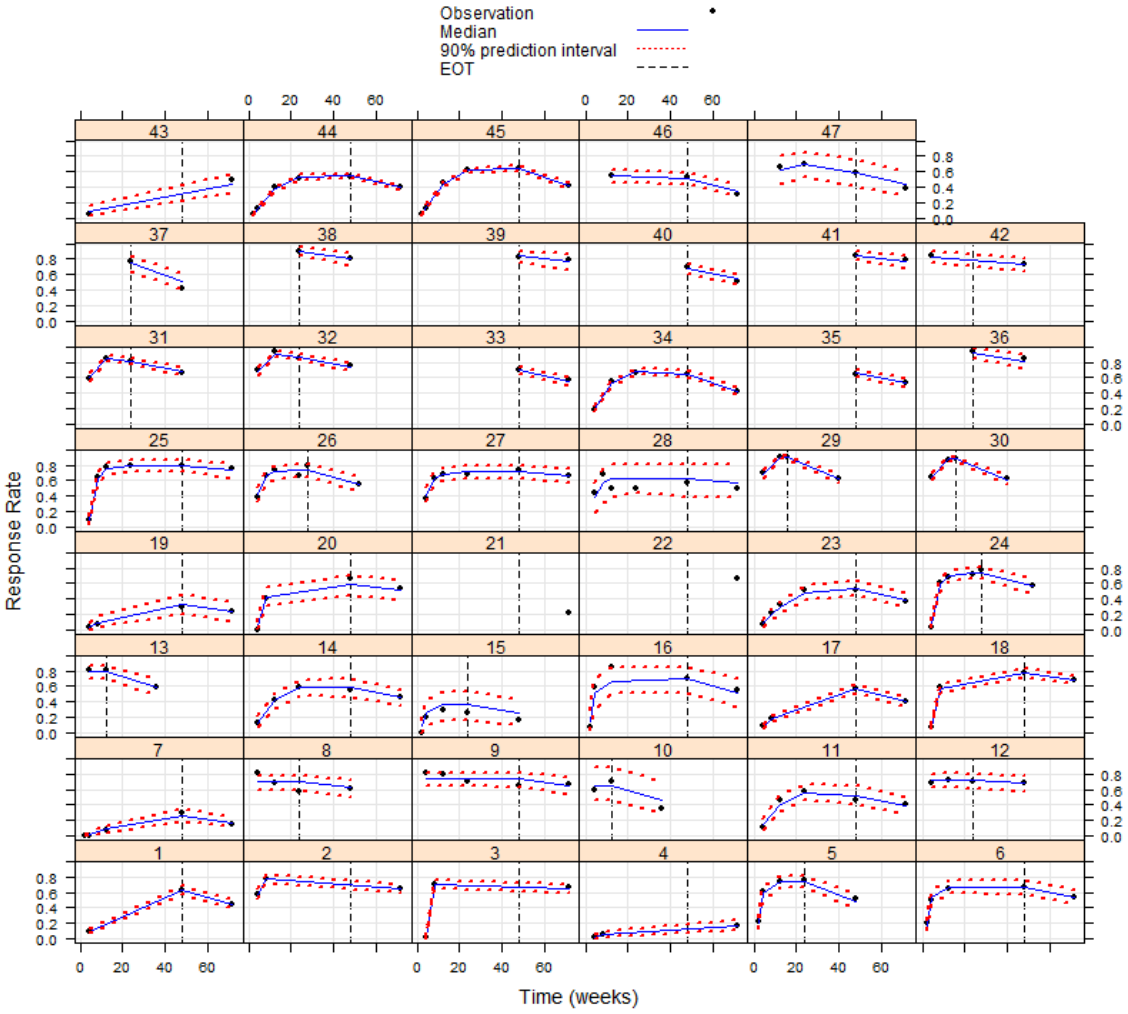
$$log(\beta_{1,ij}) = \mu_{\beta_1} + \theta_{\beta_1} \times TRI_{ij} + \eta_i^{study\,,\beta_1}$$

$$\eta_i^{study,\beta_1} \sim N\left(0, \omega_{study,\beta_1}^2\right)$$

$$tlag_{ij} = \mu_{tlag} \times (1 - TRI_{ij}) + \theta_{tlag} \times TRI_{ij}$$

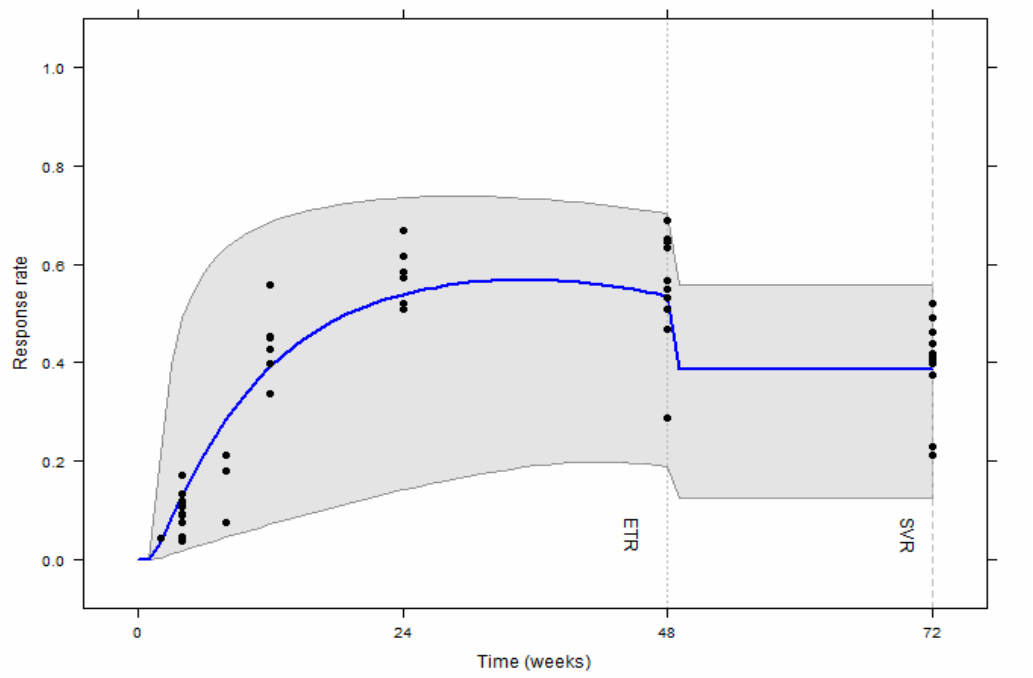
Note: PR: peginterferon alpha and ribavirin; TELA: telaprevir-based therapy; BOCE: boceprevir-based therapy; STERM: short-term therapy; TRI: triple therapy with a protease inhibitor (telparevir or boceprevir) plus PR; GTONE: proportion of patients with genotype 1 HCV infection in a treatment arm; BLK: proportion of African American patients in a treatment arm; NRESP: proportion of prior null responders in a treatment arm. TELA, BOCE, STERM and TRI are categorical covariates with two levels (yes:1 and no:0). GTONE, BLK and NRESP are continuous covariates.

Figure 6.3 Comparison of observations and individual prediction in each treatment arm.



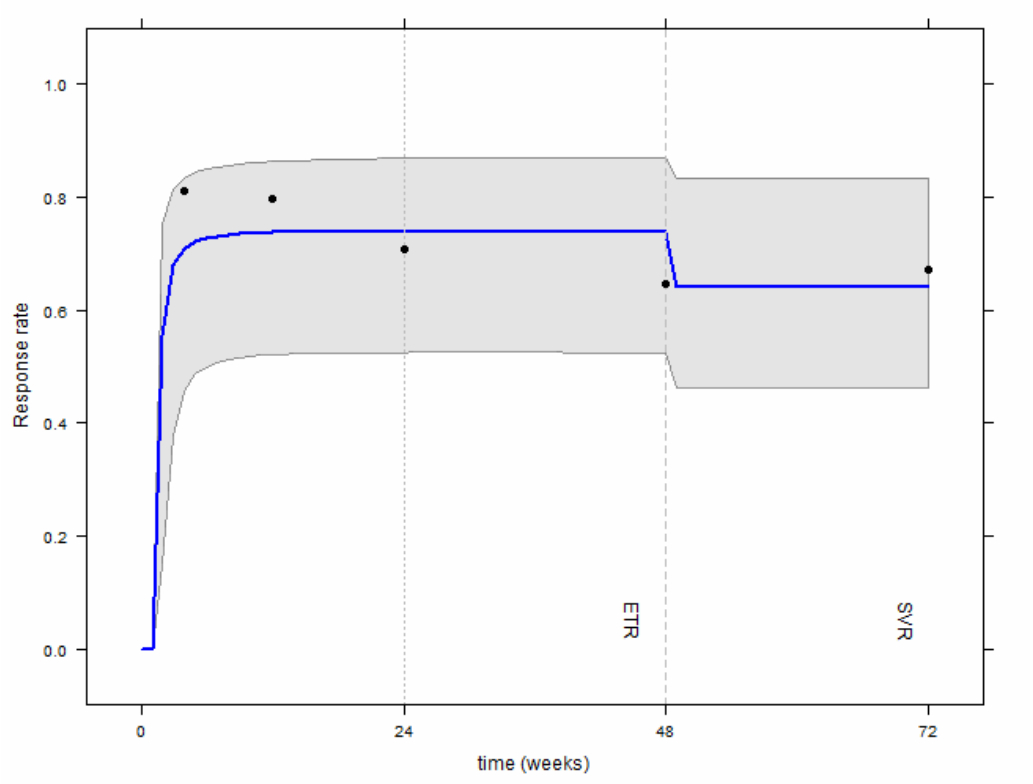
Note: EOT: end of treatment.

Figure 6.4 Model-based prediction for 48-week PR therapy in arms with certain population characteristics



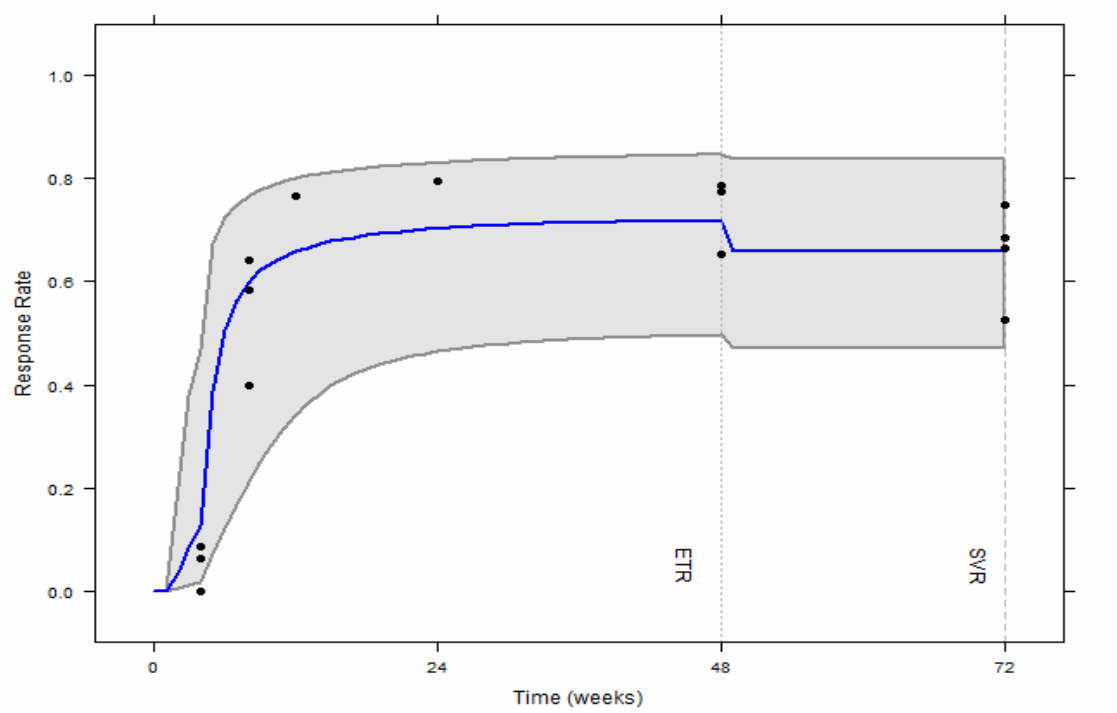
Note: The prediction was performed for treatment arms with genotype 1 patients only, no null-responders and 5.06% black patients; ETR: response rate at the end of treatment (week48); SVR: sustained virologic response (week 72); Black dot represents the observations, blue solid line represents the median of prediction and grey area represents the 90% prediction interval.

Figure 6.5 Model-based prediction for 48-week telaprevir therapy in arms with certain population characteristics



Note: The prediction was performed for treatment arms with genotype 1 patients only, no null-responders and 8.08% black patients; ETR: response rate at the end of treatment (week48); SVR: sustained virologic response (week 72); Black dot represents the observations, blue solid line represents the median of prediction and grey area represents the 90% prediction interval.

Figure 6.6 Model-based prediction for 48-week boceprevir therapy (with 4-week lead-in period) in treatment arms with certain population characteristics.



Note: The prediction was performed for treatment arms with genotype 1 patients only, no null-responders and 14.6% black patients; ETR: response rate at the end of treatment (week48); SVR: sustained virologic response (week 72); Black dot represents the observations, blue solid line represents the median of prediction and grey area resprensents the 90% prediction interval.

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CHAPTER 7
Conclusions

The current thesis work consists of two projects presenting the pharmacometric analyses of drug exposure and response data. The first project applied a population pharmacokinetic modeling approach to estimate the in vivo inhibition constant (Ki) across different CYP2C9 genotypes. In the second project, model-based meta-analysis was performed to describe the time-course of virologic response in hepatitis C clinical trials across current approved treatments.

7.1 IN VIVO INHIBITION CONSTANT

The inhibition constant (Ki) reflects the inhibitor potency of interacting with the enzyme. It is usually determined from in vitro experiments. The in vitro Ki has been used to predict the potential of drug interactions in vivo. According to the guidance of the United States Food and Drug Administration (USFDA), the need of in vivo drug interaction studies for a new molecular entity (NME) is guided by comparing the in vitro Ki of the NME to its relevant concentration in vivo. Extrapolation from in vitro data provides a time and cost saving approach to predict in vivo drug interactions. It serves as a good qualitative evaluation method.[1] However, the predictive utility of in vitro approaches can be confounded by uncertainty and variability during in vitro experiments and complexities in clinical practice.[2–6] It is challenging to quantitatively assess the in vivo drug interaction.

Given the differences between in vivo and in vitro environments and potential variability in experimental conditions, an in vitro Ki may not accurately describe inhibitor potency in vivo. To better evaluate clinical drug interactions, some studies have proposed to determine the in vivo Ki which is considered a more rational and practical indictor of the

inhibitor-enzyme interaction in vivo.[7–10] In these studies, the in vivo Ki was calculated from the formation clearances of the metabolite in the absence and presence of the inhibitor along with inhibitor plasma concentration in each study subject. The average of the in vivo Ki from all subjects was reported as the final estimate of the in vivo Ki. The major limitation of this approach is that the estimation of the in vivo Ki is affected by the inter-individual variability in formation clearance of the metabolite.

Evaluation of an in vivo drug interaction needs to consider multiple clinical factors, such as, pharmacokinetics of inhibitor and substrate and genetic polymorphisms. CYP2C9*3 is one of the mutant alleles that were found to have reduced catalytic activity from both in vitro and in vivo studies.[11,12] The presence of *3 allele leads to a lower oral clearance of the CYP2C9 substrate and also diminishes the interaction between a CYP2C9 inhibitor and the enzyme.[13] A previous study showed that the in vitro Ki varied across three CYP2C9 genotypes, suggesting the inhibitor-enzyme interaction in vivo also depends on CYP2C9 genotype.[14]

In the current work, we implemented a population pharmacokinetic modeling approach to determine the in vivo Ki of fluconazole (CYP2C9 inhibitor) for each of three CYP2C9 genotype groups (*1*1, *1*3, and *3*3). The proposed model was developed incorporating parallel elimination pathways of the CYP2C9 substrate (flurbiprofen) including CYP2C9 mediated metabolic pathway, renal clearance and residual elimination pathway. The study showed the estimates of in vivo Ki were different across the CYP2C9 genotypes. In addition, the magnitude of in vivo Ki was greater in groups with the presence of CYP2C9*3 than that in CYP2C9*1*1, suggesting the inhibition potency of fluconazole in vivo was diminished with the reduced activity in the CYP2C9*3. The in vivo estimates of Ki are comparable to those estimates from in vitro experiments. The analysis also found that the contribution of CYP2C9-mediated metabolism to the total flurbiprofen clearance was less in groups with CYP2C9*3 compared to CYP2C9*1*1. The overall magnitude of drug interaction was less significant in the CYP2C9*3 group

than the CYP2C9*1*1 group. The current population pharmacokinetic analysis allows us to estimate in vivo Ki and also provides a quantitative assessment of in vivo drug interaction. Based on the current findings, the potential of a drug interaction highly depends on the individual CYP2C9 genotypes. It suggests that CYP2C9 genotype needs to be checked when making decisions on dosage modification based on the drug interaction. The major limitation of the current study is that only few subjects with CYP2C9*3*3 were enrolled. By using a population pharmacokinetic approach, it is possible to determine the in vivo Ki and drug interaction in a large number of subjects with fewer samples of substrate and fewer levels of inhibitor. The relevant simulation is needed to optimize the study design with better sampling technique without introducing the bias and precision of the parameter estimates.

7.2 MODEL-BASED META-ANALYSIS OF HEPATITIS C RESPONSE RATE

Hepatitis C is a liver disease caused by infection with the hepatitis C virus (HCV). It can be cured by using antiviral treatment. Peginterferon alpha combined with ribavirin (PR) has been used for decades as standard care of chronic hepatitis C. With the increasing knowledge on molecular pathways of the viral life cycle, more research efforts are focused on the agents that can directly interfere the viral replication process.[15] Two protease inhibitors (PIs), telaprevir and boceprevir, were approved by the USFDA in 2011 for treating chronic HCV genotype 1 infection in adult patients. Both telaprevir and boceprevir are required to be administered along with peginterferon and ribavirin. The efficacy of HCV treatment is primarily determined based on sustained virologic response (SVR), defined as undetectable HCV RNA at week 24 after completion of the treatment. Studies showed SVR rates in PR therapy were 40-50% in patients with HCV genotype 1 infection and 70-80% in patients with genotype 2 or 3 infection.[16–20] With the addition of PI, SVR rate is improved up to 68%-75% in treatment naïve patients and to 41%-52% in patients who failed to achieve SVR in previous PR treatment.[21–24]

To better understand the therapeutic effect of antiviral treatment and minimize the potential risk, it is recommended that patient HCV RNA level be monitored along the treatment, typically at treatment weeks 4, 12 and 24, as well as at the end of treatment. Virologic responses are determined based on measurements of patient HCV RNA levels. Most clinical trials usually report these responses in form of the number of responders whose HCV RNA levels are undetectable at a given time. Several published meta-analyses or systematic reviews have conducted the comparison of treatment effectiveness. However, they were only based on the primary endpoint, the SVR data, while virologic responses at other time points were not included.[25–30]

In the current thesis work, a model-based meta-analysis was conducted to provide a mathematical model describing the time course of longitudinal virologic response during treatment and follow-up periods across approved HCV antiviral treatments including PR therapy, telaprevir combined with PR therapy and boceprevir plus PR therapy. The analysis was firstly conducted in NONMEM 7 by using a maximum likelihood algorithm. However, the initial model had several limitations and computational difficulties when trying to incorporate random effects at multiple levels. An asymptotic model was used to describe the response rate over time during treatment period. However, response rates in some arms were observed to decrease at later time points. The asymptotic model did not account for such potential response reverse. This analysis only compared the response-time profile between PI-based therapy and PR therapy. The treatment effect was not differentiated between telaprevir and boceprevir. In addition, the inter-study variability was not analyzed for all model parameters, which may inflate the corresponding inter-arm variability. This work was our first attempt and it did not include the comprehensive exploration of potential covariate effects. However, the analysis showed that treatment arms with greater proportion of genotype-1 patients tend to have the lower maximum response rate during treatment. The PI-based therapy was associated with quicker response onset than PR therapy.

To overcome these limitations, we continued our model-based meta-analysis work in a Bayesian framework using Markov Chain Monte Carlo algorithm. The potential of response reverse was incorporated into the new model of longitudinal response rate. The reduction in response reverse could be due to treatment discontinuation, inadequate virologic response and patient non-compliance to the treatment. Due to lack of the detailed information regarding these response reverse events, a latent logistic model was used to describe the probability of event occurrence as a function of time. An asymptotic model was used to describe the time course of response rate given no response reverse. Inter-study variability and inter-arm variability weighted by sample size were quantitatively analyzed for model parameters.

The analysis found the telaprevir had the fastest response onset, followed by boceprevir and PR therapy alone. The maximum response rates were lower in treatment arms with greater proportion of genotype 1 patients, black patients and prior null-responders. In the model, the SVR rate was compared to the response rate at the end of treatment. Their ratio was associated with the type and duration of the treatment. Other covariates (e.g., baseline HCV RNA level and advanced fibrosis) were also explored for their effects on model parameters but not found to be significant. It might be due to their confounding with other covariates. In addition, the cut-off value for defining high baseline HCV RNA level varies across studies. Such inconsistence also affects the analysis. The IL28B polymorphism has been demonstrated as a prognostic factor for SVR in some analyses. [31,32] This potential covariate was not included in our current work since the information regarding IL28B was generally not reported in published studies. Further analysis needs to be done to explore potential covariates with more information.

For the response reverse model, the analysis showed that probability of response reverse increased quite slow over time in PI-based therapy and was lower compared to PR therapy. However, more adverse events were observed in clinical trials of PI therapy than PR therapy. Due to the lack of detailed information of response reverse events, the model

cannot differentiate the probability from each kind of possible event. But still, including the latent response reverse model allows evaluation of the potential reduction in response rate in the later phase of treatment. Further study with more data is needed to better characterize the response reverse.

Previous one-endpoint meta-analyses[25–30] did not find the significant differences between telaprevir and boceprevir. By incorporating the longitudinal information of virologic response, the current study evaluated different effects of these two protease inhibitors on the time course of the response rate. The proposed model may be helpful to predict the virologic response for a new candidate drug with the same mechanism of action and compare it with the approved treatments. It can provide more information to support the decision making during the drug development.

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APPENDIX

Chapter 2: NONMEM Code for Population Pharmacokinetic Model

```
;Model Desc: FLUPLASMA+URINE+ META
;Project Name: flurbiprofen

$PROB RUN# 055
$INPUT C ID PID FLUCD IFLUC GT TIME NTIM EVID UVOL DV CMT AMT
$DATA FLUBCOMEAN.CSV IGNORE=C
$SUBROUTINES ADVAN6 TOL=9
$MODEL
COMP=(DEPOT)
COMP=(PLASMA)
COMP=(URINE)
COMP=(URIMETA)

$PK
;KI UMOLAR
IF(GT.EQ.1)THEN
KI=THETA(1)
ELSEIF(GT.EQ.2) THEN
KI=THETA(2)
ELSE
KI=THETA(3)
ENDIF

;INHIBITION FACTOR OF FLUCO ON 2C9META
INH=1/(1+IFLUC/KI)

;BIOFORM OH VIA CYP2C9 BY GENOTYPE
IF(GT.EQ.1) THEN
TVCLFM=THETA(4)*INH
ELSEIF(GT.EQ.2) THEN
TVCLFM=THETA(5)*INH
ELSE
TVCLFM=THETA(6)*INH
ENDIF

  CLFM=TVCLFM*EXP(ETA(1))
  CLR=THETA(7)*EXP(ETA(2))
  CLRES=THETA(8)*EXP(ETA(6))
  CL=CLFM+CLR+CLRES
  TVV=THETA(9)
  V=TVV*EXP(ETA(3))
```

```
  TVK12=THETA(10)
  K12=TVK12*EXP(ETA(4))
  ALAG1=THETA(11)*EXP(ETA(5))
  K23=CLR/V
  K24=CLFM/V
  K20=CLRES/V
  S2=V
  S3=UVOL/1000
  S4=UVOL/1000

SID=ID
TAD=NTIM

$DES
DADT(1)=-K12*A(1)
DADT(2)=K12*A(1)-K23*A(2)-K24*A(2)-K20*A(2)
DADT(3)=K23*A(2)
DADT(4)=K24*A(2)

$ERROR
ASY1=0
IF (CMT.EQ.2)ASY1=1 ; FLUPLASMA
ASY2=0
IF (CMT.EQ.3)ASY2=1; FLURINE

Y=F+F*ASY1*ERR(1)+F*ASY2*ERR(2)+F*(1-ASY1)*(1-ASY2)*ERR(3)

IPRE=F
IRES=DV-IPRE
IWRED=IPRE
IWRE=IRES; W=1


$THETA
(0, 10) ;[KI*1*1]
(0, 15) ;[KI*1*3]
(0, 30) ;[KI*3*3]
(0,0.7) ;[CLFM*1*1]
(0,0.5) ;[CLFM*1*3]
(0,0.3) ;[CLFM*3*3]
(0,0.1) ;[CLR]
(0,0.1) ;[CLRES]
(0,9)   ;[V]
(0,1.5) ;[KA]
(0,0.1) ;[TLAG]
```

```
$OMEGA
  0.1 ;[P] omega(1,1) CLM
  0.1 ;[P] omega(1,1) CLR
  0.1 ;[P] omega(2,2) V
  0.1 ;[P] omega(3,3) KA
  0.1 ;[P] omega(4,4) CLRES
  0.1 ;[P] omega(4,4) CLRES


$SIGMA
 0.1 ;[P] sigma(1,1)
 0.1 ;[P] sigma(2,2)
 0.1 ;[P] sigma(2,2)


$EST METHOD=1 INTERACTION PRINT=5 MAX=9999 NSIG=3 SIGL=9
MSFO=055.MSF

$COV PRINT=E
$TABLE ID TIME NTIM IPRE CMT FLUCD IFLUC GT KI CLFM CLR V K12 CWRES
CPRED ONEHEADER NOPRINT FILE=055.tab
$TABLE ID CLR CLRES V K12  FIRSTONLY NOAPPEND NOPRINT FILE=055.par
$TABLE ID ETA1 ETA2 ETA3  FIRSTONLY NOAPPEND NOPRINT FILE=055.eta
```

Chapter 5: NONMEM Code for model-based meta-analysis of longitudinal hepatitis C virologic response

```
$PROBLEM    RUN# 29 ;COV MODEL
$INPUT C ID STUDY UNIT=DROP ARM TIME RESP=DV POPN EOT BOD EOD STERM
TRI TELA TrtNaiv GTONE Nullresp Relap BriCir Black HRNA
$DATA ../hcvstudycovgtcon.csv IGNORE=C
$PRED
ARM1=0
ARM2=0
ARM3=0
ARM4=0
ARM5=0
ARM6=0
IF(ARM.EQ.1)ARM1=1
IF(ARM.EQ.2)ARM2=1
IF(ARM.EQ.3)ARM3=1
IF(ARM.EQ.4)ARM4=1
IF(ARM.EQ.5)ARM5=1
IF(ARM.EQ.6)ARM6=1
LEAD=0
IF(BOD.EQ.4)LEAD=1
W=1/SQRT(POPN)

TVLOGITPSS=THETA(1)+GTONE*THETA(6)+TRI*THETA(7)
LOGITPSS=TVLOGITPSS+ETA(1)+(ARM1*ETA(4)+ARM2*ETA(5)+ARM3*ETA(6)+ARM
4*ETA(7)+ARM5*ETA(8)+ARM6*ETA(9))*W
PSS=EXP(LOGITPSS)/(1+EXP(LOGITPSS))
TVLOGK=THETA(2)+TRI*THETA(8)
LOGK=TVLOGK+ETA(2)+(ARM1*ETA(10)+ARM2*ETA(11)+ARM3*ETA(12)+ARM4*ET
A(13)+ARM5*ETA(14)+ARM6*ETA(15))*W
K=EXP(LOGK)
TVLOGRR=THETA(3)
LOGRR=TVLOGRR+ETA(3)+(ARM1*ETA(16)+ARM2*ETA(17)+ARM3*ETA(18)+ARM4*
ETA(19)+ARM5*ETA(20)+ARM6*ETA(21))*W
RR=EXP(LOGRR)
TLAG=THETA(4)
IF(LEAD.EQ.1)TLAG=THETA(5)
DELPSS=PSS-EXP(TVLOGITPSS)/(1+EXP(TVLOGITPSS))
DELK=K-EXP(TVLOGK)
DELRR=RR-EXP(TVLOGRR)

MU_1=TVLOGITPSS
MU_2=TVLOGK
MU_3=TVLOGRR
```

```
IF(TIME.LT.TLAG) THEN
P=0
ENDIF
IF(TIME.LE.EOT.AND.TIME.GE.TLAG) THEN
P=PSS*(1-EXP(-K*(TIME-TLAG)))
ENDIF
IF (TIME.GT.EOT) THEN
P=PSS*(1-EXP(-K*(EOT-TLAG)))*RR
ENDIF
Y=-2*(RESP*LOG(P)+(POPN-RESP)*LOG(1-P))

$THETA
3;[LOGITPSS]
-2;[LOGK]
-1;[LOGRR]
(0,1);[TLAG]
(0,1);[TLAG]
-1;[GTONEPSS]
1;[TRIPSS]
1;[TRIK]


$OMEGA
0 FIX; [A] omega2,2
1; [A] omega2,2
0 FIX; [A] omega2,2



$OMEGA BLOCK(1) 9 ;ETA4-ETA9 BAV LOGITPSS
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) 9 ;ETA410-ETA15 BAV LOGK
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
```

```
$OMEGA BLOCK(1) 9 ;ETA416-ETA21 BAV LOGITRR
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME

$ESTIMATION MET=SAEM LAPLACE -2LL NBURN=10000 NITER=1000 CTYPE=3
PRINT=200 MSFO=./29.MSF
$ESTIMATION MET=IMP EONLY=1 ISAMPLE=3000 NITER=5
$COV PRINT=E
$TABLE NOPRINT FILE=./29.tab ID TIME POPN P K PSS RR DELPSS DELK DELRR TRI
GTONE BriCir TrtNaiv Nullresp Relap Black HRNA
```

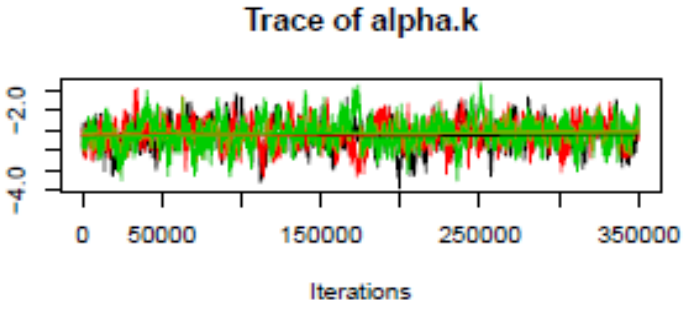
Chapter 6 JAGS Code for model-based meta-analysis of longitudinal hepatitis C virologic response

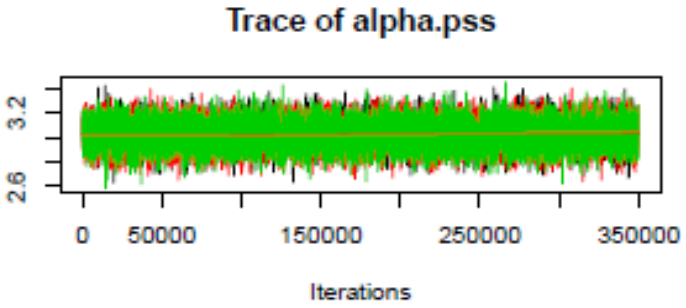
```
model
{
for (i in 1:nobs) {
nvr[i] ~dbin(pvr[i], n[i])
slop0[i]<-exp(alpha.slop+etaslopstudy[obsstudy[i]])
slop[i]<-slop0[i]*exp(beta1*tri1[i])
pbk[i]<-1/(1+exp(-(-5+slop[i]*(time[i]-2))))
pbk0[i]<-1/(1+exp(-(-5+slop0[i]*(time[i]-2))))


st1[i]<-max(0, pss[arm[i]]*(1-exp(-k[arm[i]]*(time[i]-tlag[arm[i]])))*(1-pbk[i]))
st2[i]<-pss[arm[i]]*(1-exp(-k[arm[i]]*(eot[i]-tlag[arm[i]])))*(1-1/(1+exp(-(-5+slop[i]*(eot[i]-
2)))))*rr[arm[i]]

st0[i]<-max(0, pss0[arm[i]]*(1-exp(-k0[arm[i]]*(time[i]-tlag0[arm[i]])))*(1-pbk0[i]))
pbase[i]<-max(0,pss0[arm[i]]*(1-exp(-k0[arm[i]]*(bod1[i]-tlag0[arm[i]])))*(1-1/(1+exp(-(-
5+slop0[i]*(bod1[i]-2))))))
st1bod[i]<-max(0, pbase[i]+(pss[arm[i]]-pbase[i])*(1-exp(-k[arm[i]]*(time[i]-bod1[i])))*(1-
pbk[i]))
st2bod[i]<-(pbase[i]+(pss[arm[i]]-pbase[i])*(1-exp(-k[arm[i]]*(eot[i]-bod1[i])))*(1-1/(1+exp(-(-
5+slop[i]*(eot[i]-2))))))*rr[arm[i]]

pvr[i]<-step(4-bod1[i]-0.1)*(step(time[i]-bod1[i]-0.1)*step(eot[i]-time[i])*st1[i]+step(time[i]-
eot[i]-0.1)*st2[i])
+step(bod1[i]-4)*(step(bod1[i]-time[i])*st0[i]+step(time[i]-bod1[i]-0.1)*step(eot[i]-
time[i])*st1bod[i]+step(time[i]-eot[i]-0.1)*st2bod[i])


nvrCond[i]~dbin(pvr[i], n[i])## posterior prediction for the same trtarm same study
pvrCond[i]<-nvrCond[i]/n[i]
}


for(i in 1:nstudy){
etaslopstudy[i]~dnorm(0, tau.etaslopstudy)
etastudy[i,1:3]~dmnorm(mu.eta,tau.etastudy[1:3,1:3])
etapsstudy[i]<-etastudy[i,1]
etakstudy[i]<-etastudy[i,2]
etarrstudy[i]<-etastudy[i,3]
}


for (i in 1:narm) {
```

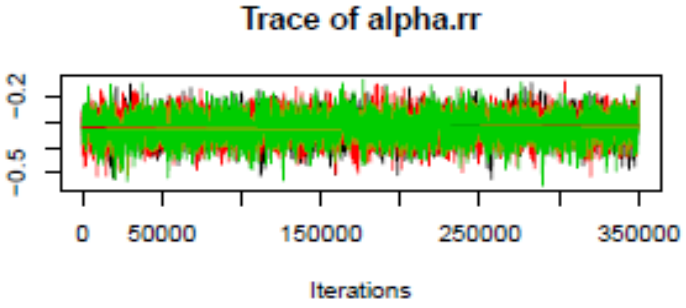
```
k0[i]<-exp(logk0[i])
logk0[i]<-mu.logk0[i]+etakarm[i]/sqrt(n[i])
mu.logk0[i]<-alpha.k+etakstudy[study[i]]

k[i]<-exp(logk[i])
logk[i]<-mu.logk[i]+etakarm[i]/sqrt(n[i])
mu.logk[i]<-mu.logk0[i]+beta3*TEL[i]+beta4*BOC[i]

pss0[i]<-1/(1+exp(-logitpss0[i]))
logitpss0[i]<-mu.logitpss0[i]+etapssarm[i]/sqrt(n[i])
mu.logitpss0[i]<-
alpha.pss+etapsstudy[study[i]]+beta2*GTONE[i]+beta7*BLACK[i]+beta9*NULLRESP[i]

pss[i]<-1/(1+exp(-logitpss[i]))
logitpss[i]<-mu.logitpss[i]+etapssarm[i]/sqrt(n[i])
mu.logitpss[i]<-mu.logitpss0[i]

rr[i]<-exp(logrr[i])
logrr[i]<-mu.logrr[i]+etarrarm[i]/sqrt(n[i])
mu.logrr[i]<-alpha.rr+etarrstudy[study[i]]+beta5*TEL[i]+beta6*STERM[i]+beta10*BOC[i]
tlag0[i]<-lagt
tlag[i]<-lagt*(1-TRI[i])+beta8*TRI[i]

etaarm[i,1:3]~dmnorm(mu.eta,tau.etaarm[1:3,1:3])
etapssarm[i]<-etaarm[i,1]
etakarm[i]<-etaarm[i,2]
etarrarm[i]<-etaarm[i,3]

}

### prior dist
alpha.slop~dnorm(-2, 1.0E-6)
alpha.pss~dnorm(3, 100)
alpha.k~dnorm(-2,1.0E-6)
alpha.rr~dnorm(-0.2, 1.0E-6)
lagt~dunif(0,2)
beta1~dnorm(0, 1.0E-2)
beta2~dnorm(0, 1.0E-6)
beta3~dnorm(0, 1.0E-6)
beta4~dnorm(0, 1.0E-6)
beta5~dnorm(0, 1.0E-6)
beta6~dnorm(0, 1.0E-6)
beta7~dnorm(0, 1.0E-6)
beta8~dunif(0,2)
beta9~dnorm(0, 1.0E-6)
beta10~dnorm(0, 1.0E-6)
```

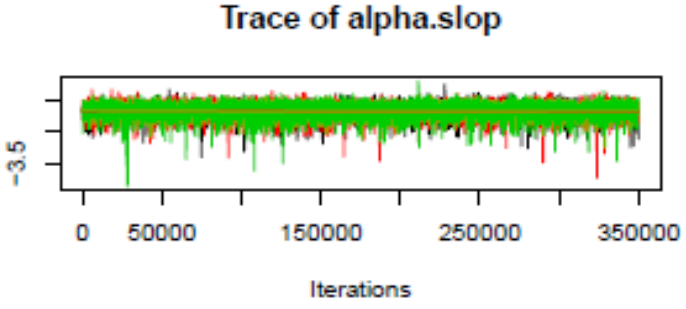
```
tau.etaarm[1:3,1:3]~dwish(R[1:3,1:3],3)
sigma.etaarm2[1:3,1:3]<-inverse(tau.etaarm[1:3,1:3])
omega.pss<-sqrt(sigma.etaarm2[1,1])
omega.k<-sqrt(sigma.etaarm2[2,2])
omega.rr<-sqrt(sigma.etaarm2[3,3])

tau.etastudy[1:3,1:3]~dwish(R[1:3,1:3],3)
sigma.etastudy2[1:3,1:3]<-inverse(tau.etastudy[1:3,1:3])
omega.psstudy<-sqrt(sigma.etastudy2[1,1])
omega.kstudy<-sqrt(sigma.etastudy2[2,2])
omega.rrstudy<-sqrt(sigma.etastudy2[3,3])

omega.slopstudy~dunif(0, 10)
tau.etaslopstudy<-1/(omega.slopstudy*omega.slopstudy)
}
```

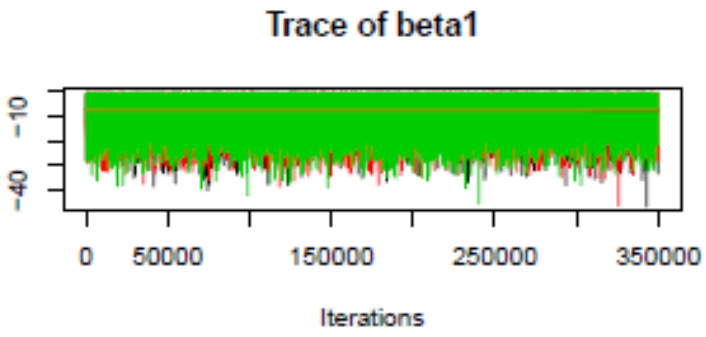
Chapter 6 Trace plots of major model parameters in Bayesian model

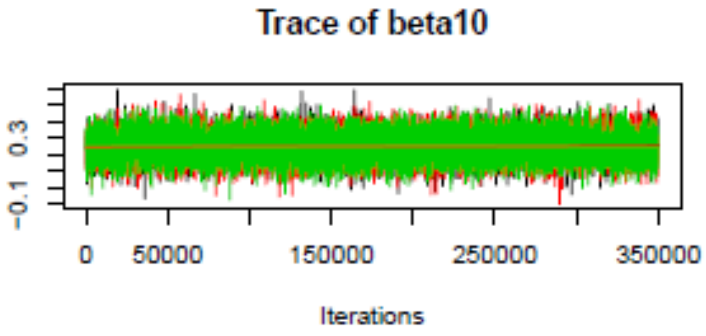


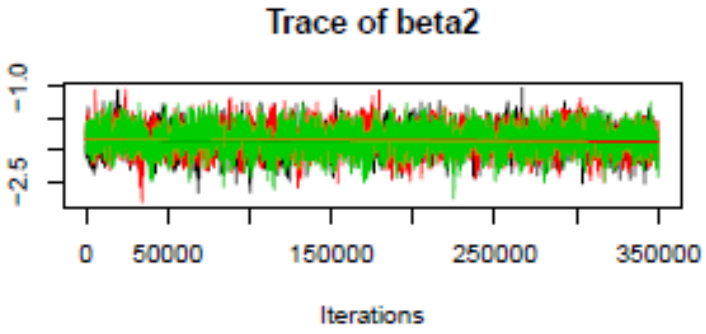




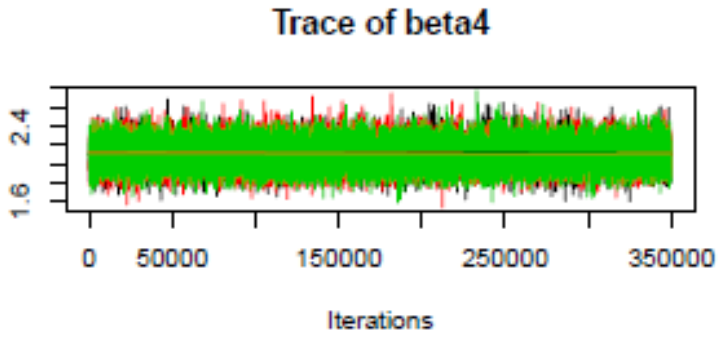


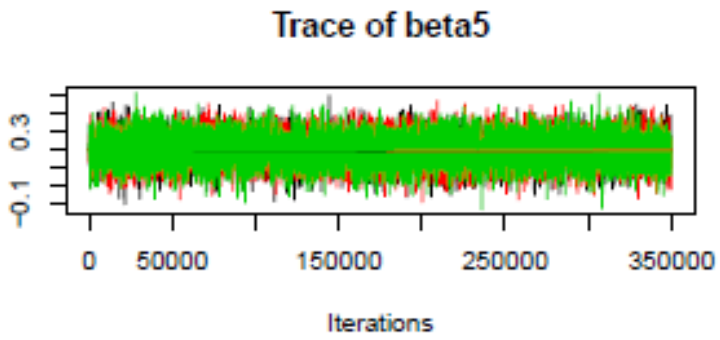

Trace of beta1
−40
−10
0
50000
150000
250000
350000
Iterations

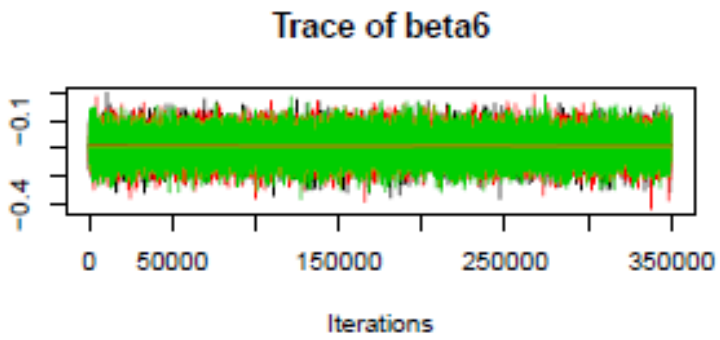

Trace of beta10
−0.1
0.3
0
50000
150000
250000
350000
Iterations

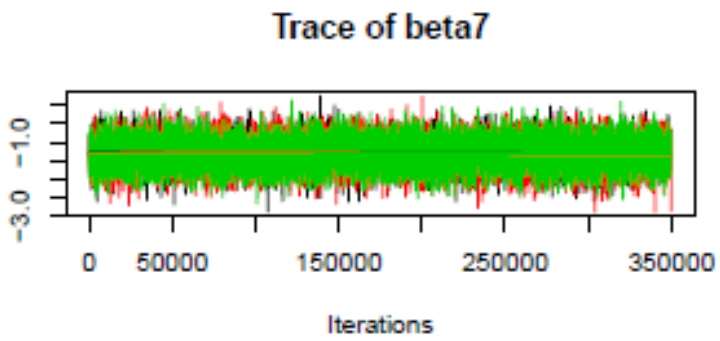

Trace of beta2
−2.5
−1.0
0
50000
150000
250000
350000
Iterations

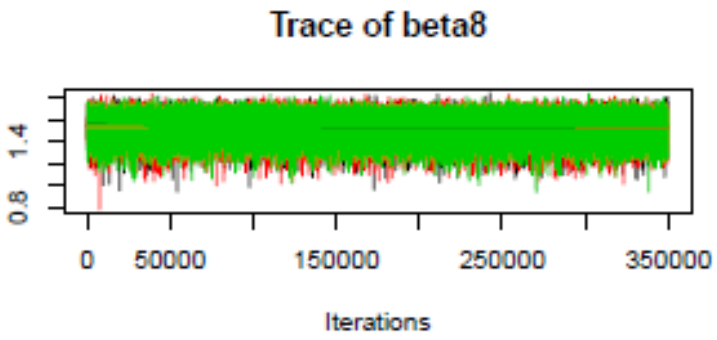

Trace of beta3
3.5
4.5
0
50000
150000
250000
350000
Iterations

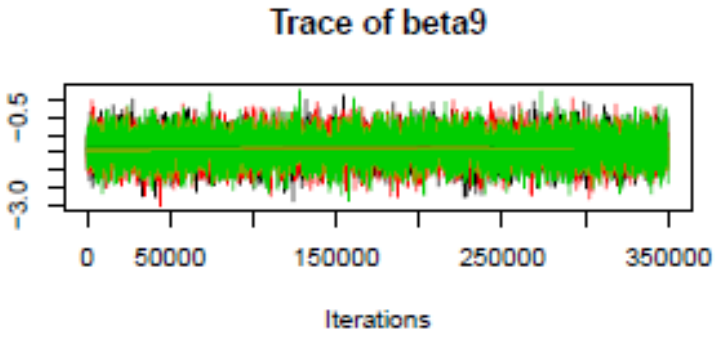

Trace of beta4
1.6
2.4
0
50000
150000
250000
350000
Iterations

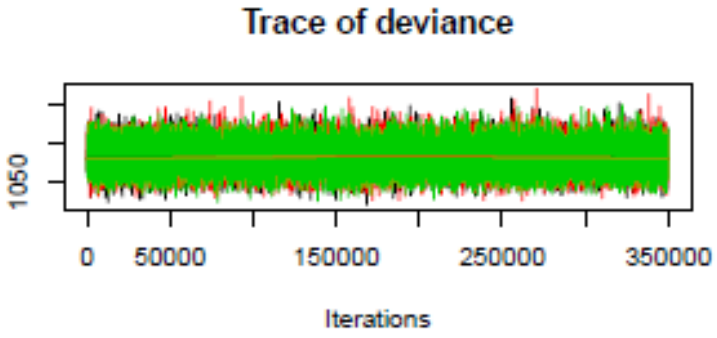

Trace of beta5
-0.1
0.3
0
50000
150000
250000
350000
Iterations


Trace of beta6
-0.4
-0.1
0
50000
150000
250000
350000
Iterations


Trace of beta7
-3.0
-1.0
0
50000
150000
250000
350000
Iterations


Trace of beta8
0.8
1.4
0
50000
150000
250000
350000
Iterations


Trace of beta9
−3.0
−0.5
0
50000
150000
250000
350000
Iterations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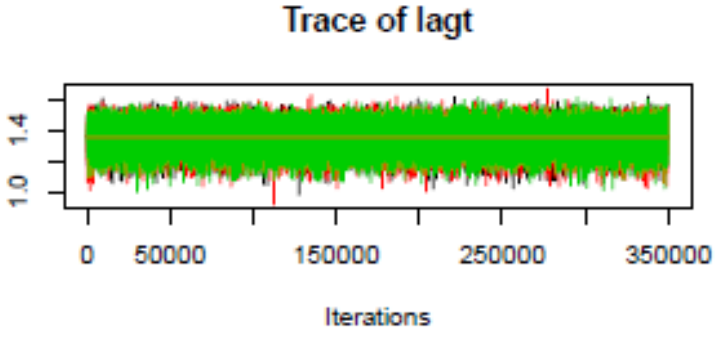
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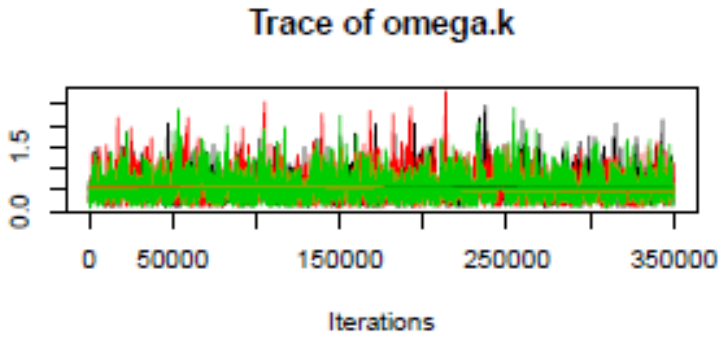
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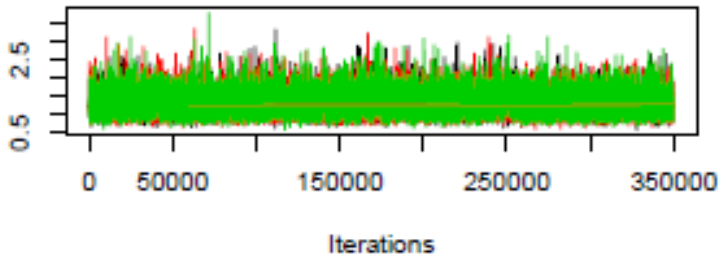
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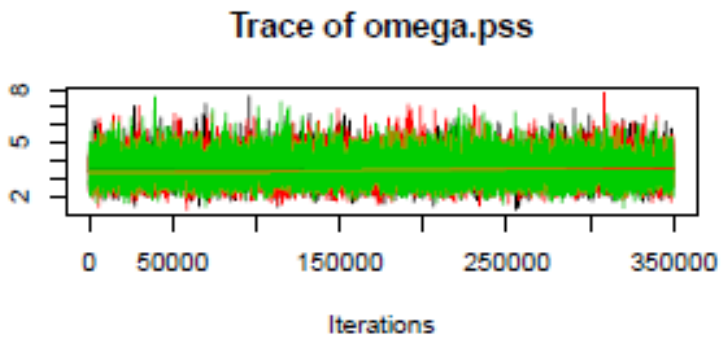
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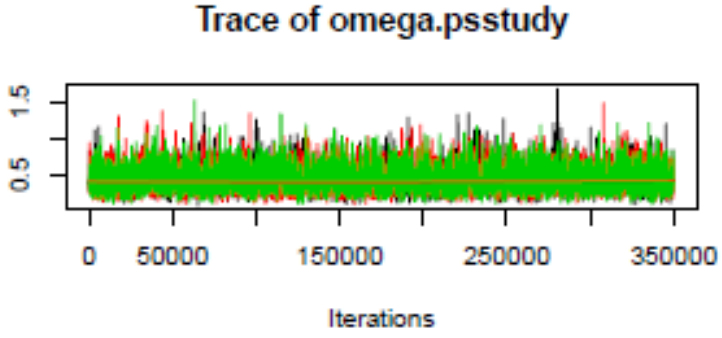
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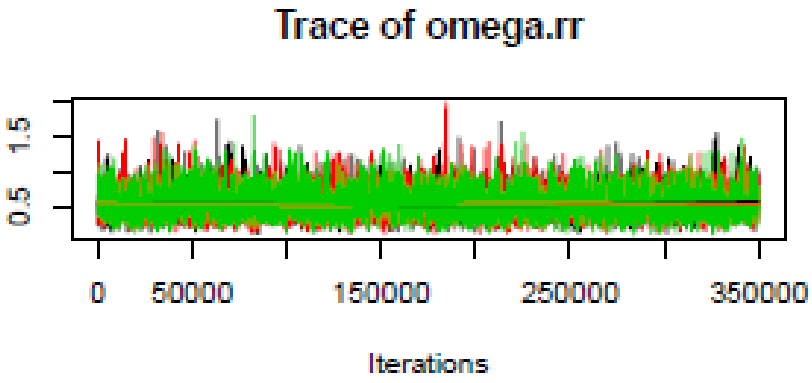
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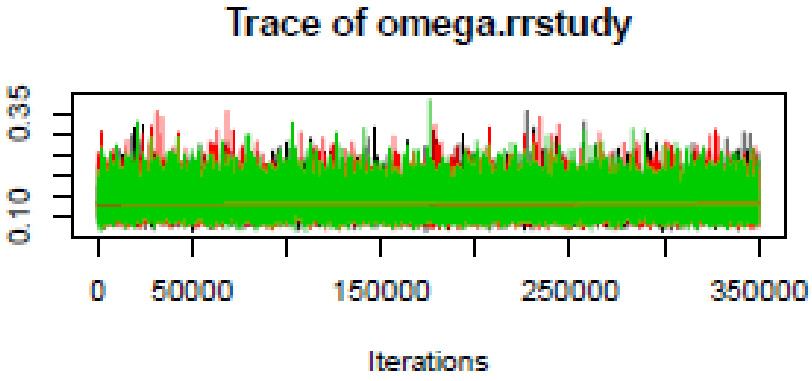
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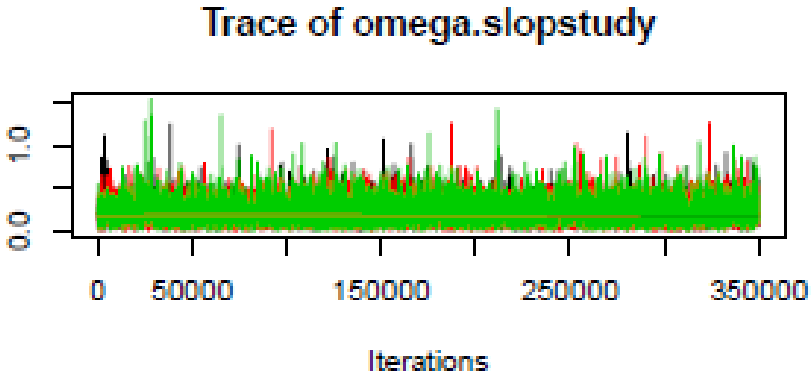
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