Model-Based Assessments of Source of Variability in Drug Exposure and Exposure-Response Relationship for Antiviral Drugs of Chronic Hepatitis C with/without Compensate Cirrhosis

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Abstract

Approximately 80-185 million individuals are infected with hepatitis C virus (HCV) worldwide and the number of HCV-infected patients is estimated to be approximately 2 million in Japan. Interferon preparations were developed for the treatment of HCV infection, and afterwards pegylated preparations of IFN (pegIFN) improved response rates and the addition of ribavirin (RBV) as combination therapy improved the rate of successful therapy. However, the sustained virologic responses of pegIFNα/RBV therapy were around 50%, and adverse drug reactions such as influenza-like symptoms and clinical hematology abnormalities were observed in more than 50% of patients.

In recent years, a direct-acting antiviral therapy without IFN has been developed. As the first direct-acting antiviral therapy, the combination regimen of daclatasvir (DCV) and asunaprevir (ASV) (referred to throughout this document as DUAL regimen) was approved for treatment of chronic HCV genotype-1 infection. However, there were still difficult-to-treat patients. The fixed-dose combination comprised of DCV, ASV and beclabuvir regimen (referred to throughout this document as 3DAA regimen) was developed, and showed a robust viral clearance.

This study was conducted to characterized the population pharmacokinetic (PopPK) models to help explain the source of variability in drug exposure for DUAL

regimen and 3DAA regimen, and to characterize the relationship between the exposures and liver-related laboratory elevations for 3DAA regimen by exposure-response (E-R) analyses to provide better understanding of safety profile in HCV infected patients.

The results from PopPK analyses showed that ASV exposure increased with cirrhosis and increasing baseline and time-varying AST/ALT values. Asian subjects had greater ASV and beclabuvir exposures than White subjects. All significant covariates included in DCV PopPK model were not considered clinically relevant. Based on the results from safety E-R analysis, higher ASV exposure was associated with increases in Grade 3 or 4 alanine aminotransferase (ALT) and Grade 3 or 4 total bilirubin (TB) elevations rates, however, the impact of ASV exposure on the ALT elevation was not clinically relevant and the effect of ASV exposure on Grade 3 or 4 TB elevation was smaller than the other significant covariates. The higher safety event rates observed in Japanese subjects were not fully explained by the difference in ASV exposure. The effect of race was the most significant covariate for both Grade 3 or 4 ALT and Grade 3 or 4 TB elevations rates, suggesting careful monitoring for the risk of severe liver disorder would be required for Japanese patients. The key covariates identified in the PopPK and E-R models help to explain the source of variability of the exposures and clinical outcome, and may guide clinical use of the drug.

LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ASV	asunaprevir
AUC	area under the concentration versus time curve
AUCss	area under the concentration versus time curve at steady state
BCRP	breast cancer resistance protein
BCV	beclabuvir
BID	twice-daily
Cavg	average concentration
Cavgss	average concentration at steady-state
CL/F	apparent oral clearance
CRCL	creatinine clearance
CYP	cytochrome P450
DAA	direct-acting antiviral
DCV	daclatasvir
E-R	exposure-response
GT	genotype
HCV	hepatitis C virus
INF	interferon
Ka	absorption rate constant
OATP	organic anion transporting polypeptide
OFV	objective function values
pcVPC	prediction-corrected visual predictive check
pegIFN	pegylated interferon
P-gp	P-glycoprotein
PK	pharmacokinetics
PopPK	population pharmacokinetics
RBV	ribavirin
TB	total bilirubin
Vc/F, V/F	apparent volume of the central compartment
Vp/F	apparent volume of the peripheral compartment

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1 INTRODUCTION

Approximately 80-185 million individuals are infected with hepatitis C virus (HCV) worldwide and the number of HCV-infected patients is estimated to be approximately 2 million in Japan.[1-3] It is estimated that 20% of patients with chronic HCV infection will develop cirrhosis.[4] In recent years, HCV treatment have evolved rapidly from peginterferon (pegIFN) plus ribavirin (RBV) to all-oral combinations of direct-acting antiviral (DAA) agents.[5, 6]

Interferon preparations were developed for the treatment of HCV infection, and afterwards pegIFN improved response rates and the addition of RBV as combination therapy incrementally improved the rate of successful therapy. It was reported that viral infection is eliminated from the body with IFN-based therapy, the development of liver cirrhosis and hepatocellular carcinoma can be prevented.[7] However, the sustained virologic responses of pegIFNα/RBV therapy were around 50%, and adverse drug reactions such as influenza-like symptoms and clinical hematology abnormalities were observed in more than 50% of patients.[8, 9] The adverse drug reactions often lead to treatment discontinuation or obstruct treatment initiation in patients who are elderly and/or have concurrent disease.

In recent years, a DAA therapy without IFN has been developed. As the first DAA therapy, the combination regimen of daclatasvir (DCV) and asunaprevir (ASV) (referred to throughout this document as “DUAL”) was approved in July 2014 for an indication for treatment of chronic HCV genotype (GT)-1 infection (including compensated cirrhosis) in patients who are ineligible-naïve or intolerant to IFN-based therapy or who have failed to respond to IFN-based therapy, and in March 2015 for the remaining patients with chronic HCV infection with/without compensated cirrhosis. DUAL regimen improved effectiveness and safety profiles/tolerability of HCV therapy with limited adverse drug reactions and high treatment adherence. However, there were still

difficult-to-treat patients. In DUAL regimen, sustained virologic response at post -treatment week 12 rates were 36.9% and 41.9% in subjects with baseline mutation of Y93H and L31F/I/M/V, respectively.[10] Therefore, highly effective treatment without resistance variants was considered necessary.

The fixed-dose combination comprised of DCV, ASV and beclabuvir (BCV) regimen (referred to throughout this document as "3DAA") was developed based on DUAL regimen by adding BCV (non-nucleoside nonstructural protein 5B inhibitor). BCV was developed only for use in combination with DCV and ASV. Results of clinical studies showed a robust viral clearance of HCV in infected subjects treated with 3DAA regimen regardless the presence or absence of drug-resistant polymorphisms.[11-13] In the phase 2 and phase 3 clinical trials, 3DAA regimen showed a high safety profile with minimal serious adverse events (AEs) and AE related discontinuations, although the safety event rates were slightly higher in Japanese HCV patients compared to non-Japanese HCV patients.[11-15] Safety profiles of 3DAA regimen and DUAL regimen were generally comparable.[13] 3DAA regimen as a fixed combination tablet was approved in Japan in 2016.

DCV is a substrate and inhibitor of the P-glycoprotein transporter (P-gp) and a substrate of, and weak inducer of, cytochrome P450 (CYP) 3A4 with minimal effects on the levels of the sensitive CYP3A4 probe midazolam in plasma.[16] DCV is excreted primarily (~88%) via feces in an unchanged form, with renal elimination accounting for a minor pathway for DCV (~ 7% of dose).[17] There was no obvious association between exposure and degree of hepatic impairment or biochemical/serological markers of liver dysfunction.[18]

ASV was also readily absorbed, with median time to maximum observed concentration ranged from 2.0 to 4.0 hour, and generally increased dose-proportionally within dose-range studied. Steady-state was generally achieved between Days 3 and 5.[19] ASV is eliminated primarily

via CYP 3A4–mediated hepatic metabolism.[20, 21] ASV is a weak inducer and sensitive substrate of CYP3A4, a moderate inhibitor of CYP2D6, a weak inhibitor and sensitive substrate of organic anion transporting polypeptide (OATP)-mediated uptake transport and a weak inhibitor of P-gp.[22] Significant food effects have been observed with the ASV tablet formulation.[23] A soft-gel capsule was developed as a food-effect mitigating formulation, and provided higher exposures (~2 fold for the area under the concentration versus time curve (AUC)) with or without food than the tablet formulation given with food.[23] Hepatic function impairment had been shown to significantly impact the steady-state pharmacokinetic (PK) of ASV.[24]

BCV is a substrate and inhibitor of P-gp and a substrate of breast cancer resistance protein (BCRP) and CYP3A4.[25, 26] There was no clinically meaningful drug-drug interaction effect among DCV, ASV and BCV.[27] BCV was readily absorbed such that median time to maximum observed concentration was achieved approximately 2 to 4 hours post dose, with a mean half-life of 7 to 9 hours, following single doses oral administration of BCV. The BCV exposures increased slightly more than proportionally over the 100 to 900 mg dose range.[28]

A significant fraction of DCV elimination is due to CYP3A4-mediated metabolism and DCV is a substrate of P-gp. ASV is cleared primarily via CYP3A4-mediated metabolism and is a substrate of P-gp and OATP1B1. BCV is a substrate of P-gp, BCRP, and CYP3A4. All three drugs were evaluated in clinical pharmacology studies, with metabolism and biliary excretion as the major clearance pathways. These results indicate that the PKs for Dual regimen and 3DAA regimen would be affected by co-administration of agents that modify CYP3A, P-gp and OATP1B1 activities, and hepatic function. Furthermore, other factors could affect exposures and/or clinical outcome for DUAL regimen and 3DAA regimen in the target population due to the variability in terms of the patient characteristics.

Population pharmacokinetics (PopPK) and exposure response (E-R) analyses are useful approach to help explain the source of variability in drug exposure by investigating the potential relationships between covariates and the PK, and to assess the effect of exposure on clinical endpoints.

The objectives of this research were to explain the source of variability in drug exposure by investigating the potential relationships between covariates and the PK parameters by means of establishing the PopPK models for DUAL regimen and 3DAA regimen, and to provide better understanding of safety by means of establishing the safety E-R models for 3DAA regimen.

2 RESEARCH 1: POPULATION PHARMACOKIETNC ANALYSIS OF DUAL REGIMEN IN HCV-INFECTED JAPANESE SUBJECTS

2.1 Objective

To develop the PopPK models for DUAL regimen in Japanese subjects with HCV infection to help explain the source of variability in drug exposure by investigating the potential relationships between covariates and the PK.

2.2 Method

DCV and ASV PopPK models were developed from 4 (AI444021: ClinicalTrials.gov identifier: NCT01016912, AI444022: NCT01017575, AI447017: NCT01051414 and AI447026: NCT01497834)[29-32] and 2 (AI447017 and AI447026) clinical studies, respectively. DCV was administered either as part of DUAL regimen with ASV or combination with pegIFN/RBV. DCV doses investigated were 10 mg and 60 mg, given once daily, and ASV doses that were investigated included 200 and 600 mg twice daily (BID) as tablet formulation, and 100 mg BID as soft-gel capsule formulation. Eligible subjects were Japanese male or female equal/greater than 20 years

of age, with chronic HCV genotype 1 infections. Subjects with compensated cirrhosis were included.

The PopPK of DCV and ASV was characterized by a nonlinear mixed-effects model. The PopPK model development was comprised of establishing a base, full and final model. A base model was developed to represent the best description of the data without considering the effect of covariates. The structural, inter-individual variability, and residual-error models were determined.

Inter-individual variability was described using a log normal distribution, as given below:

$$\theta_i = \theta_{pop} \cdot exp(\eta_i)$$

where θ_i is the parameter for the *i*th subject, θ_{pop} is the typical value of the population, η_i is an independent random inter-individual variability with mean 0 and variance of ω^2.

The residual error model for a log transform-both-sides approach was used. The residual variability was assumed to be log normally distributed, as follows:

$$ln(y_{ij}) = ln(\hat{y}_{ij}) + \theta_{ADD} \cdot \varepsilon_{ij}$$

where y_{ij} and $\hat{y}_{ij}$ represent the *j*th observed and predicted concentration, respectively, for the *i*th subject and ε is the residual intra-subject random error with the standard deviation θ_{ADD}.

The full model was developed by incorporating the effects of all pre-specified covariates on structural model parameters. **Table 2.2-1** provides a list of pre-specified covariates and their relationships to the PK parameters. The selection of the pre-specified covariates and associated PK parameters was based on clinical interest and pharmacological plausibility. All pre-specified

covariates were included in the full model without considering significance level. If correlation was observed between covariates, the most significant term was kept in the full model.

Table 2.2-1: Covariates and Related Pharmacokinetic Parameters

DCV

Covariate	Apparent Clearance (CL/F)	Apparent Volume(V/F)
Age	√	
Sex (Male / Female)	√	
Body Weight	√	√
Treatment description ([pegIFNα/RBV] / ASV [DUAL])	√	
Cirrhosis (Yes / No)	√	
Baseline creatinine clearance	√	
Patient type (Non-, Null or Partial responder / Treatment-naïve, IFN ineligible naïve or intolerant subjects)	√	
Alanine aminotransferase (ALT)	√	
Aspartate aminotransferase (AST)	√	

ASV

Covariate	Apparent Clearance (CL/F)	Apparent Volume (V/F)
Age	√	
Sex (Male / Female)	√	
Baseline body weight	√	√
Cirrhosis (Yes / No)	√	
Formulation (Phase2 formulation / Phase 3 formulation)	√	√

Covariate	Apparent Clearance (CL/F)	Apparent Volume (V/F)
Alanine aminotransferase (ALT)	√	
Aspartate aminotransferase (AST)	√	
Baseline creatinine clearance	√	
Patient Type (Non-responder , Null-responder / pegIFN/RBV or IFN-based therapy ineligible naïve or intolerant subjects)	√	
OATP haplotype	√	

The effect of continuous covariates on the PK parameters was modeled as follows:

$$\theta_i = \theta_{pop} \cdot \left(\frac{Cov_i}{Cov_{pop}}\right)^{k_{cov}}$$

where θ_i is a model parameter for *i*th subject, θ_{pop} is the typical value of a parameter, Cov_i is a continuous covariate for *i*th subject, Cov_{pop} is an index describing the typical value of this covariate in the population, k_{cov} is a coefficient describing the strength of the covariate effect.

Time-varying continuous valued covariates were assessed by evaluating the effect of both the baseline value of the covariate, as well as the effect of the change from baseline by the following relationship:

$$\theta_i = \theta_{pop} \cdot \left(\frac{Cov_{b,i}}{Cov_{b,pop}}\right)^{k_{cov,b}} \cdot \left(\frac{Cov_{t,i}}{Cov_{b,i}}\right)^{k_{cov,t}}$$

where, $Cov_{b,i}$ or $Cov_{t,i}$ is a continuous covariate at baseline or at time *t* for *i*th subject, respectively, $Cov_{b,pop}$ is an index describing the typical value of this covariate at baseline in the population, $k_{cov,b}$ and $k_{cov,t}$ is a coefficient describing the strength of the covariate effect at baseline ant time *t*, respectively.

The relationship between the typical value of a parameter and a categorical covariate was tested using the following relationship:

$$\theta_i = \theta_{pop} \cdot e^{k_{Cov} \cdot X_i}$$

where X_i is an indicator variable for *i*th subjects for categorical variable.

The final PopPK model was started from full model and obtained by removing each covariate one at a time. Model-based tests of covariate-parameter relationships were assessed with likelihood ratio test for backward elimination. The likelihood ratio test is based on the property that the difference of the objective function values (OFV) of two hierarchical models (-2 loglikelihood) is asymptotically $\chi 2$ distributed. Covariates were tested by backward elimination. A significance level of 0.001 was used for the backward elimination (which corresponds to an increase in the OFV of 10.83, 13.82 or 18.47, for 1, 2 or 4 degrees of freedom, respectively). The covariate with the smallest change in minimum OFV was removed from the model, until all remaining covariates were significant ($p < 0.001$). The 95% confidence intervals of estimated parameters were calculated by bootstrap method.

The OFV for *i*-th subject with n_i observation number can be denoted as [33]:

$$OFV_i = \sum_{i=1}^{n_i} \left(\log(\sigma_i^2) + \frac{\left(Y_i - \hat{Y}_i\right)^2}{\sigma_i^2} \right)$$

whrere, Y_i and $\hat{Y}_i$ are the measured observation and the prediction of that observation for *i*-th subjects by the model, and σ^2 is the variance of the model. The OFV is simply the summation of individual objective function values (OFV*i*).

The shrinkage estimates of inter-individual and intra-individual variability of the final PopPK model was assessed using the appropriate formula and manner.[34]

The shrinkage of inter individual variability can be denoted as:

$$sh_{\eta} = 1 - \frac{SD(\eta_{EBE})}{\omega}$$

The shrinkage of intra individual variability can be denoted as:

$$sh_{\varepsilon} = 1 - SD(IWRES)$$

where, $SD(\eta_{EBE})$ is the standard deviation of the empirical Bayes estimates distribution, ω is the standard deviation of population parameters, SD(IWRES) is the standard deviation of the individual weighted residuals and IWRES is described as $\frac{(Y_i - \hat{Y}_i)}{\sigma_i}$

High shrinkage (usually greater than 20 to 30%) would indicate the lack of information for parameter estimates.

The impact of significant covariates on the PK parameters was illustrated using forest plots. Pharmacokinetic parameters at 5th percentile and 95th percentile of the population values of the continuous covariates, or at different levels of the categorical covariates were compared with typical PK estimates. Effects of covariates at extreme values and associated 95% confidence intervals, when wholly contained within the 80% to 125% boundaries of the typical PK estimates, may suggest a lack of clinical relevance.

The diagnostic graphs include the population predicted and individual predicted mean concentrations versus observed concentrations and the conditional weighted residuals (CWRES)[35] versus the population predicted mean/the individual predicted mean and time. This set of diagnostic graphs shows whether the predicted concentrations match the observed concentrations.

Prediction-Corrected visual predictive check (pcVPC) was created to show the time course of the predicted mean and spread of concentrations (5th to 95th percentile) versus the observed

data.[36] A total of 1000 trial replicates was simulated using the observed covariates and dose regimens for each subject, the final model parameter estimates, and simulated subject-specific random effects and residual errors. The pcVPC showed the overall model fit of all PK data with different dose regimens.

The PopPK analysis was performed by nonlinear mixed effects modeling using the NONMEM computer program (Version 7.2, Icon Development Solutions). Diagnostic graphics, exploratory analyses, and post-processing of NONMEM output were performed using the S-Plus software (Version 8.1 for Linux, Insightful, Seattle, WA).

2.3 Results

2.3.1 DCV

A total of 3801 pharmacokinetic records from 336 subjects were included for model development. A one-compartment PK structure model with a first order absorption was identified as an optimal model. Inter-individual variability was estimated in apparent clearance (CL/F), apparent volume of the central compartment (V/F) and absorption rate constant (Ka), with correlation between CL/F and V/F. The residual error model was additive in log-transformed DCV plasma concentrations. To develop the full model, pre-specified covariates and those correlations were investigated. Because the correlation between age, baseline body weight and baseline creatinine clearance (CRCL), and the correlation between baseline aspartate aminotransferase (AST) and baseline alanine aminotransferase (ALT) were moderate or high, the relationships between these covariates and CL/F were separately tested. As a result, baseline CRCL and baseline ALT on CL/F showed greatest OFV change among other covariates and were retained in the full model. All other pre-specified covariates were included into the full model. Minimization and

convergence was successful. Significant covariates ($p<0.001$) remaining in the final model included sex, treatment description and baseline CRCL on CL/F and baseline body weight on V/F.

The final model parameters are presented in **Table 2.3.1-1**. Inter-individual variability of CL/F and V/F of the final model were reduced from those of the base model (39.7% to 39.4% and 40.7% to 38.1%, respectively). The residual errors were similar between the base model and the final model. The shrinkage in CL/F, V/F and Ka of subjects were 4.3%, 11.8% and 19.2 %, respectively, thus implying there is sufficient PK information from individual subjects to provide reliable individual parameter estimates for majority of the subjects.

Table 2.3.1-1: Parameter Estimates for the DCV Final PopPK Model

Name[a] [Units]	Estimate[b]	Standard Error (RSE%) [c]	95% Confidence Interval[d]
Fixed Effects			
CL/F [L/h]	5.29	0.161 (3.04)	4.98- 5.59
V/F [L]	64.2	2.04 (3.18)	60.1 - 68.2
Ka [hr]	0.865	0.0516 (5.97)	0.753 - 0.974
CL/F ~GENDER	-0.110	0.0274 (24.9)	-0.197 - -0.053
CL/F ~TX	-0.122	0.0336 (27.5)	-0.189 - 0.132
CL/F ~BCRCL	0.235	0.0462 (19.7)	0.142 - 0.333
V/F ~BBWT	0.605	0.0989 (16.3)	0.405 - 0.974
Random Effects			
CL/F	0.155 (0.394)	0.0157 (10.1)	0.0176 - 0.182
V/F	0.145 (0.381)	0.0216 (14.9)	0.0168- 0.186
KA	0.756 (0.869)	0.105 (13.9)	0.590- 0.968
CL/F:V/F	0.141 (0.941)	0.0161 (11.4)	0.00099- 0.171
σ	0.107 (0.327)	0.0193 (18.0)	0.072- 0.148
Residual Error			
σ	0.375	0.0102 (2.72)	0.358 - 0.408

[a] CL/F, apparent clearance of the central compartment; V/F, apparent volume of the central compartment; TX, treatment description; BCRCL, baseline creatinine clearance; BBWT, baseline body weight; σ, parameters for additive residual error. Random Effects and Residual Error parameter names containing a colon (:) denote correlated parameters

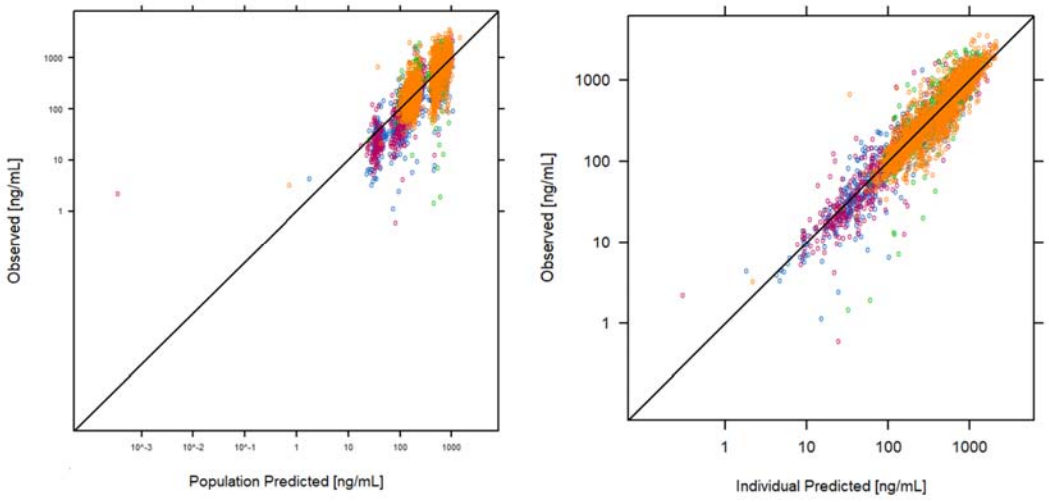
[b] Random Effects and Residual Error parameter names containing a colon (:) denote correlated parameters

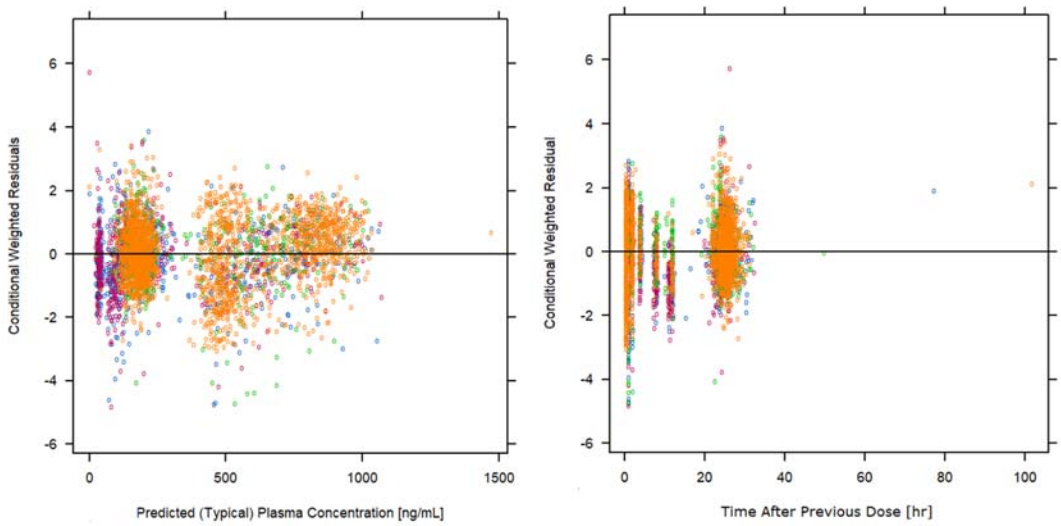
[c] Random Effects and Residual Error parameter estimates are shown as Variance (Standard Deviation) for diagonal elements and Covariance (Correlation) for off-diagonal elements

[d] All confidence intervals are from 500 bootstrap run

The diagnostic plots of the final model are represented in **Figure 2.3.1-1**, showed good agreement between the predicted concentrations and the observed concentrations, CWRES plots (bottom 2 figures) were normally distributed and unbiased with absolute value less than 6, implying there is no explicit outlier in the analysis dataset.

Figure 2.3.1-1: Diagnostic plots for the DCV Final PopPK Model

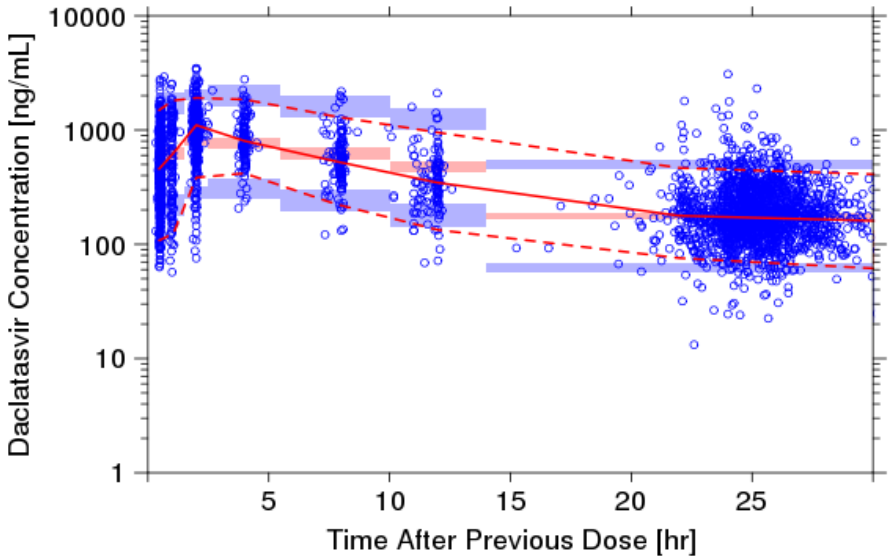




blue circle represents the samples from the subjects in AI444021, red circle represents the samples from the subjects in AI444022, green circle represents the samples from the subjects in AI447017, and orange circle represents the samples from the subjects in AI447026

The predictive performance of the developed final PPK model was assessed using pcVPC. **Figure 2.3.1-2** shows the pcVPC plotafter 14 days at steady sate. The plot showed that the model adequately described the central tendency and the spread of the observed PK at steady state.

Figure 2.3.1-2: Prediction-Corrected Visual Predictive Check for the DCV Final PopPK Model

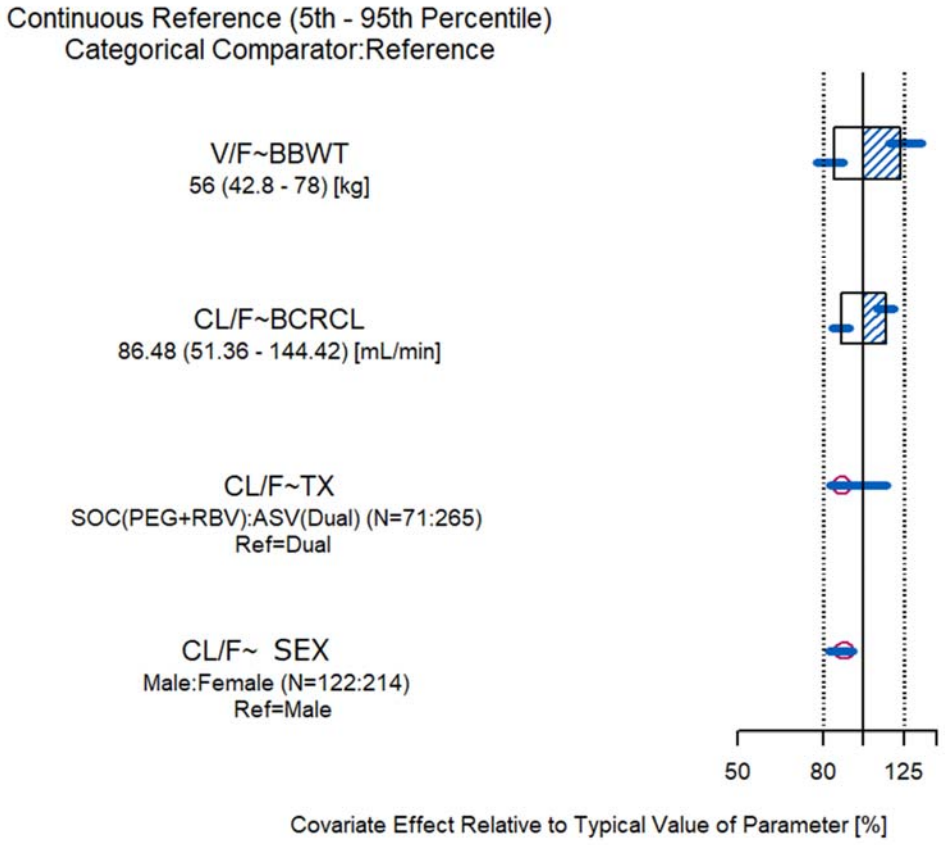


Circles are observed asunaprevir plasma concentrations, solid red line represents the median observed value and dotted red lines represent 5th percentile and 95th percentiles of the observed values. Red shaded area represent the spread of the median predicted values (5th to 95th percentile) and blue shaded areas represent the spread (5th percentile and 95th percentile) of the 5th and 95th predicted percentile concentrations. Each bin was set to cover the sampling points.

Impact of covariates on the DCV PK parameters estimated from the final PopPK model is represented in a forest plot (**Figure 2.3.1-3**). The impact of baseline body weight on V/F on the final PopPK model was overwrapped with the 80% to 125% boundary. All other covariates effects were within the 80% to 125% range. The effect of baseline body weight on V/F suggests that for subjects with the extremes of baseline body weight (5th and 95th percentile of baseline body weight were 42.8 kg and 78 kg, respectively), DCV V/F would be ~15% lower and ~22% higher, respectively, than the median (reference) body weight of 56 kg. For the effects of treatment and sex, DCV CL/F was reduced by ~10% for subjects receiving pegIFN/RBV compared to those receiving ASV combination treatment, and for females relative to males. For subjects with baseline

CRCL at the 5th or 95th percentile (51.36 mL/min or 144.42 mL/min, respectively) relative to the typical subject was reduced or increased by approximately 10%.

Figure 2.3.1-3: Covariate Effects plot for the DCV Final PopPK Model



Typical PK parameters were estimated for male, baseline body weight= 56kg, baseline creatinine clearance= 88.48 mL/min using, treatment description with administered dual (dasclatavir + asunaprevir). Categorical Covariate effects (95% CI) are represented by open symbols (horizontal lines). Continuous covariate effects (95%CI) at the 5th/95th percentiles of the covariate are represented by the end of horizontal boxes (horizontal lines). Open/shaded area of boxes represents the range of covariate effects from the median to the 5th/95th percentile of the covariate. BBWT, baseline body weight; BCRCl, baseline creatinine clearance; CL/F, apparent clearance of orally administered doses; TX, treatment description; V/F, apparent volume of distribution.

2.3.2 ASV

A total of 2626 pharmacokinetic records from 265 subjects were included for model development. Same as the DCV, a one-compartment PK structure model with a first order

absorption was identified as an optimal model. Inter-individual variability was estimated in CL/F, and V/F. The residual error model was additive in log-transformed ASV plasma concentrations. The random effect of Ka was fixed as zero because the sampling points around the peak concentrations were not enough for all subjects. Also in the ASV dataset, because the correlation between age, baseline body weight and baseline CRCL, and the correlation between baseline AST and baseline ALT were moderate or high, the relationships between these covariates and CL/F were separately tested. Age on CL/F showed the larger decrease in OFV compared to that of baseline body weight or baseline CRCL on CL/F. The time-varying effect was assessed for AST and ALT on CL/F, and was highly significant compared to incorporating only baseline AST and ALT value, with decrease -139.192 and -108.05, respectively as shown in **Table 2.3.2-1**. Both AST and ALT resulted in a significant reduction in objective function value, but AST reduced the objective function value the greatest following incorporation into the full model. As a result, AST (baseline and time-varying AST) and age were retained in the full model of ASV. All other pre-specified covariates were included into the full model.

Table 2.3.2-1: Summary of Laboratory Covariate Effects Tested on the ASV Base PopPK Model (AST and ALT)

Model No.	Model Description[a]	MIN[b]	COV[c]	OFV	ΔOFV[d]	Results[e]
1	Base Model: 1-cmpt model, first-order absorption; IIV: CL/F, V/F, Residual error: additive	Y	Y	2103.246	(REF)	
2	1+Covariate Model: BAST~ CL/F,	Y	Y	2048.881	-54.365	
3	1+Covariate Model: BALT~ CL/F	Y	Y	2072.999	-30.247	
4	1+Covariate Model: BAST+AST~ CL/F	Y	Y	1964.054	-139.192	Retain
5	1+Covariate Model: BALT+ALT~ CL/F	Y	Y	1995.196	-108.05	

[a] All models have the same base model as Base Model, Description lists the covariate effects that were tested in addition to the Base Model

[b] Minimization status(Y=successful)

[c] Covariance step status (Y=successful)

[d] Difference between OFV of model and OFV of reference model (REF)

[e] Retain =included in the full model

IIV: inter-individual variability

The final PopPK model was obtained by removing nonsignificant covariates from the full model during backward elimination. The final model parameters are provided in **Table 2.3.2-2**. For an HCV-infected subject with typical covariate values (age 62-year-old, 55 kg, female, with baseline AST of 52 U/L, no cirrhosis), CL/F was 52.1 L/h and V/F was 75.1. The first-order absorption rate constant was 0.23 $hour^{-1}$. Significant covariates ($p < 0.001$) remaining in the final model included baseline and time-varying AST, cirrhosis status, formulation on bioavailability. Inter-individual variability of CL/F and V/F of the final model were reduced from those of the base model (51.5% to 41.5% and 97.4% to 93.4%, respectively). The residual errors were similar between the base model and the final model. Shrinkage of the final model parameters was 10.0% for CL/F and 18.2% for V/F.

Table 2.3.2-2: Parameter Estimates for the ASV Final PopPK Model

Name[a] [Units]	**Estimate[b]**	**Standard Error (RSE%) [c]**	**95% Confidence Interval[d]**
Fixed Effects			
CL/F [L/h]	52.1	7.35 (14.1)	42.1 - 66.8
V/F[L]	75.1	13.5 (18.0)	56.7 - 101.0
Ka[h]	0.228	0.00395 (1.73)	0.221 - 0.234
CL/F~BAST	-0.598	0.0565 (9.45)	-0.707- -0.486
CL/F~AST	-0.382	0.0443 (11.6)	-0.458 - -0.303
F~FORM	-0.314	0.144 (45.9)	-0.551- -0.0922
CL/F~CIRHOSIS	-0.428	0.140 (32.7)	-0.753 - -0.146

Table 2.3.2-2: Parameter Estimates for the ASV Final PopPK Model

Name[a] [Units]	Estimate[b]	Standard Error (RSE%) [c]	95% Confidence Interval[d]
Random Effects			
CL/F	0.172 (0.415)	0.0232 (13.5)	0.127 - 0.213
V/F	0.872 (0.934)	0.139 (15.9)	0.663 - 1.10
σ	0.0672 (0.259)	0.0176 (26.2)	0.0364-0.0974
Residual Error			
σ	0.680	0.0193 (2.84)	0.648- 0.713

a CL/F, apparent clearance; Ka, absorption rate constant; V/F, apparent volume of distribution of central compartment, BAST; baseline aspartate aminotransferase, AST; aspartate aminotransferase at each time point, FORM; formulation (Phase 2 film-coated tablet *vs* Phase 3 softgel capsule), CIRHOSIS; cirrhosis (yes *vs*. no). Random Effects and Residual Error parameter names containing a colon (:) denote correlated parameters.

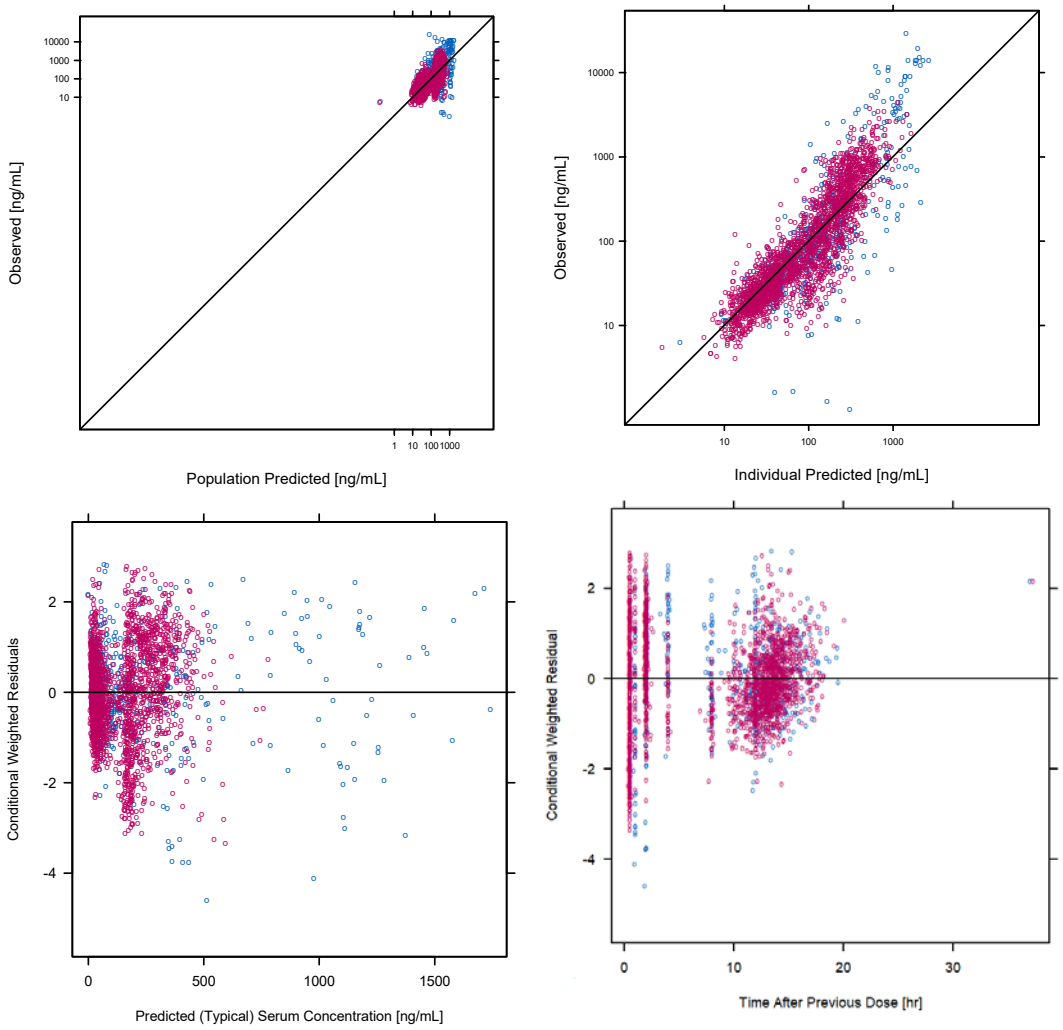
b Random Effects and Residual Error parameter estimates are shown as Variance (Standard Deviation) for diagonal elements and Covariance (Correlation) for off-diagonal elements

c RSE, relative standard error (standard error as a percentage of Estimate)

d all confidence intervals are from 500 bootstrap run

The diagnostic plots of the final model are represented in **Figure 2.3.2-1**. Diagnostic plots of final model showed good agreement between the predictions and the observations, CWRES (bottom 2 figures) that were unbiased and with absolute value less than 5, implying there is no explicit outlier.

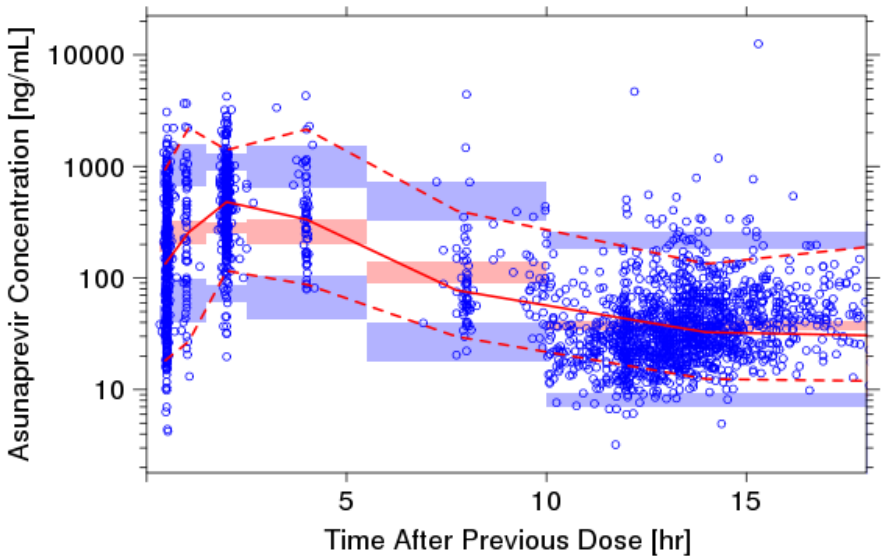
Figure 2.3.2-1: Diagnostic plots for the ASV Final PopPK Model



blue circle represents the samples from the subjects in AI447017, red circle represents the samples from the subjects in AI447026

Figure 2.3.2-2 shows the pcVPC plotat after 14 days at steady state. Overall, the pcVPC plot demonstrates that the model adequately described the central tendency and the spread of the observed PK at steady state.

Figure 2.3.2-2: Prediction-Corrected Visual Predictive Check for the ASV Final PopPK Model

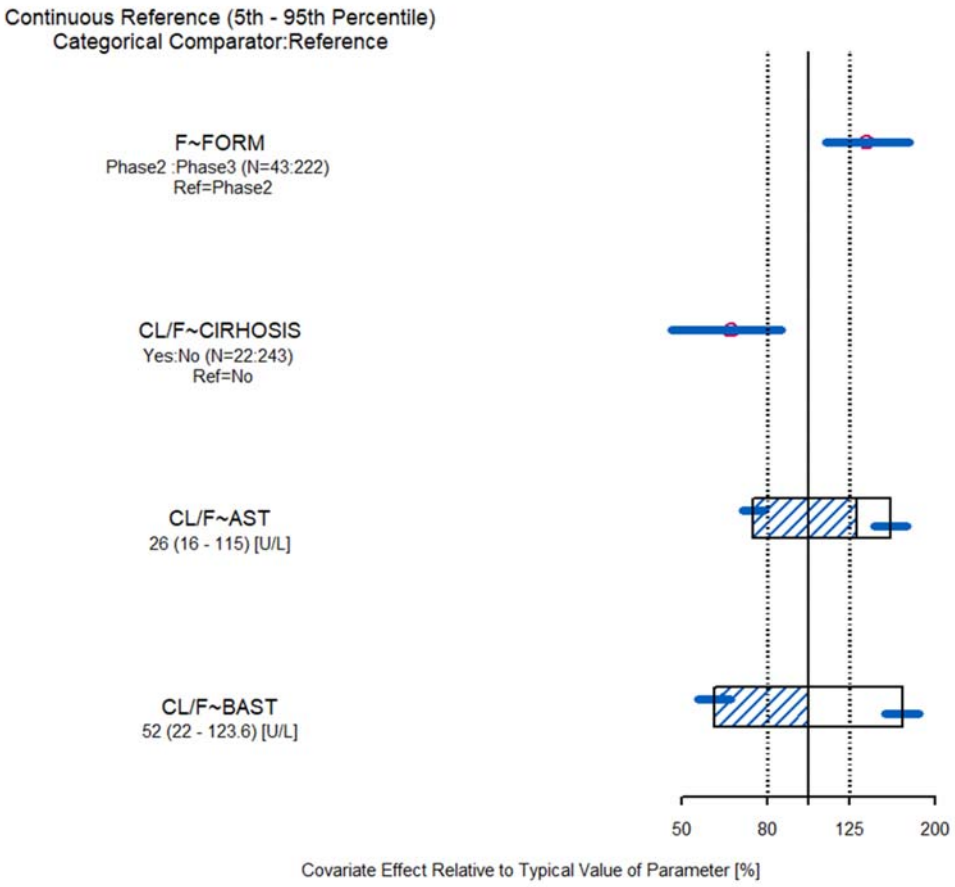


Circles are observed asunaprevir plasma concentrations, solid red line represents the median observed value and dotted red lines represent 5th percentile and 95th percentiles of the observed values. Red shaded area represent the spread of the median predicted values (5th to 95th percentile) and blue shaded areas represent the spread (5th percentile and 95th percentile) of the 5th and 95th predicted percentile concentrations. Each bin was set to cover the sampling points.

Impact of significant covariates on the ASV PK parameters estimated from the final PopPK model is illustrated in a forest plot (**Figure 2.3.2-3**). The point estimates of formulation on bioavailability and cirrhosis on CL/F exceeded 80% to 125% boundary. The bioavailability of the Phase 3 soft-gel capsule (100 mg BID) formulation was 1.37-fold higher than the tablet formulation used in Phase 2 (200 mg BID or 600 mg BID). ASV CL/F for cirrhotic subjects is expected to be 0.65-fold lower relative to subjects without cirrhosis. The effect of baseline and time-varying AST at 5th and 95th percentile on CL/F exceeds the 80%-125% range. The effect of baseline AST suggests that for subjects with the extremes of baseline AST (5th and 95th percentile of baseline AST were 22 and 123.6 U/L, respectively), ASV CL/F would be 1.67-fold higher and 0.60-fold lower, respectively, than the median baseline AST of 52 U/L. Similarly the effect of the

time-varying effect of AST suggests that for subjects with extremes of AST at the last PK sampling time point (5th and 95th percentile of AST at the last sampling time were 16 and 115 U/L, respectively), ASV CL/F would be 1.57-fold higher and 0.74-fold lower, respectively, compared to the median baseline AST.

Figure 2.3.2-3: Covariate Effects plot for the ASV Final PopPK Model



Typical PK parameters were estimated for a 62-year-old, 55 kg, female using the Phase 2 formulation with baseline AST of 52 U/L, time-varying AST of 26 U/L, no cirrhosis. The relative bioavailability was computed from CL/F (Phase 3 formulation, soft-gel capsule) / CL/F (Phase 2 formulation, film-coated tablet) or V/F (Phase 3 formulation) / V/F (Phase 2 formulation). Categorical Covariate effects (95% CI) are represented by open symbols (horizontal lines). Continuous covariate effects (95%CI) at the 5th/95th percentiles of the covariate are represented by the end of horizontal boxes (horizontal lines). Open/shaded area of boxes represents the range of covariate effects from the median to the 5th/95th percentile of the covariate.

AST, alanine aminotransferase; BAST, baseline AST, CL/F, apparent clearance of orally administered doses; F, relative bioavailability; FORM, formulation (tablet or capsule), V/F, apparent volume of distribution.

2.4 Discussion

The final model and covariate plot indicated that DCV CL/F decreased with lower baseline CRCL. These results are consistent with the results of the DCV renal impairment study, where, based on a regression analysis, DCV AUC increased with decreasing baseline CRCL, and the covariate effect was hypothesized to be a result of alteration of non-renal clearance, considering renal excretion is not a major elimination pathway for DCV. [18] In terms of hepatic function, cirrhosis status and ALT/AST levels were not identified as significant covariates of DCV PK parameters on CL/F, therefore DCV PK is not expected to be dependent on hepatic function in the subject population. These results are consistent with the results of the hepatic impairment study.[18] Overall, the effects of all significant covariates included in the final model are within or overlapped the 80% to 125% boundaries, suggesting a lack of clinical relevance. This is also supported by the fact that the overall inter-individual variability for CL/F was similar (from 39.7 % and 39.4%) and for V/F reduced only by approximately 2.6% (from 40.7% to 38.1%. This addition of the covariates to the base model suggests that the covariate effects do not contribute to inter-individual variability of DCV PK. The DCV PopPK model has been further developed by adding the data from other clinical studies including non-Japanese HCV patients after the current DCV PopPK analysis for Japanese HCV patients was performed. [37] The effects of significant covariates included in the subsequent DCV PopPK analysis are also not considered clinically relevant, which is consistent with the result from the Japanese DCV PopPK analysis.

In the final PopPK model for ASV, formulation on bioavailalibity, and baseline AST, time-varying AST and cirrhosis status on CL/F were identified as significant covariates for the PK of ASV. The effect of formulation on bioavailability of ASV, which was included in the base structural model, suggests that the bioavailability of the Phase 3 soft-gel capsule (100 mg BID) formulation was 1.37-fold higher than the tablet formulation used in Phase 2 (200 mg BID or 600

mg BID). The result was similar to the findings in AI447017 (Phase 2 study used 200mg tablet formulation) and AI447026 (Phase 3 study used 100 mg soft-gel capsule). The observed ASV AUC in AI447017 and AI447026 was 2950 ng.h/mL and 2155 ng.h/mL, respectively.[23] In addition, the trend was consistent with the result of the relative bioavailability study. The cause of the enhancement in bioavailability when administered with capsule was considered to be improved lipid solubility.

The other key covariates on ASV CL/F were AST and cirrhosis status, suggesting that ASV CL/F is a marker of hepatic function. Subjects with cirrhosis had higher baseline AST/time-varying AST, resulting in a decrease in ASV CL/F, therefore, increased exposure to ASV. The effect of liver cirrhosis on the regulation and expression of enzymes has been documented in the literature.[38] The effect of AST on CL/F has been previously reported for sildenafil and tacrolimus which are extensively metabolized by the liver.[39, 40] Note that ALT and AST were highly correlated, were both tested as covariates, and AST was included in the final model because it had a slightly greater impact although the magnitudes of the effects were very similar between these covariates. Results of the current analysis are consistent with the metabolic profile of ASV (ASV is extensively metabolized and eliminated primarily through the feces) and with the results of the hepatic impairment study (subject with moderate and severe hepatic impairment had greater ASV exposure). Hypoalbuminemia and bilirubin accumulation are frequently seen in chronic liver disease and may decrease drug protein binding. However, the results from hepatic impairment study showed that ASV protein binding was similar across all treatment groups and time post-dose, indicating that hepatic impairment had no apparent effect on ASV protein binding; therefore unbound clearance showed the same trends as total clearance and changes in ASV exposure were unexplained by protein binding.[24] The reductions of the apparent total body clearance were likely

due to the increasing fibrosis in more advanced liver impairment, which simultaneously prevented distribution to the liver as well as metabolic clearance from the liver. Furthermore, metabolic clearance is a function of both hepatic blood flow and total hepatocyte intrinsic clearance, both of which are negatively impacted by hepatic impairment and may result in higher systemic exposure. Collectively, presence of cirrhosis and increased AST, which reflect worsening hepatic function, is expected to increase exposures of ASV. Overall, cirrhosis status and AST were the significant covariates on ASV CL/F. ASV CL/F decreases with cirrhosis and increasing baseline and time-varying AST indicating an association between hepatic function and ASV CL/F.

In the process of the PopPK model development for ASV, OATP1B1 haplotypes were evaluated as a covariate on CL/F, because differences in ASV exposures between Japanese and non-Japanese subjects have been investigated previously and genetic variability in OATP1B1 mediated transport of ASV has been hypothesized to be a potential factor for the differences in exposure.[41] The impact of different OATP1B1 haplotypes on ASV CL/F was examined in order to evaluate the effect of OATP1B1 genetic variability on ASV exposure. Consequently, the 4 categories of OATP1B1 haplotypes did not show statistical significance, suggesting that in the Japanese population, genetic variability of OATP1B1 has no impact on ASV exposures. Other investigations had been evaluated by including non-Japanese data and the analyses did not reveal a relationship between OATP and ASV exposure.[23]

2.5 Conclusion

The population pharmacokinetic models for DCV and ASV characterized were characterized by nonlinear mixed effect models. All significant covariates included in DCV PopPK model were not considered clinically relevant. ASV soft-gel capsule had higher bioavailability

compared to the tablet, and ASV exposure increased with cirrhosis and increasing baseline and time-varying AST values. The significant covariates identified in ASV PopPK model is help to explain the source of variability in ASV exposure.

3 RESEARCH 2: POPULATION PHARMACOKIETNC ANALYSIS OF 3DAA REGIMEN IN HCV-INFECTED SUBJECTS

3.1 Objective

To develop the PopPK models for 3DAA regimen to help explain the source of variability in drug exposure by investigating the potential relationships between covariates and the PKs of DCV, ASV and BCV. In addition, to estimate the PK exposures of DCV, ASV and BCV for the subsequent safety E-R analyses in Research 3 and efficacy E-R analyses.[42]

3.2 Method

The PK of DCV, ASV and BCV in non-Japanese subjects who received 3DAA regimen was firstly characterized based on one phase 2 study (AI443014: NCT01455090)[14] and two phase 3 studies (AI443102: NCT01979939 and AI443113: NCT01973049) [12, 43] as the original model. The subsequent Japanese phase 3 study (AI443117: NCT02123654)[13] was added into the original model to determine the effects of interested covariates. Study AI443014 was a Phase 2, open-label, multiple dose, dose escalation study in treatment naïve subjects infected with HCV GT-1, AI443102 was a Phase 3 study in non-cirrhotic subjects with GT-1 chronic HCV infection, and AI443113 was a Phase 3 study in compensated cirrhotic subjects with GT-1 chronic HCV infection. Study AI443117 was a Japanese Phase 3 study in subjects with GT-1 chronic HCV infection, including those with compensated cirrhosis. In the treatment-naïve cohort, GT-1b subjects were randomly assigned to either 3DAA regimen or DUAL regimen. GT-1a subjects in the treatment-naïve cohort and all subjects in the treatment-experienced cohort received 3DAA regimen.

In the previous DCV PPK analyses of DUAL regimen including Japanese and non-Japanese subjects,[37] plasma PK of DCV was described by a two-compartment linear elimination model and absorption of DCV was modeled as a zero-order release followed by a first-order absorption into the central compartment. A previous ASV PPK analysis of DUAL regimen including Japanese and non-Japanese subjects, was a two-compartment model with zero-order release, first-order absorption and linear clearance.[44] Initial multiple dose ascending PK studies suggested the presence of auto-induction of CYP3A4, which occurred within 7 days. Since there was limited ASV data in the first 7 days after the start of dosing in the previous or current datasets, the dynamics of the induction process could not be estimated. Therefore, a change in clearance with time was implemented as a step change at 48 hours. The prior base models for DCV and ASV were used as a starting point of the current analysis, and appropriate structural models were selected based on the DCV and ASV PK results from the DCV 3DAA regimens.

The BCV PopPK model which could be leveraged for this research was not available. The BCV PopPK model was developed by base, full and final model steps. A base model was first developed to describe the plasma concentrations of BCV as a function of time. Different absorption models, including a zero-order release followed by 1st-order absorption, were also evaluated to better describe the absorption of BCV. An inter-individual variability model, which describes random variability in structural model parameters among individuals in the subject population, was defined for all pharmacokinetic parameters as follows:

$$\theta_i = \theta_{pop} \cdot exp(\eta_i)$$

where θ_i is the parameter for the i^{th} subject, θ_{pop} is the typical value of the parameter in the population, and η_i is a random inter-patient effect with mean 0 and variance ω^2. The ω^2 values are the diagonal elements of the inter-individual variance-covariance matrix.

Proportional plus additive error models for non-transformed concentrations and additive error models for log-transformed concentrations were considered as residual error model.

The proportional plus additive error model was given as

$$y_{ij} = \hat{y}_{ij} \cdot \left(1 + \varepsilon_{1ij}\right) + \varepsilon_{2ij}$$

The additive error models for log-transformed concentrations was given as

$$\log(y_{ij}) = \log(\hat{y}_{ij}) + \varepsilon_{ij}$$

where y_{ij} and $\hat{y}_{ij}$ represent the j^{th} observed and predicted concentration, respectively, for the i^{th} subject. ε_{1ij} and ε_{2ij} denote the residual intra-individual random errors for the constant coefficient of variation part and the additive part with respective variances σ_1^2 and σ_2^2, and ε_{ij} denotes the residual intra individual random errors with mean 0 and variances σ^2.

In the full model building step for DCV, ASV and BCV, the covariate search was performed. The covariates and related PK parameters were tested based on clinical interest, pharmacological plausibility, and the data availability. The lists of covariates and their relationships to the PK parameters in the original models are provided in **Table 3.2-1 A-C**. For highly correlated covariates that were both significant, only one of the correlated covariates was included. For categorical covariates tested in the analysis, the number of subjects in each category needed to exceed 5% of the total number of subjects. Categories of less than 5% were typically combined to increase the percentage of subjects in a category. Significant covariate-PK relationships were assessed using the likelihood ratio test at the $p < 0.05$ level of significance. All

significant covariates were included in the full model. Additionally, covariates of borderline significance were included if the covariate was highly likely to be influential, based on scientific judgment. The effect of continuous covariates on the PK parameters was modeled as follows:

$$\theta_i = \theta_{pop} \cdot \left(\frac{Cov_i}{Cov_{pop}} \right)^{k_{\mathrm{cov}}}$$

And the effect of categorical covariates was modeled as follows:

$$\theta_i = \theta_{pop} \cdot e^{k_{\mathrm{cov}} \cdot X_i}$$

where θ is a model parameter, *Cov* is a continuous covariate, *X* is a categorical variable, *i* is an index for each subject, *pop* is an index describing the typical value of this covariate in the population, and k_{cov} is a coefficient describing the strength of the covariate effect.

In the next step, the final model was derived using a stepwise backward elimination process, staring with the full model, and removing each covariate one at a time until all covariates retained were significant at the level of $p < 0.001$. In the case that the covariance step was not completed, the bootstrap estimation method was used to estimate the model uncertainty.[45]

The original PK datasets were augmented by adding the results from study AI443117. Starting from the original final PK models, the covariates of interest in updated PopPK analysis, which are provided in **Table 3.2-1 D**, were added to the original model simultaneously to develop the updated full models. Then the updated final models were derived using a stepwise backward elimination process at the level of $p < 0.001$. Covariate information in the augmented datasets is the same as the original analyses. The only new covariate was the co-administration of the 60-mg DCV tablet and the 100-mg softgel ASV capsule in DUAL regimen of Study AI443117.

Table 3.2-1 Covariates Tested in the Full Model

A: Original Model in DCV

Category	Covariates	Related PK parameter	Note
Demographics	Age	V, CL	
	Sex	V, CL	
	Baseline weight	V, CL	
	BMI	V, CL	
	Race	V, CL	White/Black/Asian/Others (including Indian-Asian)
Labs/liver function	AST	V, CL	Baseline and time-varying
	ALT	V, CL	Baseline and time-varying
	Creatinine clearance	CL	Investigate both continuous and categorical
	Cirrhosis status	V, CL	
	HCV RNA	V, CL	
Formulation	Coadministration vs FDC	ka, F	
Prognostic factors	Patient type	CL	Naive/ineligible
	Genotype	CL	GT1A/GT1B/GT4
	Host genotype	CL	CC/CT/TT
Concomitant medication	RBV	CL	
	Proton pump inhibitors	CL, ka, F	
	H2 receptor antagonists	CL, ka, F	
	CYP3A inducers	CL	
	CYP3A4 inhibitors	CL	
	P-gp inhibitors	CL	
	Calcium-channel blockers	V, CL	
	Beta-blockers	V, CL	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CL = clearance; F = bioavailability; FDC = fixed dose combination; HCV = hepatitis C virus; ka = first-order absorption rate constant; PK = pharmacokinetic; RBV = ribavirin; RNA = ribonucleic acid; V = volume.

B. Original Model of ASV

Category	Covariates	Related PK parameter	Note
Demographics	Age	Vc, CL	

Category	Covariates	Related PK parameter	Note
	Sex	Vc, CL	
	Baseline weight	Vc, Vp, CL	
	BMI	Vc, Vp, CL	
	Race	Vc, CL	White/non-White
Labs/liver function	AST	Vc, CL	Baseline and time-varying
	ALT	Vc, CL	Baseline and time-varying
	Creatinine clearance	CL	Continuous and categorical (≥□90, < 90)
Prognostic/disease-related factors	Cirrhosis status	Vc, CL, ka	No/Yes/Missing
	Baseline viral load	Vc, CL	
	Patient type	CL	Naive/ineligible
	Virus genotype	CL	GT1A/GT1B/GT4
	Host genotype	CL	CC/CT/TT
Treatment/ concomitant medication factors	Formulation	ka, F	Co-administration or FDC
	RBV	CL	
	Proton pump inhibitors	CL, ka, F	
	H2 receptor antagonists	CL, ka, F	
	CYP3A inducers	CL	ASV is a CYP3A4 substrate
	CYP3A4 inhibitors	CL	ASV is a CYP3A4 substrate
	P-gp inhibitors	CL	ASV is a P-glycoprotein substrate
	Calcium-channel blockers	Vc, CL	
	Beta-blockers	Vc, CL	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CL = clearance; F = bioavailability; FDC = fixed dose combination; HCV = hepatitis C virus; ka = first-order absorption rate constant; PK = pharmacokinetic; RBV = ribavirin; RNA = ribonucleic acid; Vc = central volume; Vp = peripheral volume; Co-administration = Co-administration of ASV, DCV, and BCV in the Phase 2 study.

C. Original Model of BCV

Category	Covariates	Related PK parameter	Note
Demographics	Age	V, CL	
	Sex	V, CL	
	Baseline weight	V, CL	
	BMI	V, CL	

Category	Covariates	Related PK parameter	Note
	Race	V, CL	White/Black/Asian/Others (including Indian-Asian)
Labs/liver function	AST	V, CL	Baseline and time-varying
	ALT	V, CL	Baseline and time-varying
	Creatinine clearance	CL	Continuous and categorical (≥ 90, < 90).
Prognostic/disease-related factors	Cirrhosis status	V, CL	No/Yes/Missing
	Baseline viral load	V, CL	
	Patient type	CL	Naive/ineligible
	Genotype	CL	GT1A/GT1B/GT4
	Host genotype	CL	CC/CT/TT
Treatment/concomitant medication factors	Formulation	ka, F	Coadministration vs FDC
	RBV	CL	
	Proton pump inhibitors	CL, ka, F	
	H2 receptor antagonists	CL, ka, F	
	CYP3A inducers	CL	
	CYP3A4 inhibitors	CL	
	P-gp inhibitors	CL	
	Calcium-channel blockers	V, CL	
	Beta-blockers	V, CL	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CL = clearance; F = bioavailability; FDC = fixed dose combination; HCV = hepatitis C virus; ka = first-order absorption rate constant; P-gp = P-glycoprotein; RBV = ribavirin; RNA = ribonucleic acid; V = volume

D. Updated Model

PK Model	Covariates	Related PK Parameter	Note
DCV	Race	V/F, CL/F	Four categories (white/black/asian/others). Japanese race will not be separated from non-Japan Asians since there were only 11/303 (3.6%) non-Japanese Asians in the dataset.
	Genotype-1b	CL/F	-
	Cirrhosis status	CL/F	-
	Patient type	CL/F	Naïve / experienced
	3DAA vs DCV+ ASV	ka, F	-
ASV	Race	Vc/F, CL/F	Similar to DCV

PK Model	Covariates	Related PK Parameter	Note
	Genotype-1b	CL/F	-
	Cirrhosis status	CL/F	-
	Patient type	CL/F	Similar to DCV
	Tablet vs FDC vs DCV + ASV	ka, F	-
BCV	Race	V/F, CL/F	Similar to DCV
	Genotype-1b	CL/F	-
	Cirrhosis status	CL/F	-
	Patient type	CL/F	Similar to DCV
	Tablet vs FDC	ka	-

Abbreviations: 3DAA = direct-acting antiviral (DCV/ASV/BCV); ALT = alanine aminotransferase; AST = aspartate aminotransferase; BCV = beclabuvir (BMS-791325); BMI = body mass index; CL = clearance; CL/F = apparent oral clearance; DCV = daclatasvir (BMS-790052); F = bioavailability; FDC = fixed dose combination (DCV 3DAA); HCV = hepatitis C virus; ka = first-order absorption rate constant; P-gp = P-glycoprotein; PK = pharmacokinetic; RBV = ribavirin; RNA = ribonucleic acid; V = volume; Vc/F = apparent volume of the central compartment; V/F = apparent volume of distribution

3.3 Results

A total of 11382, 11300 and 10728 pharmacokinetic records from 1228 subjects were included for DCV, ASV and BCV updated model development, respectively. Baseline demographics and subject characteristics are presented in **Table 3.3-1**. The majority of subjects were white (790 subjects, 84.3% in the original and 64.3% in the updated datasets, respectively). There were more GT-1b subjects in the updated data set because it was a more common genotype in the Japanese patients. Distributions of other covariates were similar to those of the original data set.

Table 3.3-1: Summary of Baseline Demographic and Characteristics

Covariate	**Original (n=937)**	**Updated (n=1228)**
Age, mean (SD), years	53 (10)	55 (11)
Weight, mean (SD), kg	80 (16)[a]	75 (18)

Table 3.3-1: Summary of Baseline Demographic and Characteristics

Covariate		Original (n=937)	Updated (n=1228)
Sex, N (%)			
	Male	362 (38.6)	672 (54.7%)
	Female	575 (61.4)	556 (45.3%)
Race, N (%)			
	White	790 (84.3)	790 (64.3%)
	Black/African American	120 (12.8)	120 (9.77%)
	American Indian or Alaska Native	7 (0.747)	7 (0.57%)
	Asian Indian	3 (0.32)	3 (0.244%)
	Japanese	1 (0.107)	292 (23.8%)
	Asian Other	11 (1.17)	11 (0.896%)
	Missing	1 (0.107)	1 (0.0814%)
	Other	4 (0.427)	4 (0.326%)
Clinical Laboratory Data			
	AST, mean (SD), U/L	58 (34)	58 (37)
	ALT, mean (SD), U/L	75 (47)	71 (47)
	CrCl, mean (SD), mL/min	111 (31)	105 (32)
Cirrhosis, N (%)			
	No	714 (76.2)	945 (77%)
	Yes	220 (23.5)	280 (22.8%)
	Unknown	3 (0.32)	3 (0.244%)
Patient type, N (%)			
	Naïve	698 (74.5)	924 (75.2%)
	Null-responder/Experienced	239 (25.5)	304 (24.8%)
Virus genotype, N (%)			
	1a	690 (73.6)	694 (56.5%)
	1b	225 (24.0)	512 (41.7%)
	4	21 (2.24)	21 (1.71%)
	6	1 (0.107)	1 (0.0814%)

[a] n=936

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CrCl, creatinine clearance; SD, standard deviation

3.3.1 DCV

The base model of DCV was described as a one-compartmental model with a zero-order release followed by first-order absorption into the central compartment. inter-individual variability was estimated for CL/F, V/F and Ka, with interaction between CL/F and V/F. The residual error model was proportional plus additive in DCV plasma concentration.

The significant covariates influenced on DCV CL/F in the updated final model, which have impact on the average steady-state exposure at steady state (Cavgss) utilized in subsequent exposure-response analyses, were sex, time-varying ALT and race (White, Black, Asian or others). These significant covariates were the same as the original model. The other covariates tested in the updated model, HCV genotype (1b or non-1b), cirrhosis status (yes, no or missing), patient type (naïve or experienced) and regimen (3DAA regimen or DUAL regimen) on CL/F or bioavailability, were removed by the backward elimination process. The parameter estimates of the original and updated final models for DCV are presented in **Table 3.3.1-1**. Overall, the original and updated analyses showed similar parameter values.

Table 3.3.1-1: Parameter Estimates of the DCV Final PopPK Models

Parameters	Original Model		Updated Model	
	Estimate (RSE%)	95% CI	Estimate (RES%)	95% CI
Fixed Effect				
CL/F (L/hr)	4.59 (1.91)	4.42, 4.76	4.6 (1.81)	4.44, 4.76
V/F (L)	66.4 (2.05)	63.7, 69.1	65.6 (2.06)	63, 68.2
ka (1/hr)	2.6 (5.54)	2.32, 2.88	2.25 (4.76)	2.04, 2.46
Duration (hr)	0.594 (5.08)	0.535, 0.653	0.645 (4.39)	0.59, 0.7
Female~CL/F	-0.233 (10.1)	-0.279, -0.187	-0.216 (9.77)	-0.257, -0.175
Female~V/F	-0.201 (14.8)	-0.259, -0.143	-0.188 (14.6)	-0.242, -0.134
Weight~V/F	0.461 (13.5)	0.339, 0.583	0.413 (14.4)	0.297, 0.529
Black~CL/F	-0.121 (25.5)	-0.181, -0.0606	-0.148 (25.1)	-0.221, -0.0751
Asian~CL/F	0.0936 (78.4)	-0.0503, 0.237	-0.0578 (43.4)	-0.107, -0.0086

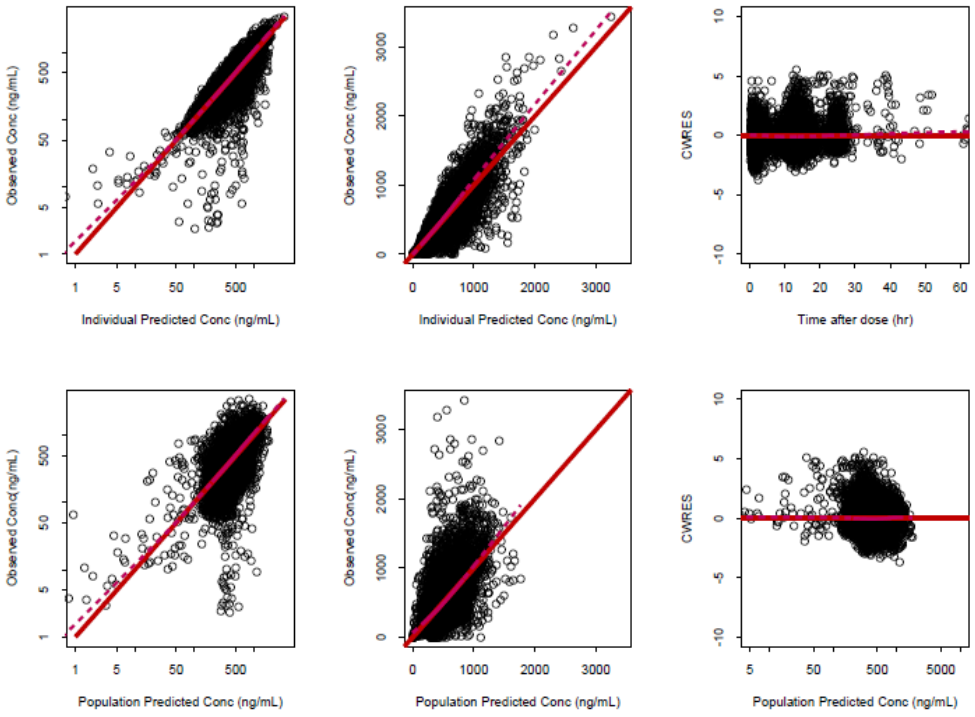
Table 3.3.1-1: Parameter Estimates of the DCV Final PopPK Models

Parameters	Original Model		Updated Model	
	Estimate (RSE%)	95% CI	Estimate (RES%)	95% CI
Race others~CL/F	0.0418 (239)	-0.154, 0.238	0.161 (72.7)	-0.0683, 0.39
ALT change~CL/F	-0.121 (9.17)	-0.143, -0.0992	-0.104 (9.37)	-0.123, -0.0849
ALT change~V/F	-0.071 (19.6)	-0.0982, -0.0438	-0.050 (27)	-0.0765, -0.0235
PPI~ka	-0.556 (25.0)	-0.828, -0.284	-	-
Black~V/F	-		-0.0506 (80.6)	-0.131, 0.0294
Asian~V/F	-		0.161 (20.7)	0.0957, 0.226
Race Other~V/F	-		0.286 (43.7)	0.041, 0.531
Random Effect				
CL/F	0.114 (5.81)	0.101, 0.127	0.112 (5.34)	0.1, 0.124
CL/F·V/F	0.0569 (11.4)	0.0442, 0.0696	0.0564 (10.7)	0.0445, 0.0683
V/F	0.0769 (11.2)	0.060, 0.0938	0.0741 (11.5)	0.0574, 0.0908
ka	0.96 (10.3)	0.766, 1.15	1.26 (9.13)	1.03, 1.49
Residual				
Additive residual	11.6 (42.2)	2.0, 21.2	12.4 (41.5)	2.31, 22.5
Proportional residual	0.319 (1.85)	0.307, 0.331	0.308 (1.8)	0.297, 0.319

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; CL/F, apparent oral clearance; DCV, daclatasvir; ka, first-order absorption rate constant; PK, pharmacokinetic; PPI, proton pump inhibitor; RSE, relative standard error; V/F, apparent volume of distribution

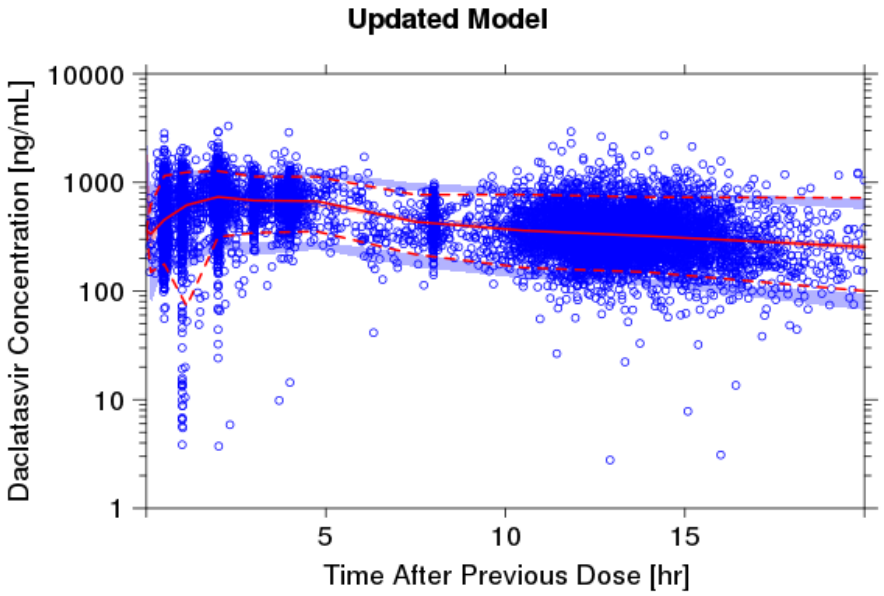
The diagnostic plots suggested no bias over predicted value and time in the updated final model (**Figure 3.3.1-1**). The pcVPC plots showed the updated final models adequately described the central tendency and the spread of the observed PK, as presented in **Figure 3.3.1-2.**

Figure 3.3.1-1: Diagnostic Plots for the Updated DCV PopPK Model



Note: Solid line is unity line, dashed line is loess line.

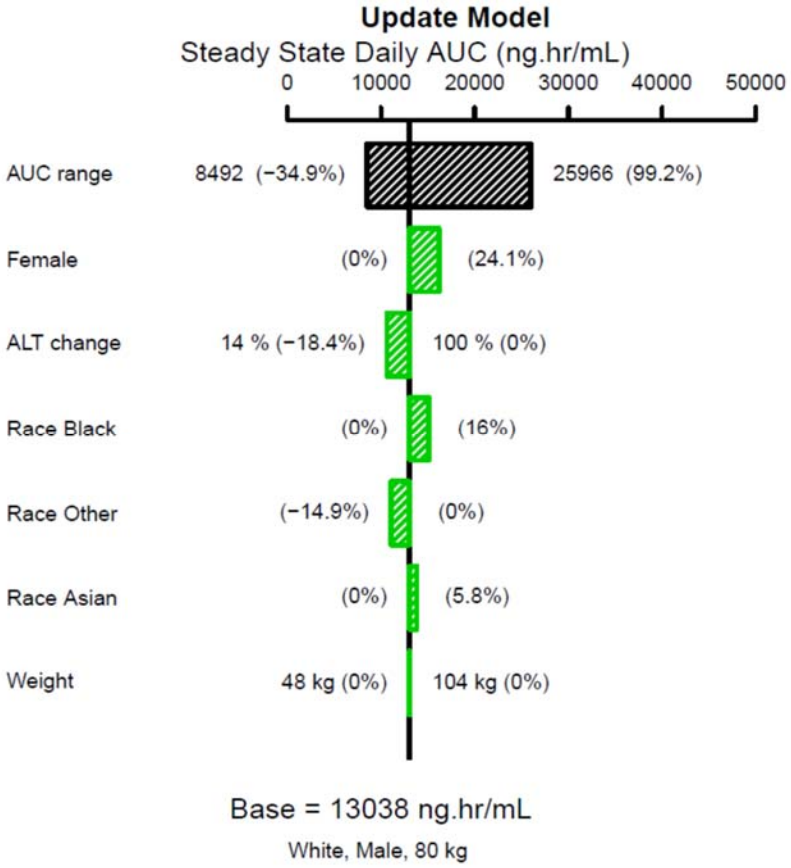
Figure 3.3.1-2: Prediction-Corrected Visual Predictive Check for the Updated DCV PopPK Model



Circles are observed concentrations, solid red lines represent the median observed value, and dashed lines represent 5th percentile and 95th percentiles of the observed values. Red shaded areas represent the spread of the median predicted values (2.5th to 97.5th percentile), and blue shaded areas represent the spread (2.5th percentile and 97.5th percentile) of the 5th and 95th predicted percentile concentrations.

The influences of statistically significant covariates on the area under the concentration versus time curve at steady state (AUC_{ss}), which was defined as the Cavgss multiply by the dosing interval, were evaluated. **Figure 3.3.1-3** shows the independent influence of each covariate on AUC_{ss} of DCV after repeat doses of 3DAA regimen for the updated model. Sex and time-varying ALT had modest impact on the DCV exposure: female had 24.1 % increase in AUC_{ss}; time-varying ALT to baseline ALT ratio of 14 % corresponds to 18.4 % decrease in AUC_{ss} comparing a patient with no change in ALT. The impact of race is small: Asian subjects had 5.8 % increase in AUC_{ss} relative to White subjects.

Figure 3.3.1-3: Effect of Covariates on Steady-State DCV Exposure



The black bar represents the 5th to 95th percentile daily exposure range calculated from post hoc parameters after 10 doses of daclatasvir 30 mg, asunaprevir 200mg and BCV 75 mg BID. The effect of a covariate was calculated by varying one covariate at a time, and fixing all other covariates to typical values. Continuous covariates were evaluated at the 5th and 95th percentiles of the population.

The covariates on DCV in the original and updated final models were evaluated, taking into account correlation of covariates among subject population. Sex was found to be the most significant covariate on the PK of DCV in both the original and updated analyses. To examine the impact of sex and the potential confounding effect of baseline body weight by stratifying sex in quartiles of body weight on AUC based on the original model, however, female subjects had higher exposures in each quartile, suggesting that the effect of sex is an independent factor (**Table 3.3.1-2**).

Table 3.3.1-2: DCV Exposure by Body Weight Quantile and Sex

	Median [5th - 95th] AUC_{ss} (ng•hr/mL)	% median difference from reference
Male (reference: 44.3-68.9 kg)		
44.3-68.9 kg (n=53)	12,883 [7,713 - 25,056]	-
69 - 80.3 kg (n=147)	13,439 [7,921 - 23,058]	4.3
80.4 - 90.7 kg (n=176)	13,629 [8,453 - 22,173]	5.8
90.8 - 126.1 kg (n=198)	12,468 [7,662 - 22,386]	-3.2
Female (reference: 44.3-68.9 kg)		
44.3-68.9 kg (n=183)	16,451 [10,224 - 27,441]	-
69 - 80.3 kg (n=83)	16,636 [10,283 - 28,278]	1.1
80.4 - 90.7 kg (n=59)	17,810 [11,577 - 27,751]	8.3
90.8 - 126.1 kg (n=35)	18,918 [11,493 - 25,211]	15.0

3.3.2 ASV

The ASV PK was described as a two-compartment model with a zero-order release from the formulation followed by the first-order absorption into the central compartment and a first order elimination. A step-wise increase in clearance after 48 hours was used to describe ASV auto-induction. Inter-individual variability was estimated for CL/F, the apparent volume of the central compartment (Vc/F), apparent volume of the peripheral compartment (Vp/F), and Ka. The residual error model was additive in log-transformed ASV plasma concentration.

The significant covariates influenced on ASV CL/F or bioavailability in the original final PopPK model, which effect on Cavgss (AUCss), were age, sex, baseline ALT, time-varying ALT, cirrhosis status, IL28B (rs12979860) genotype, co-administration (fixed dose combination tablet or all three drugs as separate tablets) and co-administration of proton pump inhibitor (yes or no). Covariate listed in **Table 3.2-1 D** were added to the original model simultaneously, and the updated model was derived using a stepwise backward elimination process at the level of $p < 0.001$.

Cirrhosis status (yes, no or missing), baseline and time-varying ALT, race (White, Black, Asian or others), age, co-administration (fixed dose combination tablet or all three drugs as separate tablets), ASV formulation (tablet or capsule) and sex (male or female) were specified as significant covariates on CL/F or bioavailability in the updated final model. Race and ASV formulation were the new covariates included in the update final PopPK model. IL28B (rs12979860) genotype on CL/F, co-administration of proton pump inhibitor (yes or no) on bioavailability and body weight on Vp/F were removed by the backward elimination process. The parameter estimates of the original and updated final models for ASV are presented in **Table 3.3.2-1.** The shrinkage of the updated final model was 10.3 % for CL/F, 33.1 % for Vc/F, 71.5 % for ka and 78.4 % for Vp/F.

Table 3.3.2-1: Parameter Estimates of the ASV Final PopPK Models

Parameters	**Original Model**		**Updated Model**	
	Estimate (RSE %)	95% CI[a]	Estimate (RSE %)	95% CI[a]
Fixed Effect				
CL/F (L/hr)	138 (9.1)	(124, 155)	113	113 (105, 123)
Vc/F (L)	170 (38)	(120, 231)	140	139 (109, 187)
ka (1/hr)	0.287 (28)	(0.253, 0.352)	0.286	0.287 (0.261, 0.349)
Q/F (L/hr)	26.9 (84)	(18.9, 40.5)	13.8	13.9 (9.62, 23.5)
Vp/F (L)	749 (97)	(535, 1050)	444	420 (308, 561)
Duration (hr)	1.97 (0.7)	(1.87, 2.09)	1.91	1.9 (1.81, 2)
Induction Effect	0.474 (39)	(0.350, 0.639)	0.412	0.415 (0.29, 0.558)
Age~CL/F	-0.626 (32)	(-0.788, -0.465)	-0.758	-0.742 (-0.891, -0.597)
Baseline ALT~CL/F	-0.312 (12)	(-0.379, -0.245)	-0.295	-0.293 (-0.35, -0.24)
ALT Change~CL/F	-0.223(11)	(-0.262, -0.18)	-0.213	-0.208 (-0.241, -0.174)
Female~CL/F	-0.200(31)	(-0.277, -0.116)	-0.187	-0.185 (-0.245, -0.116)
Cirrhosis Present~CL/F	-0.453 (18)	(-0.551, -0.336)	-0.506	-0.493 (-0.588, -0.403)
Cirrhosis Missing~CL/F	-0.788 (66)	(-2.06, 0.166)	-0.695	-0.623 (-1.97, 0.151)

Table 3.3.2-1: Parameter Estimates of the ASV Final PopPK Models

Parameters	Original Model		Updated Model	
	Estimate (RSE %)	95% CI[a]	Estimate (RSE %)	95% CI[a]
IL28B CT~CL/F	-0.0621 (140)	(-0.141, 0.0291)	-	-
IL28B TT~CL/F	-0.248 (27)	(-0.373, -0.125)	-	-
IL28B Missing~CL/F	-0.0358 (300)	(-0.257, -0.231)	-	-
Age~Vc/F	-1.28 (25)	(-1.82, -0.777)	-1.34	-1.23 (-1.65, -0.847)
ALT Change~Vc/F	0.595 (21)	(0.361, 0.844)	0.392	0.384 (0.23, 0.555)
Female~Vc/F	-0.721 (20)	(-0.991, -0.448)	-0.639	-0.62 (-0.814, -0.39)
Coadministration ~ ka	-0.322 (2.02)	(-0.421, -0.237)	-0.403	-0.409 (-0.501, -0.324)
Weight~Vp/F	2.02 (71)	(0.898, 3.1)	-	-
Coadministration~F	-0.282 (25)	(-0.382, -0.168)	-0.398	-0.407 (-0.498, -0.307)
PPI~F	0.240 (42)	(0.0858, 0.401)	-	-
Softgel~ka	-	-	0.0304	0.0298 (-0.0803, 0.517)
Softgel~F	-	-	0.264	0.253 (0.079, 0.413)
Asian~CL/F	-	-	-0.466	-0.472 (-0.571, -0.393)
Black~CL/F	-	-	-0.2	-0.198 (-0.344, -0.0622)
Race Other~CL/F	-	-	0.17	0.175 (-0.11, 0.491)
Asian~Vc/F	-	-	-0.608	-0.608 (-0.853, -0.368)
Black~Vc/F	-	-	-0.227	-0.222 (-0.603, 0.116)
Race Other~Vc/F	-	-	1.37	1.39 (0.204, 2.44)
Random Effect				
CL/F	0.251 (7.8)	(0.206, 0.287)	0.252	0.247 (0.209, 0.284)
Vc/F	1.50 (45)	(1.16, 19.5)	1.42	1.35 (1.12, 1.59)
ka	0.036 (90)	(0, 0.0985)	0.0202	0.0202 (0, 0.0627)
Vp/F	1.56 (46)	(0.894, 2.20)	1.35	1.29 (0.666, 1.99)
Residual				
Log-additive residual	0.780 (3.2)	(0.756, 0.803)	0.76	0.761 (0.743, 0.78)

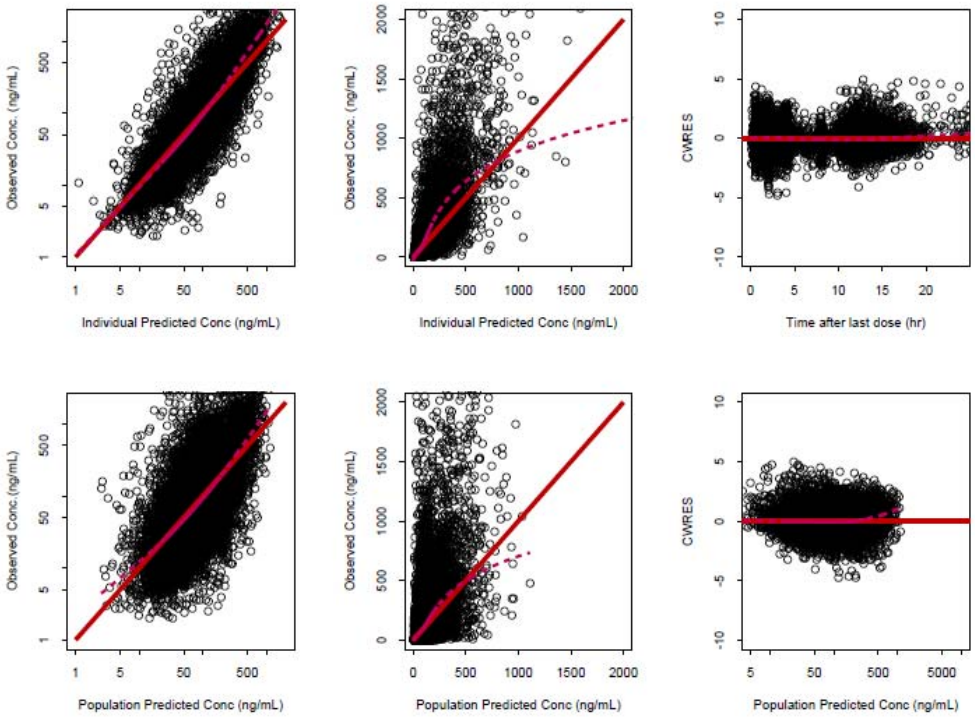
[a] Confidence intervals are calculated using bootstrap samples
[b] Minimization terminated with a rounding error, so standard errors not reported

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; CL/F, apparent oral clearance; ASV, asunaprevir; CV, coefficient of variation; F, bioavailability; ka, first-order absorption rate constant; PK, pharmacokinetic; PPI, proton pump inhibitor; Q/F, apparent inter-compartmental clearance; RSE, relative standard error; Vc/F, apparent volume of the central compartment; Vp/F, apparent volume of the peripheral compartment

Note: Coadministration means all three drugs administered simultaneously as separate tablets.

The diagnostic plots suggested no bias over original and updated models predicted value and time (**Figure 3.3.2-1**). Overall, the plots show good agreement between predictions and observations, and show that conditional-weighted residuals are unbiased over time, time-after-last-dose and population predicted concentrations. Almost all CWRES (right 2 figures) were of magnitude less than 5, implying there is no explicit outlier in the analysis dataset.

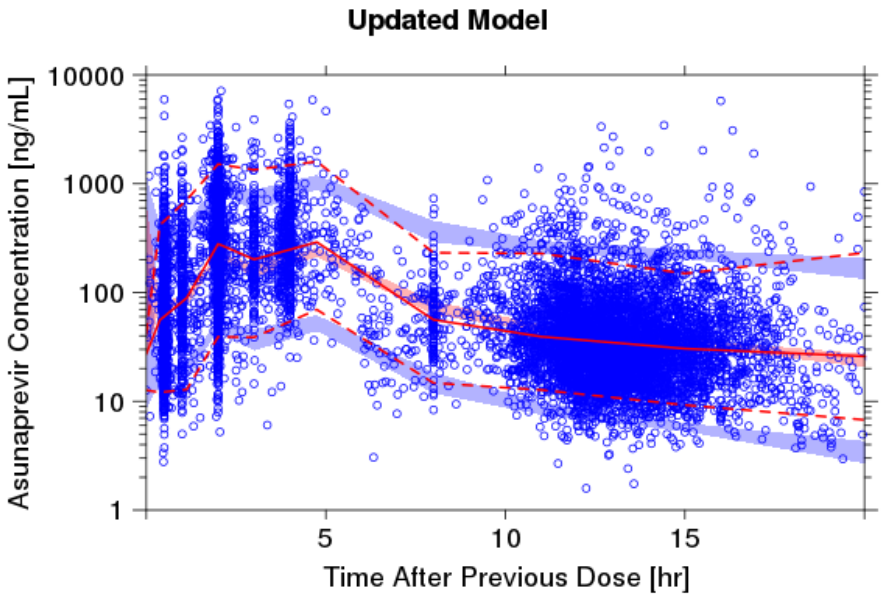
Figure 3.3.2-1: Diagnostic Plots for the Updated ASV PopPK Model



Note: Solid line is unity line, dashed line is loess line.

The pcVPC plots were done for assessing the capability of a model to reproduce the distribution of the data and showed the updated final model adequately described the central tendency and the spread of the observed PK (**Figure 3.3.2-2)**.

Figure 3.3.2-2: Prediction-Corrected Visual Predictive Check for the Updated ASV PopPK Model

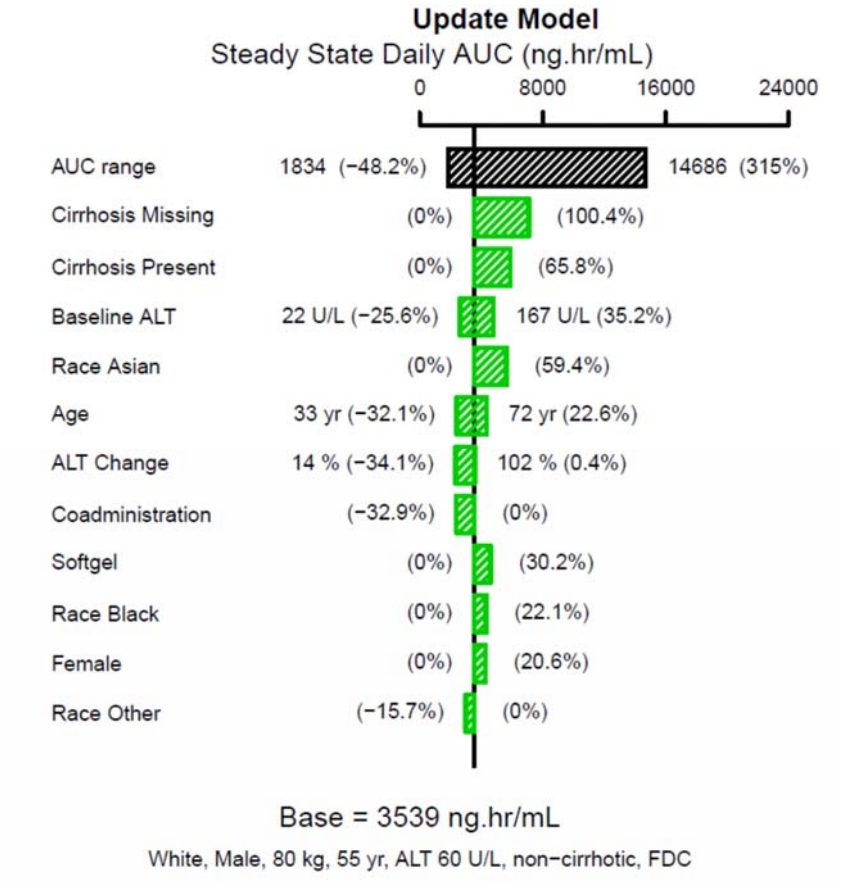


Circles are observed concentrations, solid red lines represent the median observed value, and dashed lines represent 5th percentile and 95th percentiles of the observed values. Red shaded areas represent the spread of the median predicted values (2.5th to 97.5th percentile), and blue shaded areas represent the spread (2.5th percentile and 97.5th percentile) of the 5th and 95th predicted percentile concentrations.

The effects of covariates on the final model PK exposure was evaluated by simulating the influences of statistically significant covariates on AUC_{ss}. **Figure 3.3.2-3** shows the independent influence of individual covariates on the AUC_{ss} of ASV after repeated dosing of 3DAA regimen for the updated model. Baseline ALT, cirrhosis and Race had a modest impact on ASV PK. Subjects of the updated analysis at the 95th percentile baseline ALT had 35.2 % greater AUC_{ss} and subjects at the 5th percentile of baseline ALT had 25.6 % lower AUC_{ss}. Patients with cirrhosis

had 65.8 % greater AUC_{ss}. Asian subjects had 59.4% higher AUCss compared to White subjects. Other covariates contributing more than an approximate 30% difference were age and time-varying decrease in ALT over time. When patient age having increased from 33 to 72 years, AUC_{ss} is anticipated to vary from -32.1% to 22.6% comparing to the value of a typical 55-year-old patient.

Figure 3.3.2-3: Effect of Covariates on Steady-State ASV Exposure



The black bar represents the 5th to 95th percentile daily exposure range calculated from post hoc parameters after 10 doses of daclatasvir 30 mg, asunaprevir 200mg and beclabuvir 75 mg BID. The effect of a covariate was calculated by varying one covariate at a time, and fixing all other covariates to typical values. Continuous covariates were evaluated at the 5th and 95th percentiles of the population.

The covariates on ASV in the updated final models were evaluated, taking into account correlation of covariates among subject population (**Table 3.3.2-2**). Briefly, the AUC_{ss} increased

by 90.8 % in subjects with cirrhosis. The extent of increase is greater than expected from the univariate effect (65.8%, **Figure 3.3.2-3**), in part because cirrhotic subjects had greater median baseline ALT values (80 vs 52 U/L). The effect of race on ASV AUC_{ss} increased by 62.4% in Asian subjects relative to White subjects. The extent of increase is similar to what is expected from the univariate effect (59.4%, **Figure 5.3.2-3**), despite a greater percentage of Asians being female.

Table 3.3.2-2: ASV Exposure by Race or Cirrhosis Status

	Median [5th - 95th] AUC_{ss} (ng•hr/mL)	**% median difference from reference**
ASV exposure by race (reference: White)		
White (n=790)	4,160 [1,716-12,020]	-
Black (n=120)	4,516 [1,799-17,773]	8.6
Asian (n=303)	6,756 [3,355-20,210]	62.4
ASV exposure by cirrhosis status (reference: Non-cirrhosis)		
Non-cirrhosis (n=945)	4,176 [1,723-10,435]	-
Cirrhosis (n=280)	7,969 [3,300-21,163]	90.8

3.3.3 BCV

One compartment model was selected as the structure model based on the time-concentration profile. Absorption of BCV was modeled as zero-order release of the drug followed by a first-order absorption into the central compartment. Inter-individual variability was estimated for CL/F, V/F and Ka, with interaction between CL/F and V/F. The residual error model was proportional plus additive in BCV plasma concentration. Trough PK concentrations on Day 1 were greater than the steady-state trough concentrations collected on or after Day 14 thus induction factors for clearance and bioavailability were tested and included in the model. Since there were no PK samples collected between Day 1 and Day 14, a step function was used to model the PK induction. The details of base model development are provided in **Table 3.3.3-1.**

Table 3.3.3-1: Selection of Structure Models for the BCV Base Model

Run	Model	MIN	OFV	DOF	Compare	ΔDOF	ΔOFV	P	Sig[a]
1	1st order absorption with a lag time; 1-comp linear elimination; diag eta on CL, V and Ka; proportional + additive error	RE	121966.89	10					
2	1 exclude samples collected TAD below LLOQ (54 hr)	Converge	120929.89	10					
3	2 + 2-peripheral distribution	Converge	120938.43	12	2	2	8.54	0.014	No
4	2 + Induction on CL at <= 2 days	RE	117687.5	11	2	1	-3242.39	0.000	Yes
5	4 + Induction on F1 at <=2 days	Converge	117566.11	12	4	1	-121.4	0.000	Yes
6	5 + 0 order release then first order absorption instead of Tlag	Converge	117607	12	5	0	40.9		
7	5 + BLOCK (2) for CL and V	NPSD	117520.81	13	5	1	-45.3	0.000	Yes
8	5 + BLOCK (3) for CL, V and ka	Converge	117520.16	15	5	3	-45.94	0.000	Yes
9	7 + Proportional error	Converge	117521.43	12	7	-1	0.62	0.432	No
10	7 + log additive error	Converge	2043.38	11					
11[b] (Base Model)	10 - 10 WRES outliers (N=26 samples)	Converge	857.2	11					

[a] Significant at p-value < 0.001

[b] Base Model structure: A one compartmental linear elimination model; a first-order absorption with a lag time.
Abbribiations: MIN, minimization; OFV, objective function value; DOF, degree of freedom; Δ DOF, difference of DOF from reference; Δ OFV, difference of OFV from reference; P, p-value.

Significant covariates remaining in the original final model were age, body weight, race (White, Black, Asian or others), baseline and time-varying ALT, co-administration of proton pump inhibitor (yes or no) on CL/F. The covariates included in the updated final model were the same as the original final model. Other covariates tested in the updated model, including HCV genotype (1b or non-1b), cirrhosis status (yes, no or missing) and patient type (naïve or experienced), had no significant impact on the CL/F in the updated final model. The parameter estimates of the original and updated final models for BCV are presented in **Table 3.3.3-2.** With the exception of Asian race, the BCV PK parameters were similar to the previous estimates. The shrinkage of the random effect parameters was 9.2 % for CL/F, 43.2 % for V/F, and 38.8 % for Ka.

Table 3.3.3-2: Parameter Estimates of the BCV Final Models

Parameters	Original Model		Updated Model	
	Estimate (RSE %)	95% CI[a]	Estimate (RSE %)	95% CI[a]
Fixed Effect				
CL/F (L/hr)	8.29 (2.64)	7.86, 8.72	8.29 (2.36)	7.91, 8.67
V/F (L)	78.6 (5.05)	70.8, 86.4	78.5 (4.48)	71.6, 85.4
ka (1/hr)	1.71 (7.19)	1.47, 1.95	1.83 (7.49)	1.56, 2.10
Tlag (hr)	0.446 (1.35)	0.434, 0.458	0.453 (1.04)	0.444, 0.462
Induction effect on CL	-0.616 (10.2)	-0.739, -0.493	-0.642 (9.44)	-0.761, -0.523
Induction effect on F	0.338 (15.4)	0.236, 0.440	0.320 (14.3)	0.23, 0.41
Age~CL/F	-0.253 (18.3)	-0.344, -0.162	-0.304 (14.0)	-0.387, -0.221
Female~V/F	-0.113 (28.8)	-0.177, -0.0493	-0.126 (23.3)	-0.183, -0.0686
Weight~CL/F	0.444 (16.4)	0.301, 0.587	0.420 (16.3)	0.286, 0.554
Weight~V/F	0.772 (11.6)	0.596, 0.948	0.730 (11.6)	0.564, 0.896
Black~CL/F	-0.139 (29.1)	-0.218, -0.0598	-0.177 (27.3)	-0.272, -0.0823
Asian~CL/F	0.0295 (327)	-0.16, 0.219	-0.367 (11.3)	-0.449, -0.285
Race Other~CL/F	0.0333 (235)	-0.12, 0.187	0.228 (42.6)	0.0375, 0.419
Baseline ALT~CL/F	-0.124 (16.3)	-0.164, -0.0844	-0.116 (14.9)	-0.150, -0.0821

Table 3.3.3-2: Parameter Estimates of the BCV Final Models

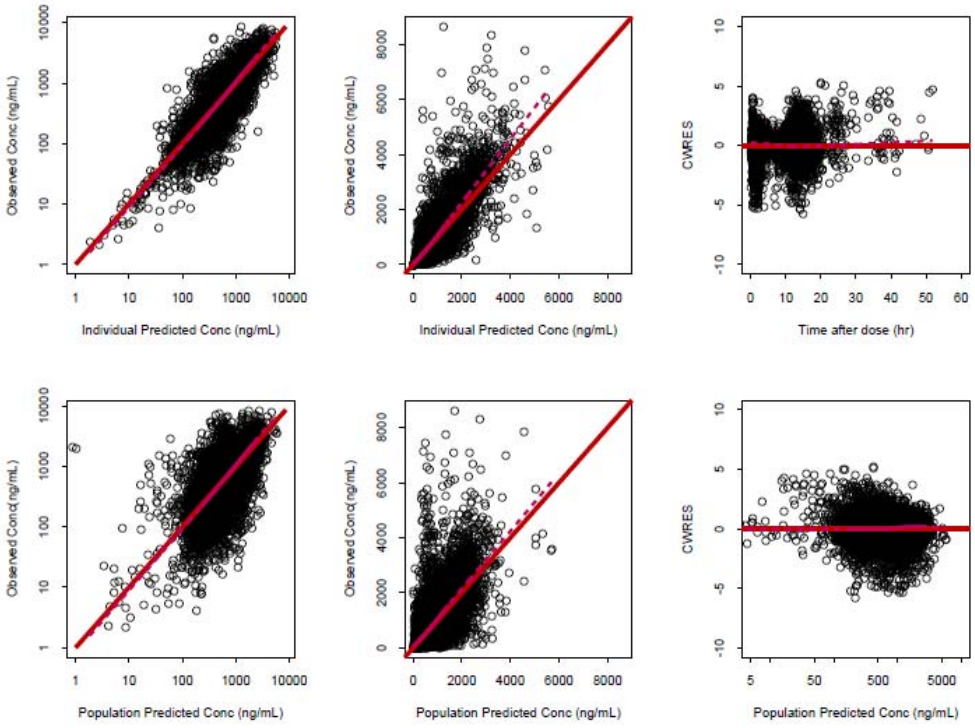
Parameters	Original Model		Updated Model	
	Estimate (RSE %)	95% CI[a]	Estimate (RSE %)	95% CI[a]
Cirrhosis Present~V/F	0.158 (24.1)	0.0833, 0.233	0.162 (21.0)	0.0954, 0.229
Cirrhosis Missing~V/F	0.274 (25.7)	0.136, 0.412	0.272 (26.0)	0.133, 0.411
Coadministration~ka	-0.485 (17.4)	-0.65, -0.32	-0.490 (16.6)	-0.65, -0.33
PPI~CL/F	-0.147 (23.8)	-0.216, -0.0784	-0.136 (25.8)	-0.205, -0.0672
ALT change~CL/F	-0.0943 (19.6)	-0.131, -0.058	-0.0881 (16.7)	-0.117, -0.0593
ALT change~V/F	0.119 (28)	0.0537, 0.184	0.113 (24.4)	0.0589, 0.167
Black~V/F	-	-	-0.0809 (55.7)	-0.169, 0.00749
Asian~V/F	-	-	-0.230 (20.0)	-0.32, -0.14
Race Other~V/F	-	-	0.374 (49.7)	0.00945, 0.739
Random Effect				
CL/F	0.133 (6.88)	0.115, 0.151	0.133 (6.65)	0.116, 0.15
CL/F•V/F	0.0618 (15.6)	0.0429, 0.0807	0.0589 (15.1)	0.0414, 0.0764
V/F	0.0739 (19.4)	0.0459, 0.102	0.0657 (18.6)	0.0418, 0.0896
ka	0.667 (11.7)	0.514, 0.82	0.738 (10.7)	0.583, 0.893
Residual				
Log-additive residual	0.278 (3.78)	0.257, 0.299	0.256 (3.39)	0.239, 0.273

Abbreviations: ALT, alanine aminotransferase; BCV, beclabuvir; CI , confidence interval; CL, clearance; CL/F, apparent clearance; F, bioavailability; ka, first-order absorption rate constant; PK, pharmacokinetic; PPI, proton pump inhibitor; RSE, relative standard error; Tlag, lag time; V/F, apparent volume of distribution

Note: Coadministration means all three drugs administered simultaneously as separate tablets.

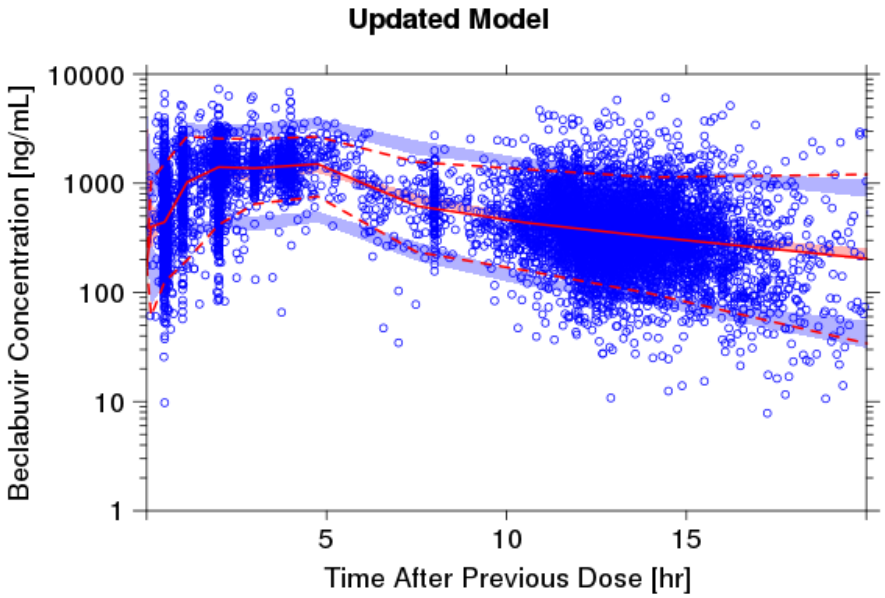
Figure 3.3.3-1 shows the diagnostic plots in the updated final model, suggesting no bias predicted value and time. The pcVPC plots showed that the updated final model adequately described the central tendency and the spread of the observed PK, as presented in **Figure 3.3.3-2**.

Figure 3.3.3-1: Diagnostic Plots of the Updated BCV PopPK Model



Note: Solid line is unity line, dashed line is loess line.

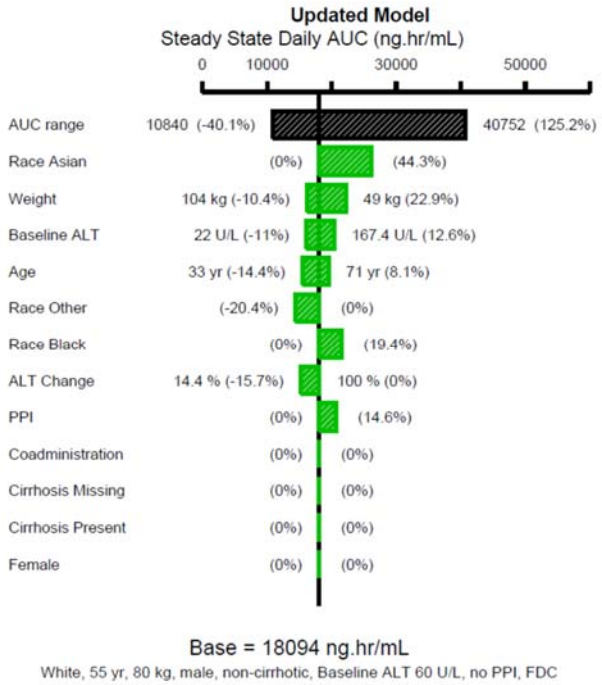
Figure 3.3.3-2: Prediction-Corrected Visual Predictive Check for the Updated BCV PopPK Model



Circles are observed concentrations, solid red lines represent the median observed value, and dashed lines represent 5th percentile and 95th percentiles of the observed values. Red shaded areas represent the spread of the median predicted values (2.5th to 97.5th percentile), and blue shaded areas represent the spread (2.5th percentile and 97.5th percentile) of the 5th and 95th predicted percentile concentrations.

Figure 3.3.3-3 shows the independent influence of each covariate on the AUC_{ss} of BCV after repeated doses of 3DAA regimen for the updated models. Asian subjects had greater impact on the BCV exposure (44.3% increase in AUC_{ss} compared to White subjects). Weight had modest impact on the BCV exposure in the updated model, where subjects at the 95th percentile weight decreased 10.4% in AUC_{ss} and subjects at the 5th percentile weight increased 22.9% in AUC_{ss}.

Figure 3.3.3-3: Effect of Covariates on Steady-State BCV Exposure



The black bar represents the 5th to 95th percentile daily exposure range calculated from post hoc parameters after 10 doses of daclatasvir 30 mg, asunaprevir 200mg and beclabuvir 75 mg BID. The effect of a covariate was calculated by varying one covariate at a time, and fixing all other covariates to typical values. Continuous covariates were evaluated at the 5th and 95th percentiles of the population.

The impact of covariates on BCV were evaluated, taking into account of correlation amongst covariates. The BCV AUC increased by 62.4% in Asian subjects compared to White subjects after taking account of correlation with other covariates (**Table 3.3.3-3**). This impact was greater than the expected univariate effect (44.3%) in **Figure 3.3.3-3**, which could be attributed to less median weight (55.6 vs 79.8 kg) in Asian subjects.

Table 3.3.3-3: BCV Exposure by Race in the Updated Model

	Median [5th - 95th] AUC_{ss} (ng•hr/mL)	% median difference from reference
ASV exposure by race (reference: White)		
White (n=790)	18,223 [10,631-33,589]	-
Black (n=120)	19,597[11,309-46,865]	7.5
Asian (n=228)	29,599 [18,726-56,188]	62.4

3.4 Discussion

In the updated DCV PopPK Model, the regimen (DUAL regimen and 3DAA regimen) on CL/F was not a significant covariate, suggesting that DCV exposure would not be altered by the addition of BCV. This result supports the previous finding that comparable overlap of DCV exposure in 3DAA regimen was observed with DCV exposure in the historical data of DUAL regimen.[27] Sex was the most important covariate on DCV exposures, and female subjects had approximately 24% greater exposures than male subjects in the updated DCV PK model. The PK difference between male and female was not explained by the body weight differences. In fact, body weight was impact on V/F but not a significant covariate for DCV CL/F. Overall, the magnitude of significant covariates were modest and not considered clinically relevant, which was consistent with the Research 1 and the previous DCV PopPK analyses.[37, 46]

The most important finding for ASV is that Asians have 62.4% greater ASV exposures than White subjects. This trend is consistent with the previous finding observed in DUAL regimen of DCV and ASV.[47] This difference could have been partly confounded by a sex effect on CL, as a greater percent of Japanese subjects were female, and females had approximately 21% greater exposures than males (**Figure 3.3.2-3**) in the updated model. The univariate effect of Asian race, which adjusted for other covariates, is 59.4%. Since ASV is a substrate of OATP1B1, the leading hypothesis was ethnic differences in the distribution of reduced function alleles among Asians.

However, the investigations evaluated the correlation of ASV with polymorphisms in liver uptake transporters did not reveal a relationship between OATP and ASV exposure.[48] The reasons for the relatively higher exposure levels of ASV in Asian subjects are not entirely clear. Racial differences in ASV exposure may be driven by more complex factors than OATP haplotypes. Other than Asian race, the ASV PK parameters and the effects of covariates were similar to the estimates in original PopPK model (**Table 3.3.2-1**). Considering the predominance of Japanese in the Asian group (292 out of 303), the effect of Asian race is likely an effect of the Japanese ethnicity rather than that of Asian race in general. Of the covariates, cirrhosis was found as an important factor for ASV exposure in both original and updated PopPK models. This result is consistent with the previous ASV PopPK analyses for DUAL regimen.[46, 49] Cirrhotic subjects have 90.8% greater exposure than non-cirrhotic subjects in the updated model. This is in part associated with the greater median baseline ALT in cirrhotic subjects, 80 U/L versus 52 U/L in non-cirrhotic subjects. After adjusting for other covariates including ALT, cirrhotic subjects would have 65.8% higher exposures than non-cirrhotic subjects (**Figure 3.3.2-3**). Overall, the impact of significant covariates were comparable to that in the previous ASV PopPK analyses for DUAL regimen, indicating that there was no obvious difference in the significant covariates on ASV PK parameters by the addition of BCV. The effect of any single covariate was well within to the wide range of exposures observed. Further consideration of ASV exposure on clinical outcome is discussed in the subsequent safety (Research 3) and efficacy E-R analyses.[42, 50]

Lastly, the results of BCV PopPK analysis showed that race, body weight, baseline and time-varying ALT, age, co-administration of proton pump inhibitor were significant covariates on CL/F. With the exception of Asian race, the BCV PK parameters are similar to the original estimates (**Table 3.3.3-2**). Asians had 44.3% greater BCV exposures than White subjects. (**Figure**

3.3.3-3). The predominant route of elimination of BCV are CYP3A4 mediated metabolism and P-gp/BCRP excretion. The polymorphisms might contribute on the exposure difference between Asian and non-Asian subjects. However, BCV exposure was not altered by co-administering with DCV and ASV, where both DCV and ASV are inhibitors of P-gp and DCV also inhibits BCRP.[27] The lack of increase in BCV exposures when co-administered with DCV and ASV suggests that BCV is not a sensitive substrate of P-gp/BCRP. In addition, BCV is not a substrate of OATP1B1 or OATP1B3. BCV will not be influenced by activity of allelic variants and allelic frequency of OATP1B1 and 1B3, although ethnic differences for these transporters are known in some cases, such as HMG-Co-A reductase inhibitors.[51] Therefore, polymorphisms of these transporters are unlikely to lead to major changes in BCV exposures.

The primary enzyme responsible for the metabolism of BCV was CYP3A4 with minor contribution by CYP3A5. In general, clinically meaningful genetic variation in CYP3A4 activity has not been found. However, recent descriptions of several polymorphisms of the CYP3A4 gene, including the CYP3A4*22 polymorphis[52] may hold promise in beginning to explain variability associated with CYP3A4 substrates. Functional CYP3A5 activity would be detectable to an appreciable degree in ~ 10% to 20% of Caucasians, 40% to 70% of Asians and > 80% of Blacks/African Americans.[53] Based on racial distributions of functional CYP3A5*1 allele, CYP3A5 could contribute to the metabolism of BCV in Black/African American subjects, have an intermediate to negligible effect in Asians, and have an insignificant effect in Whites/Caucasians. Genotyping for CYP3A5 was not performed in the BCV program, however, the general comparability of PK between Blacks/African Americans and Whites/Caucasians suggest that the effect of functional CYP3A5 is not clinically meaningful (**Figure 3.3.3-3**). In summary, genetic variations in CYP3A4 activity was not found to have any clinically meaningful

impact on the exposure, thus, it is unlikely that the differences in CYP3A4 activity to be an influential factor for the higher exposures of BCV in Japanese subjects. The mechanism of increasing exposure in BCV is not fully understood. Ethnic differences in transporters and metabolic enzymes do not appear to contribute to the observed differences.

From the results of the updated PopPK models, HCV GT-1b did not appear to be an important factor for DCV, ASV, or BCV exposure. Also, patient type, either naïve or treatment experienced, was not an important factor for DCV, ASV or BCV exposure. The PopPK models described the data, and provided the adequate estimates of individual exposures for subsequent efficacy and safety E-R analyses. The clinical relevance of exposures and covariates was assessed in the E-R analyses.

3.5 Conclusion

The effects of covariates on DCV PK were modest and not considered clinically significant. ASV exposure increased with cirrhosis and increasing baseline and time-varying ALT values. Asian subjects had greater ASV and BCV exposures than White subjects. With the exception of Asian race on ASV and BCV PK, no other parameters for DCV, ASV and BCV PopPK models were meaningfully impacted during update with Japanese subjects. The current PopPK models provided an adequate description of DCV, ASV and BCV concentration data in HCV-infected subjects.

4 RESEARCH 3: SAFETY EXPOSURE-RESPONSE ANALYSIS FOR 3DAA REGIMEN IN HCV INFECTED SUBJECTS

4.1 Objective

To characterize the E-R relationship of 3DAA regimen with treatment-emergent liver related laboratory elevations [ALT and total bilirubin (TB)] in HCV infected subjects, and to assess the impact of covariates on these E-R relationships.

4.2 Method

The safety E-R analysis was performed with combined data from HCV subjects treated with 3DAA regimen in one phase 2 study (AI443014: NCT01455090) and three phase 3 studies (AI443102: NCT01979939 and AI443113: NCT01973049 and AI443117: NCT02123654).

Grade 3 or 4 liver related laboratory elevations were modeled as the safety endpoints since they are more clinically relevant than Grade 1 or 2 elevations. The results from the previous clinical studies demonstrate that DCV did not cause any liver enzyme elevations. In addition, DCV exposure was similar whether administered as part of DUAL regimen or as part of 3DAA regimen, indicating BCV did not alter the PK of DCV.[27] Therefore, the effects of ASV and BCV exposure on Grade 3 or 4 liver related laboratory elevations were evaluated in this E-R analyses as exposure metrics.

Individual ASV and BCV exposure were calculated from the Bayesian post-hoc parameters of the final PopPK models of ASV and BCV from Research 2.[54] The Cavgss was selected as exposure metrics since the timing of onset of the laboratory abnormalities suggest that overall exposure, i.e., Cavgss, was the most relevant exposure parameter rather than a single concentration at any given time such as peak or trough concentration. This is supported by the fact that the transient rise and fall of plasma concentration is likely not reflected as significantly within the

liver, where more consistent concentrations would be expected at steady state. Furthermore, AUC which is an equivalent exposure measure to Cavg was also used to assess the relationship between drug exposure and safety event in other HCV agents. [55, 56]

Missing baseline demographic and clinical laboratory covariates were imputed as the population median (continuous) or mode (categorical) of the non-missing value, with no adjustment for study, race, or sex. There were only a few subjects (≤ 1%) with missing baseline body weight, baseline BMI, fibrosis score, race, IL28B genotype (rs12979860), cirrhosis, and prior treatment type.

Model development was conducted in three stages: the base model, covariate model and final model. First, the base model was developed. The relationship between the probability of Grade 3 or 4 liver related laboratory elevations and the Cavgss for ASV, and BCV was described using a logistic regression model, without consideration of any potential effect of covariates. The probability of AEs was given as **Equation 1**.

Equation 1

$$P(AE) = \frac{e^{\mu}}{(1 + e^{\mu})}$$

where μ is the logit transform of P(AE). The logit (log-odds) can be given as Equation 2.

Equation 2

$$\mu = \log\frac{P}{1 - P} = \beta_0 + \boldsymbol{\beta_i X}_i$$

where β_0 and $\boldsymbol{\beta}_i$ are scalar and vector parameters that represent baseline odds and the effect of the predictor variable vector $\boldsymbol{X}_i$ on the log-odds of having the events, where $\boldsymbol{X}_i$ consists of the covariate (predictor) values of subject *i*.

Pre-specified covariates tested in the E-R analyses of liver related laboratory elevations

were listed in **Table 4.2-1**.

Table 4.2-1 Pre-specified Covariates Tested in the Exposure-Response Analysis of Liver Related Laboratory Elevations

Category	Covariate
Demographic	sex (female or male), age, race (Asian or non-Asian), body weight, BMI
Baseline labs	baseline liver enzymes (ALT or TB), baseline serum creatinine
Disease related	HCV RNA(> $8x10^5$ or ≤ $8x10^5$), HCV genotype (GT-1b, GT-4 or other), IL28B genotype (rs12979860) (CC, CT or TT alleles), prior treatment type (prior peg-IFN failure, prior DAA failure, prior IFN treatment terminated due to safety issue or other), fibrosis score (F4 or F0-3), cirrhosis (yes or no/unknown), number of resistance mutations [a] (≥ 1 or 0)
Treatment	ribavirin usage (yes or no)

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; GT = genotype; HCV = hepatitis C virus; IFN = interferon; RNA = ribonucleic acid; TB = total bilirubin.

[a] NS5A polymorphism at M28, Q30, L31 or Y93

Japanese subjects were belonged to Asian race in the covariate models. We referred to race as Japanese or non-Japanese rather than Asian or non-Asian when the impact of race was discussed on the Grade 3 or 4 ALT and TB elevation rates in the Japanese study.

Among the continuous covariates evaluated in the analysis dataset, several highly correlated variables were expected. During model development, highly correlated covariates were evaluated but were not included in the same model without substantial evidence supporting their inclusion. For developing covariate model, the effects of covariates of interest on the base model were evaluated using a stepwise covariate model building approach, where covariates were tested in a forward addition ($p < 0.05$), followed by a backward elimination ($p < 0.01$) steps. Covariates were first tested on the intercept, then significant ones were further tested in the PK-exposure slopes.

Model evaluation was conducted by comparing the observed values and rate of incidence of events with the final model simulations stratified by covariates of interest. Confidence intervals of the simulation were obtained using bootstrapping methods (1000 runs). Model evaluation was also conducted using a visual predictive check of the final model [36] and presented stratified by covariates of interest.

The E-R safety analysis was performed using the NONMEM computer program (Version 7.2, level 2.0, ICON Development Solutions), compiled using Intel Fortran Compiler (Version 12.0.4, Intel Corp.). Perl-speaks-NONMEM (version 4.2, http://psn.sourceforge.net/) was used to aid the model development using NONMEM. Exploratory plots, post-processing, and visualization of NONMEM output were performed using R (Version 3.0 or later).

4.3 Results

The analysis included 1153 (99.9%) of 1154 subjects whose safety and PK data treated with 3DAA regimen were available. One subject in study AI443117 had missing PK exposure were excluded. Of note, the majority of Asian subjects were Japanese at 94.7% (216 out of 228). An overview of the Grade 3 and 4 liver related laboratory elevations, key covariates, and PK exposure are presented in **Table 4.3-1**.

Table 4.3-1 Overview of Liver Enzyme Adverse Events, Baseline Characteristics, and Exposure

Category	Variable	AI443014	AI443102	AI443113	AI443117
No. of Subjects	N	320	415	202	216
Liver related laboratory elevations	ALT Grade 3,4, N (%)	2 (0.6%)	19 (4.6%)	5 (2.5%)	30 (13.9%)
	TB Grade 3,4, N (%)	1 (0.3%)	0 (0%)	3 (1.5%)	12 (5.6%)
Subject characteristics	Median baseline ALT, U/L	60	54	79	47
	Median weight, kg	81	78	82	55
	Cirrhosis, N (%)	20 (6%)	0 (0%)	200 (99%)	46 (21%)
	Female, N (%)	117 (37%)	176 (42%)	69 (34%)	148 (69%)
	Asian Race, N (%)	3 (1%)	5 (1%)	4 (2%)	216 (100%)
Cavgss	DCV, (ng/mL)	565	529	496	600
	ASV, (ng/mL)	83	121	221	224
	BCV, (ng/mL)	959	713	738	1111

Values for Cavgss are median.

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; ASV = asunaprevir; BCV = beclabuvir; Cavg = average concentration; DCV = daclatasvir; TB = total bilirubin.

Grade 3 or 4 ALT and Grade 3 or 4 TB elevations occurred in 30 (13.9%) and 12 (5.6%) of the 216 in Japanese Phase 3 study (AI443117) who received 3 DAA regimen and who had a PK exposure result. The event rates of Grade 3 or 4 ALT and Grade 3 or 4 TB elevations in Japanese subjects were numerically greater than those in the other studies with non-Japanese subjects, which ranged from 0.6% to 4.6% and 0 % to 1.5 %, respectively. A higher number of female subjects was observed in the Japanese population (69 % female in the Japanese population compared with 34 to 42 % in the non-Japanese population). The Japanese population had a lower body weight compared to the non-Japanese population (55 kg vs. 78 to 82 kg). The ASV exposure was

approximately 2 fold higher in the Japanese population (AI443117) compared to the non-Japanese population in AI443014 and AI443102, whereas the ASV exposure in non-Japanese cirrhosis population in AI443113 was comparable to that in the Japanese population (221 ng/mL vs. 224 ng/mL) because cirrhosis is one of the covariates influencing ASV PK.[44, 46]

The base ALT model includes the effect of ASV and BCV concentration at steady-state. The final model for Grade 3 or 4 ALT elevation included the effect of race (Asian or non-Asian) and ASV exposure (C_{ASV}), and the effect of body weight in non-Asian subjects. The effect of BCV exposure was not significant and dropped from the model. The final ALT E-R model was given as:

$$\mu_i = (\theta_1 + \theta_2 \cdot (BWT - 75.9)) \cdot Non - Asian + \theta_3 \cdot Asian + \theta_4 \cdot C_{ASV,i}$$

where, θ_i indicates the strength of the covariate effect for *i*-th covariate.

The base TB model included an intercept and pre-specified linear effect of ASV and BCV exposures. Significant covariates identified during stepwise covariate modeling were race (Asian or non-Asian), fibrosis score (F4 fibrosis score or F0-F3 fibrosis score), and ASV exposure. No covariates interacting on the drug effects were identified. The effect of BCV exposure was not significant and dropped from the model. The final TB E-R model was given as:

$$\mu_i = \theta_1 + \theta_2 \cdot Asian + \theta_3 \cdot C_{ASV,i} + \theta_4 \cdot F4\ Fibrosis\ Score$$

The final model parameters and their 95% CI are provided in **Table 4.3-2**.

Table 4.3-2 Final Model Parameter Estimates

Name	Estimate	Standard Error (RSE%)[a]	95% CI[b]
Grade 3 or 4 Alanine Aminotransferase Elevation			
Intercept for non-Asian (θ_1)	-3.91	0.252 (6.44)	-4.54 , -3.52
Asian on intercept (θ_3)	1.53	0.311 (20.4)	0.865 , 2.20
Slope of ASV (ng/mL, θ_4)	0.0017	0.00065 (38.3)	0.00034 , 0.00305
BWT (kg) effect on intercept for non-Asian(θ_2)	-0.0475	0.0148 (31.2)	-0.0829 , -0.0163
Grade 3 or 4 Total Bilirubin Elevation			
Intercept for non-Asian (θ_1)	-6.79	0.658 (9.69)	-9.19 , -6.1
Slope of ASV (ng/mL, θ_2), $(ng/mL)^{-1}$	0.00321	0.000898 (28)	0.00168 , 0.00545
Asian on intercept (θ_3)	2.01	0.624 (31)	0.892 , 3.81
Fibrosis Grade 4 on intercept (θ_4)	1.64	0.563 (34.3)	0.485 , 2.97

Abbreviations: ASV = asunaprevir; BWT = baseline body weight; CI = confidence interval; RSE = relative standard error.

[a] RSE% is the relative standard error (Standard Error as a percentage of Estimate).

[b] Confidence interval values are taken from bootstrap calculations (1000 run, sampled stratified by study).

Grade 3 or 4 ALT elevation increased in Asian subjects, and decrease with increasing weight in non-Asian subjects. The final model indicated that higher ASV exposure has a modest increase of the Grade 3 or 4 ALT elevation.

The final model for Grade 3 or 4 TB elevation indicated that subjects with F4 fibrosis score (Fibro Test score greater than 0.75) had a higher rate of Grade 3 or 4 TB elevation compared to subjects with F0-3 fibrosis score, Asian subjects had a greater rate of Grade 3 or 4 TB elevation than non-Asian subjects, and Grade 3 or 4 TB elevation increased with increasing ASV exposure.

The impact of isolated effect of covariates and ASV exposure on Grade 3 or 4 ALT or Grade 3 or 4 TB elevation rate is provided in **Table 4.3-3**. Grade 3 or 4 ALT elevation rate for a "typical" baseline weight of 75.9 kg and ASV median exposure of 138 ng/mL was 2.5% (95% CI, 1.4 to 3.5) for non-Asian subjects. For an Asian subject of the same weight and exposure, the Grade 3 or 4 ALT elevation rate would be 10.4%. Increase in ASV exposures from the 5th percentile (48 ng/mL Cavgss) to the 95th percentile (472 ng/mL) was predicted to increase the rate of Grade 3 or 4 ALT elevation by 2.2%, from 2.1% at the 5th percentile to 4.3% at the 95th percentile. The Grade 3 or 4 TB elevation rate for a non-Asian with F0-3 fibrosis score and median ASV exposure of 138 ng/mL was 0.18%. The Grade 3 or 4 TB elevation rate increased to 1.3% in Asian subjects. The combined effect of Asian race and F4 fibrosis score increased the event rate to 6.4% at ASV exposure of median value of 219 ng/mL (estimated event rate of 1.7%) in Asian subjects, compared to the rate for Asian subjects alone of 1.3%.

Table 4.3-3 Impact of Covariate and Exposure

Condition		**Rate (%)**	**95% CI[a]**
	Grade 3 or 4 ALT Elevation		
Reference	non-Asian, median Cavgss exposure of ASV of 138 ng/mL, median weight of 75.9 kg	2.5	1.4, 3.5
Estimated event rate	Asian	10.4	6.3, 15.3
	ASV 5%ile, 48 ng/mL	2.1	1.2, 3.0
	ASV 95%ile, 472 ng/mL	4.3	2.1, 7.3
	Weight 5%ile, 49 kg	8.3	3.3, 16.1
	Weight 95%ile, 104 kg	0.7	0.2, 1.9
	Asian + ASV exposure of 219 ng/mL	11.8	7.6, 16.6
	Grade 3 or 4 TB Elevation		

Condition		Rate (%)	95% CI[a]
Reference	non-Asian, median Cavgss of ASV exposure of 138 ng/mL, fibrosis score 0-3	0.18	0.02, 0.33
Estimated event rate	Asian	1.3	0.20, 3.1
	ASV 5%ile, 48 ng/mL	0.13	0.01, 0.25
	ASV 95%ile, 472 ng/mL	0.51	0.07, 1.2
	Fibrosis Grade 4	0.90	0.11, 2.0
	Asian + F4 fibrosis score	6.4	1.9, 12.8
	Asian + ASV exposure of 219 ng/mL	1.7	0.29, 3.8

Abbreviations: ASV = asunaprevir; CI = confidence interval.

[a] 95% CI were calculated from bootstrap parameters (1000 run, sampled stratified by study).

The observed and model simulated Grade 3 or 4 ALT elevation stratified by race and body weight quartiles were compared to quantify the impact of significant covariates on Grade 3 or 4 ALT elevation (**Table 4.3-4**). The effect of body weight was a significant covariate in non-Asian subjects. In non-Asian subjects, simulated rate of Grade 3 or 4 ALT elevation was 6.1% for the lowest weight quartile and 0.9% for the highest weight quartile. The simulated results are consistent with the observed values.

Table 4.3-4 Grade 3 or 4 ALT Elevation Rate Stratified by Covariates

Variable	Race	Weight Quartile (kg)			
		[34.1 - 61.8]	**(61.8 - 75.9]**	**(75.9 - 88.2]**	**(88.2 - 126]**
Median WT, kg	Non-Asian	56	70	82	96
	Asian	53	67	80	90
Median ASV, ng/mL	Non-Asian	137	124	117	119
	Asian	223	216	264	149
No. of subjects	Non-Asian	124	244	273	284
	Asian	165	44	16	3
No. of events	Non-Asian	9	11	1	4
	Asian	23	8	0	0
ALT observed rate	Non-Asian	7.3%	4.5%	0.40%	1.4%
	Asian	13.9%	18.2%	0%	0%
ALT estimated rate (95% CI)[a]	Non-Asian	6.1% (2.9 , 10.2)	3.2% (1.8 , 4.4)	1.8% (0.8 , 2.7)	0.9% (0.3 , 2)
	Asian	11.9% (7.7 , 16.7)	11.7% (7.6 , 16.5)	12.6% (8.3 , 17.4)	10.6% (6.5 , 15.4)

Subjects were stratified by baseline weight quartile (eg, (61.8, 75.9] includes subjects 61.8 < bbwt ≤ 75.9 kg).

Abbreviations: ALT = alanine aminotransferase; ASV = asunaprevir; WT = weight.

[a] Model estimated and the 95% CI ALT elevation rates were calculated from bootstrap parameters using median WT and ASV of the group.

The observed and model simulated Grade 3 or 4 TB elevation rate stratified by race, fibrosis score, and ASV exposure were compared to quantify the impact of significant covariates on Grade 3 or 4 TB elevation (**Table 4.3-5**). The observed and estimated rates were both high in the subjects with F4 fibrosis score and higher ASV exposure (> 224 ng/mL), in particular, 4- to 9-fold higher in Asian subjects compared with non-Asian subjects (16.7% and 15.6% versus. 4.4% and 1.7%).

Table 4.3-5 **Grade 3 or 4 Total Bilirubin Elevation Rate Stratified by Fibrosis Score, ASV Exposure, and Race**

Variable	Race	Fibrosis Score 0-3		Fibrosis Score 4	
		ASV ≤ 127 ng/ml	ASV > 127 ng/ml	ASV ≤ 224 ng/ml	ASV > 224 ng/ml
Median ASV, ng/mL	Non-Asian	81	179	138	331
	Asian	108	215	203	448
No. of subjects	Non-Asian	453	307	97	68
	Asian	20	165	7	36
No. of events	Non-Asian	0	0	1	3
	Asian	0	6	0	6
TB observed rate	Non-Asian	0.0%	0%	1.0%	4.4%
	Asian	0.0%	3.6%	0.0%	16.7%
TB estimated rate (95% CI)[a]	Non-Asian	0.1% (0 , 0.3)	0.2% (0 , 0.4)	0.9% (0 , 0.3)	1.7% (0 , 0.7)
	Asian	1.2% (0.2 , 2.9)	1.7% (0.3 , 3.8)	7.7% (2.6 , 14.3)	15.6% (7.4 , 25.1)

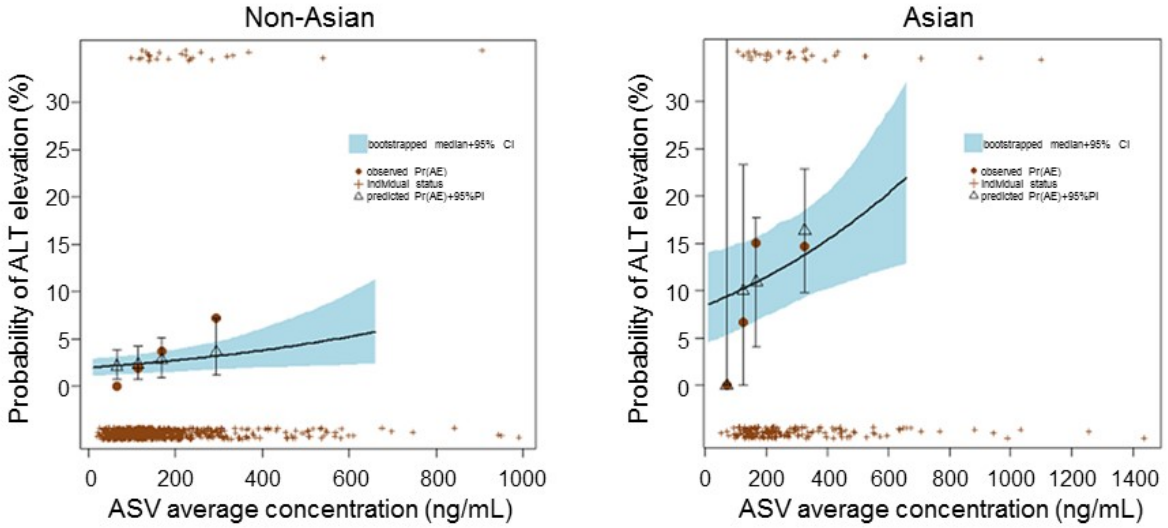
Abbreviations: ASV = asunaprevir; TB = total bilirubin.

[a] Model estimated and the 95% CI TB elevation rates were calculated from bootstrap parameters using median WT and ASV of the group. The subjects were divided by median ASV of 127 ng/mL for subjects of fibrosis grade 0-3 or median ASV of 224 ng/mL for subjects of fibrosis grade 4

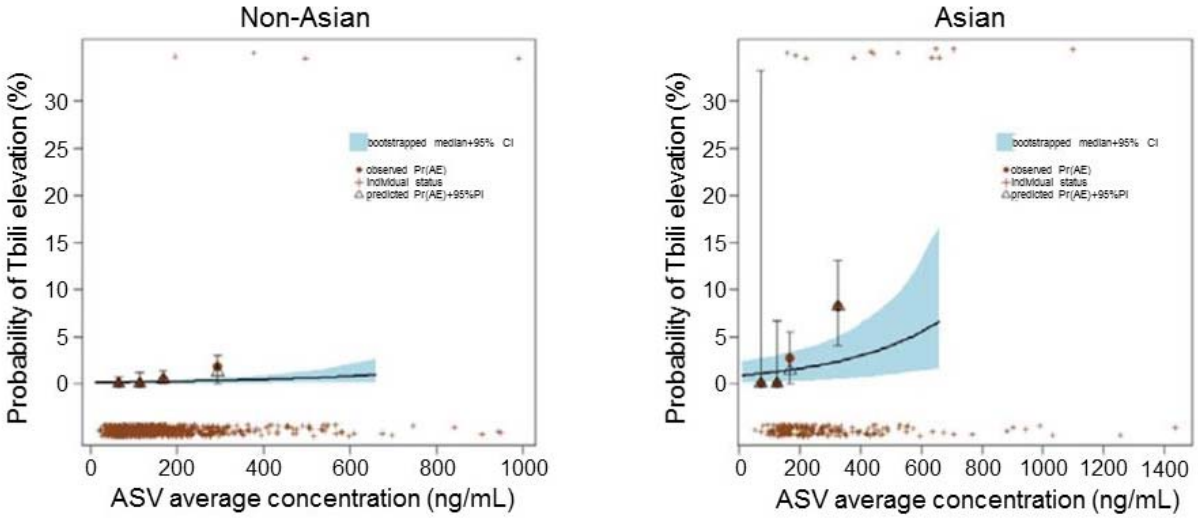
Visual predictive checks of Grade 3 or 4 ALT elevation and Grade 3 or 4 TB elevation versus ASV E-R response by race are presented in **Figure 4.3-1**. Visual predictive checks showed the ASV E-R response was generally described the data.

Figure 4.3-1: Visual Predictive Check of Grade 3 or 4 Liver Related Laboratory Elevations versus ASV Exposure and Race

A: Grade 3 or 4 ALT Elevation



B: Grade 3 or 4 TB Elevation



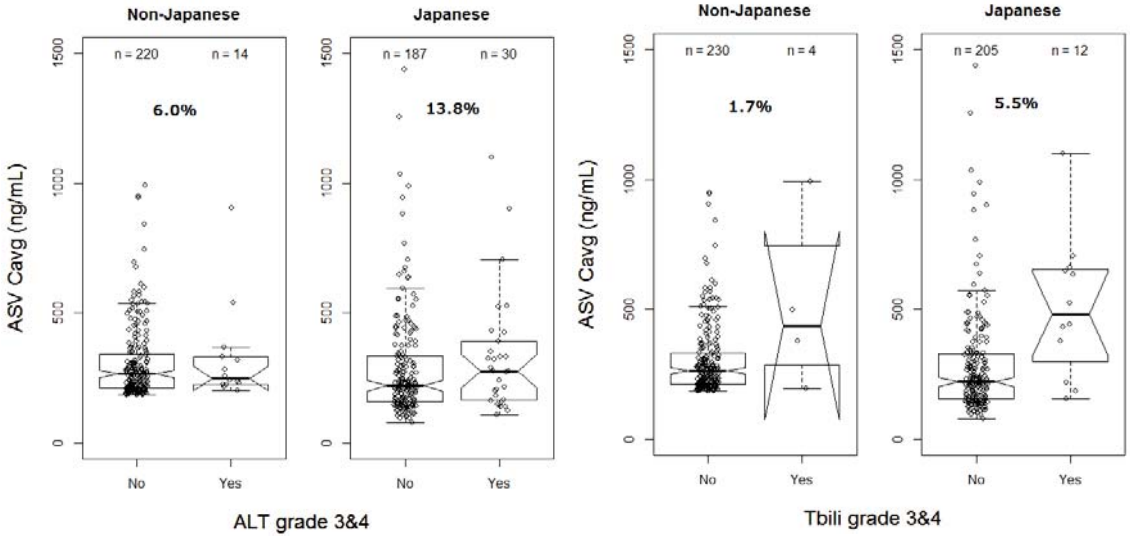
Predictions are for the reference condition of the population (median exposure and median weight of each group) except as indicted by the x-axis labels. The individual "+" symbols at the top and bottom of the plot represent individual subjects with and without AE, respectively, in each category indicated by the x-axis labels. The solid, brown circles and the open, black triangles indicate the observed and predicted AE, respectively, for each category. The vertical bars indicate the 95% prediction intervals and the blue shaded region indicates the 95% confidence interval for the bootstrapped model. The observed data include only subjects with categorical covariates consistent with the reference population, except as indicated by the x-axis labels. ALT and TB elevation rates were simulated up to 95th percentile of the ASV exposure in Asian subjects.

Abbreviations: AE = Grade 3 or 4 ALT or TB elevation event; ALT = alanine aminotransferase; ASV = asunaprevir; CI = confidence interval; PI = prediction interval; Pr(AE) = probability of an Grade 3 or 4 ALT or Tbili elevation; Tbili = total bilirubin.

4.4 Discussion

The final models for Grade 3 or 4 ALT and Grade 3 or 4 TB elevations included only the effect of ASV exposure since BCV exposure was not a significant predictor and the term for BCV exposure was dropped during the backward elimination process ($p < 0.01$). ASV exposure showed modest impact in the final ALT model (only a 2% increase in rate of events from 5th percentile to 95th percentile in ASV exposure). Asian race was the most significant factors contributing to the increase of Grade 3 or 4 ALT elevation. As shown in the clinical studies, the Grade 3 or 4 ALT elevation rate were numerically higher in Japanese study compared to other non-Japanese studies (**Table 4.3-1**). The previous ASV PopPK analysis showed that Japanese and non-Japanese Asian subjects had higher ASV exposure compared to White subjects.[44] In addition, ASV exposures would ~2 fold increase in Asian subjects compared to non-Asian subjects based on the PopPK analysis for 3DAA regimen in Research 2.[54] Although the reasons for the relatively higher exposure of ASV in Japanese or Asian subjects are not entirely clear, the higher ASV exposure might be considered as a factor of higher event rates in Japanese subjects. For further investigation, the Grade 3 or 4 ALT elevation of non-Japanese subjects at the high quartile of the ASV exposure (median ASV exposure was 265 ng/mL) were compared to all Japanese subjects (**Figure 4.4-1**).

Figure 4.4-1: Comparison of Grade 3 or 4 Liver Related Laboratory Elevation Rates in Non-Japanese With High ASV Exposure to Japanese



The line in the middle of the box is the median, the box is the inter-quartiles, the whiskers are 1.5 times the inter-quartile range.

Abbreviations: ALT = alanine aminotransferase; ASV = asunaprevir; Cavg = average concentration at steady state, Tbili= total bilirubin

The rate of Grade 3 or 4 ALT elevation in non-Japanese subjects with ASV exposure in the highest quartile was significantly lower than that in Japanese subjects (6.0% vs 13.8%). The higher event rate in Japanese subjects was not fully explained by the higher ASV exposure in Japanese subjects. This supports the interpretation of the E-R analysis that impact of ASV exposures is modest on the Grade 3 or 4 ALT elevation.

The impact of weight was estimated separately for Asian and non-Asian subjects, since the Asian subjects were concentrated at the lower weight quartiles. As presented in **Table 4.3-4**, the results of the observed and predicted ALT rates stratified by body weight and race indicate that the difference between Asians and non-Asians is not entirely explained by differences in body weight. In the lowest body weight quartile (34.1 to 61.8 kg) the observed rate of Grade 3 or 4 ALT elevation in non-Asian and Asian subjects was 7.3% and 13.9%, respectively. The model was able

to predict these reasonably with a predicted rate of 6.1% and 11.9% in non-Asian and Asian subjects, respectively. In general, these data indicate that subjects with lower body weight are at a higher risk of events which is further increased in Asian subjects with a trend towards higher rate of events at higher ASV exposures. The final model contained the body weight in the non-Asian subjects as a significant covariate. Few studies have been reported the relationship between body weight and ALT elevation. The demographics in the non-Asian subjects at each weight quantile were explored to evaluate the potential correlation with other covariates, however, there was no obvious trend to explain the higher Grade 3 or 4 ALT elevation rate in the lower weight group. The mechanism of increasing Grade 3 or 4 ALT elevation rate in the non-Asian subjects with lower body weight remains unclear, and further investigation would be needed.

ASV exposure was also included in the final E-R model for Grade 3 or 4 TB elevation. Higher ASV exposure was associated with increasing Grade 3 or 4 TB elevation. This finding was consistent with the E-R model for DUAL regimen.[57] The Grade 3 or 4 TB elevation increased in Asians and in subjects with F4 fibrosis score. Although fibrosis score was identified as a significant covariate in the final model, fibrosis score is correlated with cirrhosis status and similar results were obtained with cirrhosis status as well, indicating that the rate of Grade 3 or 4 TB was higher in subjects with cirrhosis. This trend was observed in the clinical study.[13]

The Grade 3 or 4 TB elevation was higher in the Japanese phase 3 study compared with the non-Japanese studies.[12, 13, 15] Notably, ASV exposure would be higher in Asian subjects compared to non-Asian subjects. The higher ASV exposure in Japanese subjects might be considered to contribute on higher event rate in Japanese subjects. Comparison of the ASV exposures in all Japanese subjects to non-Japanese subjects at the high quartile of the ASV exposure in **Figure 4.4-1** indicate that in non-Japanese subjects with ASV exposure at the highest

quartile, the Grade 3 or 4 TB elevation rate was significantly lower than that in Japanese subjects (1.7% versus 5.5%). These data indicate that although ASV exposure had an effect on Grade 3 or 4 TB elevation rate, the effect of comparable exposures may be higher in Asian subjects. Although ASV exposure would increase in Japanese subjects compared to non-Japanese subjects, the different event rate between Japanese and non-Japanese subjects cannot be explained only by the difference of ASV exposure.

ASV exposure was one of factors which affect Grade 3 or 4 ALT elevation and Grade 3 or 4 TB elevation. It has been previously shown and also confirmed in the PopPK analysis that ASV PK is very sensitive to changes in markers of hepatic function (ie, baseline and time varying ALT and cirrhosis status), and higher levels of ALT and presence of cirrhosis resulted in higher plasma concentrations of ASV.[44, 46, 54] Therefore it can be hypothesized that in some subjects certain events may cause higher levels of ALT or TB which subsequently lead to higher plasma concentrations of ASV.

Race is relevant for both ASV PK and the response variables. Asian subjects had higher ASV exposure and higher safety event rates than non-Asian subjects. To assess the potential confounding effect of race and ASV exposure, a sensitivity analysis was performed by excluding the non-Asian subjects from the analysis dataset, and re-estimating the effect of ASV exposure. In Asian subjects, the parameter estimates of ASV exposure on Grade 3 or 4 ALT and Grade 3 or 4 TB elevation were 0.000938 or 0.00263, respectively, which were within the 95% confidence intervals of the final model parameters (**Table 4.3-2**). A confounding factor may compromise the E-R analyses, however, the contribution of ASV exposure on Grade 3 or 4 ALT and Grade 3 or 4 TB elevation was modest in the sensitivity analysis, which was consistent with the result from final E-R models included both race and ASV exposure. The higher event rates in the Asian subjects

were not fully explained by the difference in ASV exposure, therefore, both Asian race and ASV exposure were included in the final E-R models.

4.5 Conclusion

Higher ASV exposure had a modest increase of the Grade 3 or 4 ALT and Grade 3 or 4 TB elevation rates. The magnitude of ASV exposure was smaller than the other significant covariates. Asian subjects had a greater Grade 3 or 4 ALT elevation rate than non-Asians, and Grade 3 or 4 ALT elevation rate decreased with increasing weight in non-Asian subjects. Asian subjects had a greater rate of Grade 3 or 4 TB elevation than non-Asian subjects, and subjects with F4 fibrosis score had a higher rate of Grade 3 or 4 TB elevation compared to subjects with F0-3 fibrosis score. BCV exposure was not a significant predictor for the rates of Grade 3 or 4 ALT and Grade 3 or 4 TB elevations. Considering the predominance of Japanese in the Asian group (94.7%), the effect of Asian race is likely an effect of the Japanese ethnicity rather than that of Asian race in general.

5 OVERALL CONCLUSION

The PopPK analyses for DUAL regimen and 3DAA regimen and the safety E-R analysis for 3DAA regimen in the research were the first model development to characterize the PK of DCV, ASV and/or BCV integrating multiple studies including phase 3 data and to characterize the exposure and safety relationship for 3DAA regimen.

ASV exposure would be associated with hepatic function for both DUAL regimen and 3DAA regimen. Based on the results from safety E-R analysis, higher ASV exposure was associated with increases in Grade 3 or 4 ALT and Grade 3 or 4 TB elevation rates, however, the impact of ASV exposure on the Grade 3 or 4 ALT elevation rate was not clinically relevant and the effect of ASV exposure on Grade 3 or 4 TB elevation rate was smaller than the other significant covariates. The higher safety event rates observed in Japanese subjects were not fully explained

by the difference in ASV exposure. The effect of race is the most significant covariate on Grade 3 or 4 ALT and Grade 3 or 4 TB elevation rates, suggesting careful monitoring for the risk of severe liver disorder would be required for Japanese patients. This supports the description of the Japan package insert of 3DAA regimen.[26]

In addition, the key covariates identified in the PopPK and E-R models help to explain the source of variability of the exposures and clinical outcome, and may guide clinical use of the drug.

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