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SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zepatier

International non-proprietary name: elbasvir / grazoprevir

Procedure No. EMEA/H/C/004126/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.



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List of abbreviations

ADME	Absorption, distribution, metabolism and elimination
ADY	Analysis relative day
AE	Adverse event or adverse experience
ALT	Alanine aminotransferase
APRI	Aspartate Aminotransferase to Platelet Ratio Index
ASaT	All-Subjects-as-Treated population
AST	Aspartate aminotransferase
BMI	Body mass index
BUN	Blood urea nitrogen
C2hr	Concentration 2 hours postdose
CBC	Complete blood count
CFR	Code of Federal Regulations
CHC	Chronic hepatitis C
CI	Confidence interval
CID	Component ID
CIOMS	Council for International Organizations of Medical Sciences
CLDQ-HCV	HCV-specific version of the Chronic Liver Disease Questionnaire
CPK	Creatinine phosphokinase
CQM	Clinical quality management
CRF	Case Report Form
CSR	Clinical Study Report
CT	Computerized tomography
Ctrough	Steady state concentration immediately predose
CYP	Cytochrome
DAA	Direct-Acting Antivirals
DAIDS CTC	Division of AIDS Common Toxicity Criteria
DAO	Data as observed
DNA	Deoxyribonucleic Acid
EBR	Elbasvir, MK-8742
ECG	Electrocardiogram

ECI	Event of clinical interest
eCRF	Electronic case report form
EQ-5D-5L	EuroQol 5 Dimensions health questionnaire, with 5 levels
EQ VAS	EuroQol Visual Analogue Scale
ERC	Ethical Review Committee
EOTR	End of treatment response
FACIT-Fatigue 4)	Scale Functional Assessment of Chronic Illness Therapy-Fatigue Scale (Version 4)
FAS	Full Analysis Set
FDA	Food and Drug Administration (US)
FDC	Fixed-dose combination
FUADY	follow-up analysis day
FW	Follow-up Week
GCP	Good Clinical Practice
GT	Genotype
GZR	Grazoprevir, MK-5172
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular cancer
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C virus
HSP	Hepatic Safety Population - includes all subjects who received GZR (any dose).
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen system
HRQOL	Health-related quality of life
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IFN	Interferon
IEC	Independent Ethics Committee
IL28B	Interleukin 28B (interferon, lambda 3)
INR	International Normalization Ratio
IRB	Institutional Review Board
ISP	Integrated Safety Pool - includes all subjects who received EBR (50 mg) and

	GZR (100 mg) as single entities or FDC
IVRS	Interactive voice response system
LLoQ	Lower limit of quantification
M&S	Modeling and Simulation
MCS	Mental Component Summary (part of SF-36)
MK-5172	Grazoprevir
MK-8742	Elbasvir
NASH	Nonalcoholic steatohepatitis
PCS	Physical Component Summary (part of SF-36)
PD	Pharmacodynamic(s)
PDLC	Pre-Defined Limit of Change
peg-IFN	Pegylated interferon alfa-2b
PI	Protease inhibitor
PK	Pharmacokinetic(s)
PP	Per-Protocol
PR	Preferred regimen
PRO	Patient reported outcome
QA	Quality assurance
QC	Quality control
QD	Once daily
RAP	Resistance analysis population
RAV	Resistance-associated variant
RBV	Ribavirin
RNA	Ribonucleic acid
RVR	Rapid virologic response
SAC	Scientific Advisory Committee
SAE	Serious adverse event
SAFE	Sequential algorithm for fibrosis evaluation
SAP	Statistical analysis plan
SD	Standard deviation
SF-36v2R	Short Form Health Survey, Version 2
SOC	System Organ Class

SVR	Sustained virologic response
SVR4/12/24	Sustained virologic response, having plasma HCV RNA <25 IU/mL at 4/12/24 weeks after the end of all study therapy after becoming undetectable (TND) at end of treatment
TD(q)	Target detected, quantifiable (HCV RNA .25 IU/mL)
TD(u)	Target detectable, unquantifiable (HCV RNA <25 IU/mL)
TN	Treatment naive
TND	Target not detected (HCV RNA not detected)
TW	Treatment Week
ULN	Upper limit of normal
VF	Virologic failure
WPAI: Hepatitis C	Work Productivity and Activity Impairment Questionnaire, Hepatitis C (V2.0)

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Merck Sharp & Dohme Limited submitted on 3 July 2015 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Zepatier, through the centralised procedure falling within the Article 3(1) and point 3 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 November 2014.

The applicant applied for the following indication: *treatment of chronic hepatitis C (CHC) in adults (see sections 4.2 and 5.1). For hepatitis C virus (HCV) genotype-specific activity (see section 5.1).*

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that elbasvir and grazoprevir were considered to be new active substances.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0024/2015 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0024/2015 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance elbasvir and grazoprevir contained in the above medicinal product to be considered as new active substances in themselves, as the applicant claims that either substances are not a constituent of a product previously authorised within the Union.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 18 December 2014, 25 April 2014 and 26 February 2015. The Scientific Advices pertained to insert quality, non-clinical and clinical aspects of the dossier.

Licensing status

A new application was filed in the following countries: United States of America and Canada.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Greg Markey Co-Rapporteur: Johann Lodewijk Hillege

- The application was received by the EMA on 3 July 2015.
- Accelerated Assessment procedure was agreed-upon by CHMP on 25 June 2015.
- The procedure started on 23 July 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 07 October 2015 (Annex 1). The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 9 October 2015 (Annex 2). In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.

The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 06 November 2015 (Annex 3).

- During the meeting on 19 November 2015, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 19 November 2015 (Annex 4).
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 December 2015.
- The following GMP inspection was requested by the CHMP and their outcome taken into consideration as part of the Quality assessment of the product:
 - A GMP inspection at one site responsible for the manufacture of the finished product, located in the United States of America, on 17, 18, 19, 22, 23 and 24 February 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 26 January 2016 (Annex 5).

The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 11 February 2016 (Annex 6)

- During the CHMP meeting on 25 February 2016, the CHMP agreed on a list of outstanding issues to be addressed by the applicant (Annex 7).
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 01 March 2016.

The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 15 March 2016 (Annex 8)

- During the CHMP meeting on 01 April 2016, the CHMP agreed on a second list of outstanding issues to be addressed by the applicant (Annex 9)
- The applicant submitted the responses to the CHMP second List of Outstanding Issues on 28 April

2016.

The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the second List of Questions to all CHMP members on 10 May 2016 (Annex 10).

- During the meeting on 26 May 2016, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Zepatier.

2. Scientific discussion

2.1. Introduction

Chronic hepatitis C virus (HCV) infection affects up to 170 million people worldwide. In the United States (US) and Europe, it is estimated that there are as many as 5 million and 10 million persons with chronic HCV infection, respectively.

Transmission of HCV is primarily by percutaneous exposure to infected blood through unsafe injections, inadequate sterilisation of medical equipment, transfusion of unscreened blood and blood products, and transplantation from an infectious donor. HCV is less efficiently transmitted through sex with an infected partner or birth to an infected mother. In developed countries, the peak contamination of the blood supply occurred between the 1960s and 1992, when screening was instituted. Since then, new infections have significantly declined and have been primarily associated with injection drug use and high transmission risk sexual behaviours.

Incident HCV infections occur in up to 4 million people worldwide, annually. The majority of new infections occur in the setting of persons who inject drugs (PWID) and the risk of HCV infection is highest among the young as well as new injecting drug users. Globally, an estimated 10 million (range 6 to 15) PWIDs have HCV infection. The regions with the largest HCV-infected PWID populations are Eastern Europe (2.3 million, range 1.2-3.9), East and Southeast Asia (2.6 million, range 1.8-3.6). Approximately (~) 0.7 million (range 0.5 to 1.0) and 1.7 million (range 1.1 to 2.5) PWIDs are infected with HCV in Western Europe and North America, respectively. Due to shared transmission routes of HCV and HIV, PWID are also likely to be co-infected with HIV. One-fourth to one-third of patients infected with HIV in the United States and Europe are co-infected with HCV and documented co-infection rates rise to up to 70% in parts of Eastern Europe.

There are 6 major HCV genotypes (GT) and also distinct sub-genotypes of each GT. GT1 is the most prevalent genotype, accounting for ~46% of all infections worldwide and 60-70% of infections in North America, Latin America, and Europe. GT1 is divided into two major subtypes, GT1a and GT1b. GT1b is the most common subtype worldwide and is predominant in Asia. GT1a predominates in the Americas. In the US, GT1a is more frequent (~46%) than GT1b (~26%). In Europe, the relative distributions of these subtypes vary by country. The global frequencies of GT2 to GT6 are the following: GT3 (22-30%), GT2 (9-13%), G4 (8-13%), G6 (2-5%) and G5 (1%). GT2 accounts for 10 to 15%, 5%, 10%, and 27% of infections in the US, England, France, and Italy, respectively. GT3 predominates in South Asia, but it also has global presence in part due to waves of migration from South Asia. For example, GT3 accounts for 47% of HCV cases in England. GT4, GT5 and GT6 infections are found primarily in North Africa/Middle East, sub-Saharan Africa, and Southeast Asia, respectively.

Approximately 5-25% of people who acquire HCV recover spontaneously within 2-12 weeks, while 55-85% will progress to chronic HCV infection. The most significant impact of chronic HCV infection is chronic liver inflammation that leads to cirrhosis, end stage liver disease (decompensation), hepatocellular carcinoma (HCC) and liver-related mortality. Without therapy, 16% and 41% of people with chronic HCV will develop liver cirrhosis within 20 and 30 years after infection, respectively. Approximately, 0.5 million deaths worldwide are attributable to liver cirrhosis and HCC due to HCV annually.

Therapy for chronic HCV infection aims at a sustained virologic response (SVR). Recent all-oral, interferon (IFN)-free regimens include agents that directly and specifically inhibit viral structural proteins. Four DAA classes have been developed: NS3/4A Protease Inhibitors (PIs), NS5A Inhibitors (NS5AIs), Nucleoside-Mimetic NS5B Polymerase Inhibitors (NIs), and Non-Nucleoside NS5B Polymerase Inhibitors (NNIs). Recently approved regimens continue to have deficits, notably:

1. Some require use of ribavirin (RBV)
2. Some have suboptimal efficacy (SVR12 < 90%) or require prolonged therapy in subpopulations
3. Regimens that include RBV or NIs are not optimal for use in patients with advanced CKD
4. There are limited all-oral DAA regimens approved for some genotypes

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as film-coated tablets containing 50 mg elbasvir and 100 mg grazoprevir as active substances.

Other ingredients are:

Tablet core: sodium laurilsulfate, vitamin E polyethylene glycol succinate, copovidone, hypromellose, microcrystalline cellulose, mannitol, lactose monohydrate, croscarmellose sodium, sodium chloride, colloidal anhydrous silica, and magnesium stearate

Film-coating: lactose monohydrate, hypromellose, titanium dioxide, triacetin, iron oxide yellow (E172), iron oxide red (E172), iron oxide black (E172), and carnauba wax.

The product is available in aluminium blisters sealed in a cardboard card.

2.2.2. Active Substance

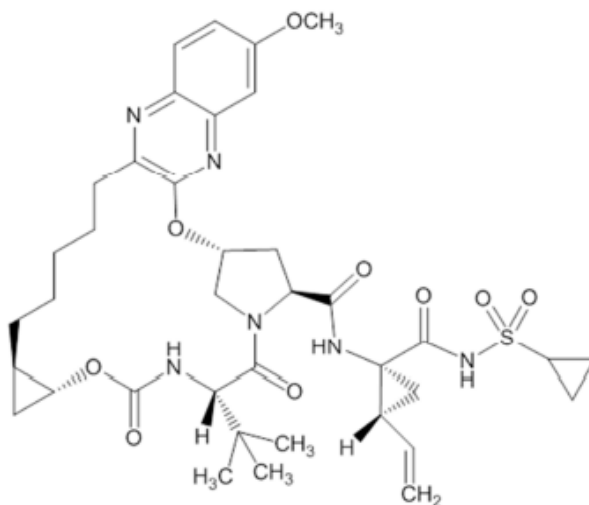
Grazoprevir

General information

The chemical name of grazoprevir is (1aR,5S,8S,10R,22aR)-N-[(1R,2S)-1-[(Cyclopropylsulfonamido)carbonyl]-2-ethenylcyclopropyl]-14-methoxy-5-(2-methylpropan-2-yl)-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8H-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-b]quinoxaline-8-carboxamide

corresponding to the molecular formula $C_{38}H_{50}N_6O_9S$ and has a relative molecular mass 766.90 g/mol and has the following structure:

Figure 1. Chemical structure of grazoprevir



The chemical structure of grazoprevir has been adequately demonstrated by infrared spectroscopy, nuclear magnetic resonance spectroscopy (1H , ^{13}C), mass spectrometry, ultraviolet absorption spectroscopy and X-ray crystallography.

Grazoprevir is a white to off-white powder; it is practically insoluble in water and is slightly hygroscopic.

Grazoprevir exhibits stereoisomerism due to the presence of seven chiral centres. Enantiomeric purity is controlled routinely by specific optical rotation.

Polymorphism has been observed for the active substance. Experiments conducted confirmed that this free acid monohydrate (III) polymorphic form is the most stable form. Grazoprevir is photosensitive in the solid state under ICH photostability stress conditions. The active substance was found to be sensitive to moisture. Particle size distribution and polymorphic form shall not affect the finished product performance Manufacture, characterisation and process controls.

Manufacture, characterisation and process controls

Grazoprevir is synthesized in eight main steps using commercially available well defined starting materials with acceptable specifications. Grazoprevir is prepared by a convergent synthesis with eight total crystallizations.

During the assessment, one of the proposed starting materials was not considered to be suitable as it carries two of the chiral centres of the final active substance and the steps involved in its synthesis are critical to ensuring its stereochemistry and the one of grazoprevir are correct. Therefore, the CHMP requested the starting material to be redefined to an earlier step of the synthesis to ensure that the important synthetic steps necessary to ensure the quality of the grazoprevir are subject to regulatory oversight. The applicant redefined the starting material to an earlier step of the synthesis which was considered satisfactory.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities, including enantiomer impurities, degradants, potential genotoxic impurities, inorganic impurities and residual solvents were well discussed with regards to their origin and characterised.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Changes introduced have been presented in sufficient detail and have been justified.

The development of the grazoprevir manufacturing process has been adequately described. For each manufacturing step, combinations of univariate and multifactor DOE studies were conducted for the major unit operations to determine acceptable ranges for the process parameters and input material attributes. No design space was claimed. No critical process parameters were identified

Grazoprevir is packaged in a package which complies with the EC directive 2002/72/EC and EC 10/2011 as amended as well as Ph. Eur. monograph 3.1.3 and 3.2.2.,

Specification

The active substance specification includes tests for description, assay (HPLC), impurities (HPLC), residual solvents (GC), water content (KF), specific rotation (Ph. Eur.), heavy metals (Ph. Eur.), and identity (IR).

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for impurities testing has been presented.

The batch data presented to support the active substance specification includes 18 commercial scale batches using the commercial process, from the commercial site. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on three commercial scale batches of grazoprevir from the proposed manufacturer stored in the intended commercial package for 12 months under long term conditions at 25 °C / 60% RH and for up to 6 months under accelerated conditions at 40 °C / 75% RH according to the ICH guidelines were provided. Photostability testing following the ICH guideline Q1B was performed on one batch. Results under stress conditions (150°C, 0.1N HCl 40°C, 0.1N NaOH 40°C, 3% H₂O₂, and azobisisobutyronitrile at 5mM in methanol) were also provided on one batch.

The following parameters were tested: description, assay, impurities (HPLC), water content (Karl Fischer titration), specific rotation, identity (IR, X-ray powder diffraction) and regioisomer purity (HPLC) and were stability indicating.

The photostability stability studies showed that grazoprevir is slightly sensitive to degradation by light. In relation to the other studies, no trends or significant changes were observed under any of the

conditions tested (150°C, 0.1N HCl 40°C, 0.1N NaOH 40°C, 3% H₂O₂, and azobisisobutyronitrile at 5mM in methanol).

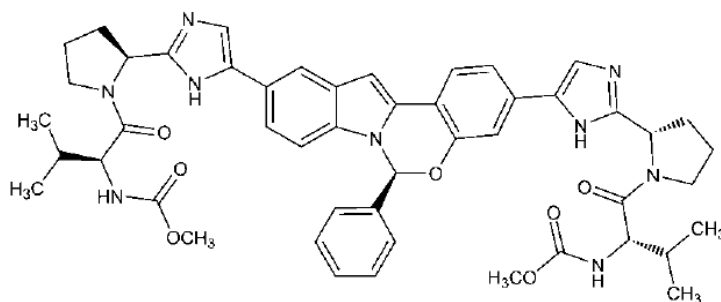
The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the re-test period of 24 months with no specific storage conditions in the proposed container

Elbasvir

General information

The chemical name of elbasvir is Dimethyl N,N' -([[(6S)-6-phenylindolo[1,2-c][1,3]benzoxazine-3,10-diyl]bis{1H-imidazole-5,2-diyl-(2S)-pyrrolidine-2,1-diyl[(2S)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate corresponding to the molecular formula C₄₉H₅₅N₉O₇ and has a relative molecular mass 882.02 g/mol and has the following structure:

Figure 2. Chemical structure of elbasvir



The chemical structure of elbasvir has been adequately demonstrated by infrared spectroscopy, nuclear magnetic resonance spectroscopy (¹H, ¹³C), mass spectrometry, ultraviolet absorption spectroscopy and X-ray crystallography.

The active substance is a white to off-white powder. It is practically insoluble in water and heptane and very soluble in ethyl acetate and acetone. Elbasvir exists as a weakly dibasic amorphous compound with no stable crystalline phase identified to date. Elbasvir is hygroscopic, but remains amorphous after absorption and desorption of moisture. Extensive polymorph screening has not identified any non-solvated crystalline forms of elbasvir

Elbasvir exhibits stereoisomerism due to the presence of five chiral centres. Enantiomeric purity is controlled routinely by specific optical rotation.

Manufacture, characterisation and process controls

Elbasvir is synthesized in six main steps using well defined starting materials with acceptable specifications.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. The development of the commercial manufacturing process for elbasvir active substance followed a systematic risk based approach following the principles outlined in ICH Q9 and ICH Q11.

Operations perceived as higher risk and or at risk of significant parameter interactions for the elbasvir active substance Critical Quality Attributes (CQAs) were studied in a systematic way by utilizing multifactor Design of Experiments (DOE) studies, first principles, prior knowledge or a combination of these elements.

Operations presenting lower risk or not expected to have multifactor interactions were studied with a traditional One Factor at A Time (OFAT) approach.

The development studies have led to the definition of the proven acceptable ranges for the elbasvir manufacturing process

No design space has been claimed. The detailed evaluation undertaken has concluded that there are no Critical Process Parameters (CPP) in the elbasvir synthetic route.

Elbasvir is packaged in a container that complies with the EC directive 2002/72/EC and EC 10/2011 as amended and Ph. Eur. monographs 3.1.3 and 3.2.2.

Specification

The active substance specification includes tests for description, assay (HPLC), impurities (HPLC), specific rotation (Ph Eur), residual solvents (GC), water content (KF), palladium, identity (IR), heavy metals (Ph Eur).

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used has been presented. No impurity reference standards are used.

Batch analysis data of 12 commercial scale of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on three commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package under long term conditions at 5 °C for up to 18 months, under accelerated conditions at 25 °C / 60% RH for up to 12 months, and at 40°C/75 %RH for up to 6 months, according to the ICH guidelines, were provided. Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions (150°C, 0.1N HCl 25°C, 0.1N NaOH 25°C, 3% H₂O₂ 25°C, azobisisobutyronitrile at 5mM in methanol 40°C) were also provide on one batch.

The following parameters were tested: description, assay (HPLC), impurities (HPLC), water content (KF), specific rotation, identity (IR) and X-ray powder diffraction.

The stability study confirmed that there was neither a change in impurities nor any significant change in the description, assay and water content during this period. The data from the solid state

thermal/humidity and photolytic stress studies indicates that elbasvir requires storage at refrigerated conditions with desiccant and protected from light.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period. 18 months storage 5°C conditions in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Zepatier is supplied as a fixed-dose combination, film-coated tablet. Each tablet contains 50 mg of elbasvir and 100 mg of grazoprevir as active substances.

Zepatier was designed as a robust, physically and chemically stable immediate-release solid dosage form using the considerations provided in the Target Product Profile. The pharmaceutical development of grazoprevir/elbasvir tablet was aimed at developing a solid oral dosage form that meets the quality requirements of the quality target product profile

During development, appropriate QTPP categories were further translated into product CQAs. The goal of the finished product development was to identify a formulation and manufacturing control strategy that robustly met these potential CQAs. The formulation strategy entailed the development of single entity formulations containing either grazoprevir or elbasvir active substances. The knowledge from the single entity development efforts was subsequently used for the development of the combination.

Excipients were selected to provide a physically and chemically stable formulation with the intended biopharmaceutical properties. The active substances and the excipients were found to be compatible by evaluating the physical and chemical stability of the finished product. All excipients are compendial grade, with the exception of the film-coat (Opadry II); however, the components of the film-coating agent are compendial. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

The control strategy was developed to achieve a manufacturing process that consistently provides a final product meeting the CQAs, and includes process parameter controls per the established proven acceptable ranges and the in-process controls. Some elements of ICH Q8-Q11, such as the application of risk management, the use of QTPP to guide development, and the use of multifactor experiments and one-factor-at-a time studies (OFAT) were used during development.

During the manufacturing development, the steps from the risk based process development plan were reviewed sequentially. The proven acceptable ranges and in-process controls developed at the commercial scale and defined for commercial production were described.

The finished product is packaged in an aluminum/aluminum blister. This package was chosen because development work indicated finished product sensitivity to moisture and active substance sensitivity to light. The material complies with Ph Eur and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product

Manufacture of the product and process controls

During the evaluation of the medicinal product, the applicant has replaced the manufacturing site which will manufacture and test grazoprevir and elbasvir spray dried intermediates (SDIs) with another manufacturing site as it was considered non-GMP compliant. The applicant has provided a detailed Risk Assessment together with appropriate updated documents to support the new manufacturing site. The risk assessment to support the validity of the data on the spray dried intermediates (SDIs) and related drug products with regard to quality, safety and efficacy is comprehensive and is sufficient to allay concerns regarding the validity of the data on the spray dried intermediates (SDIs) and related drug products with regard to quality, safety and efficacy. However the CHMP recommends a report should be presented detailing the transfer of the manufacture of the SDIs and the analytical test methods to the new site with results of validation/optimisation studies, in view of the possible need for 'optimisation' of the process following transfer, the bulk hold time for the SDIs (currently 12 m) will be revalidated on batches manufactured at the new site or a justification will be provided, the first three, consecutive commercial batches of tablets manufactured with the new manufacturing site SDIs will be placed on stability trial. Stability protocols will be in line with current guidelines and will address, in addition, address potential changes in water activity and drug substance crystallinity on storage, and provide release data from 3 batches (1 production batch and 2 pilot batches or 2 production batches) from tablets manufactured with the new manufacturing site spray dried intermediates and a comparison to tablets manufactured with the previous manufacturing site spray dried intermediates, prior to launch.

The manufacturing process consists of nine main steps: grazoprevir spray drying, grazoprevir blending and lubrication, grazoprevir roller compaction and milling, elbasvir spray drying, elbasvir blending and lubrication, elbasvir roller compaction and milling, final blending and lubrication of the grazoprevir and elbasvir granulations, tablet compression of grazoprevir/elbasvir granulations into monolithic fixed-dose combination (FDC) tablets, film coating, and packaging. The process is considered to be a standard manufacturing process.

The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed proven acceptable ranges (PARs). The process validation scheme of the manufacturing process used to manufacture the product has been provided. The process validation will be completed on commercial-scale batches prior to product being placed on the market and will be available for verification as part of routine site GMP inspections. Since the manufacturing process is a standard process, this is considered satisfactory.

The defined testing attributes to be assessed, along with the expanded testing, test methods, sampling plan, and acceptance criteria are satisfactory.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form appearance, assay for both active substances (HPLC), degradation products of the active substances (HPLC), identification for both active substances (HPLC), content uniformity (Ph Eur), dissolution (HPLC), microbial quality (Ph Eur).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for has been presented.

Batch analysis results are provided for three batches larger than the proposed commercial batch size confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data of three batches of the finished product stored for up to 18 months under long term conditions at 30 °C/75% RH and for up to 6 months under accelerated conditions at 40°C±2°C / 75%RH±5% RH according to the ICH guidelines were provided. The batches tested were manufactured at the commercial manufacturing site and were representative of the proposed commercial process, packaging and formulation. Samples were tested for assay (HPLC), related substances (HPLC), dissolution, water activity, active substance crystallinity (X-Ray power diffraction), and microbial purity (Ph Eur). The analytical procedures used are stability indicating.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products.

Based on available stability data, the proposed shelf-life 24 months with no specific temperature storage conditions as stated in the SmPC (section 6.3) are acceptable. The product should be stored in the original package until use to protect from moisture.

Adventitious agents

It is confirmed that the lactose is produced from milk from healthy animals in the same condition as those used to collect milk for human consumption and that the lactose has been prepared without the use of ruminant material other than calf rennet according to the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and veterinary medicinal products.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The finished product has been developed using single entity spray dried formulations to enhance the properties of the active substances and to mitigate decreased absorption at high gastric pH due to the steep pH dependent solubility profile.

The applicant has applied QbD principles in the development of the active substance and the finished product and their manufacturing process. However, no design spaces were claimed for the manufacturing process of the active substance, or for the finished product.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

- A report should be presented detailing the transfer of the manufacture of the SDIs and the analytical test methods to the new manufacturing site with results of validation/optimisation studies.
- In view of the possible need for 'optimisation' of the process following transfer, the bulk hold time for the SDIs (currently 12 m) will be revalidated on batches manufactured at the new manufacturing site or a justification will be provided.
- The first three, consecutive commercial batches of tablets manufactured with the new manufacturing site will be placed on stability trial. Stability protocols will be in line with current guidelines and will address, in addition, address potential changes in water activity and drug substance crystallinity on storage.
- To provide release data from 3 batches (1 production batch and 2 pilot batches or 2 production batches) from tablets manufactured with the new manufacturing site spray dried intermediates and a comparison to tablets manufactured with the previous manufacturing site spray dried intermediates, prior to launch.

2.3. Non-clinical aspects

2.3.1. Introduction

Grazoprevir is a reversibly binding macrocyclic inhibitor of Hepatitis C Virus (HCV) non-structural protein (NS) 3/4A and elbasvir is a small molecule inhibitor of HCV NS5A. These two new chemical entities are to be used in combination for the treatment of chronic HCV infection.

In vitro pharmacodynamics studies showed the Grazoprevir inhibited the replication of HCV Ribonucleic acid (RNA) with sub-nanomolar EC50 values in genotypes (GT) 1, 4, and 6. It has nanomolar potency in GT3. Elbasvir inhibited HCV replication in GT1, GT4, and GT6 with low picomolar ($EC_{50} \leq 4$ pM) activity. It is less potent in GT3 ($EC_{50} = 20$ pM). In combination, grazoprevir and elbasvir demonstrated additive effects in blocking HCV RNA replication and suppressed the emergence of resistance by creating a higher genetic barrier.

Grazoprevir and elbasvir have been developed as a fixed-dose combination tablet to provide once daily dosing, pegylated-interferon free regimen of short duration for patients chronically infected with HCV. For most HCV GT1-, 4-, or 6-infected patients, a 12-week, ribavirin-free regimen of elbasvir / grazoprevir was proposed. For some hard-to-cure patient populations (e.g., prior treatment-

experienced null responders), a 16-week, ribavirin-containing regimen is proposed. HCV-infected patients with advanced chronic kidney disease (including patients on hemodialysis) will receive elbasvir / grazoprevir without ribavirin. The recommended elbasvir / grazoprevir human dose is 100 mg/50 mg. Grazoprevir and elbasvir systemic plasma exposures in HCV-infected patients determined in clinical protocols PN060, PN061, and PN068 (pooled geometric mean steady state AUC_{0-24 hr}) are 1.97 µM.hr and 2.38 µM.hr, respectively; corresponding C_{max} values are 0.23 µM and 0.15 µM.

Grazoprevir and elbasvir were investigated as monotherapies in non-clinical toxicity studies that consisted of a battery of in vitro and in vivo genetic toxicity studies; repeat-dose oral toxicity studies of up to 1-month (elbasvir) or 3-months (grazoprevir) in mice, 6 months in rats, and 9 months in dogs; and a series of developmental and reproductive toxicity studies. In addition a 1-month oral elbasvir / grazoprevir combination dog toxicity study was conducted.

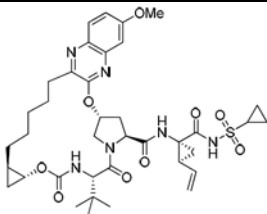
The non-clinical absorption, distribution, metabolism and elimination of grazoprevir and elbasvir as well as drug-drug interactions (DDI) were studied separately. In accordance with ICH S1A guidance, carcinogenicity studies were not conducted as clinical is expected to be less than 6 months in duration and that there is an absence of a genotoxic signal in the battery of genotoxicity studies and no evidence of a proliferative signal in the chronic toxicity studies.

Grazoprevir is also known as MK-5172 or L-002214070. Elbasvir is also known as MK-8742 or L-002469825.

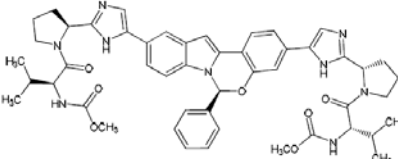
2.3.2. Pharmacology

Physical chemistry

Grazoprevir

Structure of the active substance Site of labelling (see structure)	
Molecular formula	
International Non-Proprietary Name (INN)	Grazoprevir
Systematic chemical name (IUPAC)	<i>N</i> -[[[(1 <i>R</i> , 2 <i>R</i>) -2-[5-(3-hydroxy-6-methoxy-2-quinoxaliny)pentyl]cyclopropyl]oxy]carbonyl]-3-methyl-L-valyl-(4 <i>R</i>) -4-hydroxy-L-prolyl-(1 <i>R</i> , 2 <i>S</i>) -1-amino- <i>N</i> -(cyclopropylsulfonyl)-2-ethenylcyclopropanecarboxamide cyclic (1→2)-ether

Elbasvir

Structure of the active substance	
Site of labelling (see structure)	
Molecular formula	<i>N,N'</i> -[[[(6 <i>S</i>)-6-Phenyl-6 <i>H</i> -indolo[1,2- <i>c</i>][1,3]benzoxazine-3,10-diyl]bis[1 <i>H</i> -imidazole-5,2-diyl-(2 <i>S</i>)-2,1-pyrrolidinediyl[(1 <i>S</i>)-1-(1-methylethyl)-2-oxo-2,1-ethanediy]]]bis[carbamic acid] <i>C,C'</i> -dimethyl ester
International Non-Proprietary Name (INN)	Elbasvir

Primary pharmacodynamic studies

Grazoprevir

In vitro

The *in vitro* pharmacology of grazoprevir was assessed in enzymatic and cellular replication assays. The enzymatic activity of NS3/4A was measured by monitoring the hydrolysis of a peptide substrate in a time-resolved fluorescence assay. The potency of grazoprevir was assessed in *in vitro* enzymatic assays for GTs 1-6. Cell-based replication was evaluated with a HCV replicon model using either a primer protection or a Taqman™ based quantitation method. *In vitro* testing of the combination of grazoprevir and elbasvir in suppression of resistance was assessed by monitoring the emergence of resistant colonies in GT1a replicon cells. RAVs emerging from the combined activity in the replicon cells were identified and characterised.

Activity (IC₅₀ (50% inhibitory concentration)) in a peptide hydrolysis-based enzymatic assay was sub-nanomolar across all genotypes. Activities were low pM for GT1 enzymes, and range from 0.034 to 0.135 nM for non-GT1 enzymes except for GT3a which shows an IC₅₀ of 0.690 nM.

Table 1. *In Vitro* Activity of Grazoprevir against an Enzyme Genotype Panel

Enzyme	IC ₅₀ ± SD (nM)
1a	0.007 ± 0.001
1b	0.004 ± 0.001
2a	0.067 ± 0.024
2b	0.135 ± 0.051
3a	0.690 ± 0.194
4a	0.062 ± 0.031
5a	0.067 ± 0.022
6a	0.034 ± 0.007
Chymotrypsin (selectivity)	1495 (373,750-fold)

SD: Standard deviation

In a replicon assay, grazoprevir inhibited GT1a, GT1b, GT4a, and GT6a viral replication (EC₅₀ = 0.4, 0.5, 0.3, and 0.9 nM respectively) and demonstrated a modest shift in the presence of 40% normal human serum (NHS) (EC₅₀ = 1.1 nM). It also showed potent against the full-length GT2a replicon (EC₅₀ = 2.3 nM) and chimeric replicons of GT2b (EC₅₀ = 3.7 nM), GT3a (EC₅₀ = 7.6 nM), GT5a (EC₅₀ = 1.5 nM), and GT6a (EC₅₀ = 0.9 nM). It is less potent against a full-length GT3a (S52) replicon (EC₅₀ = 35 nM) but not a chimeric replicon bearing the NS3 catalytic domain (EC₅₀ = 2.1 nM).

Grazoprevir was also shown to be potent against replicons bearing the protease gene from several patient isolates from GTs 1-6.

Resistance

In order to evaluate the potential for cross-resistance against RAVs from other classes of HCV DAAs, grazoprevir was tested against replicons bearing signature RAVs of these inhibitor classes. Grazoprevir potency was retained against a panel of RAVs commonly elicited by NS5A inhibitors and against critical resistance mutations to NS5B nucleoside and non-nucleoside inhibitors (study number pd005).

De novo resistance selection studies were conducted with grazoprevir in GTs 1-6 with concentrations up to 30-fold multiple of EC₅₀, depending on the genotype (study number PD002). In GT1b, selection pressure by grazoprevir led to the isolation of encoded mutations at both amino acid residues 156 and 168; another group of resistant genomes encoded mutations at both amino acid residues 41 and 156. Additional selections were conducted against GT1a, GT2a, GT3a, GT4a, GT5a, and GT6a replicons. Variants at position 168 were observed in all treatments. Notably, D168 A/E/G/N/V changes were seen in GT1a, GT4a, GT5a, and GT6a. For the GT2a selections, Y56H and A156T/V were the most frequently observed substitutions. For the GT3a selections, Q168R (~4-fold potency loss) was observed at all concentrations.

The antiviral activity of grazoprevir was evaluated in combination with interferon-alpha, ribavirin and elbasvir, in GT1a replicon cells and quantified using MacSynergyTM and by directly analyzing the threshold cycle number (Ct) values without conversion to RNA amounts. Grazoprevir interacted additively with interferon-alpha, ribavirin and elbasvir (study number PD008). No cytotoxic effects were observed at any of the combination concentrations tested.

Cellular cytotoxicity

Cellular cytotoxicity was low when grazoprevir was assessed in replicon-containing Huh-7 cells and HeLa cells. In both cases, the measured CC₅₀ was >60 µM (study number: pd003).

In vivo

The *in vitro* potency of grazoprevir in cell culture models was further examined in a chimpanzee model (New Iberia Research Centre, Lafayette, Louisiana, Study number PD004). Animals were infected with GT1b and given Grazoprevir at 1 mg/kg b.i.d. for 7 days. Blood was processed into plasma within 1 hour after collection. Viral load was determined by Cenetron 1210 HCV quantitative Polymerase chain reaction (PCR) assay (LOQ 15 IU/mL). Dosing of grazoprevir at 1 mg/kg b.i.d. for 7 days resulted in a 4-5 log reduction in HCV RNA in plasma in a chimpanzee infected with GT1b. In GT1a, grazoprevir administration resulted in a ~3.5 log reduction of viral load. There was a rapid rebound of circulating viruses upon cessation of dosing. Sequencing of resistant viruses showed that the primary resistant variant harboured an R155K amino acid substitution.

Grazoprevir was also quite efficacious in a chimpanzee homogeneously infected with the R155K mutant virus yielding ~2 log reduction in viral load. Post dosing liver levels of grazoprevir were measured and found to be ~1 µM or higher after oral administration of 1 mg/kg b.i.d. for 7 days. No novel mutations within the protease gene were identified. There were no changes in haematological or clinical chemistry parameters.

Elbasvir

The potency of elbasvir was assessed using a panel of sub-genomic replicon cell lines that contained NS5A sequences from all genotypes. It showed potency against HCV GT1a (EC₅₀ = 4 pM), GT1b (EC₅₀

= 3 pM), GT2a (31L) (EC₅₀ = 3 pM), GT3a (EC₅₀ = 14 pM), GT4a (EC₅₀ = 3 pM), GT5a (EC₅₀ = 1 pM), and GT6d (EC₅₀ = 3 pM) with EC₅₀ values in the picomolar range (3-14 pM) (Study number pd016). It was shown to be less active against the GT2b replicon with an EC₅₀ of 3.4 nM.

Table 2. Potency of Elbasvir in HCV Genotypes 1-6 Replicons

Replicon	EC ₅₀ ± SD ^a (nM)	EC ₉₀ ± SD (nM)
1a_H77	0.004 ± 0.002	0.006 ± 0.002
1a_H77 (40% NHS)	0.040 ± 0.013	0.082 ± 0.027
1b_con1	0.003 ± 0.001	0.006 ± 0.004
2a_JFH1	0.003 ± 0.001	0.019 ± 0.010
2b_AB030907/JFH1 ^b	3.4 ± 2.6	11 ± 4.8
3a_NC009824/con1 ^c	0.030 ± 0.010	0.120 ± 0.060
3a_S52_GU814263	0.14 ± 0.09	0.49 ± 0.19
4a_DQ418782/con1 ^c	0.003 ± 0.001	0.016 ± 0.009
4a_ED43_GU814265	0.0003 ± 0.0001	0.0005 ± 0.0001
5a_SA13_AF064490/JFH1 ^b	0.001 ± 0.001	0.002 ± 0.002
6_DQ278892/JFH1 ^b	0.009 ± 0.006	0.017 ± 0.009
6d_D84263/JFH1 ^b	0.003 ± 0.002	0.008 ± 0.005

^a Standard deviation (SD) was calculated from N ≥ 3 independent experiments.

^b This replicon has the GT2b, GT5a, GT6, and GT6d NS5A sequences in JFH1 background.

^c These replicons have the GT3a and GT4a NS5A sequences in con1 background

In addition to genotype-specific reference NS5A sequences, elbasvir was also tested against replicons generated with several patient isolates from GTs1-6. Elbasvir was potent against GT1a and GT1b patient isolates with little variation in potency with EC₅₀ values ranging from 3-10 pM). Among GT2 patient isolates, the potency of elbasvir was more varied with EC₅₀s ranging from 0.003 to 20 nM largely due to the presence of 31M in GT2b patient isolates, although, according to the Applicant, contextual sequences may also play a role (Study number pd009).

Table 3. Potency of Elbasvir in Genotype 2 Replicon Cells

Replicon Cells	EC ₅₀ ± SD ^a , (nM)	EC ₉₀ ± SD, (nM)
GT2a_JFH (31L)	0.003 ± 0.001	0.019 ± 0.01
GT2a_JCH3 (31L)	0.3	3.3
GT2a_MD2a7 (31L)	0.03	0.2
GT2a_J6CH (31M)	20	38
GT2a_MD2a1 (31M)	15	35
GT2b_0907 (31M) ^b	3.4 ± 2.6	11 ± 4.8

^a SD was calculated from N ≥ 3 independent experiments.

^b Replicon has GT2b NS5A sequence in the background of JFH1

Elbasvir was potent in GT3a patient isolates (study number pd002) with EC₅₀ values ranging from 3 pM to 0.4 nM but less potent in 3i and 3g subtypes that harboured RAVs at positions 28, 30, and 31 respectively. Elbasvir maintained potency in a diverse set of GT4 subtypes with subpicomolar EC₅₀ values for most isolates. Elbasvir is also potent in GT5a patient isolates with EC₅₀ values ranging from 0.4 – 1 pM. Against a diverse set of GT6 patient subtypes, elbasvir maintained sub-picomolar to low nanomolar activity (study number pd003). In a GT6n patient isolate, the least susceptible subtype identified with an EC₅₀ value of 2.7 nM, amino acid substitutions at positions 28, 30, and 93 were observed.

The activity of elbasvir was also assessed in replicons from HCV genotypes encoding defined mutations reported in pre-clinical or clinical studies particularly in the more studied GT1a. Elbasvir was potent against a number of the RAVs particularly those from GT1a, GT1b, and GT4a. Elbasvir was more potent than ledipasvir and ombitasvir and displayed a higher activity against RAVs from GT1b compared to

GT1a. All signature RAVs elicited by previous NS5A compounds in GT1b caused only modest potency reductions (>20-fold) against elbasvir; EC50 values were ≤ 50 pM. In contrast, the most shifted variants in GT1a caused a 2-3 log reduction in elbasvir potency. The data suggest baseline RAVs would be less impactful in GT1b than in GT1a. Elbasvir showed reduced activity particularly against Y93H substitutions in GT1a, GT1b, GT2a, and GT3a although improved over approved NS5A compounds, ledipasvir and ombitasvir.

Additional RAVs in GT1a that reduced the potency of elbasvir were L31M/V and Q30D/E/H/K/R. In GT2a, GT5a, and GT6a reduced activity was observed for substitutions at F/L28S/F and L31F (study number PD002). The presence of the natural resistance polymorph 31M in GT2b caused resistance to elbasvir. In addition to stable replicons, a more rapid transient infectious virus assay was also used to investigate defined resistance in GT1a (Study number PD010). Similar results were obtained for both cell model systems used to study RAVs.

Cell cytotoxicity

Cell cytotoxicity was low, with a measured CC50 of >25 μ M in Huh7 replicon cells, HeLa, HepG2, HEK293T, Hep3B, and MT4 cells (study number PD011).

Resistance

Cross-resistance

In order to evaluate the potential for cross-resistance versus RAVs from other classes of HCV DAA inhibitors, elbasvir was tested against replicons bearing signature RAVs from these inhibitor classes. Elbasvir maintained activity and its potency was not shifted against any of the signature RAVs selected by NS3 protease inhibitors or NS5B polymerase nucleoside and non-nucleoside inhibitors indicating no potential for cross-resistance (study number pd002)

De novo resistance selection studies were conducted with elbasvir in GTs 1-6 with concentrations up to 10,000X EC90 depending on the genotype. Generally, the number of resistant colonies decreased with increasing concentrations of elbasvir. Sequencing of RNA isolated from resistant colonies showed that variants occurred primarily at positions 28, 30, 31, and 93. Substitutions at Q30 and Y93 were mainly responsible for potency losses in GT1a. Amino acid substitutions F28S, Y93H, and Y93H in GT2a, GT2b, and GT3a respectively resulted in substantial (>1000-fold) potency losses in those genotypes. Elbasvir presented a higher genetic barrier to resistance in GT1b and GT4a where 2 nucleotide changes were generally required to elicit resistance. Amino acid substitutions, L28F and L31F, in GT5a and F28S and L31F in GT6 were the main variants responsible for resistance. Potency analysis showed the variants at these key positions caused the most elbasvir potency losses (>1000-fold) in the genotypes (study number PD002 and PD003).

Combination

The activity of the combination of elbasvir and grazoprevir was also studied on emergence of resistance in GT1a replicon cells. As independent agents, 100X and 1000X EC90 of grazoprevir and elbasvir respectively were required to suppress emergence of resistant colonies. In combination, 10X EC90 of each compound blocked the emergence of resistant colonies suggesting the combination acts, at least, additively (study number pd008). Clonal sequencing analysis of resistant colonies selected with >1X EC90 combinations of both compounds revealed mostly linked mutations with 2 or more nucleotide changes underscoring a higher genetic barrier to resistance when the inhibitors are used in combination.

Secondary pharmacodynamic studies

Grazoprevir

HCV NS3/4A is a serine protease; therefore, the inhibitory potency of grazoprevir was determined in reactions catalysed by human serine proteases. Grazoprevir showed selectivity over elastase and trypsin (IC_{50} = 100 μ M), but showed modest inhibitory potency with chymotrypsin (IC_{50} = 1495 nM) however it was 373,750-fold selective (study number pd006).

In a panel of enzyme and receptor binding displacement assays 11 targets were found in which inhibitory activity resulted in an EC_{50} = 100 μ M (study number pd007). All potencies were greater than 1 μ M, yielding in all cases a specificity index relative to the GT1b enzyme greater than 1,000,000-fold.

Table 4. Summary of Activities >100 μ M against a Panel of Enzyme or Binding Displacement Assays

Assay	IC_{50} , μ M	Selectivity of GT1b enzyme
Matrix metalloproteinase-1	1.47	367,500
Matrix metalloproteinase-12	6.89	1,722,500
Lipoxygenase 5-LO	2.84	710,000
Phosphodiesterase PDE 1	31	7,750,000
Phosphodiesterase PDE 4	81.4	20,350,000
Phosphodiesterase PDE 5	94.4	23,600,000
Phosphodiesterase PDE 6	22	5,500,000
MAKP 3 (ERK1)	45.7	11,425,000
Protein Threonine Kinase, PKA, Non-selective	55.6	13,900,000
K channel hERG	3.33	682,500
Prostanoid FP	6.49	1,622,500

pd007 - MK-5172: To evaluate, in Enzyme, and Radioligand Binding assays, the activity of compound MKW-1004 (PT #1104878)

Grazoprevir was not an inhibitor of CES1, CES2, and Cat A that are implicated in metabolism of HCV nucleotide prodrugs (Study number pd012) and so has no potential to impact CES1/2- and Cat A-mediated metabolism of pro-drugs and other therapeutic agents.

Grazoprevir was tested in a multiple round HIV infection assay in MT4 cells and in an HBV replication assay in HepG2.2.15 cells to investigate its potential activity against these viruses. Grazoprevir did not register any activity against HIV at 8.4 μ M (study number pd011) or HBV at 10 μ M (study number pd014). Thus, a specificity factor >1X104 was derived relative to its activity in GT1a replicon (EC_{50} = 0.4 nM). As a sizeable proportion of HCV patients are co-infected with HIV, grazoprevir may be co-dosed with HIV drugs. In combination studies with a set of 10 approved HIV drugs (including tenofovir, emtricitabine, darunavir, atazanavir, raltegravir, dolutegravir, efavirenz, rilpivirine, enfuvirtide, and maraviroc) in HIV replication, a concentration of 100 nM grazoprevir increased maraviroc EC_{50} 1.2-fold from 0.38 ± 0.03 nM to 0.45 ± 0.03 nM (p value = 0.03) in cell culture. There was no impact on the other HIV drugs in the presence of any concentration of grazoprevir tested (15 - 500 nM). The HIV drugs at clinically relevant concentrations did not impact the inhibitory potency of grazoprevir in GT1a replicon cells (study number: pd015).

Elbasvir

No significant activities were noted in 116 enzymatic or radioligand binding assays (study number pd001). Testing in the enzyme assays identified 3 targets with IC_{50} values <10 μ M. Only 1 target, rat Protein Serine/Threonine Kinase PKC (non-selective) gave an IC_{50} of 0.877 μ M that was <1 μ M, yielding a specificity factor of >100,000-fold relative to HCV GT1 replicon (replicon EC90).

Elbasvir was inactive against CES1, and CES2, and Cat A that are implicated in metabolism of pro-drugs (study number PD012).

As a large proportion of HCV patients are co-infected with HIV, elbasvir may be co-dosed with HIV drugs. In combination studies with a set of 10 approved HIV drugs (including tenofovir, emtricitabine, darunavir, atazanavir, raltegravir, dolutegravir, efavirenz, rilpivirine, enfuvirtide, and maraviroc) in HIV replication there was no impact on the other HIV drugs in the presence of clinically relevant concentrations of elbasvir (30 to 500 nM). Conversely, the HIV drugs did not impact the inhibitory potency of elbasvir in GT1a replicon cells at clinically relevant concentrations (study number PD015).

Safety pharmacology programme

Grazoprevir

Cardiovascular (CV)

Human ether-à-go-go-related gene (Herg) assay

Grazoprevir was tested for its effect on hERG potassium channel current (IKr), the slowly activating delayed rectifier cardiac potassium channel current (IKs) and the cardiac sodium channel hNav1.5 expressed in mammalian cells. hERG current was inhibited with IC50 value of 8.9 µM and an IC20 value of 3.5 µM. Enhanced hERG step current (while inhibiting the hERG tail current), indicated a complex activity profile. However, this complex activity profile was not considered a concern based on the high hERG current IC50 and IC20 and was consequently not explored further. There was no effect on IKs or INa at the highest concentration tested of 30 µM.

Grazoprevir was tested for effect on hERG channels heterologously expressed in CHO-K1 cells using standard whole-cell voltage-clamp techniques. Grazoprevir inhibited hERG current with an IC50 value of 25 µM and an IC20 value of 6.3 µM. In addition to the concentration-dependent inhibitory effects, apparent effects on the gating properties of the hERG current, beyond the scope of the study, were noted, most notably at actual test concentrations of 27 and 96 µM. The Applicant goes on to say that the concentration responses for hERG inhibition and enhancement were more clearly separated and even less potent when measured at physiological temperatures in the GLP hERG study.

The Applicant stated that taking into account that the hERG assay was conducted in absence of protein and that grazoprevir is >99% protein bound in humans, the IKr IC50 is >10,000X the unbound Cmax in HCV-infected patients (100 mg). Additionally, there were apparent effects on the gating properties of the hERG current at actual test concentrations of 27 µM and 96 µM. These effects were not considered a concern based on the high concentrations at the effect levels compared to the unbound Cmax at the human recommended dose of grazoprevir (100 mg).

Exploratory Cardiovascular Study in Anesthetised Dogs

In an intravenous exploratory cardiovascular study in anesthetised dogs performed at 1, 2, and 2mg/kg, there were no test article-related effects. Mean grazoprevir plasma concentrations measured following the 30-minute infusions of 1, 2 and 2 mg/kg were 12.6, 48.5, and 74.4 µM (Mean), respectively.

Cardiovascular and Respiratory

Oral Cardiovascular and Respiratory Telemetry Study in Dogs

An oral cardiovascular and respiratory telemetry study in dogs was performed at doses of 5, 20, and 600 mg/kg.

At 20 mg/kg and 600 mg/kg, increases in heart rate (peak changes: +42 bpm [42%] and +32 bpm [30%], respectively) and coincident decreases in QT interval (peak changes: -17 msec [-7%] and -20 msec [-9%], respectively) were observed approximately 3 to 20 hours post dose. Decreases in PR interval (peak changes: -8 msec [-9%]) were also observed 5 to 12 hours post dose at 600 mg/kg. There were no other test article-related effects. The no-observed-effect level (NOEL) was considered to be 5 mg/kg (approximately 50X the grazoprevir C_{max} in HCV-infected patients (100 mg) and the NOEL for respiratory function was 600 mg/kg. There were no cardiovascular changes observed at the electrocardiographic (ECG) examination or at histomorphological examination in the 1-month oral toxicity study in dogs at up to 600 mg/kg/day. C_{max} = 105 µM at 600 mg/kg/day is approximately 457X the grazoprevir C_{max} in HCV-infected patients (100 mg).

There was no test article-related QTc changes in the supratherapeutic dose QTc clinical study conducted in healthy volunteers administered a single oral dose of grazoprevir at 1600 mg.

Central nervous system (CNS)

The potential neurobehavioral effects of grazoprevir were evaluated in functional observational battery (FOB) assays was performed at doses up to the dose resulting in maximum achievable exposure, 200 mg/kg b.i.d. There were no test article-related effects. At 50 mg/kg C_{max} was 27.8 µM after a single oral dose (measured in the exploratory single and multiple dose oral toxicokinetic study in rats (Study number TT 09-1102) is approximately 121X the C_{max} in HCV-infected patients (100 mg).

Other studies

In Vivo Liver Response Assay

An exploratory liver screening assay was conducted to evaluate grazoprevir-related changes in expression of liver genes in Wistar Han rats. The extent of the changes in gene expression observed indicated that grazoprevir had limited impact on rat liver function.

Elbasvir

Cardiovascular (CV)

hERG

In the definitive assay, elbasvir minimally inhibited IK_r by 5.2% at a maximum testable concentration of 10 µM in heterologously-expressed hERG channels using standard voltage-clamp techniques. Elbasvir was also tested for its potential effect on IKs and I_{Nav1.5}. There was no effect on IKs and a minimal inhibition of I_{Nav1.5} (17%) at the maximum testable concentration, 30 µM. These assays were conducted in absence of protein. Elbasvir is >99% protein bound in humans, the IK_r IC₅₀ is >6,500X the unbound C_{max} in HCV-infected patients (50 mg).

In vivo

Conscious dogs were given oral doses of 0.5, 2, and 50 mg/kg, there were no cardiovascular findings. The NOEL for cardiovascular changes was considered to be 50 mg/kg. No pharmacokinetics was collected in telemetrised dogs; however, at a single dose of 25 mg/kg, the dose of the 14-day oral toxicity study in dogs C_{max} = 0.594 µM and represents approximately 4X the C_{max} in HCV-infected patients (50 mg).

Respiratory

Conscious dogs were given single doses of 0.5, 2, and 50 mg/kg. No test article-related changes in respiratory function or body temperature were observed. The no-observed-effect level (NOEL) for respiratory and body temperature effects was considered to be 50 mg/kg.

CNS

A FOB study was conducted after a single dose in rats (oral doses of 100, 300 mg/kg, and 1000 mg/kg b.i.d. (total daily dose, 2000 mg/kg). There were no test article-related neurobehavioral changes. No pharmacokinetics was collected after a single day of administration at 1000 mg/kg b.i.d. However, after 7 daily doses of 750 mg/kg in the exploratory 7-day tolerability study in male rats, C_{max} = 1.14 µM and represents approximately 8X the C_{max} in HCV-infected patients (50 mg).

Other studies

Additional screening studies were performed to determine elbasvir serum biochemical profile in rats and dogs to identify any potential elbasvir-related changes in gene expression indicative of changes in pathways associated with various physiologic or pathologic functions in some major rat organs or tissues. No effects were noted.

2.3.3. Pharmacokinetics

The pharmacokinetic parameters of grazoprevir and elbasvir have been studied in mice, Wistar Han (WI) or, pigmented Dutch-Belted rabbits, beagle dogs and cynomolgus monkeys. A battery of studies has been conducted to determine the absorption, distribution, metabolism, and excretion profile of grazoprevir and elbasvir in multiple non-clinical species including those used for both pharmacological (mouse and rat) and toxicological (rat, rabbit and dog) evaluations. Grazoprevir and elbasvir were administered as ¹⁴C labelled, [³H]-labelled or un-labelled drug. The routes of administration were intravenous or oral.

Methods of analysis

Grazoprevir

Plasma and liver concentrations of grazoprevir for the evaluation of the PK/ADME (non-GLP pharmacokinetic experiments of the compound were determined by LC/MS/MS following protein precipitation using Turbo Ion Spray in positive ion mode. Radioactivity was determined by direct liquid scintillation counting (LSC) of samples or HPLC eluent (the latter with an in-line radiochemical flow detector). For samples with limited total radioactivity, the HPLC eluent was fractionally collected into scintillation plates and the plates were dried and counted using a microbeta counter. Reverse-phase HPLC combined with radiometric detection was used to obtain [³H]grazoprevir or [¹⁴C]grazoprevir metabolite profiles. Metabolite structures were determined by mass spectrometry. For toxicokinetic studies, plasma and liver concentrations of grazoprevir were determined by a validated LC MS/MS method in accordance with Good Laboratory Practice (GLP).

Elbasvir

Both plasma and liver concentrations of elbasvir were determined by LC/MS/MS following protein precipitation using Turbo Ion spray in positive ion mode for the characterization of the PK/ADME (non-GLP studies) of the compound. Radioactivity was determined by direct LSC of samples and of HPLC eluent using radiochemical flow detectors or in cases of low total radioactivity, by fractionally collecting

samples into plates and then counting the dried plates using a microbeta detector. The metabolite profiles were obtained using reverse-phase HPLC combined with radiometric detection and metabolite structures were determined using mass spectrometry. Validated LC/MS/MS methods in accordance with GLP were used for the toxicokinetic determinations of elbasvir plasma and liver concentrations.

Absorption

Grazoprevir

Following intravenous administration of grazoprevir, the mean plasma clearance (CL_p) in rats and dogs was 27.8 mL/min/kg and 5.2 mL/min/kg, the elimination half-life (t_{1/2}) was 1.4 and 3.0 hours, and the steady state volume of distribution (V_{dss}) was 3.1 L/kg and 0.7 L/kg in the rat and dog, respectively (Study Report PK001, 043M94). Following oral administration of grazoprevir, the bioavailability (%F) in both of these species was low (12-13%). Based on studies conducted in bile duct-cannulated (BDC) rats and dogs, the fraction absorbed was estimated to be ~40%, suggesting that the low oral bioavailability likely results from incomplete absorption and a hepatic first pass effect. In female Dutch Belted rabbits orally administered 200 mg/kg grazoprevir, the mean C_{max}, T_{max}, and AUC_{0-∞} were 8.0 µM, 1.5 hr, and 68 µM·hr, respectively (Study Report PK004, 043M94).

In a toxicokinetics study, the effect of multiple oral dosing and gender on grazoprevir pharmacokinetics was also evaluated in SD rats. In a 14 day study, grazoprevir oral exposures in rat were similar following single (101 µM·hr) or multiple (70.0 µM·hr) oral doses of 50 mg/kg. In another study, the AUC_{0-24hr} was 10.1 µM·hr in males and 7.44 µM·hr in female WI rats after multiple oral doses of 25 mg/kg for 1 month. The AUC_{0-24hr} was 16.5 µM·hr in males and 29.6 µM·hr in female WI rats after multiple oral doses of 50 mg/kg for 1 month.

Elbasvir

Elbasvir pharmacokinetics was studied in WI rats, Dutch Belted rabbits, beagle dogs, and cynomolgus monkey. In rat, dog, and monkey, respectively, elbasvir had a moderate plasma clearance (24 mL/min/kg, 8.4 mL/min/kg, and 5.2 mL/min/kg), volume of distribution (5.0 L/kg, 3.0 L/kg, and 2.7 L/kg) and half-life (4.2, 7.7, and 16 hours) (Study Report PK001, 03YQQP). The oral bioavailability of elbasvir was low in rats (3-9%) and moderate in dogs (~35%). The estimated fraction absorbed in those species was ~0.1-0.4 based on the totality of intravenous and oral pharmacokinetics as well as excretion data in BDC animals. T_{max} was prolonged, occurring at 3-5 hours. In both rats and dogs, the oral exposures were less than dose-proportional, possibly due to solubility-limited absorption (Study Report PK001, 03YQQP; 2.6.6.B.3.8).

Multiple dose oral exposures were also studied in rats, and comparison of the single dose (300 mg/kg) AUC_{0-∞} of 7.8 µM·hr to the multiple dose (300 mg/kg) AUC_{0-∞} of 11.5 (Table 2.6.6: B.9) indicates some potential for accumulation. Exposures in male and female rats were comparable.

In dogs administered repeated oral doses of 2, 25, and 1000 mg/kg, the comparison of AUC_{0-∞} on Day 1 to Week 5 showed no potential for accumulation or autoinduction of elbasvir. The multiple dose studies were conducted in both male and female dogs and the data indicated a lack of gender-dependence on the oral PK of elbasvir.

Distribution

Grazoprevir

Rat

The tissue distribution of grazoprevir was assessed in Wistar Han (WI; non-pigmented) and Long Evans (LE; pigmented) rats by quantitative whole body autoradiography (QWBA) following a single oral dose (50 mg/kg) of [¹⁴C]grazoprevir (Study Report PK015, 03Z4SS). The distribution pattern of grazoprevir in the WI and LE rats was similar. Grazoprevir tissue distribution was limited and radioactivity was located primarily in the gastrointestinal (GI) tract and liver. Selective distribution to the liver was consistent with grazoprevir being a rat OATP1B2 substrate. Grazoprevir and related radioactivity had limited distribution to the brain, as anticipated for a P-gp substrate. In the pigmented LE rats, low levels (494 ng radioequivalents/g tissue) of radioactivity were detected in the uveal tract of the eye at 8 hours; however, the radioactivity was below the limit of quantification by 24 hours, indicating that grazoprevir and related radioactivity does not bind to melanin. Elimination of radioactivity was nearly complete from most tissues by 24 hours post dose and in all tissues, including the GI tract and liver, by 168 hours post dose, demonstrating that grazoprevir and related radioactivity is effectively eliminated.

In a separate study, the concentration of grazoprevir in the liver and plasma of rats was studied as a function of time at several oral doses of 5, 25, and 100 mg/kg (Study Reports PK001, 043M94; PK005, 042YRZ). At all-time points, the liver concentrations exceeded the plasma concentrations. The liver to plasma concentration ratios ranged from ~400 at plasma concentrations less than ~0.1 µM and decreased to 5 at a plasma concentration of 51.1 µM, suggesting some saturable uptake process. While the plasma exposures (AUC_{0-∞}) increased in a greater than dose proportional manner, the liver exposures were proportional between the 5 mg/kg and 25 mg/kg oral doses and less than dose proportional from the 25 mg/kg to 100 mg/kg doses. At the 25 mg/kg dose which has a well-defined curve, the terminal plasma and liver concentrations declined in parallel with an estimated half-life of ~7 hours.

Dog

Liver and plasma concentrations were also studied in dog following oral doses of 1 or 5 mg/kg [Study Reports PK001, 043M94; PK005, 042YRZ). The results in dog mimic the results in rat with liver concentrations exceeding the plasma concentrations at all times. Also like rat, the dog liver to plasma concentration ratios decreased as the plasma concentrations increased, indicating potential saturable uptake into liver. The liver to plasma concentration ratios ranged from ~70 at plasma concentrations less than ~0.04 µM and decreased to 5 at a plasma concentration of 8.33 µM. In this species, the terminal plasma clearance described the terminal liver clearance with an elimination half-life of ~9 hours. Grazoprevir elimination from the dog liver was complete by ~72 hours.

Placental Transfer in Rat and Rabbit

Grazoprevir crosses the placenta of pregnant rats and rabbits. The foetal to maternal plasma concentration ratios range from 0.0138 to 0.894 in rat and 0.0128 to 0.0706 in rabbit.

In Vitro Plasma Protein Binding and Blood-to-Plasma Concentration Ratio

Grazoprevir was bound extensively to plasma proteins. The extent of binding was similar in all species tested with a fraction unbound of 0.016, 0.014, 0.009, and 0.012 in rat, rabbit, dog, and human,

respectively (Study Reports PK003, 0436QZ; PK004, 043M96). The unbound concentration was constant over the range tested (0.1 – 10 μ M). Grazoprevir bound to both human serum albumin and α 1-acid glycoprotein and the binding to these proteins was not saturable at concentrations up to 10 μ M (Study Report PK021, 03XQKZ).

An in vitro analysis of plasma from renal disease patients and healthy matched control subjects indicated that renal disease does not alter grazoprevir plasma protein binding; the unbound fractions were 0.022, 0.018, and 0.017 in patients with severe renal impairment, patients with end stage renal disease (ESRD) on dialysis, and in healthy matched control subjects, respectively (Study Report PK021, 03XQKZ). In vitro plasma protein binding was also conducted on samples from varying degrees of hepatic insufficiency and showed that hepatic insufficiency did not change grazoprevir plasma protein binding.

The unbound fractions were 0.017 and 0.012 in mild hepatic insufficient and matched controlled subjects, respectively, 0.021 and 0.017 in moderate hepatic insufficient and matched controlled subjects, respectively, and 0.019 and 0.017 in severe hepatic insufficiency and matched controlled subjects, respectively (Study Report PK021, 03XQKZ).

The in vitro equilibrium blood-to-plasma concentration ratio was independent of the grazoprevir concentrations (0.1-10 μ M) tested, with mean values of 0.6, 0.5, and 0.7 for rat, dog, and human, respectively (Study Report PK003, 0436QZ), indicating that grazoprevir does not extensively partition into blood cells and that total blood clearance is higher than plasma clearance in these species.

Elbasvir

The tissue distribution of [14 C]elbasvir was studied using QWBA in both pigmented Long Evans (LE) rats and non-pigmented Wistar-Kyoto (WK) rats following a single 30 mg/kg P.O. dose (Study Report PK008, 03LRMP). Elbasvir distributed well into most tissues including the liver, but had limited distribution to the brain; elbasvir was shown to be a P-gp substrate (Study Report PK008, 03LRMP; PK004, 03Y5VM). Total radioactivity was below the limit of quantification in all tissues except the spleen, kidney cortex, liver, and Harderian gland by 7 days post dose and in all tissues by 28 days post dose, except the uveal tract of the eye in pigmented rats. At an early time point, the concentration of total radioactivity in the uveal tract of the eye was low and the same in both the pigmented and non-pigmented rats, but as time progressed the elimination of radioactivity from uveal tract of the pigmented rats was slower. The retention of radioactivity in the uveal tract of the pigmented rats indicated a potential for elbasvir to bind to melanin which is sometimes related to phototoxicity. However, elbasvir phototoxicity potential was studied in preclinical species and found to not be of concern.

The distribution of elbasvir to the liver and brain was also examined using LC/MS/MS analysis following a 30 mg/kg P.O. dose to WK rats. The results show a brain to plasma AUC_{0-24hr} ratio of 0.3 and a liver to plasma AUC_{0-24hr} ratio of 193. The limited distribution to the brain is consistent with the QWBA analysis; however, the LC/MS/MS-based analysis indicates a higher distribution of elbasvir into the liver (liver to plasma AUC_{0-24hr} = 193) as compared to the QWBA analysis (liver to plasma concentration ratio 12-20). The reason for the quantitative difference in elbasvir distribution to the liver based on the two different methods of analysis remains unknown. However, the results suggest that elbasvir distributed relatively well into the liver, the target organ for HCV-treatment.

Placental Transfer in Rat and Rabbit

Elbasvir placental transfer was studied in pregnant rats and rabbits following 1000 mg/kg/day oral dosing from Gestation Day (GD) 6/7 to GD 20 with placental transfer evaluated on GD 20. Elbasvir

crossed the placenta of both rats and rabbits with fetal plasma concentrations being 0.6 to 5% that of the maternal plasma.

In Vitro Plasma Protein Binding and Blood-to-Plasma Concentration Ratio

Elbasvir binding to plasma proteins was extensive. In mouse, rat, dog, monkey, and human the unbound fraction was <0.001 and independent of the concentration tested (Study Reports PK002, 03XZ42; PK005, 03Y9KC). The unbound fraction in rabbit plasma was higher at 0.012 (Study Report PK005, 03Y9KC). Elbasvir bound to both human serum albumin and α 1-acid glycoprotein and binding to both of these plasma proteins was considered to contribute to the low unbound fraction in plasma (Study Report PK012, 03YB6T). In in vitro evaluations, elbasvir plasma protein binding was unchanged in both renal insufficient and hepatically impaired patients (Study Report PK0012, 03YB6T).

Elbasvir did not preferentially partition into blood cells with blood to plasma concentration ratios of 0.61, 0.94, 0.56, and 0.62 in rat, dog, monkey, and human, respectively (Study Report PK002, 03XZ42). The total blood clearance was, therefore, slightly higher than the reported plasma clearances for these species.

Metabolism

Grazoprevir

In Vivo Metabolism in Rat, Rabbit, Dog, and Human

The in vivo metabolism of [3H] or [14C]grazoprevir was studied in the rat, rabbit, dog and human. The studies indicated that in all preclinical species tested, both metabolism and biliary excretion of intact compound contributed to grazoprevir elimination. Across the preclinical species tested and humans, oxidative metabolism was the major route of biotransformation and little or no circulating metabolites observed.

The metabolism of [3H] or [14C]grazoprevir was determined in BDC male Wistar rats following a 5 mg/kg P.O. dose, in BDC male Beagle dogs following a 0.5 mg/kg intravenous dose or 1 mg/kg P.O. dose, and in intact female Dutch Belted rabbits following a 200 mg/kg oral dose (Study Reports PK002, 03Z77Z; PK004, 043M96; PK018, 03ZXFB). In rats, radioactivity recovered in bile and feces represented 28.1% and 72.8%, respectively, of the administered dose and a negligible amount (0.4%) of the radioactive dose was recovered in urine (Study Report PK002, 03Z77Z). The metabolites characterized in rat bile and feces consisted of several minor metabolites resulting from oxidative metabolism (M1a, M3, M4a, M4b, M5, M7a, M9) or oxidative metabolism and the addition of glutathione (M8) (Study Report PK002, 03Z77Z). The elimination and biotransformation of grazoprevir in dogs was similar to rats. Following an intravenous dose to dogs, grazoprevir was eliminated in bile (74% of the dose) as approximately equivalent amounts of oxidative metabolites and grazoprevir, in feces (22% of the dose) and in urine ($<1\%$ of the dose) (Study Report PK018, 03ZXFB). The metabolites detected in dog bile (M3, M4a, M4b, M7a) were also detected in rat bile, indicating that the metabolism across species was similar. The radioactivity in feces following oral dosing was profiled and only grazoprevir and a metabolite likely formed via bacterial metabolism in the gut, M10, were identified. Only grazoprevir was detected in the plasma of rats and dogs following oral administration of [3H] or [14C]grazoprevir. Similar to rats and dogs, grazoprevir was the major component of the radioactivity eliminated in rabbit feces and several minor oxidative metabolites (M1a, M3, M4a, M4b, M5, M7a, and M9) were also observed. The

predominant radioactive peak in rabbit plasma was grazoprevir along with minor amounts of metabolites M3, M4a, M4b, and M7.

Overall, the metabolism of grazoprevir in preclinical species and human was similar. Following an oral dose of 186-188 mg (~200 µCi) of [14C]grazoprevir to humans, essentially the entire radioactive dose (110%) was excreted in feces, with urinary recovery contributing only 0.29% [PK016, 03N2K3]. Based on the fecal metabolic profile, grazoprevir and M10 (considered a likely bacterial product of grazoprevir formed and retained within the alimentary canal) accounted for 80% of the administered dose and oxidative metabolites (and their bacterial products) account for 22% of the administered dose (Study Report PK009, 03ZXF3). A minor oxidative metabolite observed only in human feces, M14 (4% of the dose), was structurally similar to the metabolite M5 (M14 without hydroxylation) formed in vivo in rats and rabbits and in vitro in rats, rabbits, dogs and humans.

Based on the absolute oral bioavailability study in human, the human fraction absorbed was estimated to be ~0.5 with wide variability [see Clinical Report] and coupled with the metabolism data suggests that biliary excretion and metabolism contribute almost equally to the elimination of grazoprevir in human. The oxidative metabolites observed in human feces were observed in the preclinical species (Study Reports PK002, 03Z77Z; PK004, 043M96; PK018, 03ZXFB). Also, similar to rats and dogs, grazoprevir was the only radioactive peak detected in human plasma (Study Report PK009, 03ZXF3).

In Vitro Metabolism in Mouse, Rat, Rabbit, Dog, and Human

The in vitro metabolism of grazoprevir was studied in liver microsomes and hepatocytes from rat, rabbit, dog, and human and hepatic microsomes from mice (Study Reports PK003, 0436QZ; PK004, 043M96; PK009, 03ZXF3; PK026, 03YBN6). The in vitro metabolism across species was similar with oxidative metabolism being the predominant biotransformation pathway. In rat, rabbit, dog, and human liver preparations, 8 oxidative metabolites were identified. The metabolites resulted from hydroxylation (M1a, M4a, M4b, M7a, and M9), oxidative O-dealkylation (M3), oxidative loss of the vinylcyclopropylamide (M5) and oxidation and addition of glutathione (M8). The metabolites formed in vitro were predictive of the metabolites formed in vivo, with the exception of the metabolites proposed to result from bacterial metabolism, M10, M11a, and M11b. In mouse liver microsomal incubations, minor amounts of M3, M4a, M4b, and M10 were observed. The metabolites identified in human liver preparations were also seen in the rat, rabbit, and dog liver preparations.

The potential for grazoprevir to form chemically reactive metabolites was assessed using [3H] or [14H]grazoprevir with rat or human liver microsomes or human hepatocytes. Grazoprevir showed some NADPH-dependent covalent protein binding in both species and the covalent protein binding was decreased with the addition of glutathione (GSH) or ritonavir [an approved antiviral protease inhibitor (Study Report PK012, 03XQKP)]. The reduced covalent protein binding with the addition of ritonavir or GSH and in the absence of NADPH suggests that the bioactivation is CYP-dependent and that endogenous cellular antioxidants/nucleophiles can serve a protective role. The absence of metabolites indicative of bioactivation in human excreta suggests that formation of chemically reactive intermediates is minor, if any, in human.

Elbasvir

In Vivo Metabolism in Rat, Rabbit, Dog, and Human

Following a 5 mg/kg intravenous dose of [14C]elbasvir to BDC male Wistar rats, elbasvir was eliminated predominantly as parent compound in bile (20.1%), urine (16.2%) and feces (20.2%), totalling 56.5%

of the administered dose [Study Report PK017, 03YK0B; PK018, 03YQFJ]. Oxidative metabolites totalling 15.4% of the administered dose were also recovered in the bile as M2 and M3. The metabolites M2 and M3 are simple hydroxylation products of elbasvir. Following a 1 mg/kg intravenous dose of [14C]elbasvir to BDC dogs, only a minor amount (~4%) of the dose was recovered as the oxidative metabolites M2 and M3 in dog bile and feces (Study Reports PK011, 03Y9QY; PK016, 03Y7QS). Profiling of plasma from rats and dogs that received an oral dose of elbasvir indicated that only elbasvir circulates in these species (Study Reports PK003, 03YQYY; PK003, 03YQYY).

Following a 100 mg/kg oral dose of [14C]elbasvir to intact Dutch Belted rabbits the majority of the dose was recovered in feces (70%) with very little drug related material recovered in urine (1%) [Study Report PK005, 03Y9KC]. The radioactivity extracted from rabbit feces was profiled and the only radioactive peak observed was elbasvir (Study Report PK005, 03Y9KC). Several minor oxidative metabolites (M1a, M1b, M2, M2a, M2b, M2c, M3, and M4) were detected using mass spectrometry only. Rabbit plasma was also profiled and elbasvir was the only radioactive peak observed with minor amounts of the oxidative metabolites M2b and M4 found using mass spectrometry [Study Report PK005, 03Y9KC].

The metabolism of elbasvir in preclinical species was representative of the metabolism in human. The oxidative metabolites, M2 and M3, found in the excreta of preclinical species were also the only two metabolites detected in human excreta (feces) (Study Reports PK010, 040346; PK013, 03WD9D). Elbasvir was the only dose-related material detected in human plasma (Study Reports PK010, 040346; PK013, 03WD9D). Since the metabolites formed in vivo in human (M2 and M3) are also formed in the rat, rabbit, and dog, these preclinical species are suitable toxicology species (Study Reports PK002, 03XZ42; PK005, 03Y9KC).

In Vitro Metabolism in Mice, Rat, Rabbit, Dog, and Human

Elbasvir demonstrated low turnover in mouse, rat, dog, and human liver microsomes and/or hepatocytes. Qualitatively, metabolites observed in human were also observed in animal liver preparations. In rat, rabbit, dog, and human liver preparations 2 hydroxylation products, M2 and M3, were identified (Study Reports PK002, 03XZ42; PK005, 03Y9KC). A trace amount of a double oxidation metabolite, M1, was also detected in rat, dog, and human hepatocytes (Study Reports PK002, 03XZ42; PK005, 03Y9KC). In mouse hepatocytes, M2 was detected.

Excretion

Grazoprevir

In the preclinical species (Wistar rats, Dutch belted rabbits, and Beagle dogs) and humans, the major mode of grazoprevir elimination was biliary and/or faecal route, with little renal elimination. Following a 5 mg/kg P.O. dose of [3H] grazoprevir to BDC rats, the radioactivity was recovered in bile (28.1%), faeces (72.8%), and urine (0.4%) totalling 101.3% (Study Report PK002, 03Z77Z). In intact rabbits following a 200 mg/kg oral dose, the radioactivity was eliminated in faeces (77.3%) and urine (1.4%) totalling 78.7% of the dose over 0-72 hours (Study Report PK004, 043M96). In BDC dogs following a 0.5 mg/kg intravenous dose, the dose was recovered in bile (74.3%), faeces (22.0%) and urine (0.5%) and also in bile (27.8%), faeces (26.3%) and urine (3.4%) following a 1 mg/kg oral dose (Study Report PK018, 03ZXFB). Following a 186-188 mg oral dose to healthy human volunteers, grazoprevir was eliminated almost entirely into faeces (110%) with very little of the dose recovered in urine (0.29%) (Study Report PK016, 03N2K3).

Excretion in Milk of Rats

Grazoprevir excretion into the milk of lactating rats was studied by determining the concentration of drug in maternal plasma and milk on Lactation Day (LD) 14 at 2 and 8 hours following 25 mg/kg/day, 100 mg/kg/day, or 200 mg/kg b.i.d. from GD 6 to LD 14. Maternal plasma concentrations of grazoprevir ranged from 3.81 to 22.3 µM and milk concentrations from 1.70 to 31.7 µM. The milk to maternal plasma concentration ratios ranged from 0.538 to 0.866 indicating that grazoprevir is excreted into the milk of lactating rats.

Excretion in Milk of Rats

The excretion of elbasvir into the milk of lactating rats was investigated by measuring the concentration of drug in maternal plasma and milk on Lactation Day (LD) 14 following a 1000 mg/kg/day oral dose from GD 6 to LD 14 (n=4). The concentrations in plasma and milk were determined at 2 hours after the last dose on LD 14. The elbasvir concentration in maternal plasma and milk at 2 hours was 1.40 µM and 5.75 µM, respectively, with a milk to plasma concentration ratio of 4.15. The results showed the excretion of elbasvir into the milk of lactating rats.

Pharmacokinetic drug interactions

Grazoprevir

The potential for grazoprevir to be a victim of drug-drug interactions (DDI) was studied in vitro. The oxidative metabolism of grazoprevir was shown to be catalysed primarily by CYP3A (Study Reports PK003, 0436QZ; PK011, 044RQK). Grazoprevir was also shown to be a substrate for OATP1B1, OATP1B3, and P-glycoprotein (P-gp) (Study Report PK003, 0436QZ). Therefore, grazoprevir was considered to have a potential to be a victim of drug-drug interactions by compounds known to inhibit or induce CYP3A and P-gp or inhibit OATP1B1 and/or OATP1B3. In vitro in LLC-PK1 cells, grazoprevir showed an apparent permeability (Papp) of 19x10⁻⁶ cm/s (Study Report PK003, 0436QZ).

The potential for grazoprevir to perpetrate DDIs was also studied in vitro. Grazoprevir showed some potential to inhibit intestinal CYP3A (IC₅₀ = 73 µM), but not any of the other major CYPs, UGT1A1, CES1, CES2, or Cat A (PK013 (Study Reports PK013, 042TXL; PK008, 03Y9RJ).

Grazoprevir did not show evidence of time-dependent inhibition of either CYP3A or CYP2C8 (Study Reports PK003, 0436QZ; PK025, 03YPR5). In incubations with primary human hepatocytes, grazoprevir showed no potential to induce CYP3A4, CYP1A2, or CYP2B6 (Study Report PK003, 0436QZ). In in vitro studies, grazoprevir demonstrated time- and temperature-dependent uptake in rat and human hepatocytes, suggesting that a carrier (transporter)-mediated system(s) is involved in grazoprevir uptake into human and rat hepatocytes (Study Report PK003, 0436QZ).

Based on in vitro data, grazoprevir is not predicted to inhibit P-gp, OATP1B1, or OATP1B3, but has some potential to inhibit intestinal BCRP (Study Reports PK003, 0436QZ; PK010, 03Y34H). Grazoprevir also showed some in vitro inhibition of BSEP, MRP2, MRP3, and MRP4 (Study Reports PK007, 03Y34C; PK017, 03DVGM).

It is noted that in drug interaction studies conducted with grazoprevir, no information on the effects of organic anion transporter (OAT) 1, OAT3, organic cation transporter (OCT) 1 or OCT2 has been provided. The applicant is requested to provide this information

Clinical interaction studies showed that grazoprevir is a CYP3A/P-gp and OATP1B1/3 substrate and weak inhibitor of CYP3A and BCRP.

Elbasvir

Based on in vitro evaluations using human liver microsomes, elbasvir has a potential to be a victim of CYP3A/P-gp inhibition and induction. In human liver microsomes with specific chemical inhibitors and in experiments with recombinant CYP isoforms, elbasvir oxidative metabolism was shown to occur primarily via CYP3A (PK002, 03XZ42). In cell based assays expressing select transporters, elbasvir was shown to be a substrate for P-gp (PK002, 03XZ42; PK004, 03Y5VM; PK015, 03Y4Z0). In vitro, elbasvir appeared not to be a substrate for OATP1B1 or OATP1B3 (PK002, PK004, PK015). Due to poor passive permeability ($0.7\text{--}4.7 \times 10^{-6}$ cm/sec) in the host cell line (Madin-Darby canine kidney (MDCK) II cells), it could not be determined whether or not elbasvir is a substrate for BCRP (PK004; 03Y5VM).

Based on the in vitro evaluation, elbasvir was shown to have a low potential for perpetrating drug-drug interactions. In human liver microsomes, elbasvir did not inhibit any major CYP isoform (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4) (PK020, 03Y5BH). Elbasvir was also not a time-dependent inhibitor of CYP3A (PK002, 03XZ42). In vitro, elbasvir was a weak inhibitor of UGT1A1 (70.9 μM) at two-orders of magnitude above the clinically relevant C_{max} (0.151 μM) (PK009, 03MBKP; see Clinical Report). Therefore, elbasvir was considered unlikely to be a clinically-relevant inhibitor of UGT1A1. In incubations with human hepatocytes, elbasvir did not cause induction of CYP3A4, CYP1A2 or CYP2B6 (PK020, 03Y5BR). In vitro, elbasvir showed some potential to inhibit human P-gp, OATP1B1, OATP1B3, and BCRP (PK002, 03XZ42; PK004, 03Y5VM; PK021, 03YS0W).

Clinical interaction studies confirmed that elbasvir is a CYP3A/P-gp substrate and also that elbasvir is a mild inhibitor of P-gp and BCRP, but not OATP1B1 or OATP1B3.

2.3.4. Toxicology

Single dose toxicity

Grazoprevir

No formal single dose toxicity studies have been conducted. However, consistent with ICH M3 (R2) guidance, acute toxicity was assessed from the repeat-dose toxicity studies, in which oral doses of grazoprevir up to 500 mg/kg/day in mice, 1000 mg/kg/day in rats (with highest AUC_{0-24 hr} at 200 mg/kg b.i.d., *i.e.* total daily dose of 400 mg/kg/day) and up to 600 mg/kg/day in dogs were administered.

Elbasvir

No formal acute toxicity studies have been conducted. However, consistent with ICH M3 (R2) guidance, acute toxicity after a single dose was assessed from the repeat-dose oral toxicity studies, where oral doses of elbasvir up to 1000 mg/kg in mice, 1000 mg/kg b.i.d. in rats, and 1000 mg/kg in dogs were given. There was no evidence of acute toxicity in these species.

Repeat dose toxicity

Grazoprevir

Grazoprevir was investigated in a number of repeat-dose toxicity studies in mice, rats, and dogs, including oral range finding studies of up to 3-months in mice, toxicity studies of up to 6 months in rats and 9 months in dogs. Systemic plasma exposures were unexpectedly low in the initial 1-month rat study. It was subsequently determined that grazoprevir, when administered in a solution, provided higher exposures than in suspension. As a result a 1-month rat study was repeated at doses up to the maximum feasible dose in solution (200 mg/kg b.i.d). Unless otherwise indicated, routine Necropsy

parameters (physical signs, body weight, food consumption, ophthalmic, and clinical pathology determinations; and electrocardiographic examinations in dogs only) and postmortem parameters (organ weights, gross observations and histomorphologic examinations) were evaluated.

Mice

One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild- Type Mice (Hybrid)

Male and female Jic:CB6F1-nonTgrasH2@Jcl mice were given 20, 100, 200, or 500 mg/kg/day of grazoprevir, supplied as a spray-dried formulation. In addition to routine endpoints, Transmission electron microscopy (TEM) examination was performed on liver slides, and grazoprevir concentrations in liver-gallbladder homogenate were determined. Recovery assessment was not included in this study.

At 500 mg/kg/day, there were body weight loss in both sexes, decreases in total white blood cell and lymphocyte counts (-37% and -43%, respectively) in females and increases in total white blood cell, neutrophil, lymphocyte, and eosinophil (+63, +53, +61, +350%) counts in males, minor increases in red blood cell and decreases in other erythroid parameters (mean corpuscular volume, haemoglobin, and haemoglobin concentration), minor increases in platelet counts in both sexes, compared to controls. Increases in total/direct bilirubin (up to 47-fold), aspartate aminotransferase (AST; up to 2-fold), alanine aminotransferase (ALT; up to 5-fold), and alkaline phosphatase (ALP; >2-fold), and minor decreases in serum total protein, albumin and globulin, and minor increases in glucose, phosphorus, cholesterol in both sexes, and in blood urea nitrogen in a single male, compared to controls. At 200 mg/kg/day decreases in total white blood cell and lymphocyte counts were seen in females (-41% for both parameters) and minor increases in platelet count was noted in males. Serum biochemical changes consisted of increase in total/direct bilirubin in both sexes (up to 8-fold), and minor increases in serum ALT (>2-fold), ALP (individual) and phosphorus, and minor decreases in serum total protein, albumin and globulin in females only, and minor increases in cholesterol in both sexes. At 100 mg/kg/day, test article-related changes consisted of minor decreases in total white blood cell and lymphocyte counts in females only, and minor increases in cholesterol in both sexes and in total bilirubin in 1 male.

At 500 mg/kg/day degeneration of the renal tubular epithelium of the kidney (very slight in females and very slight to moderate in males), very slightly to slightly increased hepatocellular size in both sexes, and slight periportal hepatocellular cytoplasmic rarefaction (consistent with increased hepatocellular glycogen content, as confirmed by PAS staining and TEM) in females, and villous atrophy (mostly restricted to the duodenum; very slight in females and very slight to slight in males) in the small intestine, were noted. The increased hepatocellular size at 500 mg/kg/day correlated with increased mean liver weights and increased organ size in both sexes and with prominent lobular pattern in males. At 100 mg/kg/day and 200 mg/kg/day in females and at 200 mg/kg/day in males mean liver weight increases with very slightly to slightly increased hepatocellular size was seen in males at 200 mg/kg/day (of overall lower incidence and mean severity than at 500 mg/kg/day). Additional histomorphologic changes were observed in other tissues from animals from both sexes at 500 mg/kg/day and in 2 males at 200 mg/kg/day, but were considered secondary to reduced general condition of these animals. The NOEL was considered to be 20 mg/kg/day in both sexes. However, the applicant argues that the changes noted at 100 mg/kg/day were limited to very slight to slight haematological/serum biochemical changes and increased liver weights, and are considered of minimal toxicological significance based on their small magnitude, the lack of impact on general condition of the animals, and the lack of histomorphologic correlates. The NOEL was considered to be 20 mg/kg/day in both sexes. The NOAEL was considered to be 100 mg/kg/day. 100 mg/kg/day provided a plasma systemic exposure (AUC_{0-24 hr} = 164 µM.hr & C_{max} of 40.8 µM) approximately 83X and 177X over the grazoprevir exposure in HCV-infected patients (100 mg), respectively.

A high inter-animal variability was observed in plasma concentrations at all doses. There were no substantial (*i.e.*, greater than 2-fold) sex-related differences in mean systemic exposure (AUC_{0-24 hr}) or mean C_{max} at all doses.

Three-Month Study in CD1 Mice

CrI:CD1(ICR) mice were given 20, 100, 200, or 500 mg/kg/day of grazoprevir, supplied as a spray-dried formulation. Recovery assessment was not included as part of this study. At 500 mg/kg/day in both sexes decreases in red blood cell count, and in other erythroid parameters (haemoglobin concentration, haematocrit, mean corpuscular volume, haemoglobin, haemoglobin concentration); and increases in serum glucose, ALT, and ALP (ALT and ALP >2-fold relative to controls), and moderate to marked increases in total/direct bilirubin (up to 32-fold relative to controls) were observed compared to controls. In addition at 500 mg/kg/day there were increases in white blood cell count, due to increases in neutrophil and lymphocyte counts, and in serum cholesterol in females, and decreased total body weight gain in males. At 200 mg/kg/day increases in serum ALP (<2-fold) and total bilirubin (up to 3-fold) were noted in both sexes, along with increased in serum ALT (<2-fold) in females; and increases in serum glucose in males, compared to controls. At 100 mg/kg/day increases in serum total/direct bilirubin were seen in males (up to 2-fold).

At 500 mg/kg/day slight to marked degeneration of the renal tubular epithelium of the kidney, and very slightly to moderately increased hepatocellular size which correlated with increases in mean liver weights and/or increased organ size were seen in both sexes and with prominent lobular pattern in males. Increased hepatocellular size was associated with very slight to slight periportal hepatocellular cytoplasmic rarefaction was observed in the liver of females. TEM examination of livers from 2 females and 2 males at 500 mg/kg/day showed cytoplasmic accumulation of large amounts of glycogen in periportal and centrilobular hepatocytes, which was more prominent in females. In the gallbladder, very slight or slight focal inflammation was observed in 1 female and 1 male. At 200 mg/kg/day, very slight to slight increased hepatocellular size associated with, in 1 female, very slight peri-portal hepatocellular cytoplasmic rarefaction correlated with mean liver weight increases in both sexes.

The NOEL was considered to be 100 mg/kg/day in females and 20 mg/kg/day in males. However, the Necropsy changes noted at 200 mg/kg/day (the serum biochemical changes) were considered of minimal toxicological significance based the small magnitude and the lack of histomorphologic correlates. The post-mortem changes noted at 200 mg/kg/day (very slight to slight increased hepatocellular size associated with very slight peri-portal hepatocellular cytoplasmic rarefaction and correlated with mean liver weight increases) were not considered adverse based on their slight severity and the lack of evidence of necrosis or inflammation. Therefore, the NOAEL was considered to be 200 mg/kg/day (AUC_{0-24 hr} = 820 ± 109 µM.hr). 200 mg/kg/day provided a plasma systemic exposure (AUC_{0-24 hr} = 820 µM.hr) approximately 416X over the grazoprevir exposure in HCV-infected patients (100 mg). At 100 mg/kg/day the approximate exposure cover would be 237X.

A high inter-animal variability was observed in plasma concentrations at all doses. There were no substantial sex-related differences in mean systemic exposure (AUC_{0-24 hr}) or mean C_{max} at all doses. Mean systemic exposure (AUC_{0-24 hr}) and C_{max} were greater than dose proportional between 20 mg/kg/day and 100 mg/kg/day, and approximately dose proportional between 100 mg/kg/day and 500 mg/kg/day.

Rats

One-Month Oral Toxicity Study in Rats

Male and female CrI:WI(Han) rats were given 25, 50, or 1000 mg/kg/day of grazoprevir. There were no relevant effects. Recovery assessment was not included as part of this study. The NOAEL was considered to be 1000 mg/kg/day (AUC_{0-24 hr}: $24.3 \pm 4.09 \mu\text{M}\cdot\text{hr}$). There were no substantial (*i.e.*, greater than 2-fold) sex-related differences in systemic exposure (AUC_{0-24 hr}) or C_{max}. Systemic exposure and C_{max} were slightly greater than dose-proportional between 25 mg/kg/day and 50 mg/kg/day, and were similar at 50 mg/kg/day and 1000 mg/kg/day, suggesting a saturation of absorption at doses greater than 50 mg/kg/day. Grazoprevir concentrations in plasma from control group animals at 2 hours post dose were below the LLQ of the bioanalytical method.

One-Month Oral Toxicity Study in Rats with a 2-Week Interim Necropsy.

The purpose of this study was to evaluate the potential toxicity and toxicokinetic profile of grazoprevir when administered orally for approximately 2 weeks and 1 month. Male and female CrI:WI(Han) were given 50, 200 mg/kg/day, or 200 mg/kg b.i.d. (a minimum of 6 hours between the 2 daily doses) of grazoprevir or vehicle (dosed b.i.d.; a minimum of 6 hours between the 2 daily doses) only. 200 mg/kg/day was the highest feasible dose in solution. Recovery assessment was not included as part of this study. Liver drug concentrations were also measured. There were no relevant effects. The NOAEL was considered to be 200 mg/kg b.i.d. (AUC_{0-24 hr} = $212 \pm 63.0 \mu\text{M}\cdot\text{hr}$).

A high variability in plasma concentrations was observed at all doses and each collection day. There were no substantial (*i.e.*, greater than 2-fold) sex-related differences in mean systemic exposure (AUC_{0-24 hr}) or mean C_{max} in Study Weeks 2 and 4 at all doses. Taking into account the inter-individual variability, mean systemic exposure was approximately dose proportional between 50 mg/kg/day and 200 mg/kg/day, and mean C_{max} was considered similar at 50 mg/kg/day, 200 mg/kg/day and 200 mg/kg b.i.d. Mean systemic exposure and C_{max} were considered similar in Study Weeks 2 and 4 at 50 mg/kg/day. The apparent 2-fold difference in mean systemic exposure and mean C_{max} was attributed to the high inter animal variability. Systemic exposure and C_{max} were considered similar in Study Weeks 2 and 4 at 200 mg/kg b.i.d. Grazoprevir concentrations in plasma from controls 2 hours post dose were below the LLQ of the bioanalytical method in Study Weeks 2 and 4.

High variability in liver concentrations was observed at all doses at interim (Study Week 3) and final necropsies (Study Week 5). Mean liver concentrations at interim and final necropsies were considered similar at 50 mg/kg/day, 200 mg/kg/day and 200 mg/kg b.i.d. Low levels of the test substance were measured in liver at necropsy in 3 control animals. All concentration values for grazoprevir in the liver controls were less than 0.9% of the mean value at 50 mg/kg/day (low dose), in Weeks 3 and 5, respectively. Based on the low concentrations of the test substance observed in the liver of the control animals compared to the higher concentrations that were measured in the liver at 50 mg/kg/day, the low levels of the test substance that were observed in the control liver were not considered to have compromise the validity or reliability of the toxicokinetic evaluation or of the toxicity assessment.

Six-Month Oral Toxicity Study in Rats

Male and female CrI:WI(Han) rats were given 50 mg/kg or 200 mg/kg once daily, or 200 mg/kg twice daily (200 mg/kg b.i.d.) of grazoprevir. Recovery assessment was not included as part of this study.

Individual increases in serum total bilirubin was noted in one female at 200 mg/kg/day (0.3 mg/dL vs up to 0.2 mg/dL in controls) and in both sexes at 200 mg/kg b.i.d. (0.3 mg/dL or 0.4 mg/dL in 4 of 15 females, and in 2 of 15 males vs up to 0.2 mg/dL in both sexes in controls), and decreases in serum sodium, chloride, and potassium was seen in females at 200 mg/kg b.i.d. The clinical pathology

changes were considered of minimal toxicological significance based on their small magnitude and the lack of histological correlates. Focal haemorrhage in the glandular mucosa of the stomach was seen in males at 200 mg/kg b.i.d.

Since the systemic exposure to grazoprevir at 200 mg/kg b.i.d. was similar in males and females and also similar to the systemic exposure at 200 mg/kg/day (once daily), this focal haemorrhage was considered due to prolonged daily direct contact of the gastric mucosa to grazoprevir administered at 200 mg/kg twice daily. The gender difference was attributed to the heavier males received larger dosing volumes than females at 200 mg/kg b.i.d. In the absence of chronic changes after 6 months of treatment, this finding in males was considered of minimal toxicological significance. The NOAEL was considered to be 200 mg/kg b.i.d. ($AUC_{0-24 \text{ hr}} = 445 \pm 88.5 \mu\text{M}\cdot\text{hr}$). This dose provides an exposure ($AUC_{0-24 \text{ hr}} = 445 \mu\text{M}\cdot\text{hr}$) approximately 226X over the grazoprevir exposure in HCV-infected patients (100 mg).

High inter-animal variability was observed in plasma concentrations at all doses. There were no substantial sex-related differences in mean systemic exposure ($AUC_{0-24 \text{ hr}}$) or mean C_{max} . In Weeks 4, 9, and 13, between 50 mg/kg/day and 200 mg/kg/day, mean systemic exposure was approximately dose proportional and mean C_{max} was less than dose proportional. At 200 mg/kg/day and 200 mg/kg b.i.d., mean systemic exposure and mean C_{max} were similar, for the 3 collection intervals. Mean systemic exposure and mean C_{max} were similar in Study Weeks 4, 9, and 13 for each dose group, suggesting that steady state is maintained until Study Week 13. Grazoprevir concentrations in plasma from all control animals at 2 hours post dose were below the LLQ of the bioanalytical method in Study Weeks 4, 9, and 13. There were no sex-related differences in mean liver concentrations at Week 27 (final necropsy) and taking into account the high inter-animal variability, these mean concentrations were similar over the dose range.

Low level of the test substance was measured in liver at necropsy in 1 control animal, and was less than 1% of the mean liver concentration of the low-dose group (50 mg/kg/day). Based on the low concentration of the test substance observed in the liver of the control animal compared to the higher concentrations that were measured at 50 mg/kg/day, the low level of the test substance that was observed in the control liver was not considered to compromise the validity or reliability of the toxicokinetic evaluation or of the toxicity assessment.

Dog

One-Month Oral Toxicity Study in Dogs

Beagle dogs were given 5, 20, or 600 mg/kg/day of grazoprevir or vehicle only. 50 mg/kg/day was expected to produce toxicity based on results from the preliminary tolerability study and to result in systemic plasma exposure that is high multiples (>50-fold) the human exposure. Recovery assessment was not included as part of this study.

Emesis, decreases in erythroid parameters (red blood cell count, haemoglobin, reticulocytes, and haematocrit), slight to moderate increases in total bilirubin (up to 5-fold compared to controls), and degeneration of the seminiferous epithelium of the testes was seen at 600 mg/kg/day. The Applicant goes onto say that changes were considered minimally adverse. Emesis did not impact the physical condition of the animals; the haematological changes were of very slight severity and did not have any histomorphologic correlates; the bilirubin increase although of moderate severity was not associated with changes in any other biomarkers of liver toxicity (AST, ALT, ALP) and did not have any histomorphologic correlates; the degeneration of the seminiferous epithelium of the testes noted in all animals at 600 mg/kg/day was of very slight to slight severity. The change was widely distributed throughout the testes and was characterised by exfoliation of degenerate and frequently

multinucleated spermatogenic epithelial cells into the lumen with thinning of spermatogenic epithelium, occasional sertoli cell vacuolation, and retained spermatids. The epididymides of affected animals had a corresponding increase in cellular debris and exfoliated cells within tubular lumens. There were no findings in the testes and epididymides in animals at 20 mg/kg/day. The Applicant argues that these findings are expected to be reversible based on a literature reference (Ref. 4.3: 03RKQY] Creasy Dianne M. Pathogenesis of male reproductive toxicity. Toxicological Pathology 2001;29(1):64-76).

Increases in total bilirubin in 1 male (2-fold compared to pre-test) was seen at 20 mg/kg/day and was considered of minimal toxicological significance based on their low amplitude, the lack of associated increases in any other biomarkers of hepatobiliary toxicity (AST, ALT, ALP) and the lack of histomorphologic correlates. The NOAEL was considered to be 20 mg/kg/day which provided a plasma systemic exposure (AUC_{0-24 hr} = 497 µM.hr) which is approximately 252X over the grazoprevir exposure in HCV-infected patients (100 mg).

There were no substantial (*i.e.*, greater than 2-fold) sex-related differences in systemic exposure (AUC_{0-24 hr}) or C_{max}. Systemic exposure was greater than dose proportional between 5 mg/kg/day and 20 mg/kg/day and less than dose proportional between 20 mg/kg/day and 600 mg/kg/day on Study Day 1 and in Study Week 5. C_{max} was approximately dose proportional between 5 mg/kg/day and 20 mg/kg/day and less than dose proportional between 20 mg/kg/day and 600 mg/kg/day, on Study Day 1 and in Study Week 5. Systemic exposure and C_{max} were similar at the low and mid doses on Study Day 1 and in Study Week 5, suggesting that steady state toxicokinetics at these doses were attained within the first day of dosing and were maintained until Study Week 5. At 600 mg/kg/day systemic exposure and C_{max} were slightly greater in Study Week 5 than on Study Day 1 suggesting slight accumulation probably due to slow elimination at this dose. Grazoprevir concentrations in plasma from all control group animals on Study Day 1 and in Study Week 5 were below the LLQ of the bioanalytical method.

Nine-Month Oral Toxicity Study in Dogs with a 3-Month Interim Necropsy.

Beagle dogs were given 5 mg/kg/day or 15 mg/kg/day for approximately 38 weeks, or 300 mg/kg/day followed by 100 mg/kg/day for approximately 11 and 27 weeks, respectively, of grazoprevir, or vehicle only. 15 mg/kg/day was expected to produce toxicity and to result in systemic plasma exposure at high multiples (>50-fold) of the human exposure. At 300 mg/kg/day, excessive body weight losses (up to -22% relative to pre-test) due to unsatisfactory food consumption/inappetence led to the sacrifice of 2 animals (1 male and 1 female in Study Weeks 7 and 11, respectively), and to the lowering of the high dose to 100 mg/kg/day in Study Week 12. Recovery assessment was not included as part of this study.

At 300 mg/kg/day increases in the incidence and frequency of unformed/liquid faeces (females only) and post-dosing emesis compared to controls; yellow discoloration of the faeces and, in a single animal, skin and eyes (bulbar conjunctiva); decreases in erythroid parameters and increases in fibrinogen and platelets; increases in serum total bilirubin (up to 11-fold compared to controls; predominantly direct bilirubin in the most affected animals), increases in serum ALP (<4-fold; males only), and minor transient decreases in serum cholesterol; and increases in urine bilirubin, were observed.

Necropsy findings during the 27-week period at 100 mg/kg/day were generally similar to the findings noted in the first 11 weeks at 300 mg/kg/day and included increases in the incidence and frequency of unformed/liquid faeces (females only) and post-dosing emesis (females only), yellow discoloration of the faeces; body weight loss and unsatisfactory food consumption/inappetence (in a single male); decreases in most erythroid parameters and increases in fibrinogen and platelets; increases in serum

total bilirubin (up to 7-fold compared to controls; predominantly direct bilirubin), increases in serum ALP (<4-fold; males and transiently in a single female), and transient decreases in serum cholesterol (in a single male); and increases in urine bilirubin (in a single male). Also at 100 mg/kg/day, along with the decreased erythroid parameters, increase in mean erythrocyte count (males in Study Week 38) and/or in mean or individual reticulocyte count, and the presence of nucleated erythrocytes in some animals were suggestive of a regenerative response. At 15 mg/kg/day, increases in serum total bilirubin was noted in both sexes (up to 3-fold compared to controls).

There were gross and microscopic changes at 300 mg/kg/day (both sexes) in animals killed early in Study Weeks 7 and 11, respectively. Gallbladder distention with abundant bile content was noted grossly in both animals but did not correlate with any specific histomorphologic changes. Yellowish discoloration of the mucosae was also noted in the male. Histomorphologic changes in the male consisted of seminiferous tubule degeneration correlating grossly with pale and flaccid testes, pigmentation (hemosiderin) in the liver (sinusoidal cells) and bone marrow, focal inflammation of the duodenum, and myeloid hyperplasia in the bone marrow. Pigmentation (hemosiderin) was seen in the bone marrow. At 300/100-mg/kg/day distended gallbladder with abundant bile content was seen in all dogs. In addition, 2 of 3 males at this dose had yellowish discoloration of the gingival mucosae and white adipose tissue and/or the aorta (without any specific histomorphologic correlate in the tissues examined).

Mean liver weights (without a specific histomorphological correlate) were increased in both sexes at 300/100 mg/kg/day only, and dose-dependent decreases in mean testicular weights at was seen ≥ 15 mg/kg/day. There were histomorphologic changes in the liver, the gallbladder and the testes at ≥ 15 mg/kg/day, and the spleen, bone marrow, and the epididymides at 300/100 mg/kg/day (see later text). In the liver, there was very slight to slight pigmentation in sinusoidal cells (hemosiderin) in both sexes at 300/100 mg/kg/day only. Very slight microlithiasis was noted occasionally in the lumina of larger bile ducts at ≥ 15 mg/kg/day. Very slight microlithiasis was also observed in the lumen of the gallbladder in both sexes at 300/100 mg/kg/day and in 1 female at 15 mg/kg/day. Both in the bile ducts and in the gallbladder, the luminal microlithiasis was not associated with any alteration of the tissues. At 300/100 mg/kg/day, there was increased pigmentation (hemosiderin) in the spleen in males, whereas increased extramedullary erythropoiesis was observed in both sexes. Hyperplasia of the erythroid lineage was observed in the bone marrow from a single male at 300/100 mg/kg/day. There was degeneration of testicular seminiferous tubules at ≥ 15 mg/kg/day that was dose-dependent in incidence and grade (very slight in 1 male at 15 mg/kg/day; moderate to marked in 3 males at the 300/100 mg/kg/day), correlated with decreases in testicular weights, and at higher grades coincident with decreased amounts of sperm in the lumina of the otherwise unaltered epididymidal ducts.

The mean liver grazoprevir concentrations (sexes combined) were 4.82 μM at 5 mg/kg/day at the 3-month interim necropsy, and 6.14, 39.3, and 136 μM at 5, 15, and 100 mg/kg/day, respectively, at the final necropsy. Liver grazoprevir concentrations in control animals were below the LLQ.

In the 2 animals that were killed early at 300 mg/kg/day, the individual toxicokinetic parameters following the last administration of grazoprevir in Study Week 7 or 11 were comparable to those obtained in surviving animals at this dose level (AUC_{0-24 hr} of 3610/3240 $\mu\text{M}\cdot\text{hr}$, C_{max} of 171/149 μM , and T_{max} of 4.0/8.0 hr in the male/female, respectively). At the time of sacrifice, the individual liver grazoprevir concentrations were 363/356 μM in the male/female, respectively.

High inter-animal variability was observed at 100 mg/kg/day and 300 mg/kg/day. There were no substantial sex-related differences in mean systemic exposure (AUC_{0-24 hr}) and in mean C_{max} values. On Day 1 and in Weeks 4 and 12, mean systemic exposure was greater than dose proportional between 5 mg/kg/day and 15 mg/kg/day, and mean C_{max} was approximately dose proportional. On

Day 1 and in Week 12, mean systemic exposure and mean C_{max} were less than dose proportional between 15 mg/kg/day and 300 mg/kg/day. Mean systemic exposure and mean C_{max} were similar on Day 1 and in Weeks 4 and 12 at 5 mg/kg/day and 15 mg/kg/day, and on Day 1 and in Weeks 3 and 12 at 300 mg/kg/day, suggesting that steady state in toxicokinetics was attained on Study Day 1. Mean systemic exposure and mean C_{max} values at 100 mg/kg/day were similar in Study Weeks 16 and 26, and similar to the values obtained at 300 mg/kg/day.

All grazoprevir concentrations in plasma from control group animals were below the LLQ (LLQ = 0.013/0.014 µM) on Study Day 1 and in Study Weeks 4 and 12. There were no substantial (*i.e.*, greater than 2-fold) sex-related differences in mean liver concentrations. The mean liver concentrations at 5 mg/kg/day in Study Weeks 14 and 39 (interim and final necropsy, respectively) were similar. In Study Week 39, the mean liver concentration at 100 mg/kg/day was approximately 3-fold greater than the mean liver concentration at 15 mg/kg/day, which was approximately 6-fold greater than the mean liver concentration at 5 mg/kg/day. All grazoprevir concentrations in liver from control group animals were below the LLQ (LLQ = 0.104 µM) in Study Week 39.

Based on these results, the NOEL was considered to be 5 mg/kg/day (AUC_{0-24 hr} = 69.3 ± 6.0 µM.hr). The Applicant goes on to say that at 15 mg/kg/day, the bilirubin increase was of slight severity, had not progressed with treatment duration, was not associated with changes in any other biomarkers of hepatobiliary toxicity (AST, ALT, ALP increases) and did not correlate with histomorphologic changes. The lumen microlithiasis in the gallbladder and larger bile ducts was considered of minimal toxicological significance based on the very slight severity and the lack of histomorphologic changes in the gallbladder or liver. Once again the Applicant argues that the degeneration of the testicular seminiferous tubules characterised by loss of germ cell population confined to postspermatogonial cell types was also considered of minimal toxicological significance, based on its very slight severity, and expected reversibility (Ref. 4.3: 03RKQY Creasy Dianne M. Pathogenesis of male reproductive toxicity. Toxicological Pathology 2001;29(1):64-76). See over-all Assessor toxicology comments on recovery. Therefore, the NOAEL was considered to be 15 mg/kg/day (AUC_{0-24 hr} = 367 ± 65.0 µM.hr), which provides a systemic plasma exposure (AUC_{0-24 hr} = 367 µM.hr) approximately 186X over the grazoprevir exposure in HCV-infected patients (100 mg). There were no test article-related changes at 5 mg/kg/day. This dose provides a systemic plasma exposure (AUC_{0-24 hr} = 69.3 µM.hr) approximately 35X over the grazoprevir exposure in HCV-

Elbasvir

Elbasvir was investigated in a number of repeat-dose toxicity studies in rats and dogs of duration up to 6 months and 9 months, respectively. Additionally, a 1-month range-finding study in rasH2 wild-type mice was conducted to support dose selection for a potential short-term carcinogenicity study in rasH2 transgenic mice. The high dose was generally the limit dose according to ICH M3 (R2) guidance, 1000 mg/kg/day. Routine antemortem parameters were measured.

Rodent

One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice.

Jic:CB6F1-nonTgrasH2@jcl mice were given 10, 50, 300, and 1000 mg/kg/day elbasvir and 1 control group of 10/5 females and 10/5 males that received the vehicle only. There were no effects. The NOEL was considered to be 1000 mg/kg/day (AUC_{0-24 hr} 151 ± 15.1 µM.hr) approximately 63X over the human exposure in HCV-infected patients (50 mg).

There were no substantial sex-related differences in mean systemic exposure (AUC_{0-24 hr}) or mean C_{max} at all doses. Mean systemic exposure and C_{max} were approximately dose-proportional between

10 mg/kg/day and 50 mg/kg/day, and less than dose-proportional across 50, 300, and 1000 mg/kg/day. *In vitro*, in mouse hepatocytes, only M2 was detected. In general, there were no substantial sex-related differences in mean liver concentrations at 4, 8, and 24 hours post dose, except at 1000 mg/kg/day at 24 hours where mean liver level was approximately 11.5-fold greater in males (correlated with approximately 21-fold greater mean 24-hour plasma level in males due to slower elimination), and the mean concentrations increased with the dose.

Fourteen-Day Oral Toxicity Study in Rats with Micronucleus and Functional Observational Battery Assays

Crl:WI(Han) rats were given 100 mg/kg/day, 300 mg/kg/day, or 1000 mg/kg b.i.d. (total daily dose: 2000 mg/kg) or vehicle only. In addition to routine endpoints, a FOB assay was performed after the first dose in male rats, a micronucleus assay was performed in both sexes (see Section 4.3) and liver elbasvir concentrations were determined in samples at all doses. At 1000 mg/kg b.i.d. white discoloration of the faeces (thought, most likely due to the faecal elimination of non-absorbed test article). Compared to controls, decreases in body weight gain (-19% and -25% in females and males, respectively) with transient decreased food consumption in both sexes, and increases in neutrophils (slight; +60%) in females only and serum cholesterol (very slight, +36%) in males only, were noted. At 300 mg/kg/day decreased body weight gain (-14%) and increase in neutrophils (+71%) in males only were seen. Overall, these antemortem changes were considered minimal, without a histomorphologic correlate and so were of minimal toxicological significance. There were no test article-related postmortem findings. There were no test article-related variations in the FOB parameters. Based on these results, the NOEL was considered to be 300 mg/kg/day in females and 100 mg/kg/day in males. However, the changes observed in the study were considered of minimal toxicological significance and so the NOAEL was considered to be 1000 mg/kg administered twice daily (AUC_{0-24 hr}: 21.3 ± 2.15 µM.hr), approximately 9X over the elbasvir exposure in HCV-infected patients (50 mg).

There were no substantial sex-related differences in mean systemic exposure (AUC_{0-24 hr}) or mean C_{max} at 300 mg/kg/day and 1000 mg/kg b.i.d. Mean systemic exposure and C_{max} were less than dose proportional between 300 mg/kg/day and 1000 mg/kg b.i.d. Mean elbasvir liver concentrations increased in a less than dose-proportional manner between 100 mg/kg/day and 1000 mg/kg b.i.d.

Three-Month Oral Toxicity Study in Rats

Crl:WI(Han) rats were given 50, 300, or 1000 mg/kg/day of elbasvir or vehicle only. Transient post dosing salivation was noted females at ≥300 mg/kg/day and in males at all doses. Due to the minimal and transient nature of this change generally observed immediately post dose, it was considered to be a palatability issue rather than a centrally-mediated effect of the compound and consequently of minimal toxicological significance. There were no effects on gross observations, organ weight, or histomorphologic changes. The NOAEL was considered to be 1000 mg/kg/day (AUC_{0-24 hr} 17.3 ± 1.23 µM.hr).

There were generally no substantial sex-related differences in mean systemic exposure (AUC_{0-24 hr}) or mean C_{max} at all doses. Mean systemic exposure and C_{max} were less than dose-proportional across all doses. Mean elbasvir liver concentrations were similar in females and males at all doses, and increased in a dose related manner.

Six-Month Oral Toxicity Study in Rats

Crl:WI(Han) rats were given 30, 300, or 1000 mg/kg/day of elbasvir or vehicle only. Antemortem changes were observed at all doses. In both sexes salivation, very slight to slight (non-dose related)

increases in urine volume (up to +71%) generally with very slight decreases in urine specific gravity (up to -1.2%), were observed. In addition, in males at ≥ 300 mg/kg/day decreased body weight gain (up to -17% at 1000 mg/kg/day) associated with occasional decreased food consumption was noted. This had no impact on the physical condition of the animals. Based on their nature and in the absence of correlating histomorphological changes, these findings were considered of minimal toxicological significance. The NOEL was considered to be 30 mg/kg/day. However, the changes noted were of minimal toxicological significance, and so the NOAEL was considered to be 1000 mg/kg/day (AUC_{0-24 hr}: 21.9 ± 1.16 $\mu\text{M}\cdot\text{hr}$) approximately 9X over the elbasvir exposure in HCV-infected patients (50 mg).

Mean systemic exposure and C_{max} were less than dose-proportional across all doses. Mean elbasvir liver concentrations were approximately 2- to 3.4-fold greater in males than in females at all doses, and increased in a dose related manner. Additionally, the toxicokinetic parameters were comparable to previous studies, when similar doses were compared.

Dog

One-Month Oral Toxicity Study in Dogs with a Three-Month Treatment-Free Period

Female beagle dogs were given 1000 mg/kg/day of elbasvir. Six elbasvir-treated dogs were necropsied following an approximately 3-month treatment-free period. Originally, a 6-month treatment-free period was scheduled in the study. However, elbasvir-related changes observed after the 1-month dosing period had resolved following the 3-month treatment-free period, and the 6 remaining elbasvir-treated dogs originally planned to be necropsied following the 6-month treatment-free period were returned to the animal colony without post-mortem examinations.

White discoloration of the faeces, emesis, and body weight loss was noted during the dosing period. Decreased skin turgor and/or thin appearance were observed in 2 animals and this correlated with the body weight loss. These ante-mortem changes were limited to the 1-month dosing period, and resolved in the 3-month treatment-free period. Postmortem changes noted at the end of the 1-month dosing period consisted of vacuolation in the gastrointestinal-associated lymphoid tissues of the small intestine (Peyer's patches) and large intestine, as well as in the spleen and/or lymph nodes. The changes characterised by the presence of an increased number of large vacuolated macrophages, most prominent within the follicular areas of the lymphoid tissues and ultrastructurally by the presence of an increased number of lysosomes containing myelin figures in affected macrophages were consistent with phospholipidosis. There were no postmortem findings noted at necropsy, histomorphological or TEM examination at the end of the 3-month treatment-free period, indicating reversibility of the elbasvir-related phospholipidosis.

Three-Month Oral Toxicity Study in Dogs

Beagle dogs were given 2, 25, or 1000 mg/kg/day of elbasvir or vehicle only. White/pale brown discoloration of the faeces in all animals at 1000 mg/kg/day, most likely due to the fecal elimination of non-absorbed test article.

There were no gross observations or organ weight changes. An increased number of large vacuolated macrophages in the gut-associated lymphoid tissue of the small intestine (Peyer's patches) and/or large intestine, lymph nodes, and/or spleen in females and males at 1000 mg/kg/day and in 1 male at 25 mg/kg/day, were observed. The vacuolation was ultrastructurally consistent with lysosomal phospholipid accumulation (phospholipidosis). The severity of these changes ranged from very slight to slight at 1000 mg/kg/day and was very slight in males at 25 mg/kg/day. The applicant stated the vacuolation observed, was not associated with any other effects in the lymphoid tissues and in particular not with lymphoid depletion or inflammation/necrosis, or with any haematological changes

(i.e., no decreased white blood cell or lymphocyte counts, no abnormal circulating blood cells). Therefore, based on the very slight to slight severity, the absence of other effects in the lymphoid tissue or in circulating blood cells, this histomorphologic finding was considered of minimal toxicological significance. Elbasvir-related phospholipidosis was shown to be reversible in a 1-month oral toxicity study in dogs with a 3-month treatment-free period (see above). Based on these results, the NOEL was considered to be 2 mg/kg/day. The changes noted in the study were of minimal toxicological significance and the NOAEL was considered to be 1000 mg/kg/day ($AUC_{0-24 \text{ hr}}: 19.3 \pm 2.19 \mu\text{M}\cdot\text{hr}$) approximately 7X over the elbasvir exposure in HCV-infected patients (50 mg).

There were generally no substantial sex-related differences in mean systemic exposure ($AUC_{0-24 \text{ hr}}$) or mean C_{max} . Mean systemic exposure was greater than dose-proportional between 2 and 25 mg/kg/day and less than dose-proportional between 25 and 1000 mg/kg/day while mean C_{max} was approximately dose-proportional between 2 and 25 mg/kg/day and less than dose-proportional between 25 and 1000 mg/kg/day. Mean systemic exposure and C_{max} were not substantially different on Study Day 1 and in Study Weeks 5 and 13, suggesting that steady state toxicokinetics were attained after the first dose. All elbasvir liver concentrations were below the LLQ at 2 mg/kg/day. Mean elbasvir liver concentrations at 25 and 1000 mg/kg/day were greater in males than in females, and increased as the dose increased.

Nine-Month Oral Toxicity Study in Dogs

Beagle dogs were given 5, 25, or 1000 mg/kg/day of elbasvir or vehicle only. At 1000 mg/kg/day sporadic emesis, frequent discoloration of faeces and occasional presence of substance in pan (mainly white or pale brown for both observations and most likely due to presence of test article in faeces) were noted. At the beginning of the study, transient or intermittent body weight losses, and occasional decreases in food consumption was seen. The applicant goes onto say that these changes had no major impact on the general condition of the animals, no histomorphological correlated and are considered of minimal toxicological significance.

There were no test article-related changes in clinical pathology parameters or at in electrocardiographic and ophthalmologic examinations.

In both sexes vacuolation was observed at 1000 mg/kg/day (8/8 dogs; generally very slight to slight) and 25 mg/kg/day (2/8 dogs; very slight). The applicant goes onto say that the vacuolation was generally observed in the follicular areas of the lymphoid tissues, in the gut-associated lymphoid tissue of the stomach, small intestine (Peyer's patches), large intestine, in solitary lymphoid follicle in the gallbladder and/or in lymph nodes. The nature and severity of the changes (previously shown to be ultrastructurally consistent with phospholipidosis) were generally comparable to those previously observed in dogs following administration of elbasvir during 3 months indicating no progression of this finding with longer duration of dosing. No other effects were seen, in particular no lymphoid depletion, were observed in the lymphoid tissue. Therefore, similarly to the 3-month toxicity study, these histological findings are considered of minimal toxicological significance. Based on these findings, the NOEL was considered to be 5 mg/kg/day. However, the changes noted in this study are of minimal toxicological significance, and the NOAEL was therefore considered to be 1000 mg/kg/day ($AUC_{0-24 \text{ hr}}: 16.6 \pm 3.06 \mu\text{M}\cdot\text{hr}$) approximately 7X over the elbasvir exposure in HCV-infected patients (50 mg).

There were generally no substantial sex-related differences in mean systemic exposure ($AUC_{0-24 \text{ hr}}$) or mean C_{max} at all doses. Mean systemic exposure and mean C_{max} were approximately dose-proportional between 5 mg/kg/day and 25 mg/kg/day and less than dose-proportional between 25 mg/kg/day and 1000 mg/kg/day. Generally, mean systemic exposure and C_{max} were not substantially

different on Study Day 1 and in Study Weeks 4, 13, and 26 at all doses, suggesting that steady state toxicokinetics were attained after the first dose.

Genotoxicity

Grazoprevir

Grazoprevir was not genotoxic in the microbial mutagenesis assay and was negative in the chromosomal aberration assay in Chinese Hamster Ovary cells. The top concentrations used in these studies were the limit concentration or limited by cytotoxicity, respectively.

Grazoprevir was negative in the *in vivo* micronucleus assay in rats up to the limit dose of 1000 mg/kg/day and up to the dose resulting in the maximum achievable exposure, 200 mg/kg b.i.d., total daily dose, 400 mg/kg/day. 400 mg/kg/day provides a plasma systemic exposure ($AUC_{0-24\text{ hr}} = 212\text{ }\mu\text{M}\cdot\text{hr}$) 108X over the grazoprevir exposure in HCV-infected patients (100 mg).

Elbasvir

Elbasvir was not genotoxic in the microbial mutagenesis assay, and was negative in the assay for chromosomal aberrations in Chinese Hamster ovary cells and in an exploratory assay for micronucleus induction in Chinese Hamster ovary cells. The top concentrations used in these studies were the limit concentration or limited by solubility.

Elbasvir was negative in *in vivo* micronucleus assays in rats up to 1000 mg/kg b.i.d. (total daily dose: 2000 mg/kg). The high dose was the limit dose, 1000 mg/kg administered twice daily.

Carcinogenicity

In accordance with ICH S1A guidance, carcinogenicity studies were not conducted given that the human use of grazoprevir and elbasvir is less than 6 months in duration and that there is an absence of a genotoxic signal in the battery of genotoxicity studies and no evidence of a proliferative signal in the chronic toxicity studies. The CHMP considered this as acceptable.

Reproduction Toxicity

Studies were conducted to determine the developmental and reproductive effects of grazoprevir in rats and rabbits. An IV formulation was developed for the rabbit embryo-fetal developmental study as no oral formulations achieved reproducible systemic plasma exposures at multiples of human exposure.

Fertility and early embryonic development

Grazoprevir

An oral fertility study in sexually mature female and male rats was performed at doses up to 200 mg/kg b.i.d., dose resulting in the highest achievable exposure.

There were no effects on female and male fertility parameters and in general toxicity parameters and the NOEL for both female and male fertility parameters and general toxicity parameters was considered to be 200 mg/kg b.i.d. (total daily dose: 400 mg/kg/day). Based on these findings, the NOEL for female/male fertility parameters in rats and for general toxicity parameters in female and male rats was 200 mg/kg b.i.d. ($AUC_{0-24\text{ hr}} = 212 \pm 63.0\text{ }\mu\text{M}\cdot\text{hr}$ based on Study Week 4 toxicokinetic parameters from the 1-month oral toxicity study in rats).

Embryo-fœtal development

Grazoprevir

An oral developmental toxicity study in rats with prenatal evaluation was performed at doses up to 200 mg/kg b.i.d. (total daily dose: 400 mg/kg/day), dose resulting in the highest achievable systemic plasma exposure. There was no test article-related maternal or developmental toxicity and the NOEL for both maternal and developmental toxicity was 200 mg/kg b.i.d. This dose provides a plasma systemic exposure ($AUC_{0-24 \text{ hr}} = 217 \mu\text{M}\cdot\text{hr}$) approximately 110X over the grazoprevir exposure in HCV-infected patients (100 mg).

In a preliminary oral developmental toxicity study in rabbits, pregnant rabbits were given grazoprevir at doses of 50 mg/kg/day, 200 mg/kg/day, 200 mg/kg/b.i.d. (total daily dose: 400 mg/kg). The exposure was limited to $3.61 \mu\text{M}\cdot\text{hr}$, and according to the Applicant, excessive vehicle-related toxicities were observed in the control and at 200-mg/kg/b.i.d, due to total volume of PEG administered (2 mL/kg/day).

As a consequence, an intravenous nanosuspension was developed for the studies in rabbits. In a preliminary intravenous developmental toxicity study, dose limiting maternal toxicity was achieved at the 200 mg/kg/day with mortality of 2 dams and discontinuation of this dose-group.

In the definitive intravenous developmental rabbit (Dutch Belted) toxicity study, animals were given 25, 50, and 100 mg/kg/day nanosuspension of grazoprevir or vehicle only by IV injection. The incidence of foetuses with a cervical rib at 100 mg/kg/day group was slightly above the highest historical control group incidence (litter mean of 2.9% [3 foetuses in 1 litter] and 1%, respectively). The Applicant argued that due to this low incidence and no other effects on skeletal morphology, this finding was considered non-test article-related.

The maternal and developmental NOEL was considered to be 100 mg/kg/day. This dose provides a plasma systemic exposure ($AUC_{0-24 \text{ hr}} = 76.1 \mu\text{M}\cdot\text{hr}$) approximately 39X over the grazoprevir exposure in HCV-infected patients (100 mg). The exposure cover over the clinical exposure (100 mg) at 50 mg/kg/day ($AUC_{0-24 \text{ hr}} = 24.4$) is approximately 12x, which is an acceptable safety margin.

On GD 15, mean systemic exposure ($AUC_{0-24 \text{ hr}}$) was greater than dose proportional between 25 mg/kg/day and 100 mg/kg/day and, mean C_{max} was greater than dose proportional between 25 mg/kg/day and 50 mg/kg/day and approximately dose proportional between 50 mg/kg/day and 100 mg/kg/day.

Elbasvir

Oral Fertility Study in Female and Male Rats

The potential effects of elbasvir on the fertility of F0 female and male rats were evaluated following once daily oral administration for 15 days prior to cohabitation, during cohabitation, and through GD 7 for the females or until the day prior to scheduled sacrifice (approximately 6 weeks total) for the males. CrI:WI(Han) sexually mature rats were assigned to 4 groups of 20 rats per sex each that received 50, 300, or 1000 mg/kg/day of elbasvir or vehicle only. At 300, or 1000 mg/kg/day there were transient decreases in bodyweight and food consumption. Decreased sperm count per gram cauda epididymis was seen at 1000 mg/kg/day (14% below controls); however, there were no effects on reproductive parameters as assessed by mating performance, fertility, embryonic/fetal survival, mean testicular weight, and sperm motility. There was no reproductive toxicity at 1000 mg/kg/day in females or animals of both sexes at 50 and 300 mg/kg/day.

Based on these findings, the NOEL for female fertility parameters was considered to be 1000 mg/kg/day. The NOEL for male fertility parameters was considered to be 300 mg/kg/day, based on the slight decrease in mean sperm count. Given the lack of any coincident effects on reproductive performance as assessed by mating performance, fertility, and embryonic/foetal survival, mean testicular weight, and sperm motility and of any histomorphological testicular changes in the chronic rat toxicity study (Study number: TT 12-6033) and also in the chronic dog toxicity study (Study number TT 12-6030), this finding was not considered adverse and the NOAEL for female/male fertility parameters was therefore considered to be 1000 mg/kg/day (AUC0-24 hr: $17.3 \pm 1.23 \mu\text{M}\cdot\text{hr}$, measured at Study Week 13 in the 3-month oral toxicity study in rats (study number: TT 11-6024), approximately 7X over the human exposure in HCV-infected patients (50 mg), respectively. The NOEL for general toxicity parameters was 50 mg/kg/day both sexes. However, the changes in general toxicity parameters were considered of minimal toxicological significance and the so the NOAEL was considered to be 1000 mg/kg/day.

Oral Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rats

From GD 6 through 20, females were given 50, 300, or 1000 mg/kg/day of elbasvir or vehicle only. At 1000 mg/kg/day a decrease in mean maternal body weight gain from GD 6 to 21 was noted (13% below controls), and from GD 6 to 21 adjusted for total foetal weight (19% below control). Based on its low severity, this decrease in maternal body weight is considered of minimal toxicological significance. There were no other effects. The NOEL for maternal toxicity was considered to be 300 mg/kg/day. However, the maternal changes noted in the study were of minimal toxicological significance and the NOAEL for maternal toxicity was considered to be 1000 mg/kg/day. The NOAEL for developmental toxicity was considered to be 1000 mg/kg/day (AUC0-24 hr: $21.8 \pm 1.79 \mu\text{M}\cdot\text{hr}$) approximately 9X over the human exposure in HCV-infected patients (50 mg).

Mean systemic exposure (AUC0-24 hr) and C_{max} were less than dose proportional across all doses. Additionally, mean AUC0-24 hr and C_{max} in pregnant rats were consistent with those achieved in non-pregnant rats at the same doses.

Oral Embryo-Foetal Developmental Toxicity and Toxicokinetic Study in Rabbits

The potential developmental toxicity of elbasvir was evaluated in rabbits following oral administration from GD 7 through 20. From GD 7 through 20, females were given 30, 100, or 1000 mg/kg/day of elbasvir, or vehicle only. There were no effects. Mean systemic exposure (AUC0-24 hr) was approximately dose-proportional across the 3 doses, while mean C_{max} was less than dose-proportional. The NOEL for both maternal and developmental toxicity was considered to be 1000 mg/kg/day (AUC0-24 hr: $39.4 \pm 9.20 \mu\text{M}\cdot\text{hr}$) approximately 17X over the human exposure in HCV-infected patients (50 mg).

Prenatal and postnatal development, including maternal function

Grazoprevir

The potential effects of grazoprevir on development, growth, behaviour, reproductive performance, and fertility of F1 generation in CrI:WI(Han) rats following oral administration of 25, 100, or 200 mg/kg b.i.d. (total daily dose: 400 mg/kg) to F0 females were evaluated. There were no changes in the F0 and F1 generation (postimplantation survival, pup external morphology, mortality, clinical signs, body weights, developmental signs (vaginal opening and preputial separation), behavioural tests (passive avoidance, auditory startle habituation, open-field motor activity), reproductive performance, or fertility) at any dose and the NOEL for both maternal toxicity in the F0 generation and developmental toxicity in the F1 generation was considered to be 200 mg/kg b.i.d. The highest mean

maternal plasma systemic exposure achieved at 100 mg/kg/day ($AUC_{0-24\text{ hr}} = 155\text{ }\mu\text{M}\cdot\text{hr}$) represents approximately 79X the human exposure in HCV-infected patients (100 mg).

Elbasvir

Oral Pre- and Postnatal Developmental Toxicity Study in Rats

The potential effects of elbasvir on development, growth, behaviour, reproductive performance, and fertility of the F1 generation following oral administration to F0 female rats from GD 6 through LD 20 were evaluated. From GD 6 through LD 20, females were given 50, 300 or 1000 mg/kg/day of elbasvir or vehicle only once daily by oral gavage.

There were no test article-related deaths or clinical signs in the F0 or F1 generations at any dose. In the F0 generation, there were no gross observations. At 1000 mg/kg/day, there were transient decreases in mean maternal body weight gain between GD 6 and 8 (47% below control) and mean food consumption on GD 8 (13% below control). There were no effects on body weights or food consumption at 50 or 300 mg/kg/day. In the F1 generation, there were no effects on post-implantation survival, pup external morphology, clinical signs, body weights, developmental signs (vaginal opening and preputial separation), behavioural tests (passive avoidance, auditory startle habituation, open-field motor activity), reproductive performance, or fertility at any doses.

The NOEL for maternal toxicity in the F0 generation was considered to be 300 mg/kg/day; however, the changes noted in the F0 generation were not considered adverse and so the NOAEL was considered to be 1000 mg/kg/day ($AUC_{0-24\text{ hr}}: 21.8 \pm 1.79\text{ }\mu\text{M}\cdot\text{hr}$, measured in the oral embryo-fetal developmental toxicity and toxicokinetic study in rats approximately 9X over the human exposure in HCV-infected patients (50 mg)). In the F1 generation, there were no effects on development, growth, behaviour, reproductive performance, or fertility, so the NOEL for developmental toxicity in the F1 generation was considered to be 1000 mg/kg/day.

Local Tolerance

Local tolerance studies (bovine corneal opacity and permeability assay (BCOP) and acute dermal irritation/corrosion study in rabbits) were conducted. In a bovine corneal opacity and permeability assay, grazoprevir and elbasvir were classified as a non-irritant. Grazoprevir and elbasvir were not dermal irritants in New Zealand White Rabbits.

Other toxicity studies

Antigenicity

Grazoprevir

There were no observations or changes considered to be due to potential antigenicity induced by grazoprevir or elbasvir in the routine repeat-dose toxicity studies. Therefore, no antigenicity evaluations were conducted.

Immunotoxicity

The Applicant states that there were no indications of immunotoxic potential as outlined in Section 2.1.1 of the ICH guideline Immunotoxicity Studies for Human Pharmaceuticals (S8, Step 4), such as notable haematological changes (i.e. in leucocytes or lymphocytes), alterations in organ weight and/or histology of the spleen, thymus, lymph nodes or bone marrow, changes in serum globulins indicative

changes in serum immunoglobulins, increased incidence of infections, or increased occurrence of tumours. As stated in Section 2.2, Weight of Evidence Review, the triggers for additional immunotoxicity studies would be a finding of significant magnitude in a single area or findings from two or more factors. Since the toxicity studies conducted with elbasvir / grazoprevir in rats and dogs lacked triggers of immunotoxicity or any findings indicating immunotoxic potential, additional studies were not conducted.

A Local Lymph Node Assay in Mice (LLNA) in mice was conducted in support of the occupational safety program. In this study, elbasvir did not cause dermal irritation and was not a dermal sensitizer.

Dependence

The results of the QWBA in non-pigmented and pigmented rats following single oral administration of [¹⁴C]-grazoprevir at 50 mg/kg and elbasvir demonstrated that they does not readily cross the blood brain barrier. Additionally, there was no indication that they have a pharmacologic profile, including off-target profile, consistent with drug abuse liability potential, and there was no evidence in the routine repeat dose toxicity studies of psychoactive effects (*i.e.*, sedative or stimulant effects). As such, no animal abuse potential studies were conducted.

Metabolites

Grazoprevir

No circulating metabolites were detectable in human plasma. Therefore, the ICH M3 (R2) guidance requirements relative to metabolite safety assessment have been met with grazoprevir and no studies were conducted with any individual metabolites. Additionally, all metabolites of grazoprevir that were present in the human excreta are also formed in rats, rabbits or dogs, with the exception of a minor (4% of the dose) metabolite, M14, detected in human faeces only. This minor metabolite M14 is proposed to be a hydroxylated product of the metabolite M5, which was found in rat bile and faeces and rabbit faeces.

Elbasvir

No circulating metabolites were detectable in human plasma and all human metabolites of elbasvir (identified *in vitro* in liver microsomes or present in the excreta) are also formed in rats or dogs. No further studies were conducted.

Studies on impurities

Grazoprevir and Elbasvir

No nonclinical studies were conducted with any individual impurities. All impurities were qualified by the presence in batches evaluated in the nonclinical toxicity studies or by levels allowable under ICH guideline Impurities in New Drug Substances [ICH Q3A (R2)]. Additionally, impurities have been assessed for potential mutagenicity according to ICH M7 guidance.

Phototoxicity

Due to grazoprevir light absorbance in the 290 to 700 nm range, a phototoxicity study was conducted in pigmented rats at 50 mg/kg/day and 200 mg/kg b.i.d. There were no findings in the eyes and/or skin indicating that grazoprevir does not pose a risk for phototoxicity.

Due to elbasvir light absorbance in the 290 to 700 nm range, a phototoxicity study was conducted in pigmented rats at 100 mg/kg/day and 1000 mg/kg/day. There were no findings in the eyes and/or skin indicating that elbasvir does not pose a risk for phototoxicity.

Combination Toxicity Studies

Beagle dogs given elbasvir as a 25-mg/kg/day formulation and/or grazoprevir as a 5-mg/kg/day formulation, as follows: grazoprevir followed by elbasvir; grazoprevir followed by elbasvir vehicle; grazoprevir vehicle followed by elbasvir. The fourth group was given grazoprevir vehicle followed by elbasvir vehicle. Mortality, clinical observations, body weights, food consumption, electrocardiographic and ophthalmology examinations, and clinical and anatomic pathology evaluations were conducted. Elbasvir and/or grazoprevir concentrations in plasma and liver homogenate were determined.

There were no ante-mortem changes. In females lymphoid tissue vacuolation was observed in the lymph nodes and the gut-associated lymphoid tissue of the small (Peyer's patches) and/or large intestine at 25/5-mg/kg/day elbasvir/grazoprevir and at 25/0-mg/kg/day elbasvir/grazoprevir and was attributed to elbasvir administration

In females lymphoid tissue vacuolation was observed in the lymph nodes and the gut-associated lymphoid tissue of the small (Peyer's patches) and/or large intestine at 25/5-mg/kg/day elbasvir/grazoprevir and at 25/0-mg/kg/day elbasvir/grazoprevir and was attributed to elbasvir administration. These changes were morphologically consistent with phospholipidosis, and were consistent with findings noted in toxicity studies conducted in dogs with elbasvir. This finding was considered of minimal toxicological significance based its low severity (Study number TT 14-1031) and the lack of other effects in the lymphoid tissue (i.e., no lymphoid depletion) and of haematological changes. Additionally, the elbasvir-related phospholipidosis was shown to be reversible in a 1-month oral toxicity in dogs with a 3-month treatment-free recovery period (Study number: TT 14-1031). There was no evidence of a toxicologically significant difference in the post-mortem results when elbasvir and grazoprevir were combined in this study. The NOAEL for the elbasvir / grazoprevir combination was considered to be 5/25 mg/kg/day (approximately 31/3X in HCV-infected patients, 100 mg)

There was no evidence of interaction on the toxicokinetic profiles of each compound as mean systemic exposures and mean C_{max} of elbasvir and grazoprevir were similar at all doses.

2.3.5. Ecotoxicity / environmental risk assessment

Grazoprevir

For grazoprevir, a slight risk to the sediment compartment cannot be ruled out. After refinement using Simpletreat, the PEC/PNEC was marginally above 1.

Elbasvir

For elbasvir, the risk to the sediment compartment has not been fully evaluated. The Applicant is has committed to refine the PECSW using the outcome of the ready biodegradability study (OECD 301) as input for Simpletreat instead of the outcome of the OECD 314 study. The study on aerobic and anaerobic transformation in soil (OECD 307) revealed a DT₅₀ > 1,000 days in soil. Based on the results of the provided OECD 307 study elbasvir should be classified as very persistent in soil.

The applicant committed to submit and updated ERA with data from the OECD 225 (sediment toxicity test with *Lumbriculus variegatus*) and OECD 218 (repeat toxicity test with the chironomid, (*Chironomus riparius*)) studies post-authorisation.

Table 5. Grazoprevir - Summary of main study results

Substance (INN/Invented Name): (1aR,5S,8S,10R,22aR)-N-[(1R,2S)-1-[(Cyclopropylsulfonamido)carbonyl]-2-ethenylcyclopropyl]-14-methoxy-5-(2-methylpropan-2-yl)-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8H-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-b]quinoxaline-8-carboxamide hydrate					
CAS-number (if available): 1350462-55-3					
PBT screening			Result		Conclusion
Bioaccumulation potential- log K _{ow}	OECD107	> 4.00 (pH 5) <u>3.72 (pH 7)</u> 1.71 (pH 9)		There is some potential for bioconcentration in aquatic species at an environmentally relevant pH	
PBT-assessment:					
Fish Bioconcentration, steady state, total residues	OECD 305	BCF _{ss, total residues} = 3.09 (low concentration) BCF _{ss, total residues} = 7.62 (high concentration)		Species: <i>Lepomis macrochirus</i> Is not expected to bioaccumulate in fish	
Phase I					
Calculation	Value	Unit		Conclusion	
PEC _{surfacewater} default F _{pen} used DOSE _{ai} = 100 mg/inh-day	0.5	µg/L		> 0.01 threshold (Y)	
Other concerns (e.g. chemical class)	-	-		(N)	
Phase II Physical-chemical properties and fate					
Study type	Test protocol	Results		Remarks	
Dissociation Constant (pKa)		<2.0 4.68			
Water solubility	OECD 105	0.07 mg/L (pH 5) 13.6 mg/L (pH 7) 85.9 mg/L (pH 9)		Column elution Column elution Shake-flask	
Adsorption-Desorption Constant (log K _{oc})	OECD 106	4.01 (DU Soil) 3.97 (RMN Soil) 3.88 (MSL Soil) 3.41 (ROE Soil) 3.34 (Wareham Sludge) 3.15 (New Bedford Sludge)		3.31% oc 0.93% oc 1.98% oc 3.31% oc Low mobility in soil. < 10,000 Phase II Tier B trigger	
Aerobic Biodegradation in Sludge	OECD 314B	k _e = 0.0355 days ⁻¹ DT ₇₅ = 39 days DT ₉₀ = 65 days Overall half-life = 20 days (biotic)		The degradation in the biotic sludge is due to biodegradation.	
Aerobic Biodegradation in Sediment/Water (total system)	OECD 308	Taunton River: DT ₅₀ = 76.25 days Weweantic River: DT ₅₀ = 59.09 days			
Phase IIa Effect studies					
Study type	Test protocol	Endpoint	value	Unit	Remarks
Algae, toxicity to Green Algae	OECD 201		NOEC = 10 LOEC = >10	mg/L	Species: <i>Pseudokirchneriella subcapitata</i> Additional testing to further define the EC values was not conducted since the highest concentration tested

					(10 mg/L) approximates the solubility limit of MK-5172 under testing conditions.
<i>Daphnia</i> sp. Reproduction Test	OECD 211		NOEC= 5.0 LOEC= 10	mg/L	Species: <i>Daphnia magna</i>
Fish, Early Life Stage Toxicity Test/ <i>Species</i>	OECD 210		NOEC= 0.98	mg/L	Species: <i>Pimephales promelas</i>
Activated Sludge Respiration Inhibition Test	OECD 209		EC ₅₀ >1000 EC ₁₀ = 1.3 (NOEC)	mg/L	
Midge Emergence	OECD 218		NOEC= 6.4	mg/kg	Species: <i>Chironomus riparius</i>

PNECmicro-organisms=0.13 mg/L; PNECSW=0.098 mg/L; PNECGW=0.5 mg/L; PNECSED=0.246 mg/kg; PECGW=0.12 µg/L; PECSED=0.08 mg/kg

Table 6. Elbasvir - Summary of main study results

Substance (INN/Invented Name): Dimethyl N,N'-([[(6S)-6-phenylindolo[1,2-c][1,3]benzoxazine-3,10-diyl]bis{1H-imidazole-5,2-diyl-(2S)-pyrrolidine-2,1-diyl}[(2S)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate					
CAS-number (if available): Not available					
PBT screening		Result		Conclusion	
Bioaccumulation potential- log K _{ow}	OECD107	6.54		Potential PBT (Y)	
PBT-assessment: The logKow value for elbasvir was < 6.54, therefore screening for PBT is required as it meets the criteria for classification as a PBT compound.					
Fish Bioconcentration, steady state, total residues	OECD 305	BCF = 73.2 (low concentration) BCF = 48.2 (high concentration)		Unlikely to bioconcentrate in fish and therefore does not meet the criteria to be PBT compound	
Phase I					
Calculation	Value	Unit		Conclusion	
PEC _{surfacewater} , default Fpen used. DOSEai = 50 mg/inh-day.	0.25	µg/L		> 0.01 threshold (Y)	
Other concerns (e.g. chemical class)				(N)	
Phase II Physical-chemical properties and fate					
Study type	Test protocol	Results		Remarks	
Dissociation Constant (pKa)		4.81 5.88			
Water solubility	OECD 105	pH 5 = 0.05 mg/L pH 7 = 0.008 mg/L pH 9 = 0.02 mg/L			
Adsorption-Desorption	OECD 106	K _{oc} = DU Soil (3.14% oc): 5.08 RMN Soil (0.99% oc): 5.21 MSL Soil (1.74% oc): 5.24 ROE Soil (3.31% oc): 5.02 Wareham Sludge: 4.81 New Bedford Sludge: 4.48		Low mobility in soil. Exceeds the trigger of 10,000 Phase II Tier B risk assessment	
Aerobic Biodegradation in Sludge	OECD 314B	Half-life (biotic) = 45 days K _e = 0.0154 days			
Aerobic Biodegradation in Sediment/Water (total system)	OECD 308	Half-life = 43 days (Taunton River) Half-life = 91 days (Weweantic River)			
Phase IIa Effect studies					
Study type	Test protocol	Endpoint	value	Unit	Remarks
Algae, Growth Inhibition Test/ <i>Species</i>	OECD 201		NOEC: 81 µg/L	µg/L	Species: <i>Pseudokirchneriella subcapitata</i>
<i>Daphnia</i> sp. Reproduction Test	OECD 211		NOEC: 840 µg/L	µg/L	
Fish, Early Life Stage Toxicity Test/ <i>Species</i>	OECD 210		NOEC: 2.3 µg/L	µg/L	Species: <i>Pimephales promelas</i>
Activated Sludge, Respiration Inhibition Test	OECD 209		EC50 > 1000 mg/L The EC10: 271.9 mg/L	µg/L	No effects on emergence
Midge emergence (sediment-dwelling organisms)	OECD 218		NOEC: 50 mg/kg LOEC: 100 mg/kg		
Phase IIb Studies					
Aerobic and anaerobic transformation in soil	OECD 307	DT50 %CO ₂	DT50 > 1.000		Very persistent in soil

Soil Micro organisms: Nitrogen Transformation Test	OECD 216		EC50 > 10 mg/kg (NOEC = 10 mg/kg).	mg/kg	Below the guideline trigger value of 25% difference from the control after 28 days of exposure
Terrestrial Plants, Growth Test/ <i>Species</i> corn, oat, oilseed rape, perennial ryegrass, radish and soybean	OECD 208	NOEC: 25		mg/kg	most sensitive endpoint fresh shoot weight in the corn and oilseed rape
Earthworm, Acute Toxicity Tests	OECD 207	NOEC: 100 mg/kg		mg/kg	
Collembola, Reproduction Test	ISO 11267/ OECD 232	NOEC: 1000		mg/kg	

2.3.6. Discussion on non-clinical aspects

Grazoprevir and elbasvir were investigated as monotherapies in nonclinical toxicity studies including a battery of in vitro and in vivo genetic toxicity studies; repeat-dose oral toxicity studies of up to 1-month (elbasvir) or 3-months (grazoprevir) in mouse, 6 months in rats, and 9 months in dogs; and a series of developmental and reproductive toxicity studies. A 1-month oral grazoprevir/elbasvir combination dog toxicity study was conducted. Overall, the nonclinical monotherapy programs did not reveal any overlapping toxicity between grazoprevir and elbasvir and there was no evidence of interaction on the toxicological or toxicokinetic profiles of each compound when they were co-administered in dogs.

Safety margins have been calculated using data from clinical protocols PN060, PN061, and PN068 (pooled geometric mean steady state AUC_{0-24 hr}) are 1.97 µM.hr and 2.38 µM.hr, respectively; corresponding C_{max} values are 0.23 µM and 0.15 µM.

In the reproductive toxicity studies in rats conducted up to 200 mg/kg b.i.d., there were no changes in female and male fertility parameters for Grazoprevir (approximately 108X over the grazoprevir exposure in HCV-infected patients (100 mg)). In addition teratogenicity was not observed in pregnant rats or rabbits administered grazoprevir during the period of organogenesis. In the definitive IV developmental rabbit toxicity study conducted at 25, 50, and 100 mg/kg/day, the maternal and developmental no-observed-effect level (NOEL) was ≥ 100 mg/kg/day (AUC_{0-24 hr} = 76.1 µM.hr represents approximately 39X grazoprevir exposure in HCV-infected patients). In the developmental study in rats with postnatal evaluation conducted at 25, 100 mg/kg/day, and 200 mg/kg b.i.d., there were no changes in the F0 and F1 generation at any dose (79X grazoprevir exposure in HCV-infected patients (100 mg) – compared to maternal exposure).

In the reproductive toxicity studies with elbasvir, female and male fertility was evaluated in sexually mature female and male rats at 50, 300, and 1000 mg/kg/day. No teratogenicity was observed following elbasvir administration during the period of organogenesis to pregnant rats or rabbits up to the limit dose, 1000 mg/kg/day (AUC_{0-24 hr} = 21.8 µM.hr and 39.4 µM.hr, respectively represent approximately 9X and 17X the elbasvir exposure in HCV-infected patients (50 mg), respectively). No effects on F1 generation in rats were observed when dams were administered elbasvir up to the limit dose, 1000 mg/kg/day during organogenesis and lactation. This dose provides a systemic plasma exposure, AUC_{0-24 hr} = 21.8 µM.hr (based on toxicokinetic results of the embryo-foetal developmental study in rats) approximately 9X over the elbasvir exposure in HCV-infected patients (50 mg).

Both Grazoprevir and elbasvir were shown to be non-genotoxic in a standard battery of genotoxicity studies. In accordance with ICH S1A guidance, carcinogenicity studies were not conducted given that the human use of elbasvir is less than 6 months in duration and that there is an absence of a genotoxic signal in the battery of genotoxicity studies and no evidence of a proliferative signal in the chronic toxicity studies.

Recovery was not investigated as part of any of the pivotal safety studies. A 12 oral exploratory study, with 12 week recovery period was conducted in rash2 wild-type that only investigated the hepatobiliary changes previously seen in shorter term study in the same species/strain. Fully pathology was not conducted as part of this study. A 1-month oral toxicity study in dogs with 3-month treatment free recovery period was conducted at of 1000 mg/kg/day elbasvir to evaluate the reversibility of the phospholipidosis observed in the repeat-dose study in dogs. Given that suitable margins of exposure cover exist for all findings seen in all species compared to clinical exposures, the value of repeating in vivo study to investigate reversibility at this stage would be limited.

The applicant committed to submit an updated ERA with data from the OECD 225 (sediment toxicity test with *Lumbriculus variegatus*) and OECD 218 (repeat toxicity test with the chironomid, (*Chironomus riparius*)) post-authorisation.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical package is considered acceptable and there are no outstanding concerns. The applicant committed to provide an updated ERA with data from the OECD 218 and OECD 225 studies post-authorisation.

2.4. Clinical aspects

2.4.1. Introduction

Grazoprevir (GZR) and Elbasvir (EBR) have been developed only for use in a fixed dose combination tablet. The clinical pharmacology programme is summarised in Table 13.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table 7. Clinical pharmacology studies

Study Number	Study Description	Elbasvir (EBR) / Grazoprevir (GZR) Doses	Treatment duration
Biopharmaceutics Studies			
5172-P027	Effects of Famotidine and of a High-fat Meal on the PK of Grazoprevir	100 mg GZR fasted, fed and with famotidine	Single doses
5172-P040	Microdose Absolute Bioavailability Study of Single and Multiple Doses of Grazoprevir	25 mg, 200 mg GZR & 14C grazoprevir microdose	Single and multiple doses & single microdoses
5172-P069	Food Effect on the PK of Grazoprevir/Elbasvir FDC	50 mg/100 mg EBR/GZR FDC2 with and without food	Single doses in each treatment period

Study Number	Study Description	Elbasvir (EBR) / Grazoprevir (GZR) Doses	Treatment duration
8742-P005	Effect of Famotidine on the PK of Elbasvir	100 mg EBR with and without famotidine	Single doses
8742-P018	Food Effect on the PK of Elbasvir	50 mg EBR with and without famotidine	Single doses
Biocomparison / Bioequivalence Studies			
5172-P002	Formulation Biocomparison for Grazoprevir	600 mg GZR Formulations 1-4	Single doses
5172-P008	Comparative Bioavailability of Two Different Grazoprevir Tablet Formulations	600 mg GZR formulation #5 600 mg GZR formulation #6 600 mg GZR formulation #1	Single dose for each treatment
5172-P045	Comparative Bioavailability of Two Elbasvir Tablet Formulations and One Elbasvir/Grazoprevir Fixed	Groups 1-3: 100 mg GZR (Formulation 1) Group 1: 50 mg EBR (PMF1) with and without famotidine Group 2: 50 mg EBR (PMF 2) with and without famotidine Group 3: 50 mg/100 mg EBR/GZR FDC tablet Reference: Groups 1-3: 100 mg GZR (Formulation 1) & 50 mg EBR (FFP)	Single doses each in 3 treatment periods per group
5172-P055	Grazoprevir/Elbasvir Fixed Dose Combination Comparative Bioavailability	100 mg/50 mg GZR/EBR 100 mg GZR 50 mg EBR	Single dose of each treatment
Healthy Subject PK and Initial Tolerability Studies			
5172-P001	Single-Dose and Multiple- Dose Study of Grazoprevir	Part I: 2 to 1,600 mg GZR Part II: 100 to 1,000 GZR mg Part III: 100 mg GZR with and without ketoconazole	Part I: Single doses Part II: 10 days Part III: Single dose
5172-P007	[14C]Grazoprevir Absorption, Metabolism, and Excretion (AME)	Actual doses ranged: 186 – 188 mg [195 - 197 µCi]	Single dose
8742-P001	Single-Dose and Multiple- Dose Study of Elbasvir	Part I: 5 to 400 mg EBR Part II: 10 to 200 mg EBR	Part I: Single doses Part II: 10 days
8742-P006	Extended Dose Study of Elbasvir	50 mg EBR	28 days
8742-P014	[14C]Elbasvir Absorption, Metabolism, and Excretion (AME)	Actual doses ranged: 50.3-51.4 mg [202 – 206 µCi]	Single dose
Patient PK and Initial Tolerability Studies			
5172-P004	Multiple Dose of Grazoprevir in HCV-Infected Patients	10 to 800 mg GZR	7 days
8742-P002	Multiple Dose of Elbasvir in HCV-Infected Males Part I: GT1 (GT1a or GT1b) HCV Part II: GT3 HCV Part III: GT1a HCV	Part I: 5 to 50 mg EBR Part II: 10 to 100 mg EBR Part III: 10 to 50 mg EBR	Part I: 5 days Part II: 5 days Part III: 5 days
Intrinsic Factor PK Studies			
5172-P009	Japanese PK of Grazoprevir	100, 400, 800, 1,200 mg GZR	Single doses of 100, 400, 800 and 1,200 mg and multiple doses of 400 and 800 mg for 10 days
5172-P013	Grazoprevir PK in Subjects with Hepatic Insufficiency Part I: Mild (Child-Pugh 5-6) Part II: Moderate (Child-Pugh 7-9) Part II: Severe (Child-Pugh 10- 15) Parts I-III: healthy match	200 mg GZR Part I 100 mg GZR Part II 50 mg GZR Part III	10 days
5172-P014	Grazoprevir PK in Healthy Elderly Males and Females	400 mg GZR	7 days
5172-P042	Chinese PK of Grazoprevir	100 mg GZR or 200 mg GZR	10 days

Study Number	Study Description	Elbasvir (EBR) / Grazoprevir (GZR) Doses	Treatment duration
5172-P050	Grazoprevir PK in Subjects with Renal Insufficiency	100 mg GZR & 50 mg EBR	10 days
8742-P004	Elbasvir PK in Healthy Elderly Males and Females	100 mg EBR	Single dose
8742-P009	Elbasvir PK in Subjects with Hepatic Insufficiency Part I: Mild (Child-Pugh 5-6) Part II: Moderate (Child-Pugh 7-9) Part III: Severe (Child-Pugh 10-15) Part IV: healthy match	50 mg EBR	Single dose
7009 P050	Elbasvir PK in Subjects of Asian Ethnicity	Part I: 10, 50 & 100 mg EBR Part II: 50 mg EBR with MK-7009	Part I: Single doses Part II: Multiple doses for 10 days

2.4.2. Pharmacokinetics

Formulations

GZR has low solubility and high permeability whereas EBR has low solubility and low permeability. The solubility of both GZR and EBR are pH-dependent. Five formulations were developed and used in clinical studies, including:

- Single entity fit-for-purpose (FFP) formulations of each of GZR and EBR
- Single entity preliminary market (PMF) formulations of each of GZR (PMF1) and EBR (PMF2)
- A fixed-dose combination formulation tablet (EBR/GZR FDC2) that was used in the core Phase 3 studies (060/C-EDGE TN; 061/C-EDGE COINFECTION and 068/C-EDGE TE).
- The final market image (FMI) formulation has not been tested clinically. On the basis that the FMI tablet is identical to the Phase 3 EBR/GZR FDC formulation except for a change in the film coat colour and the debossed marking no bioequivalence study was considered to be necessary.

Analytical methods

Concentrations of GZR and EBR in plasma and dialysate were determined in various laboratories (sponsor's facilities and CRO) using validated liquid chromatography-tandem mass spectrometric detection (LC-MS/MS) methods.

Absorption

Bioavailability

GZR

Study P001V01 - Study Title: A Single-Dose and Multiple-Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of MK-5172 (Grazoprevir)

In Study 001 in healthy subjects, GZR was not measurable in plasma after doses < 25 mg. At higher doses the T_{max} generally occurred at 2-4 h. C_{12h} values of at least 28 nM and AUC_{0-∞} of at least 3.2 µM·hr (targets calculated from results with another PI and adjusted based on relative activity vs. GZR) were attained with single doses ≥ 800 mg and multiple doses ≥ 400 mg (AUC) and 700 mg/day (AUC and C_{max}). At 100 - 400 mg/day the accumulation ratio based on plasma AUC₀₋₂₄ was ~3-fold. Plasma GZR increased in a greater than dose-proportional manner over the range tested and was time-dependent.

After single and multiple doses a biphasic plasma elimination profile was observed with a terminal elimination half-life of 16.9-24.7 h.

Study P040 - Study Title: A 14C-microdose, Absolute Bioavailability Study of Single and Multiple Doses of Grazoprevir (MK-5172) in Healthy Adult Volunteers

In Study 040 in healthy subjects - GZR absolute bioavailability was estimated to be in the range ~10% to 40% at doses from 25 to 200 mg (using 25 and 100 mg tablets). The applicant attributed the wide range to first-pass hepatic uptake and metabolism as well as incomplete absorption.

Assuming that intestinal availability (F_g) has a value of ~1 (since ketoconazole had a minimal effect on GZR C_{max}; see 2.1.10), together with the mean absolute bioavailability, the mean systemic plasma clearance of ~20-40 L/h and accounting for the blood to plasma ratio of 0.7, a human oral fraction absorbed (F_a) range of ~0.2-1.0 was estimated. This F_a range encompasses the F_a of ~0.5 estimated from a PB-PK model and the minimum F_a of 0.22 estimated from the human ADME study.

Study MK-5172 004 – Study Title: “A Multiple Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Grazoprevir (MK-5172) in Hepatitis C Infected Male Patients”

In Study 004 in HCV-infected male patients – As in healthy subjects, T_{max} was at 2-4 h and elimination was biphasic with t_{1/2} ~25-45 h, longer at lower doses. The 7-day accumulation ratios based on AUC₀₋₂₄ were 2-4-fold. GZR steady state (reached in ~ 5 days) plasma exposure was ~2-fold higher in HCV patients than in healthy subjects, such that measurable concentrations occurred with 10 mg/day dosing. GZR doses ≥ 400 mg/day were required to meet the pre-defined targets (described above). Sections 2.2.3 and 2.2.5 report the antiviral efficacy by dose and PK-PD analysis.

Table 8. Statistical Summary of Plasma Pharmacokinetics and Accumulation Ratios of Grazoprevir (MK-5172) Following the Administration of Multiple Oral Doses of 10 to 800 mg Grazoprevir (MK-5172) QD on Days 1 - 7 in GT1 and 100 to 800 mg Grazoprevir (MK-5172) QD on Days 1 - 7 in GT3 HCV-Infected Male Patients

Pharmacokinetic Parameter	Dose (mg)	Day 1		Day 7		Day 7/Day 1 GMR (90% CI)	rMSE
		N [†]	GM (95% CI)	N [†]	GM (95% CI)		
AUC ₀₋₂₄ (μM·hr) [†]	10	5	0.0159 (0.00804, 0.0313)	5	0.0628 (0.0318, 0.124)	3.96 (2.50, 6.25)	0.433
	30	5	0.0770 (0.0390, 0.152)	5	0.260 (0.132, 0.513)	3.37 (2.14, 5.33)	
	50	5	0.134 (0.0679, 0.265)	5	0.419 (0.212, 0.827)	3.12 (1.98, 4.93)	
	100	10	0.492 (0.304, 0.796)	10	1.16 (0.716, 1.87)	2.36 (1.70, 3.25)	
	200	10	0.965 (0.596, 1.56)	10	3.21 (1.98, 5.19)	3.33 (2.41, 4.60)	
	400	10	8.04 (4.97, 13.0)	10	18.2 (11.2, 29.4)	2.26 (1.64, 3.13)	
	600	10	17.2 (10.6, 27.8)	10	41.9 (25.9, 67.7)	2.44 (1.76, 3.37)	
	800	20	31.7 (22.6, 44.6)	18	72.5 (51.1, 103)	2.29 (1.80, 2.90)	
C _{max} (μM) [†]	10	5	0.00136 (0.000672, 0.00274)	5	0.00377 (0.00186, 0.00761)	2.77 (1.56, 4.93)	0.544
	30	5	0.0161 (0.00796, 0.0325)	5	0.0235 (0.0116, 0.0474)	1.46 (0.82, 2.59)	
	50	6	0.0134 (0.00704, 0.0254)	5	0.0430 (0.0216, 0.0855)	3.22 (1.83, 5.65)	
	100	10	0.0842 (0.0512, 0.138)	10	0.141 (0.0859, 0.232)	1.68 (1.12, 2.52)	
	200	10	0.161 (0.0978, 0.264)	10	0.684 (0.416, 1.12)	4.25 (2.83, 6.38)	
	400	10	1.68 (1.02, 2.76)	10	3.38 (2.06, 5.56)	2.02 (1.34, 3.03)	
	600	10	3.18 (1.93, 5.23)	10	7.69 (4.68, 12.6)	2.42 (1.61, 3.63)	
	800	20	5.85 (4.11, 8.31)	18	11.3 (7.81, 16.2)	1.92 (1.43, 2.60)	
C ₂₄ (nM) [†]	10	5	0.490 (0.248, 0.971)	5	2.41 (1.22, 4.78)	4.92 (3.44, 7.04)	0.339
	30	5	2.03 (1.03, 4.01)	5	7.20 (3.64, 14.2)	3.55 (2.48, 5.08)	
	50	5	4.14 (2.09, 8.19)	5	12.7 (6.40, 25.1)	3.06 (2.14, 4.37)	
	100	10	11.3 (6.99, 18.3)	10	20.1 (12.4, 32.6)	1.78 (1.38, 2.29)	
	200	10	13.5 (8.34, 21.9)	10	22.2 (13.7, 35.9)	1.64 (1.28, 2.11)	
	400	10	43.8 (27.0, 71.0)	10	70.2 (43.3, 114)	1.60 (1.25, 2.07)	
	600	10	70.9 (43.8, 115)	10	93.2 (57.6, 151)	1.31 (1.02, 1.69)	
	800	20	96.9 (68.9, 136)	18	174 (123, 247)	1.80 (1.49, 2.17)	

EBR

The absolute bioavailability of EBR is being assessed in an ongoing study. Meanwhile, the fraction absorbed (fa) of EBR was estimated from the PB-PK model to be ~0.4.

Study MK-8742-P001 – Study Title: A Single and Multiple Dose Study to Evaluate the Safety and Pharmacokinetics of MK-8742 (Elbasvir)

In Study MK-8742-P001, after single and multiple doses, T_{max} was from 2.5 to 4.0 h. The target C₂₄ (3.0 nM; derived from daclatasvir data and expected to provide a 3 log₁₀ decline in HCV RNA) was attained at ≥ 10 mg/day. Steady state occurred within 1-2 days. The accumulation ratios for AUC₀₋₂₄ ranged from 0.981 at 100 mg/day to 2.05 at 10 mg/day. On day 10, based on dosing with multiples of 1 and 10 mg capsules, AUC and C_{max} increased in an approximately dose proportional to slightly less than dose proportional fashion over 10 mg to 100 mg/day and EBR PK appeared to be time-independent. Plasma concentrations declined bi-exponentially with t_{1/2} ~20 h after 10 mg to 200 mg daily dosing.

On dosing in the fasted state with 50 mg/day (as 5 x 10 mg capsules) for 28 days, Study 006 gave very comparable AUC, C_{max} and C₂₄ values vs. those observed on day 10 in Study 001 at 50 mg/day. T_{max} was 4 h and the accumulation ratios were 1.39 for AUC₀₋₂₄, 1.20 for C_{max} and 1.46 for C₂₄. There was no relationship detected between plasma exposures and abnormalities in LFTs.

Table 9. Summary Statistics of Plasma Elbasvir Pharmacokinetics Following Administration of Multiple Doses (Daily for 10 Days) of 10 mg to 200 mg Elbasvir to Healthy Male Subjects (Part II)

Pharmacokinetic Parameters	10 mg (1 x 10 mg) (N = 6)	50 mg (5 x 10 mg) (N = 6)	100 mg (10 x 10 mg) (N = 6)	200 mg (10 x 10 mg + 1 x 100 mg) (N = 6)
Day 1 (First Dose)				
AUC ₀₋₂₄ (nM*hr) [†]	180 (113, 286)	1210 (764, 1930)	2450 (1540, 3900)	3240 (2040, 5140)
C _{max} (nM) [†]	14.2 (8.65, 23.2)	96.5 (58.9, 158)	220 (134, 361)	297 (181, 487)
C ₂₄ (nM) [†]	4.64 (2.94, 7.32)	30.1 (19.1, 47.4)	55.9 (35.4, 88.2)	74.5 (47.2, 117)
T _{max} (hr) [‡]	3.00 (2.00, 8.00)	3.50 (3.00, 6.00)	3.00 (2.00, 4.03)	4.00 (3.00, 4.00)
Day 10 (Last Dose)				
AUC ₀₋₂₄ (nM*hr) [†]	369 (232, 586)	1510 (948, 2400)	2400 (1510, 3820)	3540 (2230, 5630)
C _{max} (nM) [†]	32.4 (19.8, 53.2)	108 (65.7, 176)	190 (116, 311)	281 (171, 460)
C ₂₄ (nM) [†]	8.40 (5.33, 13.3)	38.0 (24.1, 60.0)	58.1 (36.8, 91.6)	82.2 (52.1, 130)
T _{max} (hr) [‡]	2.51 (2.00, 4.00)	4.00 (3.00, 4.00)	4.00 (3.00, 6.00)	4.00 (2.00, 4.00)
Apparent t _{1/2} (hr) [§]	18.83 (19.73)	20.65 (17.65)	19.67 (5.91)	20.09 (6.69)
Accumulation Ratio (Day 10/Day 1) [†]				
AUC ₀₋₂₄ (nM*hr)	2.05 (1.46, 2.88)	1.24 (0.885, 1.74)	0.981 (0.699, 1.38)	1.09 (0.780, 1.53)
C _{max} (nM)	2.29 (1.59, 3.3)	1.12 (0.775, 1.61)	0.862 (0.599, 1.24)	0.945 (0.657, 1.36)
C ₂₄ (nM)	1.81 (1.31, 2.5)	1.27 (0.917, 1.75)	1.04 (0.752, 1.43)	1.10 (0.800, 1.52)

[†] Back-transformed least-squares mean and 95% confidence interval from linear mixed effects model performed on natural logtransformed values.

[‡] Median (min, max) reported for T_{max}.

[§] Geometric mean with %CV reported for apparent t_{1/2}.

[†] Back-transformed least-squares mean differences and 90% confidence interval from mixed effects model performed on natural logtransformed values.

Square root of conditional mean squared error (residual error) from linear mixed effects model = 0.340, 0.366, and 0.324 for AUC₀₋₂₄,

C_{max}, and C₂₄ based on a linear mixed effects model performed on log-transformed values, respectively. When multiplied by 100, provides estimates of pooled within-subject %CV on the raw scale.

MK-8742-P002 – Study title: A Multiple Dose Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of MK-8742 (Elbasvir) in Hepatitis C Infected Males

In Study 002 in HCV-infected male patients – T_{max} was at 2-3 h and t_{1/2} was ~20-24 h. The 5-day accumulation ratios based on AUC₀₋₂₄ and dosing in the fasted state were 1.5-1.9-fold. Steady state was reached in ~ 2-3 days. At 50 mg/day and 100 mg/day the mean plasma exposures (AUC₀₋₂₄) on day 5 were 1.49 and 2.20 μM.h, respectively. These values in HCV patients are very similar to those reported above on Day 10 for the same daily doses in healthy subjects.

Table 10. Statistical Summary of Plasma Pharmacokinetic Parameter Values and Accumulation Ratios of Elbasvir (MK-8742) Following the Administration of Multiple Oral Doses of 5 to 100 mg Elbasvir QD on Days 1 - 5 in GT1, GT1a, and GT3 HCV-Infected Male Patients

Pharmacokinetic Parameter	Dose (mg)	Day 1		Day 5		Day 5/Day 1		rMSE [†]
		N	GM (95% CI)	N	GM (95% CI)	N	GMR (90% CI)	
AUC _{0-24 hr} (hr*μM) [†]	5	5	0.101 (0.0596, 0.172)	5	0.155 (0.0909, 0.263)	5	1.53 (0.94, 2.47)	0.452
	10	15	0.0770 (0.0567, 0.105)	15	0.149 (0.110, 0.203)	15	1.94 (1.47, 2.56)	
	50	15	0.719 (0.529, 0.977)	15	1.36 (1.00, 1.85)	15	1.89 (1.43, 2.50)	
	100	5	1.41 (0.831, 2.40)	5	2.08 (1.22, 3.53)	5	1.47 (0.91, 2.38)	
C _{max} (μM) [†]	5	5	0.00962 (0.00542, 0.0171)	5	0.0126 (0.00711, 0.0224)	5	1.31 (0.75, 2.30)	0.525
	10	15	0.00608 (0.00436, 0.00847)	15	0.0109 (0.00779, 0.0151)	15	1.79 (1.29, 2.47)	
	50	15	0.0635 (0.0456, 0.0884)	15	0.106 (0.0758, 0.147)	15	1.66 (1.20, 2.30)	
	100	5	0.133 (0.0749, 0.236)	5	0.170 (0.0958, 0.302)	5	1.28 (0.73, 2.24)	
C _{24 hr} (nM) [†]	5	5	2.37 (1.44, 3.90)	5	3.89 (2.36, 6.41)	5	1.64 (1.07, 2.50)	0.396
	10	15	2.21 (1.66, 2.95)	15	4.08 (3.06, 5.44)	15	1.84 (1.44, 2.36)	
	50	15	18.1 (13.6, 24.1)	15	34.3 (25.7, 45.8)	15	1.90 (1.49, 2.42)	
	100	5	36.9 (22.4, 60.7)	5	56.2 (34.1, 92.5)	5	1.52 (1.00, 2.33)	

†Geometric mean and geometric mean ratio back-transformed from the linear mixed effects model analyzed on natural log scale.

‡Median (minimum, maximum) reported for T_{max}.

§Geometric mean and percent geometric CV reported for apparent t_{1/2}.

¶rMSE: Square root of mean squared error (residual error) from the linear mixed effect model. rMSE*100% approximates the within subject % CV on the raw scale.

Pharmacokinetic data from GT1, GT1a and GT3 HCV infected patients were pooled for analysis.

Influence of food and pH

GZR

Study MK-5172 P001 – Study Title: A Single-Dose and Multiple-Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of MK-5172 (Grazoprevir)

In Study MK-5172 P001 the GMRs (fed/fasted) and 90% CIs after single 50 mg doses were 1.06 (0.58, 1.93), 1.19 (0.69, 2.04) and 0.67 (0.48, 0.94) for AUC_{0-∞}, C_{max} and C_{24h}, respectively.

Study MK-5172 P027 – Study Title: A 3-Period, Partial Crossover Study to Assess the Effects of Famotidine and of a High-Fat Meal on the Single-Dose Pharmacokinetics of MK-5172 in Healthy Adult Subjects

In this study using a single dose of a 100 mg tablet:

- o A high fat breakfast did not affect the AUC_{0-∞} (GMR 1.11; 90% CI 1.01, 1.21) or C₂₄ (0.98; 0.84, 1.14) but the effect on C_{max} was much more variable (CV% 58.9) with a GMR of 1.61 and 90% CI (0.99, 2.62). T_{max} was delayed by ~ 40 minutes in the fed state.
- o When dosing was in the fasted state and after two doses of 20 mg famotidine (at 10 h and 2 h prior to GZR) the GMRs (90% CI) for AUC_{0-∞}, C_{max} and C₂₄ were 1.22 (1.03, 1.44), 1.63 (1.23 and 2.16) and 1.03 (0.84, 1.25), respectively, indicating a mean 22% increase in exposure and a mean 63% increase in C_{max}.

EBR

Study MK-8741-P001 – Study Title: A Single and Multiple Dose Study to Evaluate the Safety and Pharmacokinetics of MK-8742 (Elbasvir)

In this study, administration of single 50 mg doses with a high-fat meal resulted in ~30% to 40% decreases in EBR AUC_{0-∞}, AUC₀₋₂₄, C_{max} and C₂₄ with GMRs of 0.67, 0.63, 0.56 and 0.69, respectively.

Study MK-5172 005 – Study Title: A 2-Period, Fixed Sequence Study to Assess the Effect of Famotidine on a Single Dose of MK-8742 in Healthy Adult Subjects

In Study 005 using single doses of 100 mg administered as multiple 10 mg capsules, dosing in the fasted state after two doses of famotidine (as in GZR 027 above) significantly lowered the plasma exposures (by ~ 60%) as shown below but the delay in T_{max} was only ~45 minutes.

Table 11. Statistical Summary of Plasma Pharmacokinetics of MK-8742 Following the Administration of a Single Oral Dose of 100 mg MK-8742 Alone and Following the Administration of a Single Oral Dose of 100 mg MK-8742 With Multiple Oral Doses of 20 mg Famotidine in Healthy Adult Male Subjects

	MK-8742 Alone			MK-8742 With Famotidine			(MK-8742 With Famotidine/ MK-8742 Alone)		
Pharmacokinetic Parameter	N	GM	95% CI	N	GM	95% CI	GMR	90% CI	Pseudo Within Subject %CV [‡]
AUC _{0-∞} [†] (uM.hr)	10	3.77	(2.90, 4.90)	10	1.57	(1.25, 1.99)	0.42	(0.33, 0.53)	28.328
AUC ₀₋₂₄ [†] (uM.hr)	10	2.42	(1.76, 3.32)	10	0.938	(0.720, 1.22)	0.39	(0.30, 0.51)	32.226
C _{max} [†] (nM)	10	216	(150, 310)	10	76.2	(54.6, 106)	0.35	(0.27, 0.47)	34.916
C ₂₄ [†] (nM)	10	55.4	(42.0, 73.2)	10	23.8	(18.4, 30.8)	0.43	(0.32, 0.57)	34.333
t _{max} [§] (hr)	10	3.25	(2.00, 4.01)	10	4.00	(3.50, 4.00)			.
Apparent terminal t _{1/2} (hr)	10	17.07	23.03	10	17.45	34.55			.

[†]Back-transformed least squares mean and confidence interval from linear mixed effect model performed on natural log transformed values

[‡]Pseudo Within-Subject %CV = $100 \times \sqrt{(\sigma^2 A + \sigma^2 B - 2 \times \sigma AB) / 2}$, where $\sigma^2 A$ and $\sigma^2 B$ are the estimated variances on the log scale for the two treatment groups, and $\sigma^2 AB$ is the corresponding estimated covariance, each obtained from the linear mixed effects model

[§]Median (min, max) reported for t_{max}

^{||} Geometric arithmetic mean and percent geometric coefficient of variation reported for apparent terminal t_{1/2}

GM = Geometric least-squares mean; GMR = Geometric least-squares mean ratio; CI = Confidence interval

In Study 018 using single doses of 50 mg film-coated tablets, a high fat breakfast did not affect T_{max} but reduced plasma exposures by ~ 15%. The GMRs (90% CI) for AUC_{0-∞}, AUC_{0-t} and C_{max} were 0.85 (0.67, 1.08), 0.85 (0.66, 1.08) and 0.90 (0.67, 1.20), respectively, with C₂₄ GMR of 0.87 (0.67, 1.12).

EBR/GZR

Study MK-5172 069 – Study Title: A Relative Bioavailability Study to Assess the Effect of Food on the Pharmacokinetics of Both MK-5172 and MK-8742 Following the Administration of the Fixed Dose Combination FDC2 of MK-5172A to Healthy Subjects

In Study 069, using the FDC2 formulation (GZR 100 mg/EBR 50 mg), a high fat breakfast increased exposure to GZR by ~1.5-2-fold but slightly decreased exposure to EBR. The applicant concluded that the FDC2 tablet could be taken with or without food.

Table 12. Summary of Plasma Pharmacokinetics of MK-5172 Following a Single Dose of 50 mg MK-8742/100mg MK-5172 to Healthy Subjects (N=26)

Pharmacokinetic Parameter	MK-5172 Fasted ¹			MK-5172 Fed ²			MK-5172 Fed /MK-5172 Fasted		Pseudo Within-Subject %CV ⁸
	N ⁴	GM	95% CI	N	GM	95% CI	GMR	90% CI	
AUC _{0-∞} (nM·hr)	17 ^{3,4}	548	(392,623)	22 ^{3,4}	742	(653,882)	1.54	(1.34,1.76)	21.4
AUC _{0-t} (nM·hr)	25	342	(268,438)	25 ⁴	657	(570,754)	1.91	(1.67,2.18)	27.3
C _{max} (nM)	25	30.8	(23.7,39.8)	26	87.1	(68.2,111)	2.83	(2.16,3.72)	56.7
C _{24hr} (nM)	25	4.20	(3.50,5.09)	26	6.95	(6.05,7.99)	1.65	(1.47,1.85)	23.3
C _{2hr} ⁵ (nM)	25	18.8	(2.88, 188)	26	46.9	(0.00,134)	-	-	-
T _{max} ⁶ (h)	25	3.00	(1.00,6.00)	26	2.00	(1.00,6.00)	-	-	-
t _{1/2} ⁷ (h)	17 ³	35.80	35.6	22 ^{3,4}	30.98	23.3	-	-	-

Table 13. Summary of Plasma Pharmacokinetics of MK-8742 Following a Single Dose of 50 mg MK-8742/100mg MK-5172 to Healthy Subjects (N=26)

Pharmacokinetic Parameter	MK-8742 Fasted ¹			MK-8742 Fed ²			MK-8742 Fed /MK-8742 Fasted		Pseudo Within-Subject %CV ⁶
	N ³	GM	95% CI	N	GM	95% CI	GMR	90% CI	
AUC _{0-∞} (nM·hr)	25	2300	(2020,2630)	25 ³	2060	(1800,2350)	0.891	(0.817,0.971)	17.8
AUC _{0-t} (nM·hr)	25	2250	(1980,2570)	25 ³	2000	(1750,2270)	0.885	(0.812,0.964)	17.7
C _{max} (nM)	25	128	(111,145)	26	108	(96.4,121)	0.852	(0.772,0.941)	20.4
C _{24hr} (nM)	25	35.2	(31.0,39.9)	26	32.6	(28.5,37.3)	0.926	(0.850,1.01)	17.7
C _{2hr} (nM)	25	92.2	(78.1,111)	26	48.2	(32.9,70.6)	0.518	(0.353,0.762)	81.0
T _{max} ⁴ (h)	25	3.50	(2.00, 4.00)	26	3.00	(1.50, 6.00)	-	-	-
t _{1/2} ⁵ (h)	25	17.72	12.2	25 ³	17.79	13.5	-	-	-

Bioequivalence studies

GZR

The minor differences between GZR **FFP** and GZR **PMF1** were not expected to have any impact on in-vivo performance of the formulations and no BE study was conducted.

Study MK-5172-P002 – Study Title: A Formulation Biocomparison Study for MK-5172 (Grazoprevir) in Healthy Adult Subjects

Study MK-5172-P008 – Study Title: An Explorative Biocomparison Study to Compare the Pharmacokinetics of Two Different MK-5172 Tablet Formulations With the Phase I Fit-For-Purpose (FFP) Formulation in Healthy Volunteers.

Studies 002 and 008 compared dosing with multiples of 100 mg FFP tablets and multiples of test formulations (three 200 mg tablet formulations of varying drug load, a 150 mg and 200 mg PMF tablet) to deliver 600 mg single doses of GZR in the fasted state. Test formulations showed lower bioavailability compared to the FFP tablet except for slightly higher bioavailability with the 150 mg PMF tablet. These alternative formulations/tablets were not further pursued clinically.

EBR/GZR

Study MK-5172-P045 – Study Title: A Study to Evaluate the Comparative Bioavailability of Two New MK-8742 Tablet Formulations and One MK-8742/MK-5172 (MK-5172A) Fixed Dose Combination in the Absence and Presence of Famotidine in Healthy Subjects

Study 045 was conducted wholly in the fasting state. Famotidine was given as described in study 027.

In the comparison between 50 mg EBR administered as 5 x 10 mg **FFP** capsules (A) vs. 1 x 50 mg **PMF1** tablet (B), each given with 1 x 100 mg GZR **FFP** tablet, EBR bioavailability was much lower with (B) vs. (A). In the comparison between 50 mg EBR administered as (A) vs. 1 x 50 mg **PMF1** tablet given after two prior doses of famotidine (C), each administered with 1 x 100 mg GZR **FFP** tablet, EBR bioavailability was also much lower with (C) vs. (A) but slightly greater than observed with (B). The **EBR PMF1** tablet was not further pursued clinically.

In the comparison between 50 mg EBR administered as (A) vs. 1 x 50 mg **PMF2** tablet (D), each given with 1 x 100 mg GZR **FFP** tablet, EBR bioavailability was only slightly lower for (D) vs. (A), with lower 90% CI in the range 0.70 to 0.74.

Distribution

GZR

Binding of [3H]GZR to human plasma proteins was low and was concentration-independent up to 10 µM. However, the unbound plasma fraction is reported as 0.012.

Protein binding was similar in the plasma of patients without HCV but with severe renal impairment (97.8%) or ESRD on haemodialysis (98.4%) vs. healthy matched controls (98.3%). Protein binding was similar in patients with mild (98.3%), moderate (97.9%) and severe hepatic impairment (98.1%) without HCV infection vs. controls (98.3 – 98.8%).

The mean blood/plasma concentration ratio was 0.7 in vitro, indicating that it does not preferentially distribute into erythrocytes and that blood clearance may be ~40% higher than plasma clearance.

Passive permeability in LLC-PK1 cells was 19×10^{-6} cm/s, indicating good passive permeability. It was found to be a P-gp substrate in transfected cells. It was not possible to determine whether it is a substrate of BCRP due to endogenous transport in the host cell line.

GZR demonstrated time- and temperature-dependent uptake into human hepatocytes. It is a substrate of OATP1B1 and OATP1B3 in transfected cells with a low K_m (0.4 and 0.2 µM, respectively), suggesting a potential for saturable uptake into liver.

Based on intravenous administration in healthy subjects and non-compartmental analysis the absolute volume of distribution (V_d) at steady-state was estimated to be ~3600 L following a single dose of 25 mg and ~1400 L following single and once-daily doses of 200 mg GZR. The latter finding is proposed to reflect saturation of liver uptake at the 200 mg dose, which reduces the relative amount of drug distributing to the liver compared to plasma and therefore reduces V_d .

Active liver uptake transport via OATP1B with liver metabolism and excretion means that not all of the GZR taken up into liver returns to plasma before elimination. Hence, the estimated V_d may not fully account for the volume associated with the liver and the true V_d may be greater.

EBR

EBR is extensively (>99.9%) bound to human plasma proteins in vitro over the concentration range of 1 to 10 μ M. There was no evidence of saturation of plasma protein binding within the analytical limits of the assay. Binding is to albumin and to α 1-acid glycoprotein. Renal insufficiency (including ESRD patients on haemodialysis) and hepatic insufficiency had no effect on plasma protein binding in subjects not infected with HCV.

The mean blood/plasma concentration ratio in vitro was 0.62 indicating that it does not bind preferentially to erythrocytes and total blood clearance may be ~60% higher than plasma clearance.

In-vitro studies concluded that it is a P-gp substrate but not an OATP1B1 or OATP1B3 substrate. It was not possible to determine whether it is a substrate of BCRP due to endogenous transport in the host cell line and high non-specific binding.

Based on the POPPK analysis, the apparent V_d ($V_c + V_p$) for a typical individual is ~680 L with inter-individual variability of V_c ~26%. The V_c in HCV-infected patients was estimated to be ~30% higher than in healthy subjects.

Elimination

Excretion

GZR Study MK-5172-P007 - Study Title: A Study to Investigate the Absorption, Distribution, Metabolism, Excretion, and Mass Balance of MK-5172

Six fasted male subjects received a single oral nominal dose of 200 mg (~200 μ Ci) [14 C]GZR. Total radioactivity in plasma was < LLOQ of 40.9 ng equivalents/g (53.330 nM equivalents) in all subjects by 24 h post-dose and plasma GZR was < LLOQ of 1.00 ng/mL (1.30 nM) in all subjects by 168 h.

Through the first 8 h the GZR plasma exposure (AUC_{0-8} and C_{max}) was similar to total radioactivity with a GMR (95% CI) for AUC_{0-8} of 0.71 (0.59, 0.87) and GMR for C_{max} of 0.81 (0.69, 0.96). No metabolites of GZR were found in plasma. The T_{max} values were similar for radioactivity and GZR (2.59 and 3.01 h).

Table 14. Statistical Comparisons of Plasma Pharmacokinetic Parameters for MK-5172 Compared to Total Radioactivity and of the Recovery of the Radioactive Dose Following Administration of a Single Oral Dose of 200 mg (~200 µCi) [¹⁴C]MK-5172 in Healthy Male Subjects

Pharmacokinetic Parameter/ Analyte	N ^{††}	GM [†]	95% CI	GMR [‡]	95% CI	rMSE [§]
AUC_{0-8hr} (nM•hr or nM equivalents•hr)						
MK-5172	6	467	(270, 808)	0.71	(0.59, 0.87)	0.111
Total Radioactivity ^{§§}	5	655	(379, 1130)			
C_{max} (nM or nM equivalents)						
MK-5172	6	135	(65.2, 278)	0.81	(0.69, 0.96)	0.094
Total Radioactivity	5	166	(80.6, 344)			
T_{max} (hr)						
MK-5172	6	2.59	(2.01, 5.01)			
Total Radioactivity	5	3.01	(2.01, 5.01)			
AUC_{0-∞} (nM•hr or nM equivalents•hr)[†]						
MK-5172	6	1010	(711, 1440)			
Apparent terminal t_½ (hr)^{††}						
MK-5172	6	23.75	(74.14)			
Proportion of the Radioactivity Dose Recovered						
		Urine	Feces	Total		
Fraction of Radioactivity Recovery (%)		0.29	109.77	110.30		
95% Confidence Interval		(0.22, 0.36)	(93.26, 126.27)	(93.61, 126.99)		

†Back-transformed least squares mean and confidence interval from linear mixed effect model performed on natural log-transformed values.

‡GMR = Ratio of geometric least squares means (MK-5172/Total Radioactivity)

§rMSE: Square root of conditional mean squared error (residual error) from the linear mixed-effect model.

rMSE*100% approximates the within-subject %CV on the raw scale.

||Median (min, max) reported for T_{max}.

† Geometric mean and 95% confidence interval from the summary statistics were reported for AUC_{0-∞}.

††Geometric mean (GM) and geometric coefficient of variation (GCV) presented for apparent terminal t_½.

CI = Confidence Interval.

‡‡Subject AN 0002 had all BLQ values for total radioactivity. Therefore, all pharmacokinetic parameters for total radioactivity from Subject AN 0002 could not be quantified and were excluded from total radioactivity statistics.

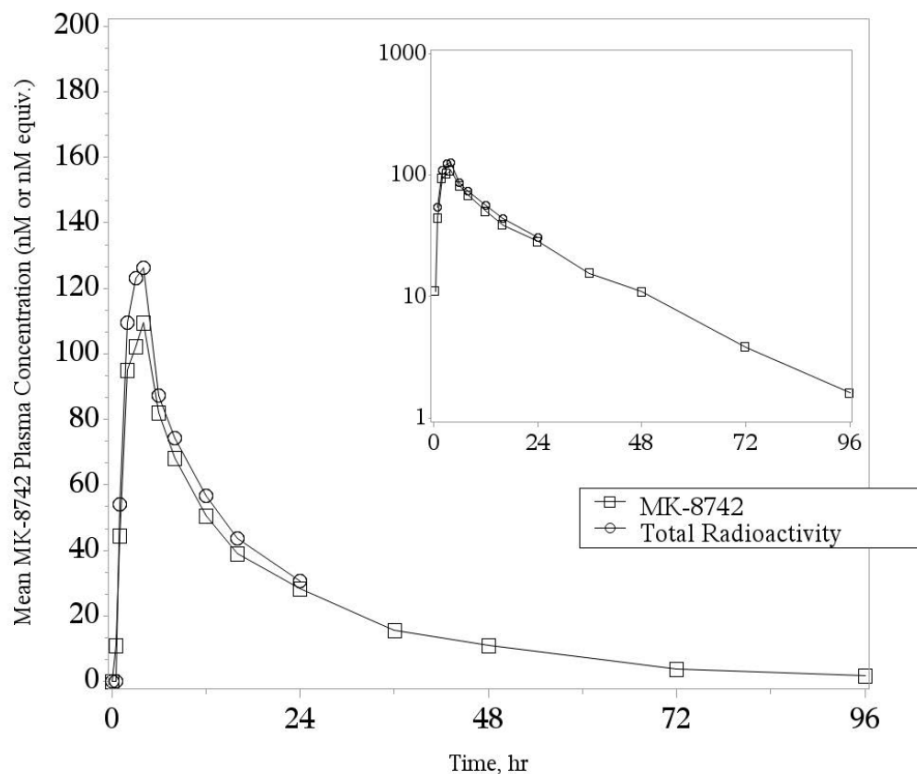
§§AUC_{0-8hr} for total radioactivity was extrapolated for Subject AN 0004 due to a BLQ value at the 8-hour time point.

Radioactivity in urine fell below the LLOQ of 9.90 ng equivalents/g (12.909 nM equivalents) in all subjects by 216 h while radioactivity in faeces was quantifiable up to at least 288 h in all subjects. On average, by 168 h the majority of the radioactive dose appeared to have been excreted in faeces (102%), with less than 0.3% being excreted in urine. Overall, 110.30% (95% CI: 93.61% - 126.99%) of the radioactive dose was recovered, with 109.77% in faeces (95% CI: 93.26% - 126.27%) and 0.29% in urine (95% CI: 0.22% - 0.36%).

EBR Study 014 – Study Title: A Study to Investigate the Absorption, Metabolism, Excretion, and Mass Balance of MK-8742

Six fasted male subjects received a single oral nominal dose of 50 mg (~200 µCi) [¹⁴C]EBR. Total radioactivity in plasma was < LLOQ of 22.64 ng equivalent/g EBR in all subjects by 36 h post-dose. The plasma EBR LLOQ was 0.25 ng/mL (0.28 nM) and concentrations were quantifiable in all subjects through the entire 96 h sampling interval. The AUC₀₋₁₆ of total radioactivity in plasma was similar to that of intact EBR (see Figure 8).

Figure 3. Arithmetic Mean Plasma Concentration-Time Profiles of MK-8742 and Total Radioactivity in Plasma Following the Administration of a Single Oral Dose of Approximately 50 mg [¹⁴C]MK-8742 (~200 µCi) in Healthy Adult Male Subjects (Inset = Semi-log Scale) (N = 6)



Radioactivity in urine fell to < LLOQ of 5.58 ng equivalent/g for all subjects by 48 h while levels in faeces appeared quantifiable from 24 h to at least 96 h in all subjects. The majority (94.2%) of radioactivity was excreted in the first 168 h. Overall, 94.3% (95% CI: 88.3% - 100%) of the radioactive dose was recovered, with 94.1% in faeces (95% CI: 88.1% - 100%) and 0.175% in urine (95% CI: 0.118% - 0.232%).

Metabolism and excretion

GZR ADME Study 007

Proposed biotransformation pathway is shown in Figure 9.

The applicant proposes a biotransformation pathway, mediated mainly by CYP3A as shown in Figure 10.

In the GZR ADME study radioactivity in plasma was < LLOQ in all subjects by 24 h post-dose and plasma GZR was < LLOQ (< 1.00 ng/mL [1.30 nM]) in all subjects by 168 h. T_{max} values were similar for radioactivity and GZR (2.59 and 3.01 h). Through the first 8 h the GZR plasma exposure (AUC₀₋₈ and C_{max}) was similar to total radioactivity with a GMR (95% CI) for AUC₀₋₈ of 0.71 (0.59, 0.87) and GMR for C_{max} of 0.81 (0.69, 0.96). No metabolites of GZR were found in plasma.

Radioactivity in urine was < LLOQ in all subjects by 216 h while radioactivity in faeces was quantifiable for at least 288 h. By 168 h the majority of the radioactive dose appeared to have been excreted in faeces (102%), with less than 0.3% being excreted in urine. Overall, 110.30% of the radioactive dose was recovered, with 109.77% in faeces and 0.29% in urine. [14C]GZR-related radioactivity was present in faeces as unchanged GZR (44.75%), the gut bacterial reductive metabolite M10 (33.93%) and 6 oxidative metabolites (total ~21% radioactivity; M4a, M4b, M7a, M11a, M11b and M14).

The applicant concluded that $\geq 21\%$ of the oral GZR dose is absorbed and, since it is a P-gp substrate, some of the drug in faeces likely came from biliary secretion. The applicant concluded that GZR elimination is mediated by oxidative metabolism, mainly via CYP3A, and by biliary secretion. The between-subject variability in GZR steady-state exposure (~55% for AUC and ~64% for C_{max} in non-cirrhotic HCV-infected patients) and the within-subject variability (~26% for AUC and ~45% for C_{max}) have been attributed to variability in saturable liver uptake and first pass metabolism.

In the EBR ADME study radioactivity in plasma was < LLOQ in all subjects by 36 h. The plasma EBR LLOQ was 0.25 ng/mL (0.28 nM) and concentrations were quantifiable in all subjects through the entire 96 h sampling interval. The AUC₀₋₁₆ of total radioactivity in plasma was similar to that of intact EBR.

Radioactivity in urine fell to < LLOQ for all subjects by 48 h while levels in faeces appeared quantifiable from 24 h to at least 96 h in all subjects. The majority (94.2%) of radioactivity was excreted in the first 168 h. Overall, 94.3% of the radioactive dose was recovered, with 94.1% in faeces and 0.175% in urine. [14C]EBR-related radioactivity was present in faeces as M2 and M3 mono-oxidative metabolites (~19% of the administered dose) and EBR (~75% of the administered dose).

The between-subject variability in EBR steady state exposure was generally within ~37-48% for AUC and ~34-61% for C_{max} in HCV-infected patients. The within-subject variability was estimated to be ~27% for AUC and ~35% for C_{max}.

Dose proportionality and time dependencies

GZR

GZR plasma exposure increases in a greater than dose-proportional manner in keeping with saturation of liver uptake processes during first-pass elimination. The AUC₀₋₂₄ exponent from the power model was ~1.7 in healthy and HCV-infected populations at steady state with similar terminal elimination slopes across all doses, supporting a conclusion that non-linearity in first pass is the primary driver of the greater than proportional behaviour rather than non-linearity in elimination. In addition, greater than dose proportional increases in exposure were observed for POPPK model-estimated C_{max} and AUC for HCV-infected patients in Phase 2 and 3 studies who received 25 to 800 mg QD. PB-PK model simulations performed with/without activation of CYP3A or OATP1B saturation showed that the

nonlinear kinetics likely reflect interplay between CYP3A and hepatic uptake transporters such as OATP1B. The GZR linearity index is ~1.2-2.5 across a 100 to 400 mg dose range in healthy subjects reflecting time-dependent PK, which is ascribed to the same saturable processes.

EBR

EBR PK appeared to be linear and time-independent.

Intra- and inter-individual variability

GZR exhibited consistent moderate to high variability in PK parameters within and across individual clinical studies. When serial pharmacokinetic samples were collected the overall between-subject variability in GZR steady-state exposure ranged from 39% to >100% for AUC and 42% to >100% for Cmax in healthy subjects across Phase 1 studies and ~55% for AUC and ~64% for Cmax in non-cirrhotic HCV-infected patients (Study 059). The within-subject variability was estimated to be ~26% for AUC and ~45% for Cmax in Study 055 using a partial replicate design. The applicant attributes the variability to saturable liver uptake and metabolism of GZR, particularly during first pass.

EBR PK exhibited consistently low to moderate variability within and across individual clinical studies. When serial pharmacokinetic samples were collected the overall between-subject variability (geometric coefficient of variation) in EBR steady state exposure was generally within the range of ~25-62% for AUC and ~22-68% for Cmax in healthy subjects and ~37-48% for AUC and ~34-61% for Cmax in HCV-infected patients. The within-subject variability was estimated to be ~27% for AUC and ~35% for Cmax in Study 055.

Pharmacokinetics in target population

GZR POPPK analysis

The final model is a two-compartment open model with first order elimination. For GZR 100 mg QD the actual and predicted steady-state exposures in a reference population of HCV-infected patients (i.e. without baseline factors that increase exposure [cirrhosis, Japanese race or severe CKD]) were similar.

Table 15. Summary Statistics of Steady State Grazoprevir Pharmacokinetics in Non- Cirrhotic HCV- Infected patients Following Once Daily Administration of 100 mg GZR/ 50 mg EBR for 12 Weeks

PK Parameter	5172-P059				Population PK Modeling	
	N	Range	GM [†]	95% CI	GM	90 % CI
AUC ₀₋₂₄ (uM*hr)	10	1.03 - 6.69	1.84	(1.18, 2.86)	1.86	(1.83,1.99)
Cmax (uM)	10	0.15 - 1.24	0.32	(0.20, 0.52)	0.22	(0.21, 0.23)
C ₂₄ (nM)	10	7.48 - 54.11	19.89	(11.23, 35.24)	23.4	(23.2, 25.9)
Tmax (hr)	10	0.5 – 3.0	2.0	-	-	-

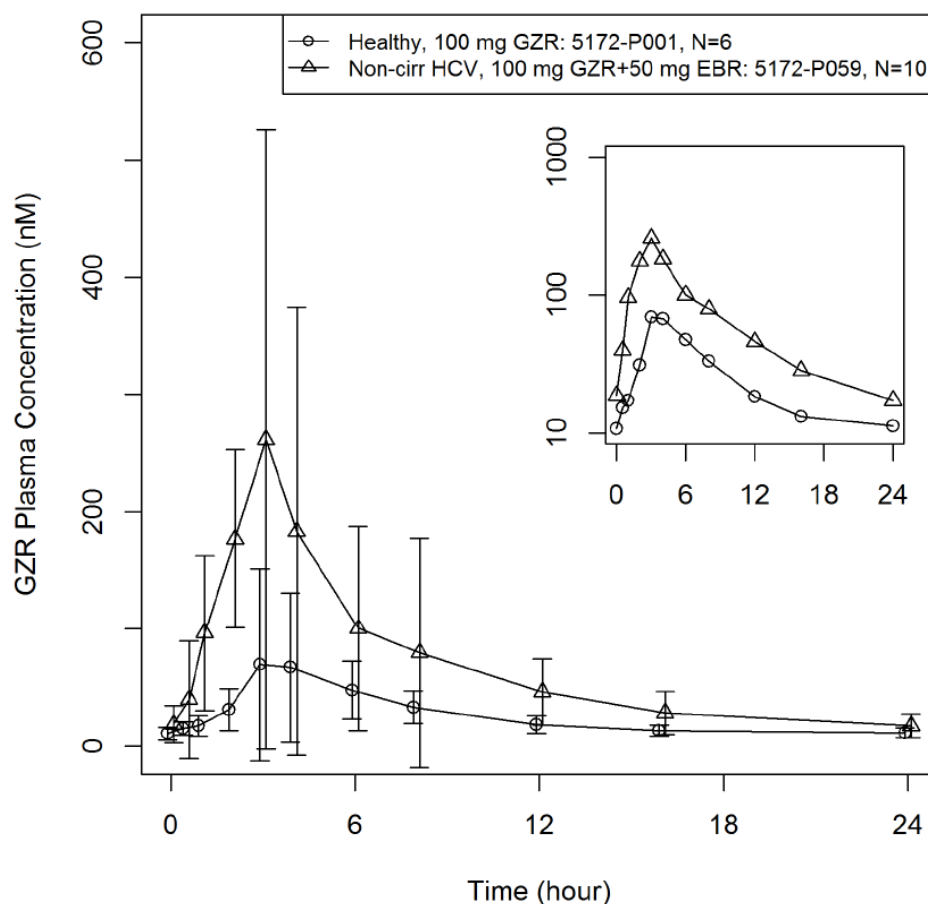
5172-P059 PK parameters represent the Day 28 Intensive PK data for each patient.

[†] Geometric mean for 5172-P059 back-transformed from a linear fixed effect model analyzed on log scale with fixed effect for population. CI: confidence interval.

Median, min and max reported for Tmax.

In addition, the gmean AUC and Cmax values (estimated from the POPPK model) for the 950 HCV-infected patients with PK data in Phase 3 studies (060, 061 and 068; including cirrhotic and non-cirrhotic patients) were 1.972 µM•hr and 0.228 µM, respectively. Steady-state exposure in non-cirrhotic HCV-infected patients was ~2-fold higher vs. healthy subjects but concentration-time profiles were similar.

Figure 4. Greater GZR Concentrations in Non-Cirrhotic HCV-Infected Patients (5172-P059) Than in Healthy Subjects (5172-P001) Following Once Daily 100 mg GZR or 100 mg GZR + 50 mg EBR Administration: Mean $\pm$ SD GZR Concentration-Time Profiles (Inset: Semi-log)



The PBPK model was used to explore the potential mechanistic basis for the effect of HCV disease on GZR exposure. The model-predicted effect of HCV disease was consistent with the observed clinical data, supporting a conclusion that the higher exposure in HCV-infected patients is likely due to change in first-pass processes and therefore bioavailability (F) caused by impaired liver function. The reduced functional liver mass and OATP1B transporter abundance in HCV-infected patients has the potential to result in saturable liver uptake of GZR via OATP1B1 at lower concentrations than in healthy subjects. The applicant concluded that GZR PK properties in healthy subjects can be extrapolated to HCV-infected patients given the consistency of ADME processes and similar concentration-time plasma profiles.

Across Phase 2 and 3 studies Ctrough with GZR 100 mg QD gradually increased up to ~Day 7 and then declined to reach stable values at ~Week 3 in cirrhotic and non-cirrhotic patients. Since GZR is not predicted to induce CYP3A4/P-gp this has been attributed to on-treatment improvement of liver function.

EBR POPPK analysis

A two-compartment model with lagged first order absorption was used to characterise EBR PK. No statistically significant influence of HCV genotype, cirrhosis, Child-Pugh B status, dialysis or use of strong CYP3A/P-gp inhibitors was identified. For 50 mg QD actual and predicted steady-state exposures in a reference population of HCV-infected patients (as above) were similar. The POPPK estimated gmean AUC and Cmax values in patients were 2.383 $\mu\text{M}\cdot\text{hr}$ and 0.151 μM , respectively. In

the POPPK model HCV was a significant covariate only for Vc. Analyses indicated that steady-state is generally achieved within ~5 days at 50 mg/day in HCV-infected patients. The gmean accumulation ratio (AUC ratio) ranged from 1.24-1.39 in healthy subjects compared to 1.89 in HCV-infected patients.

Pharmacokinetic interaction studies

In clinical study protocols, the applicant's pre-defined acceptance criteria for concluding no effect of concomitant medications on GZR and/or EBR were generally 90% CI around GMRs of (0.5, 2.0) for GZR and EBR. Corresponding criteria for concluding no effect of GZR and/or EBR on co-administered medications varied but were mostly 90% CI around GMRs of (0.70, 1.43) or (0.5, 2.0).

In vivo

DDI studies were conducted with dosing of all agents in the fasted state at least on PK sampling days. Please note that the doses of GZR used were often higher than proposed for the final FDC because 200 mg in healthy subjects was thought to reflect exposures with 100 mg in HCV-infected patients. The EBR dose was most often 50 mg.

Table 16. Effects of concomitant medications on GZR and EBR

Coadministered Drug	Regimen of Coadministered Drug	Regimen of GZR or/and EBR	N	Geometric Mean Ratio [90% CI] of GZR and EBR PK with/without Coadministered Drug (No Effect=1.00)			
					AUC [†]	C _{max}	C ₂₄
Antifungal							
Ketoconazole	400 mg QD	GZR 100 mg SD	8	Grazoprevir	3.25 (2.53, 4.18)	1.13 (0.77, 1.66)	--
	400 mg QD	EBR 50 mg SD	7	Elbasvir	1.80 (1.41, 2.29)	1.29 (1.00, 1.66)	1.89 (1.37, 2.60)
Antimycobacterial							
Rifampin	600 mg PO QD	GZR 200 mg QD	12	Grazoprevir	0.93 (0.75, 1.17)	1.16 (0.82, 1.65)	0.10 (0.07, 0.13)
	600 mg IV SD	GZR 200 mg SD	12	Grazoprevir	10.21 (8.68, 12.00)	10.94 (8.92, 13.43)	1.77 (1.40, 2.24)
	600 mg PO SD	GZR 200 mg QD	12	Grazoprevir	8.35 (7.38, 9.45) [‡]	6.52 (5.16, 8.24)	1.62 (1.32, 1.98)
	600 mg SD IV	EBR 50 mg SD	14	Elbasvir	1.22 (1.06, 1.40)	1.41 (1.18, 1.68)	1.31 (1.12, 1.53)
	600 mg SD PO	EBR 50 mg SD	14	Elbasvir	1.17 (0.98, 1.39)	1.29 (1.06, 1.58)	1.21 (1.03, 1.43)
HCV Antiretroviral							
EBR	20 mg QD	GZR 200 mg QD	10	Grazoprevir	0.90 (0.63, 1.28)	0.87 (0.50, 1.52)	0.94 (0.77, 1.15)
GZR	200 mg QD	EBR 20 mg QD	10	Elbasvir	1.01 (0.83, 1.24)	0.93 (0.76, 1.13)	1.02 (0.83, 1.24)
HIV Protease Inhibitor							
Atazanavir/r	300 mg/100 mg QD	GZR 200 mg QD	12	Grazoprevir	10.58 (7.78, 14.39)	6.24 (4.42, 8.81)	11.64 (7.96, 17.02)
	300 mg/100 mg QD	EBR 50 mg QD	10	Elbasvir	4.76 (4.07, 5.56)	4.15 (3.46, 4.97)	6.45 (5.51, 7.54)
Darunavir/r	600 mg/100 mg BID	GZR 200 mg QD	13	Grazoprevir	7.50 (5.92, 9.51)	5.27 (4.04, 6.86)	8.05 (6.33, 10.24)
	600 mg/100 mg BID	EBR 50 mg QD	10	Elbasvir	1.66 (1.35, 2.05)	1.67 (1.36, 2.05)	1.82 (1.39, 2.39)
Lopinavir/r	400 mg/100 mg BID	GZR 200 mg QD	13	Grazoprevir	12.86 (10.25, 16.13)	7.31 (5.65, 9.45)	21.70 (12.99, 36.25)
	400 mg/100 mg BID	EBR 50 mg QD	10	Elbasvir	3.71 (3.05, 4.53)	2.87 (2.29, 3.58)	4.58 (3.72, 5.64)
Ritonavir [‡]	100 mg BID	GZR 200 mg SD	10	Grazoprevir	2.03 (1.60, 2.56)	1.15 (0.60, 2.18)	1.88 (1.65, 2.14)
HIV Integrase Strand Transfer Inhibitor							
Dolutegravir	50 mg SD	GZR 200 mg+EBR 50 mg QD	12	Grazoprevir	0.81 (0.67, 0.97)	0.64 (0.44, 0.93)	0.86 (0.79, 0.93)
	50 mg SD	GZR 200 mg+EBR 50 mg QD	12	Elbasvir	0.98 (0.93, 1.04)	0.97 (0.89, 1.05)	0.98 (0.93, 1.03)
Raltegravir	400 mg BID	GZR 200 mg QD	11	Grazoprevir	0.89 (0.72, 1.09)	0.85 (0.62, 1.16)	0.90 (0.82, 0.99)
	400 mg SD	EBR 50 mg SD	10	Elbasvir	0.81 (0.57, 1.17)	0.89 (0.61, 1.29)	0.80 (0.55, 1.16)
HIV Non-Nucleoside Reverse Transcriptase Inhibitor							
Efavirenz	600 mg QD	GZR 200 mg QD	12	Grazoprevir	0.17 (0.13, 0.24)	0.13 (0.09, 0.19)	0.31 (0.25, 0.38)
	600 mg QD	EBR 50 mg QD	10	Elbasvir	0.46 (0.36, 0.59)	0.55 (0.41, 0.73)	0.41 (0.28, 0.59)
Ralpivirine	200 mg QD	GZR 200 mg+EBR 50 mg QD	19	Grazoprevir	0.98 (0.89, 1.07)	0.97 (0.83, 1.14)	1.00 (0.93, 1.07)
	200 mg QD	GZR 200 mg+EBR 50 mg QD	19	Elbasvir	1.07 (1.00, 1.15)	1.07 (0.99, 1.16)	1.04 (0.98, 1.11)
HIV Nucleotide Reverse Transcriptase Inhibitor							
Tenofovir disoproxil fumarate	300 mg QD	GZR 200 mg QD	12	Grazoprevir	0.86 (0.65, 1.12)	0.78 (0.51, 1.18)	0.89 (0.78, 1.01)
	300 mg QD	EBR 50 mg QD	10	Elbasvir	0.93 (0.82, 1.05)	0.88 (0.77, 1.00)	0.92 (0.81, 1.05)
Immunosuppressant							
Cyclosporine	400 mg SD	GZR 200 mg+EBR 50 mg QD	14	Grazoprevir	15.21 (12.83, 18.04)	17.00 (12.94, 22.34)	3.39 (2.82, 4.09)
	400 mg SD	GZR 200 mg+EBR 50 mg QD	14	Elbasvir	1.98 (1.84, 2.13)	1.95 (1.84, 2.07)	2.21 (1.98, 2.47)
Mycophenolate mofetil	1000 mg SD	GZR 200 mg+EBR 50 mg QD	14	Grazoprevir	0.74 (0.60, 0.92)	0.58 (0.42, 0.82)	0.97 (0.89, 1.06)
	1000 mg SD	GZR 200 mg+EBR 50 mg QD	14	Elbasvir	1.07 (1.00, 1.14)	1.07 (0.98, 1.16)	1.05 (0.97, 1.14)
Prednisone	40 mg SD	GZR 200 mg+EBR 50 mg QD	14	Grazoprevir	1.09 (0.95, 1.25)	1.34 (1.10, 1.62)	0.93 (0.87, 1.00)
	40 mg SD	GZR 200 mg+EBR 50 mg QD	14	Elbasvir	1.17 (1.11, 1.24)	1.25 (1.16, 1.35)	1.04 (0.97, 1.12)
Tacrolimus	2 mg SD	GZR 200 mg+EBR 50 mg QD	16	Grazoprevir	1.12 (0.97, 1.30)	1.07 (0.83, 1.37)	0.94 (0.87, 1.02)
	2 mg SD	GZR 200 mg+EBR 50 mg QD	16	Elbasvir	0.97 (0.90, 1.06)	0.99 (0.88, 1.10)	0.92 (0.83, 1.02)

Correction: Rilpivirine dose was 25 mg

Coadministered Drug	Regimen of Coadministered Drug	Regimen of GZR or/and EBR	N	Geometric Mean Ratio [90% CI] of GZR and EBR PK with/without Coadministered Drug (No Effect=1.00)			
					AUC [†]	C _{max}	C ₂₄
Opioid-Substitution Therapy							
Buprenorphine/ naloxone	8 - 24 mg/2 - 6 mg QD	GZR 200 mg QD	12	Grazoprevir	0.80 (0.53, 1.22)	0.76 (0.40, 1.44)	0.69 (0.54, 0.88)
	8 mg/2 mg SD	EBR 50 mg SD	15	Elbasvir	1.22 (0.98, 1.52)	1.13 (0.87, 1.46)	1.22 (0.99, 1.51)
Methadone	20-150 mg QD	GZR 200 mg QD	12	Grazoprevir	1.03 (0.53, 1.97)	0.88 (0.36, 2.14)	0.77 (0.56, 1.04)
	20-150 mg QD	EBR 50 mg QD	10	Elbasvir	1.71 (1.16, 2.51)	1.93 (1.30, 2.86)	1.86 (1.22, 2.83)
Acid-Reducing Agent							
Famotidine	20 mg SD	GZR 100 mg/EBR 50 mg SD	16	Grazoprevir	1.10 (0.95, 1.28)	0.89 (0.71, 1.11)	1.12 (0.97, 1.30)
	20 mg SD	GZR 100 mg/EBR 50 mg SD	16	Elbasvir	1.05 (0.92, 1.18)	1.11 (0.98, 1.26)	1.03 (0.91, 1.17)
Pantoprazole	40 mg QD	GZR 100 mg/EBR 50 mg SD	16	Grazoprevir	1.12 (0.96, 1.30)	1.10 (0.89, 1.37)	1.17 (1.02, 1.34)
	40 mg QD	GZR 100 mg/EBR 50 mg SD	16	Elbasvir	1.05 (0.93, 1.18)	1.02 (0.92, 1.14)	1.03 (0.92, 1.17)
Phosphate Binder							
Calcium acetate	2668 mg SD	GZR 100 mg+EBR 50 mg SD	12	Grazoprevir	0.79 (0.68, 0.91)	0.57 (0.40, 0.83)	0.77 (0.61, 0.99)
	2668 mg SD	GZR 100 mg+EBR 50 mg SD	12	Elbasvir	0.92 (0.75, 1.14)	0.86 (0.71, 1.04)	0.87 (0.70, 1.09)
Sevelamer carbonate	2400 mg SD	GZR 100 mg+EBR 50 mg SD	12	Grazoprevir	0.82 (0.68, 0.99)	0.53 (0.37, 0.76)	0.84 (0.71, 0.99)
	2400 mg SD	GZR 100 mg+EBR 50 mg SD	12	Elbasvir	1.13 (0.94, 1.37)	1.07 (0.88, 1.29)	1.22 (1.02, 1.45)
Statin							
Atorvastatin	20 mg SD	GZR 200 mg QD	9	Grazoprevir	1.26 (0.97, 1.64)	1.26 (0.83, 1.90)	1.11 (1.00, 1.23)
Pitavastatin	1 mg SD	GZR 200 mg QD	9	Grazoprevir	0.81 (0.70, 0.95)	0.72 (0.57, 0.92)	0.91 (0.82, 1.01)
Pravastatin	40 mg SD	GZR 200 mg+EBR 50 mg SD	12	Grazoprevir	1.24 (1.00, 1.53)	1.42 (1.00, 2.03)	1.07 (0.99, 1.16)
	40 mg SD	GZR 200 mg+EBR 50 mg SD	12	Elbasvir	0.98 (0.93, 1.02)	0.97 (0.89, 1.05)	0.97 (0.92, 1.02)
Rosuvastatin	10 mg SD	GZR 200 mg QD	11	Grazoprevir	1.16 (0.94, 1.44)	1.13 (0.77, 1.65)	0.93 (0.84, 1.03)
	10 mg SD	GZR 200 mg+EBR 50 mg QD	11	Grazoprevir	1.01 (0.79, 1.28)	0.97 (0.63, 1.50)	0.95 (0.87, 1.04)
	10 mg SD	GZR 200 mg+EBR 50 mg SD	11	Elbasvir	1.09 (0.98, 1.21)	1.11 (0.99, 1.26)	0.96 (0.86, 1.08)
Abbreviations: GZR, grazoprevir; EBR, elbasvir; IV, intravenous; PO, oral; SD, single-dose administration; QD, once daily administration; GZR+EBR, administration of GZR							

Correction: Methadone dose range was 20 – 120 mg.

Table 17. Effects of GZR or EBR on concomitant medications

Coadministered Drug	Regimen of Coadministered Drug	GZR or/and EBR Administration	GZR or/and EBR Regimen	N	Geometric Mean Ratio [90% CI] of Coadministered Drug PK with/without GZR or/and EBR (No Effect=1.00)		
					AUC [†]	C _{max}	C _{trough} [‡]
P-gp Substrate							
Digoxin	Digoxin 0.25 mg SD	EBR	50 mg QD	18	1.11 (1.02, 1.22)	1.47 (1.25, 1.73)	--
CYP3A Substrate							
Midazolam	Midazolam 2 mg SD	GZR	200 mg QD	11	1.34 (1.29, 1.39)	1.15 (1.01, 1.31)	--
CYP2C8 Substrate							
Montelukast	Montelukast 10 mg SD	GZR	200 mg QD	23	1.11 (1.01, 1.20)	0.92 (0.81, 1.06)	1.39 (1.25, 1.56)
HCV Antiretroviral							
GS-331007	Sofosbuvir 400 mg SD	GZR+EBR	200 mg+50 mg QD	16	1.13 (1.05, 1.21)	0.87 (0.78, 0.96)	1.53 (1.43, 1.63)
Sofosbuvir	Sofosbuvir 400 mg SD	GZR+EBR	200 mg+50 mg QD	16	2.43 (2.12, 2.79) [¶]	2.27 (1.72, 2.99)	--
HIV Protease Inhibitor							
Atazanavir/r	Atazanavir 300 mg/r 100 mg QD	GZR	200 mg QD	11	1.43 (1.30, 1.57)	1.12 (1.01, 1.24)	1.23 (1.13, 1.34)
	Atazanavir 300 mg/r 100 mg QD	EBR	50 mg QD	8	1.07 (0.98, 1.17)	1.02 (0.96, 1.08)	1.15 (1.02, 1.29)
Darunavir/r	Darunavir 600 mg/r 100 mg BID	GZR	200 mg QD	13	1.11 (0.99, 1.24)	1.10 (0.96, 1.25)	1.00 (0.85, 1.18)
	Darunavir 600 mg/r 100 mg BID	EBR	50 mg QD	8	0.95 (0.86, 1.06)	0.95 (0.85, 1.05)	0.94 (0.85, 1.05)
Lopinavir/r	Lopinavir 400 mg/r 100 mg BID	GZR	200 mg QD	13	1.03 (0.96, 1.16)	0.97 (0.88, 1.08)	0.97 (0.81, 1.15)
	Lopinavir 400 mg/r 100 mg BID	EBR	50 mg QD	9	1.02 (0.93, 1.13)	1.02 (0.92, 1.13)	1.07 (0.97, 1.18)
HIV Integrase Strand Transfer Inhibitor							
Dolutegravir	Dolutegravir 50 mg SD	GZR+EBR	200 mg+50 mg QD	12	1.16 (1.00, 1.34)	1.22 (1.05, 1.40)	1.14 (0.95, 1.36)
Raltegravir	Raltegravir 400 mg BID	GZR	200 mg QD	11	1.43 (0.89, 2.30)	1.46 (0.78, 2.73)	1.47 (1.09, 2.00)
	Raltegravir 400 mg SD	EBR	50 mg SD	10	1.02 (0.81, 1.27)	1.09 (0.83, 1.44)	0.99 (0.80, 1.22) [§]
HIV Non-Nucleoside Reverse Transcriptase Inhibitor							
Efavirenz	Efavirenz 600 mg QD	GZR	200 mg QD	11	1.00 (0.96, 1.05)	1.03 (0.99, 1.08)	0.93 (0.88, 0.98)
	Efavirenz 600 mg QD	EBR	50 mg QD	7	0.82 (0.78, 0.86)	0.74 (0.67, 0.82)	0.91 (0.87, 0.96)
Rilpivirine	Rilpivirine 200 mg QD	GZR+EBR	200 mg+50 mg QD	19	1.13 (1.07, 1.20)	1.07 (0.97, 1.17)	1.16 (1.09, 1.23)
HIV Nucleotide Reverse Transcriptase Inhibitor							
Tenofovir disoproxil fumarate	Tenofovir disoproxil fumarate 300 mg QD	GZR	200 mg QD	12	1.18 (1.09, 1.28)	1.14 (1.04, 1.25)	1.24 (1.10, 1.39)
	Tenofovir disoproxil fumarate 300 mg QD	EBR	50 mg QD	10	1.34 (1.23, 1.47)	1.47 (1.32, 1.63)	1.29 (1.18, 1.41)
Immunosuppressant							
Cyclosporine	Cyclosporine 400 mg SD	GZR+EBR	200 mg+50 mg QD	14	0.96 (0.90, 1.02)	0.90 (0.85, 0.97)	1.00 (0.92, 1.08) [§]
Mycophenolic acid	Mycophenolate mofetil 1000 mg SD	GZR+EBR	200 mg+50 mg QD	14	0.95 (0.87, 1.03)	0.95 (0.67, 1.07)	--
Prednisolone	Prednisone 40 mg SD	GZR+EBR	200 mg+50 mg QD	14	1.08 (1.01, 1.16)	1.04 (0.99, 1.09)	--
Prednisone	Prednisone 40 mg SD	GZR+EBR	200 mg+50 mg QD	14	1.08 (1.00, 1.17)	1.05 (1.00, 1.10)	--
Tacrolimus	Tacrolimus 2 mg SD	GZR+EBR	200 mg+50 mg QD	16	1.43 (1.24, 1.64)	0.60 (0.52, 0.69)	1.70 (1.49, 1.94) [§]
Oral Contraceptive							

Ethinyl estradiol (EE)	0.03 mg EE/ 0.15 mg LNG SD	GZR	200 mg QD	20	1.10 (1.05, 1.14)	1.05 (0.98, 1.12)	--
		EBR	50 mg QD	20	1.01 (0.97, 1.05)	1.10 (1.05, 1.16)	--
Levonorgestrel (LNG)		GZR	200 mg QD	20	1.23 (1.15, 1.32)	0.93 (0.84, 1.03)	--
		EBR	50 mg QD	20	1.14 (1.04, 1.24)	1.02 (0.95, 1.08)	--
Opioid Substitution Therapy							
Buprenorphine	Buprenorphine 8 - 24 mg/Naloxone 2 - 6 mg QD	GZR	200 mg QD	12	0.98 (0.81, 1.19)	0.90 (0.76, 1.07)	--
	Buprenorphine 8 mg/Naloxone 2 mg SD	EBR	50 mg QD	15	0.98 (0.89, 1.08)	0.94 (0.82, 1.08)	0.98 (0.88, 1.09)
R-Methadone	Methadone 20-150 mg QD	GZR	200 mg QD	12	1.09 (1.02, 1.17)	1.03 (0.96, 1.11)	--
		EBR	50 mg QD	10	1.03 (0.92, 1.15)	1.07 (0.95, 1.20)	1.10 (0.96, 1.26)
S-Methadone		GZR	200 mg QD	12	1.23 (1.12, 1.35)	1.15 (1.07, 1.25)	--
		EBR	50 mg QD	10	1.09 (0.94, 1.26)	1.09 (0.95, 1.25)	1.20 (0.98, 1.47)
Statin							
Atorvastatin	Atorvastatin 20 mg SD	GZR	200 mg QD	9	3.00 (2.42, 3.72)	5.66 (3.39, 9.45)	--
	Atorvastatin 10 mg SD	GZR+EBR	200 mg+50 mg QD	16	1.94 (1.63, 2.33)	4.34 (3.10, 6.07)	0.21 (0.17, 0.26)
Pitavastatin	Pitavastatin 1 mg SD	GZR	200 mg QD	9	1.11 (0.91, 1.34)	1.27 (1.07, 1.52)	--
Pravastatin	Pravastatin 40 mg SD	GZR+EBR	200 mg+50 mg QD	12	1.33 (1.09, 1.64)*	1.28 (1.05, 1.55)	--
Rosuvastatin	Rosuvastatin 10 mg SD	GZR+EBR	200 mg+50 mg QD	12	2.26 (1.89, 2.69)**	5.49 (4.29, 7.04)	0.98 (0.84, 1.13)
		GZR	200 mg QD	12	1.59 (1.33, 1.89)**	4.25 (3.25, 5.56)	0.80 (0.70, 0.91)
Abbreviations: GZR, grazoprevir; EBR, elbasvir; IV, intravenous; PO, oral; SD, single-dose administration; QD, once daily administration; GZR+EBR, administration of GZR and EBR as separate pills							
*AUC0-inf for SD administration; AUC0-24 for QD administration; AUC0-12 for BID administration							

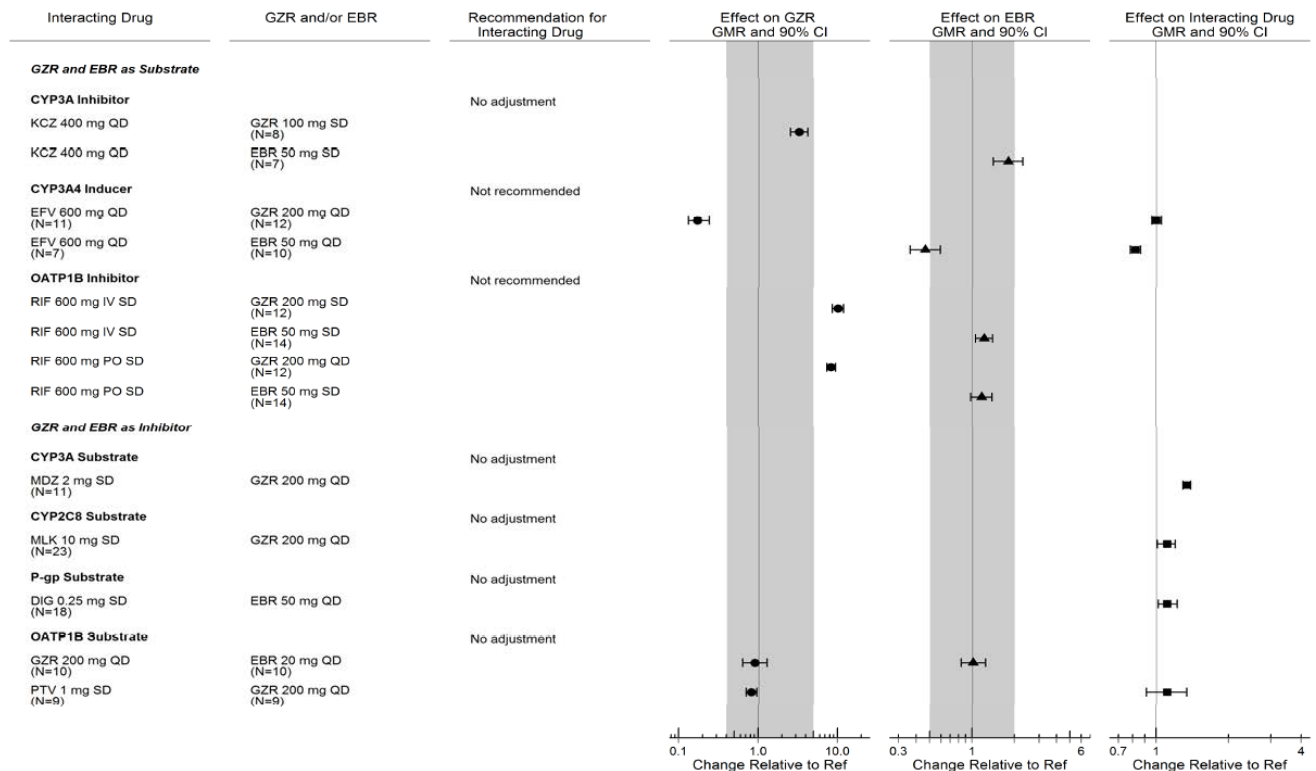
During the procedure the applicant reported results from study 081 in which the effects of EBR/GZR on TDF were assessed. The TFV GMR [90% CI] for AUC0-24 was 1.27 [1.20, 1.35], indicating that the effect of the FDC was not greater than that of EBR alone and that no dose adjustment was needed.

Summary of applicant's conclusions from clinical DDI studies

- GZR and EBR show clinically relevant decreases in exposure with moderate and strong CYP3A inducers but no clinically relevant increases in exposure with CYP3A inhibitors.
- GZR exposure increases to a clinically relevant extent with OATP1B inhibitors.
- P-gp and BCRP play a limited role in the absorption of GZR and EBR.
- GZR is a weak inhibitor of CYP3A, an intestinal BCRP inhibitor, but not an inhibitor of OATP1B.
- EBR is not an inhibitor of OATP1B.
- EBR is an inhibitor of intestinal BCRP and intestinal P-gp.

Figure 12 summarises the applicant's initial position regarding need for dose adjustments by mechanism of interaction, noting that the grey areas represent the applicant's bounds. The applicant contraindicated use of EBR/GZR with inhibitors of OATP1B and inducers of CYP3A during the procedure.

Figure 5. Effect on AUC of GZR, EBR, and Probe Substrates, Inducers, or Inhibitors of Metabolic Enzymes and Transporters when Coadministered, with Recommendations for Interacting Drug when Coadministered with EBR/GZR



KCZ: ketoconazole, EFV: efavirenz, RIF: rifampin, MDZ: midazolam, MLK: montelukast, DIG: digoxin, PTV: pitavastatin

2.4.3. Pharmacodynamics

Mechanism of action

Grazoprevir (GZR; MK-5172), is an inhibitor of the NS3/4A protease (i.e. it is a PI). NS3/4A-mediated cleavage of the polyprotein formed by translation of the HCV RNA genome is essential for replication.

Elbasvir (EBR; MK-8742) is an inhibitor non-structural protein 5A (NS5A). NS5A is a pleiotropic protein with important roles in HCV viral replication and modulation of the physiology of the host cell.

Primary pharmacology

GZR

In a peptide hydrolysis-based enzymatic assay GZR inhibited NS3/4A from GT 1 – 6 with IC_{50} values < 1 nM. The IC_{50} range was from picomolar levels for GT-1 to 0.034-0.135 for non-GT1 enzymes except for GT3 (0.69 nM).

In the replicon assay, GZR inhibited GT1a, 1b, 2a, 2b, 4a, 5a and 6a viruses with a mean EC_{50} range 0.2 – 2.9 nM. The EC_{50} was 0.4 nM for GT1a and 0.5 nM for GT1b replicon cell lines with a similar value for the full-length GT4a replicon (0.3 nM) and GT6a (0.2 nM). The EC_{50} was higher for a homologous GT2a (2.3 nM) replicon and for chimeric replicons encoding NS3/4As from GT2b (3.7nM) or GT5a (1.5nM).

Least activity was observed for the chimeric GT3a (GLA) replicon (EC_{50} 7.6 nM) and full-length GT3a (S52) replicon (35 nM) although the value for the chimeric replicon with only the S52 protease domain was 2.1 nM. Also, the EC_{50} in representative GT3a patient isolates ranged from 2-7 nM.

The EC_{50} shifted by <3-fold when estimated in the presence of 40% normal human serum. Using engineered replicons activity was not affected by RAVs associated with treatment failure to other protease inhibitors including V36A/L/M or T54A/S (telaprevir) and Q80K/R (simeprevir). Amino acid substitutions associated with the most substantial potency shifts were R155G/T/W, A156T/V and D168A/F/G/H/I/K/L/T/V/Y for GT1b and Y56H and D168A/G/H/I/K/T/V for GT1a. RAVs identified in GT3 did not confer large potency shifts (≤ 7 -fold).

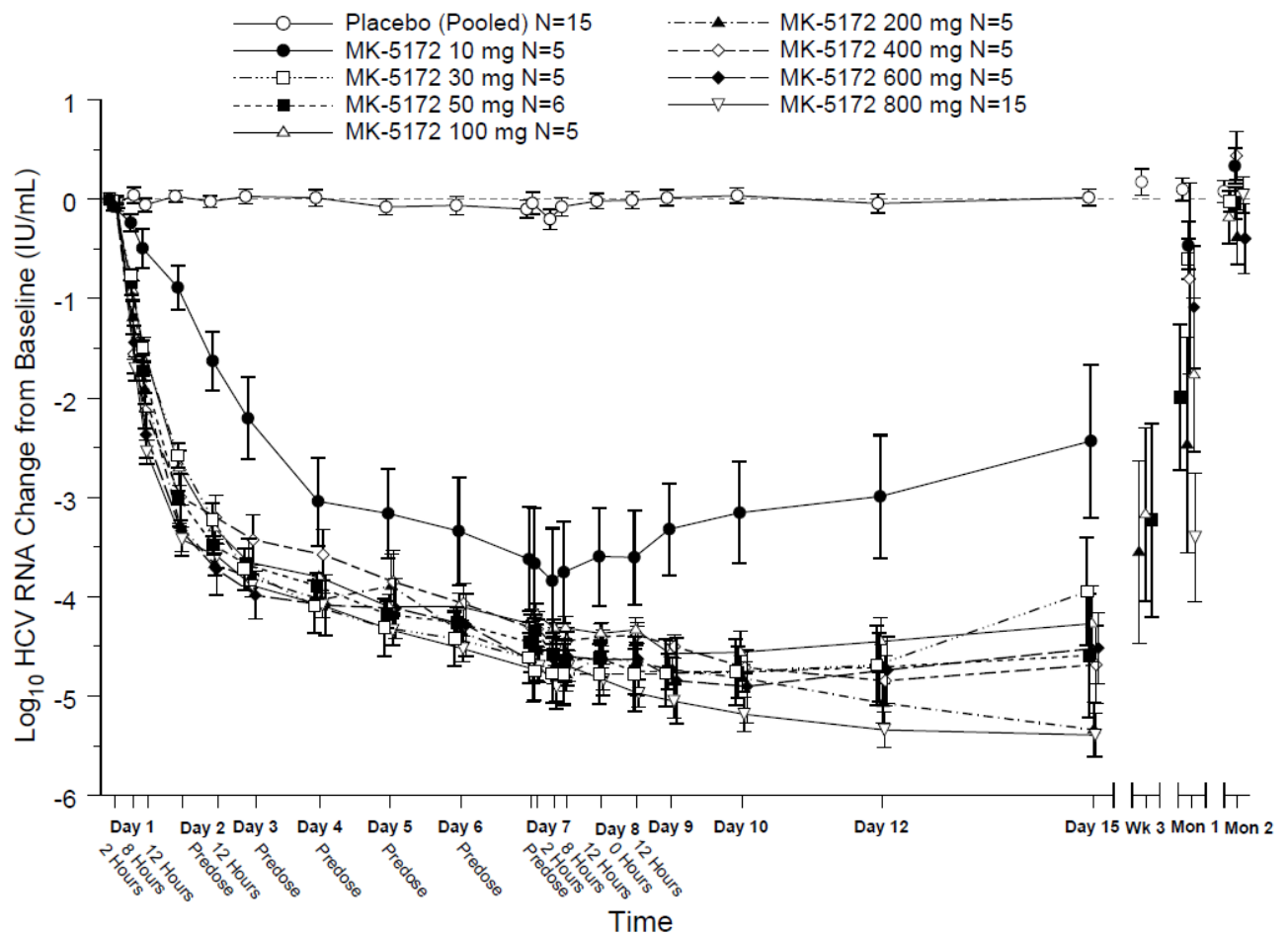
GZR showed an additive effect with interferon-alpha, ribavirin and EBR with an effect that was additive to synergistic with SOF. No cytotoxic effects were observed at any of the combination concentrations tested.

The emergence of resistance in GT1a replicon cells was assessed on exposure to the combination of GZR and EBR. Tested alone concentrations 100 X and 1000 X EC_{90} were required to suppress emergence of resistant colonies but 10 X EC_{90} of each tested in combination blocked the emergence of resistant colonies. Clonal sequencing analysis of resistant colonies selected with $>1X$ EC_{90} combinations of both compounds revealed mostly linked mutations (≥ 2 in both protein targets), indicating a higher genetic barrier to resistance when GZR and EBR are used in combination.

Study MK-5172-P004 – Study title: A Multiple Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Grazoprevir (MK-5172) in Hepatitis C Infected Male Patients

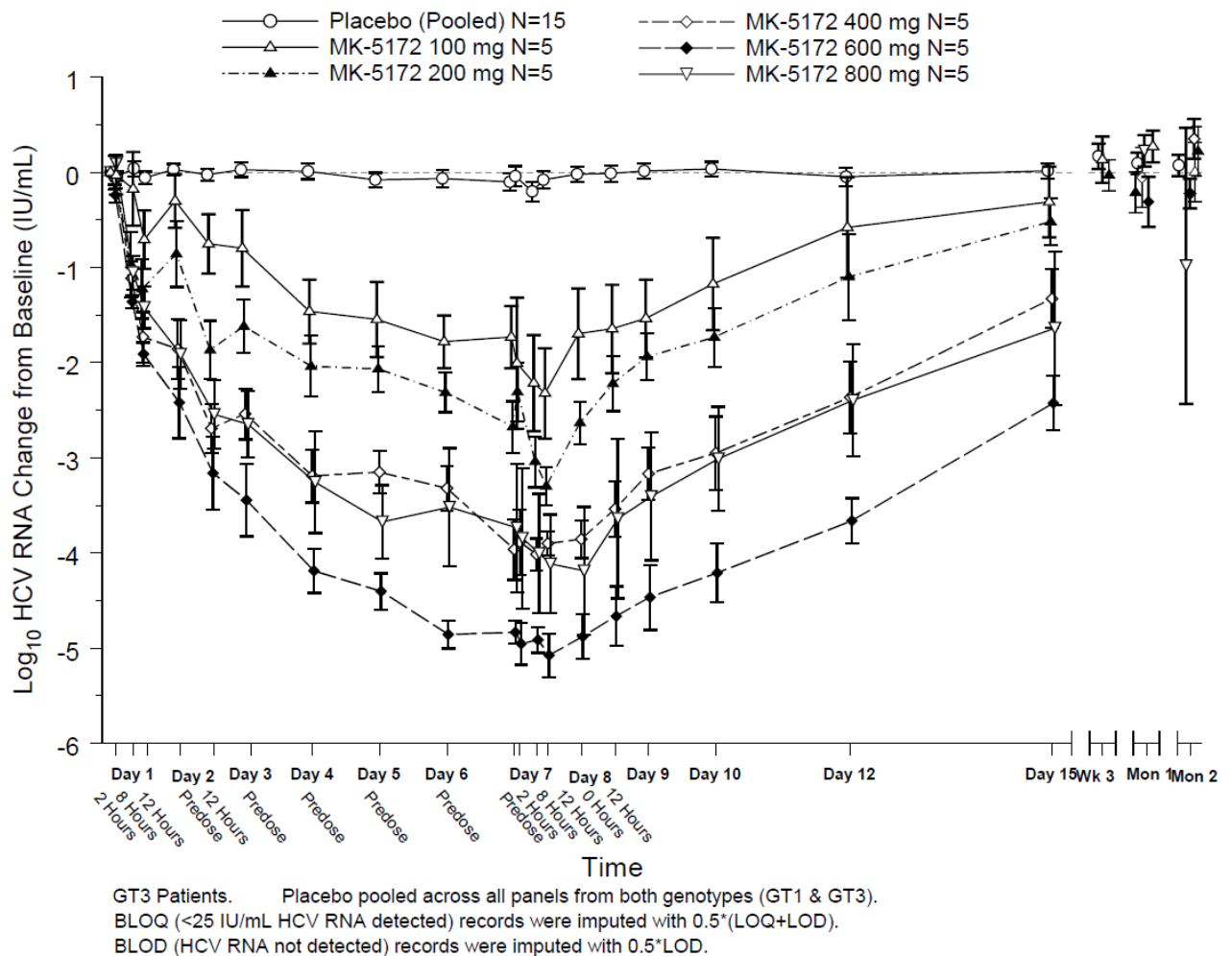
In Study MK-5172-P004 in HCV-infected male patients the maximum $\log_{10}$ HCV RNA reduction vs. placebo was 5.34 IU/mL, which occurred with 800 mg/day in GT1 patients. In GT3 patients the largest reduction was 4.98 IU/mL, which occurred with 600 mg/day. Significant differences vs. placebo occurred at all GZR doses for both genotypes tested, as summarised in Figures 13 and 14 for each genotype below, which suggest more rapid recovery after day 7 for GT3 (it seems all were GT3a) vs. GT1. GT1a and GT1b responded similarly and showed similar relationships with PK parameters.

Figure 6. Arithmetic Mean ($\pm$ SE) of log₁₀ HCV RNA Change From Baseline (IU/mL) Following the Administration of Multiple Oral Doses of 10 to 800 mg Grazoprevir (MK-5172) QD in GT1 HCV-Infected or Placebo QD in GT1 and GT3 HCV-Infected Male Patients on Days 1 – 7



GT1 Patients. Placebo pooled across all panels from both genotypes (GT1 & GT3).
 BLOQ (<25 IU/mL HCV RNA detected) records were imputed with $0.5 \times (\text{LOQ} + \text{LOD})$.
 BLOD (HCV RNA not detected) records were imputed with $0.5 \times \text{LOD}$.

Figure 7. Arithmetic Mean ($\pm$ SE) of log₁₀ HCV RNA Change From Baseline (IU/mL) Following the Administration of Multiple Oral Doses of 100 to 800 mg Grazoprevir (MK-5172) QD in GT3 HCV-Infected or Placebo QD in GT1 and GT3 HCV-Infected Male Patients on Days 1 – 7



Baseline and post base-line resistance analysis was conducted in 71/74 GZR patients. The NS3/4A gene from samples with > 1000 IU/mL was subjected to PCR and population and selective clonal sequencing. On average 45 clones per sample were used for the clonal sequencing analysis. The sensitivity of variant detection in clonal sequencing was between 5 - 10%. Post-baseline variants in > 10% of the samples were noted and NS3/4a variants with more than 5-fold reduced susceptibility were considered as RAVs.

Substitutions at amino acids 168, 156, 56 and 155 were observed in > 10% of GZR patients and amino acid variants were also observed at other positions but examination of sequences in Genbank database showed that most of these positions are highly polymorphic and their role in resistance is unclear. In general, there was no notable difference in terms of the types of RAVs and the prevalence of RAVs selected by different dose levels within each genotype. RAVs at amino acids 168, 156, 56 and 155 occurred more often with GT1a vs. GT1b patients (e.g. at 168: 65% vs. 52% GT1b; at 156: 26.9% vs. 19%; at 56: 26.9% vs. 9.5%; at 155: 23% vs. 19%).

In HCV GT1a replicons, substitution Y56H resulted in 16-fold reduced susceptibility and D168A, D168E, D168K led to 81-, 14- and 212-fold reduced susceptibility, respectively. In GT1b, variants Y56H, A156T, and D168K resulted in 12.6-, 280-, and 121-fold reduced susceptibility, respectively.

Clonal sequence analysis of 13 GT1a and 6 GT1b samples showed that RAVs were consistent with those identified by the population analysis. The R155W/A156G double mutation, which confers a > 3000-fold reduction in susceptibility, was not found up to 2-month follow-up.

The most frequent baseline polymorphisms were:

- GT1a - at 80 (42%) followed by 122 (15%), 168 or 170 (7.6%)
- GT1b - at 56 (36%) or 170 (32%)
- GT3 at 166 (37.5%)

None of the baseline polymorphisms impacted on viral response.

EBR

In a panel of sub-genomic replicon cell lines that contained NS5A sequences the mean EC₅₀ values were almost all in the range 3-14 pM and specifically GT1a (4 pM), GT1b (3 pM), GT2a (31L) (3 pM), GT3a (14 pM), GT4a (3 pM), GT5a (1 pM) and GT6d (3 pM). The EC₅₀ was markedly higher for the GT2b replicon (3.4 nM), which was largely due to amino acid substitution of L31M within the GT2 NS5A sequence.

There was a 10-fold shift in potency in the presence of 40% NHS.

Against replicons generated with several patient isolates the EC₅₀ values for GT1a and GT1b were from 3-10 pM. For GT2 values ranged from 0.003 to 20 nM, largely reflecting the presence of 31M in GT2b. For GT3a the range was from 3 pM to 0.4 nM with least activity against 3i and 3g subtypes that harbored RAVs at positions 28, 30 and 31. In a diverse set of GT4 and GT6 subtypes there were sub-picomolar EC₅₀ values for most isolates. The least susceptible was a GT6n isolate for which the EC₅₀ was 2.7 nM and in which amino acid substitutions were found at positions 28, 30 and 93. The range for GT5a was from 0.4-1 pM.

EBR activity was also assessed in engineered replicons encoding defined RAVs, including those from GT1a, GT1b and GT4a. RAVs elicited by other NS5A inhibitors in GT1b caused <20-fold reductions in EBR activity and EC₅₀ values were ≤ 50 pM. In contrast, the most shifted variants in GT1a caused a 2-3 log reduction in activity.

Reduced activity was observed particularly against Y93H substitutions in GT1a, GT1b, GT2a and GT3a. Additional RAVs in GT1a that reduced EBR activity were L31M/V and Q30D/E/H/K/R. In GT2a, GT5a and GT6a reduced activity was observed for substitutions at F/L28S/F and L31F.

The presence of the natural resistance polymorph 31M in GT2b caused EBR resistance. Similar results were obtained when a more rapid transient infectious virus assay was used to study defined resistance in GT1a instead of stable replicons.

In *de novo* resistance selection studies RNA isolated from resistant colonies revealed that variants occurred primarily at positions 28, 30, 31 and 93. Substitutions at Q30 and Y93 were mainly responsible for potency losses in GT1a. Substitutions F28S, Y93H and Y93H in GT2a, GT2b and GT3a, respectively, resulted in >1000-fold loss of activity in those genotypes.

EBR presented a higher genetic barrier to resistance in GT1b and GT4a in which two nucleotide changes were generally required to elicit resistance. Substitutions L28F and L31F in GT5a and F28S and L31F in GT6 were the main variants responsible for resistance.

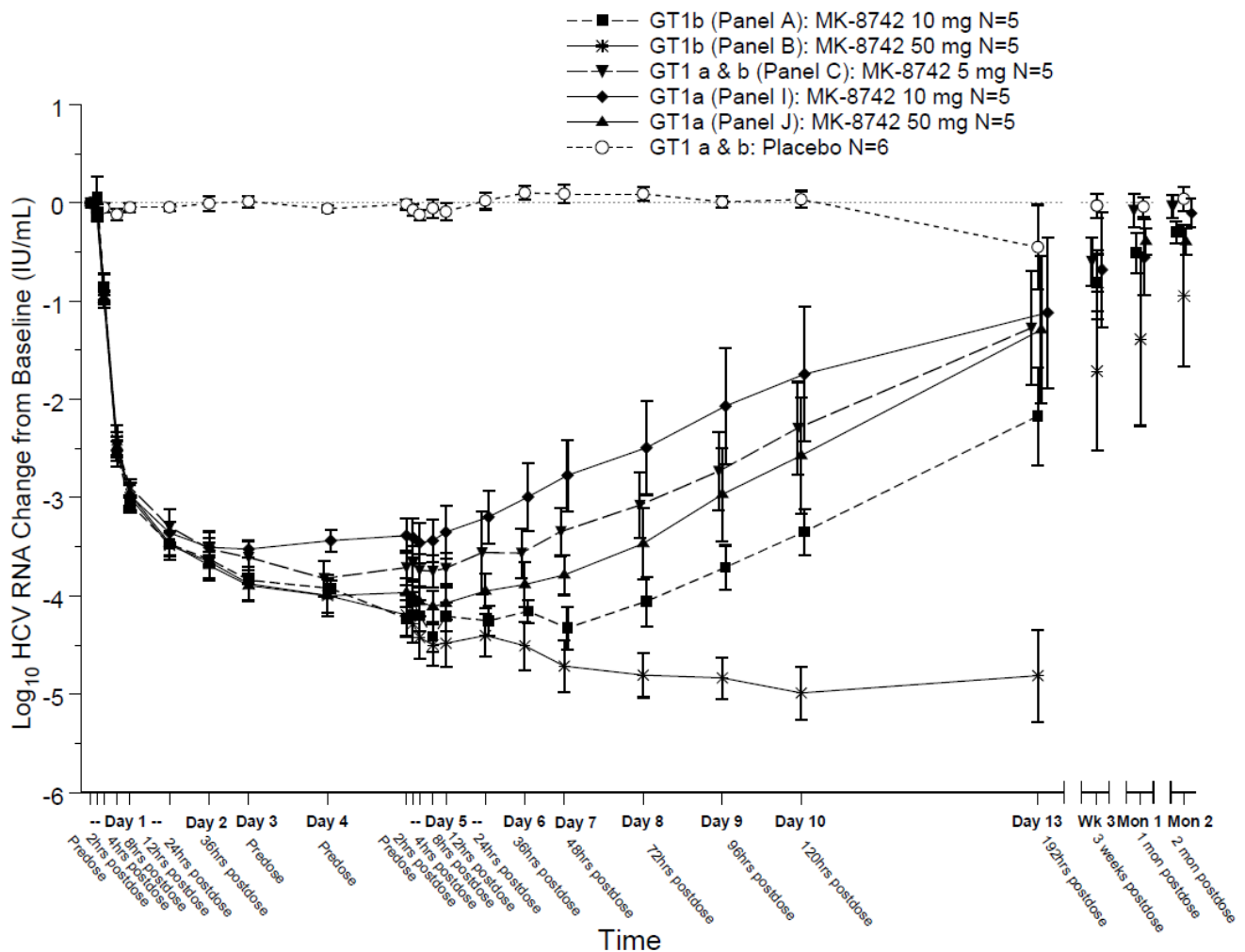
There was additive to synergistic activity with ribavirin, sofosbuvir and GZR.

Study MK-8742-P002 – Study title: A Multiple Dose Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of MK-8742 (Elbasvir) in Hepatitis C Infected Males

In the EBR monotherapy Study 002 in HCV-infected male patients the maximum log₁₀ HCV RNA reduction vs. placebo was 4.41 IU/mL, seen with 50 mg/day in GT1 patients. In GT1a and GT3 the largest reductions were 3.95 IU/mL at 50 mg/day and 2.27 at 100 mg/day, respectively. Significant differences vs. placebo occurred only at 50 mg/day and 100 mg/day for GT3.

Baseline resistance analysis was conducted in 44 EBR patients using population sequencing and polymorphisms at amino acid loci prone to resistance selection by NS5A inhibitors (including EBR) were evaluated (positions 28, 30, 31, 58 and 93). Seven patients had HCV with baseline polymorphisms at one or more of these loci (2 GT1a, 2 GT1b and 3 GT3), with reduced susceptibility to EBR in HCV replicons.

Figure 8. Profiles of the Change From Baseline in log₁₀ HCV RNA for GT1 HCV-Infected Male Patients After Receiving Multiple QD Doses of Elbasvir/Placebo for 5 Days

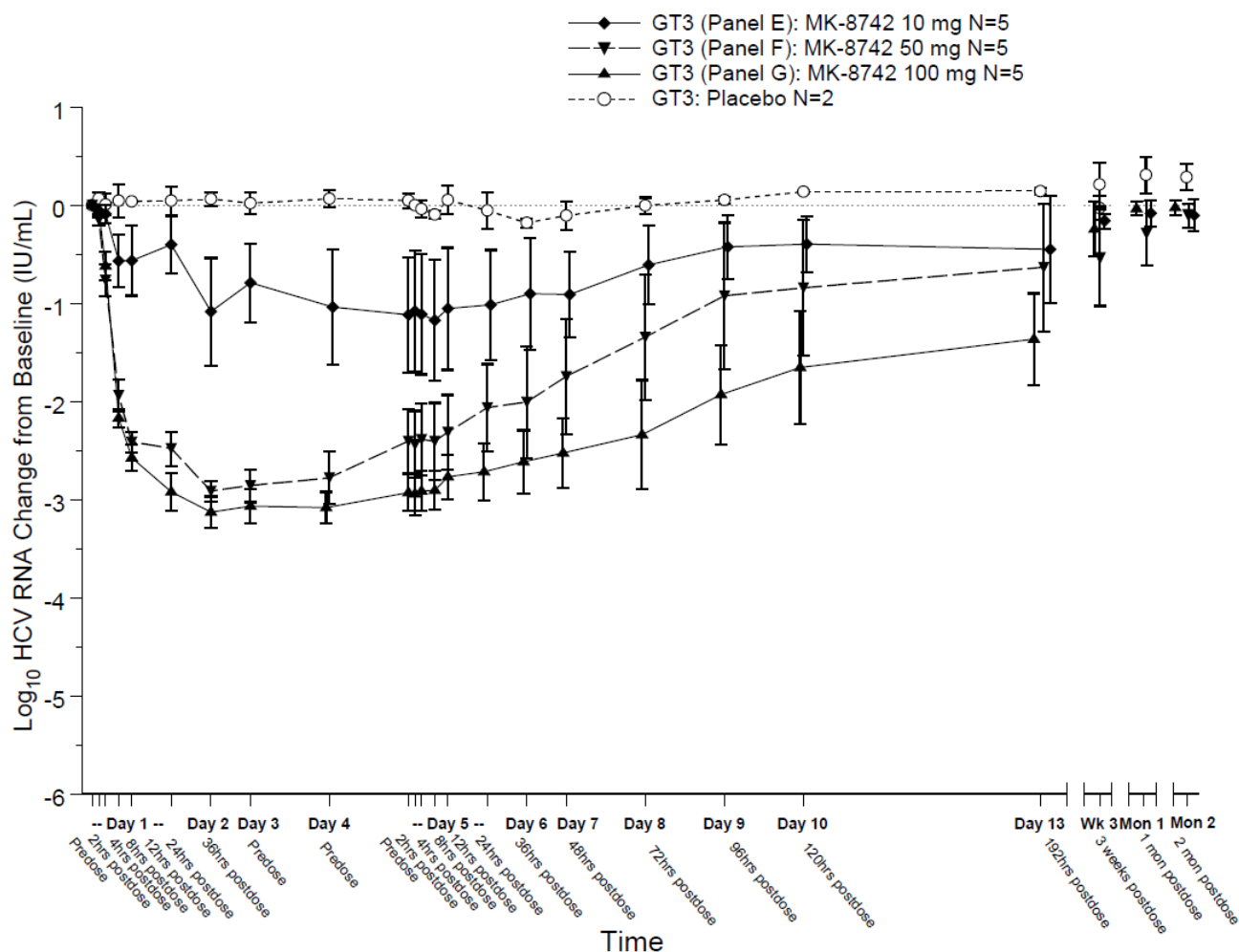


* BLOQ (< 25 IU/ml HCV RNA detected) records were imputed with $0.5 \cdot (\text{LOQ} + \text{LOD})$.

* BLOD (HCV RNA not detected) records were imputed with $0.5 \cdot \text{LOD}$.

* LOQ = 25 IU/ml, LOD = 3.8 IU/ml.

Figure 9. Profiles of the Change From Baseline in $\log_{10}$ HCV RNA for GT3 HCV-Infected Male Patients After Receiving Multiple Once Daily Doses of Elbasvir/Placebo for 5 Days



* BLOQ (<25 IU/ml HCV RNA detected) records were imputed with $0.5 \times (\text{LOQ} + \text{LOD})$.

* BLOD (HCV RNA not detected) records were imputed with $0.5 \times \text{LOD}$.

* LOQ = 25 IU/ml, LOD = 3.8 IU/ml.

Post-baseline analysis of virus variants suggested that M28T, Q30R, L31V and Y93H in GT1a, L31V and Y93H in GT1b and A30K, L31F and Y93H in GT3 were the major RAVs selected by EBR based on population sequencing. Variants with these substitutions showed reduced susceptibility to EBR in HCV replicons. Detailed clonal sequencing revealed that most of the variants contained double or triple linked mutations. Additional polymorphisms were also noted. Three of 5 GT3 patients treated with 10 mg EBR did not show any antiviral response and clonal sequencing detected NS5A RAVs.

Secondary pharmacology

Two QTc studies were conducted as shown in Table 24. All dosing was in the fasted state and in each study moxifloxacin 400 mg was shown to be an adequate positive control.

Table 18. Thorough QTc studies

Protocol number	Active substance	Dose	Design	Subjects
5172-P049	Grazoprevir Thorough QTc study	1600 mg GZR	Single dose	41/39

Protocol number	Active substance	Dose	Design	Subjects
8742-P015	Elbasvir Thorough QTC study	Part I: 200, 400, 800 mg EBR Part II: 700 mg EBR (determined in part I)	Single doses	Part I: 11/8 Part II: 42/36

Study MK-5172-P049- Study title: A Single Dose Study to Assess the Effect of MK-5172 on the QTc Interval of Healthy Adult Subjects

In Study 049 the gmean GZR AUC after a 1600 mg dose was 77 $\mu\text{M}\cdot\text{h}$ but the range was very large (23-326 $\mu\text{M}\cdot\text{h}$). The gmean C_{max} was 14 μM with a range from 5.46-44.6 μM . The applicant does not discuss the possible reasons for such a wide range to occur in healthy fasted subjects. Nevertheless, the gmean GZR AUC and C_{max} values (estimated from the POPPK model) for the 950 HCV-infected patients with PK data in Phase 3 studies were 1.972 $\mu\text{M}\cdot\text{h}$ and 0.228 μM , respectively, indicating that the exposures achieved with 1600 mg considerably exceeded those predicted to be typical for HCV-infected patients.

Table 19. Summary of Plasma Pharmacokinetics of MK-5172 Following a Single Oral Dose of 1600 mg MK-5172 in Healthy Volunteers

	MK-5172 Pharmacokinetic Parameters				
	AUC ₀₋₂₄ ($\mu\text{M}\cdot\text{hr}$)	C _{max} (μM)	T _{max} (hr)	C ₂ (μM)	C ₂₄ (nM)
N [†]	40	40	40	41	40
AM	97.2	16.0	4.87	5.86	248
SD	71.5	8.98	1.96	4.74	375
ACV	73.5	56.1	40.32	80.9	150.8
GM	77.0	14.1	4.54	3.67	125
GCV	78.1	52.6	38.82	209.5	147.6
Med	76.6	13.8	4.08	4.57	101
Min	23.0	5.46	2.08	0.0116	25.7
Max	326	44.6	12.00	22.1	1610

1600 mg MK-5172: A single supratherapeutic dose of 1600 mg MK-5172 (16 x 100 mg tablet) on Day 1

AN = Allocation number; AM = Arithmetic mean; SD = Standard deviation;

ACV = Arithmetic coefficient of variation is calculated in the original scale with the equation: $100 \times (\text{SD}/\text{AM})$.

Med = Median; Min = Minimum; Max = Maximum;

GM = Geometric mean; GCV = Geometric coefficient of variation is calculated in the natural log-scale with the equation: $100 \times \sqrt{\exp(s^2) - 1}$, where s^2 is the observed variance on the natural log-scale.

[†]Subject AN 0034 was terminated early in Period 1.

The maximum mean difference from placebo in QTcF change from baseline occurred at 8 h [-0.48 ms; 90% CI (-2.54, 1.58)], indicating no prolongation of the QTc interval to a clinically significant degree. The population-corrected QTc (QTcP) gave similar results. There were no QTcF changes from baseline ≥ 30 ms and ≤ 60 ms or > 60 ms. There were 3 post-GZR instances of QTcF > 450 and ≤ 480 ms compared to one occurrence after placebo vs. 19 after moxifloxacin. There were no important effects of GZR on other ECG parameters.

Study MK-8742-P015 - Study title: A Single Dose Trial to Assess the Effect of MK-8742 on QTc Interval in Healthy Adult Volunteers

In Study 015 Part 1 was used to select a dose of EBR for Part 2. In Part 1 the 200 mg, 400 mg and 800 mg doses gave plasma exposures in healthy subjects as shown in Table 26.

Table 20. Part 1: Statistical Analysis of Pharmacokinetics Primary Objective Estimation of Geometric Means for Pharmacokinetic Parameters Following a Single Oral Dose Administration of MK-8742 to Fasted Healthy Adult Subjects for Each Level of Treatment (Per-Protocol Population)

Parameter (unit)	Treatment	N	Geometric LS Mean	Geometric Mean 90% CI
C _{max} (nM)	200 mg MK-8742	6	265	(210, 334)
	400 mg MK-8742	6	423	(336, 532)
	800 mg MK-8742	6	654	(519, 824)
AUC _{0-24hr} (nM.h)	200 mg MK-8742	6	3120	(2530, 3850)
	400 mg MK-8742	6	5300	(4300, 6530)
	800 mg MK-8742	6	8130	(6600, 10000)
C _{24hr} (nM)	200 mg MK-8742	6	84.6	(67.6, 106)
	400 mg MK-8742	6	149	(119, 187)
	800 mg MK-8742	6	239	(191, 300)

In Part 2 the 700 mg dose gave a gmean C_{max} of 567 nM (range 245-1240 nM) and AUC₀₋₂₄ of 6200 nM.h (range 2690-14,500 nM.h). In comparison, the POPPK estimated gmean AUC and C_{max} values in HCV-infected patients dosed at 50 mg/day were 2383 nM.h and 151 nM, respectively. In Part 2 there were 14 placebo patients with measurable EBR from the previous treatment period but concentrations were from 0.287-8.43 nM (i.e. $\leq 1.5\%$ of gmean C_{max}). A test for first-order carryover included in the model used in the primary evaluation of QTcF change from baseline was not statistically significant at the 0.10 level.

After 700 mg EBR the largest mean change from baseline of QTcF vs. placebo was 0.856 (-1.06, 2.78), occurring at 1.5 h post-dose. No subject had average QTcF readings >450 ms and all had a QTcF change from baseline ≤ 30 ms at all times.

Relationship between plasma concentration and effect

In Study MK-5172-p004 the GT3 patients showed a trend for greater viral response across the 100 to 800 mg dose range, with mean differences in maximum log₁₀ HCV RNA reduction that increased from 2 to 5 log₁₀. A dose-dependent relationship was not detected in GT1a or GT1b patients since maximum log₁₀ HCV RNA reduction was consistently in the range of 4 to 5 log₁₀ and patient exposures across the 30 - 800 mg dose range appeared to be on the PK-PD plateau.

In Study mk-8742-p002 there was no trend to suggest a relationship between EBR PK and reduction in HCV GT1 RNA. The analysis for GT3 was complicated by the lack of response in 3/5 patients at 10 mg/day and no conclusions could be drawn.

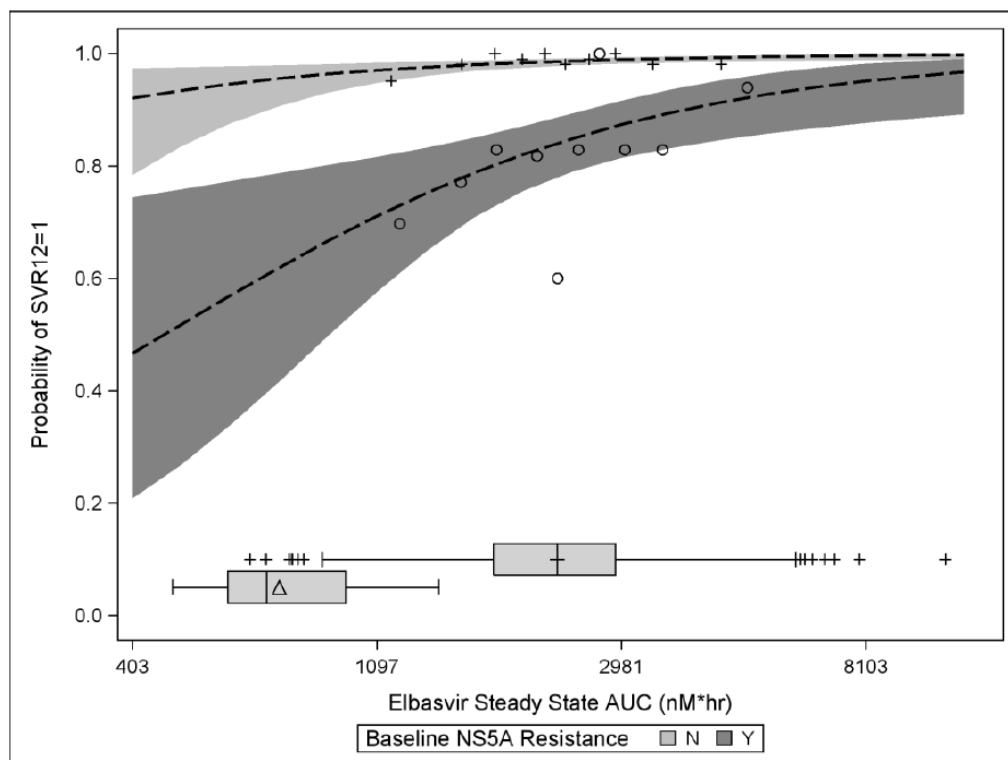
Exposure-response analyses based on Phase 2/3 efficacy and PK data showed that:

- **GZR** AUC₀₋₂₄ and C_{trough} were not associated with SVR12 ($p > 0.2$), suggesting that exposures at the 100 mg dose were on the maximal response plateau of the E-R curve for efficacy.
- **EBR** AUC₀₋₂₄ and EBR C_{trough} were both associated with SVR12 ($p = 0.003$ and 0.006).

Based on area under the ROC curve, the final model for EBR AUC₀₋₂₄ as the pharmacokinetic endpoint was a slightly better predictor of SVR12 than the model for C_{trough}. Significant covariates ($p < 0.001$) were treatment duration, baseline log₁₀ HCV RNA and presence or absence of baseline resistance (of any degree) to NS5A inhibitors. In general, SVR12 rates were lower for short treatment durations (8 weeks), higher baseline log₁₀ HCV RNA and in patients who had baseline resistance to NS5A inhibitors. NS5A RAVs that result in > 5-fold shift in EBR were associated with a greater reduction in SVR12 vs. those associated with < 5-fold shift. Also, the presence of baseline NS5A resistance had a greater impact on SVR12 for GT1a compared to the other genotypes.

Figure 17 shows that EBR exposures associated with the 50 mg dose (with 100 mg GZR) are generally on the plateau of the E-R curve, with a subset with baseline resistance to NS5A inhibitors being more sensitive to reductions in AUC_{0-24} compared to those without baseline resistance to NS5A inhibitors.

Figure 10. Predicted SVR12 Probability from PK-SVR12 Model as a Function of EBR Steady State AUC_{0-24} , Demonstrating Near Maximal Response Across 50 mg Exposures with Increased Sensitivity to Exposure in Patients with NS5A Resistance. Separate Curves Are Presented for Patients With and Without Baseline NS5A Resistance, Assuming a 12- Week Treatment Duration and the Median Value of Baseline HCV RNA



Note: The SVR12 predicted probability is calculated by fixing at the geometric mean of GZR therapeutic dose AUC_{0-24} , mean Baseline $\log_{10}$ HCV RNA and at 12 week treatment duration.
 +: Baseline NS5A resistance = "N"; o: Baseline NS5A resistance="Y"; the observed EBR AUC_{0-24} values associated with a 50 mg EBR dose were divided into 10 bins (separately for subjects with and without baseline NS5A resistance) and the associated SVR12 rate calculated for the subjects in each bin. It should be noted that this approach to binning the observed values does not account for differences in baseline HCV RNA between subjects, which may also influence the efficacy outcome.
 Boxplot with Δ : dose=20 mg; Boxplot with +: dose=50 mg.

Simulations showed that reducing the dose of either GZR or EBR by half still resulted in high projected SVR12 rates, even in the worst-case scenario of development of on-treatment resistance to both compounds simultaneously in GT1a.

Exposure-safety analyses explored GZR exposure and Late ALT/AST Elevations. AUC_{0-24} and C_{max} were obtained from POPPK and C_2 was the GM of individual values from Day 7 onwards. All three PK parameters were well correlated with the Late ALT/AST Elevation Events, with AUC_{0-24} appearing to be slightly more predictive based on AUC of ROC.

A 5-fold increase in AUC_{0-24} relative to the reference population corresponded to a predicted population Late ALT/AST Elevation Event rate of ~2%. A rate of 5% was predicted at a GM AUC_{0-24} ~ 23.7 $\mu M \cdot hr$, which represents an exposure margin ~14-fold the GM with 100 mg doses in the reference population and ~11-fold that in a broader population. Since AUC_{0-24} increases in a greater than dose proportional manner, a 5-fold increase results in AUCs similar to those observed with a 200 mg GZR dose and a 14-fold increase results in AUCs between those observed with 200 and 400 mg. The applicant considered

that a 5% incidence rate of Late ALT/AST Elevation Events was the highest clinically acceptable rate and this boundary was applied to dose selection for Phase 2b and 3 studies.

Table 21. Observed and Predicted Population Late ALT/AST Elevation Event Rate at Various GZR Dose Levels - AUC0-24 Based Results

Dose / Population	GZR AUC0-24hr Values in Patients with PK Data			Observed Rate in Patients with Safety Data			Predicted Rate (%; 95% CI)
	N (PK) [†]	GM AUC0-24 (nM·hr)	GMR [‡]	N (Event) [§]	N (Safety)	Rate (%; 95% CI)	
25 mg	29	242	0.14	0	28	0.0 (0.0, 12.3)	0.1 (0.0, 0.2)
50 mg	89	1303	0.76	0	88	0.0 (0.0, 4.1)	0.4 (0.2, 0.7)
100 mg	1270	1721	1.00	7	1273	0.5 (0.2, 1.1)	0.5 (0.3, 0.8)
Reference							
100 mg Other	770	2818	1.64	7	769	0.9 (0.4, 1.9)	0.8 (0.5, 1.2)
200 mg	39	8498	4.94	1	65	1.5 (0.0, 8.3)	2.1 (1.2, 3.1)
400 mg	42	31062	18.05	4	64	6.3 (1.7, 15.2)	6.2 (3.4, 9.9)
800 mg	40	113645	66.05	6	58	10.3 (3.9, 21.2)	16.6 (8.3, 27.8)

[†] N (PK): Number of patients with available PK data.

[‡] GMR = geometric mean ratio of PK relative to the reference population at 100 mg.

[§] N (Event): Number of patients with occurrence of the Late ALT/AST Elevation Event.

^{||} N (Safety): Number of patients with available safety information for the evaluation of Late ALT/AST Elevation Event.

The reference population included non-cirrhotic, non-severe CKD, non-Asian HCV-infected patients in the 100 mg dosing arms of the Phase 2 and 3 studies/arms included in this analysis.

The analyses above were used to derive the applicant's comparability bounds applied to interpretation of PK data documenting effects of intrinsic and extrinsic factors. The applicant's rationale for the selection of EBR/GZR 100/50 mg once daily for variable durations for HCV-infected sub-populations in Phase 2b/3 studies is based mainly on the following:

GZR

The GZR monotherapy study (004) indicated that ≥ 50 mg doses were on the maximum response plateau of the dose-response curve for GT1a and GT1b.

In study 003 SVR12 rates were 89.4% in TN non-cirrhotic GT1 patients treated with 100 mg GZR + PR vs. 79.3% to 93.0% with 200, 400 or 800 mg + PR, indicating no need for > 100 mg. In study 038 similar SVR12 rates were obtained with 50 mg + PR (21/25, 84%) and 100 mg + PR (23/26, 89%) but the rate was 13/24 (54%) for the 25 mg + PR regimen in the PP analysis. Elevations of ALT and/or AST were observed after TW4 at doses from 200 mg but not at 100 mg.

Results of Studies 003 and 038 suggested that 100 mg GZR is associated with maximal efficacy and is associated with an acceptable risk of Late ALT/AST Elevation Events.

EBR

The EBR monotherapy study (002) provided evidence that 10 and 50 mg EBR were associated with similar efficacy but 50 mg may provide better sustained suppression of GT1a vs. 10 mg, consistent with in-vitro data suggesting that the EBR exposures at 50 mg are more likely to cover more common NS5A RAVs than exposures at 10 mg.

In Part A of the Phase 2 study 035, 12 weeks EBR 50 mg provided similar efficacy to 20 mg with SVR12 rates of 100% (22/22) and $> 95\%$ (23/24), respectively, in TN non-cirrhotic, GT1-infected patients.

The applicant considered that the 50 mg dose gives a margin for potential decreases in EBR PK without impacting efficacy, particularly in more difficult to treat patients (e.g. with cirrhosis).

RAVs in Phase 2/3 clinical failures

During the procedure the applicant updated the information to describe 31 GT1/4 virologic failures with 12 weeks EBR/GZR. Samples were assayed using population sequencing.

At failure treatment-emergent NS3 RAVs were present in 17/31 (54.8%). At FUW24, 29/31 had NS3 sequences and treatment-emergent NS3 RAVs were detected in 6/29.

Treatment-emergent NS5A RAVs were present in 27/31 (87.1%) at the time of failure. At FUW24, 29/31 had NS5A sequences and RAVs were detected in 23/29 (79.3%).

There were 14/31 (45.2%) with treatment-emergent NS3 and NS5A RAVs at the time of failure. Four of 29 with sequences at FUW24 had treatment-emergent NS3 and NS5A RAVs.

These results indicate a relatively rapid disappearance of treatment-emergent NS3 RAVs and a slower decline in NS5A RAVs, which is consistent with data reported for other compounds in the same classes.

For GT1a treatment-emergent NS3 RAVs were detected in 64% who experienced virologic failure but they were present in 22% by FUW24. Treatment-emergent NS5A RAVs were detected in 88% who experienced virologic failure (88%) and most were detectable at FUW24 (87%). Also, 56% of GT1a patients had treatment-emergent NS3 and NS5A RAVs at failure, and 17% still had these RAVs at FUW24.

For GT1b treatment-emergent NS3 RAVs were detected in 1/4 virologic failures and the RAVs (Y56F and V107I) persisted to FUW24. Treatment-emergent NS5A RAVs were detected in 3/4 virologic failures and persisted through FUW24.

Treatment-emergent NS3 RAVs were not detected in the two GT4 virologic failures but both had treatment-emergent NS5A RAVs, which were not detectable at FUW24.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetics

Summary of PK properties of GZR

GZR is a relatively insoluble compound that has limited oral bioavailability (at least 22% is absorbed in healthy subjects) that is thought to reflect incomplete absorption and first-pass hepatic uptake. Taking GZR with food increases the GZR AUC ~1.5-fold and C_{max} ~2.8-fold. The DDI studies were almost all conducted in the fasted state. In the clinical efficacy studies EBR/GZR was taken without regard to food but many potentially interacting concomitant medications were forbidden. Variability in exposure associated with the dosing conditions (fasting/fed) was taken into account when predicting the effects of extrinsic and intrinsic factors on exposure. For example, use of a strong CYP3A inhibitor (~3-fold increase in GZR AUC) with GZR in the fed state (~1.5-fold increase in GZR AUC) would result in a ~4.5-fold increase in GZR exposure. Taking into account the contraindications and warnings that were introduced to the SmPC during the procedure, the recommendation that EBR/GZR may be taken without regard to food was accepted.

Plasma clearance (85 L/h in HCV-infected; IIV of 42% at 100 mg/day) is dose-dependent, which is thought to reflect saturable liver uptake via OATP1B and saturable CYP3A metabolism. With a blood:plasma ratio of 0.7, total blood clearance may be ~40% higher than plasma clearance. Subsequent liver metabolism via CYP3A4 and excretion means that not all of the GZR returns to

plasma before elimination in faeces as unchanged drug (~80%) and oxidative metabolites (~20%). Therefore the Vd derived from plasma levels may be an under-estimate. As oral bioavailability is unknown, it cannot be excluded that extensive biliary excretion of unchanged drug occurs, in which case P-gp and BCRP are likely to be involved.

HCV-infected patients showed increases in plasma levels up to ~day 7 and then a decline to a stable level after several weeks, which was thought to reflect improvement in hepatic function as HCV was cleared. Steady-state exposure in non-cirrhotic HCV-infected patients was ~2-fold higher than in healthy subjects although the concentration-time profile was similar to that in healthy subjects.

The PBPK model supported the conclusion that the difference between HCV-infected and uninfected individuals likely reflects a combination of reductions in CYP3A and OATP1B abundance, hepatic blood flow and functional liver mass, resulting in saturable liver uptake of GZR at lower concentrations in HCV-infected patients vs. healthy subjects. The lack of dose-proportionality and the time-dependency observed have been ascribed to the interplay between first pass metabolism due to CYP3A and hepatic uptake via OATP1B.

After daily dosing with GZR 200 mg in mild, 100 mg in moderate and 50 mg in severe hepatic insufficiency, the day 10 GMRs indicated that respective AUCs were 1.66, 4.82 and 11.68 vs. the control group. In the clinical trials of efficacy using EBR/GZR 100/50 mg/day patients were restricted to Child-Pugh A. Use is contraindicated in patients with CP-B and CP-C.

With low solubility and the potential for various factors to affect systemic exposure it is not really a surprise that between-subject variability in GZR steady-state exposure ranged from ~55% for AUC and ~64% for Cmax in non-cirrhotic HCV-infected patients and that within-subject variability was estimated to be ~26% for AUC and ~45% for Cmax. Age was an important covariate.

The POPPK modelling for GZR was comprehensive and allowed for a semi-mechanistic understanding of the pharmacokinetics. The PBPK modelling for GZR provided useful supporting data for the effect of disease on exposure but this model cannot be used to derive dose adjustments in patients.

Summary of PK properties of EBR

EBR is also a relatively insoluble compound. The lack of dose proportionality observed at > 100 mg has been ascribed to low solubility limiting its absorption. An absolute oral bioavailability study indicated that about one third of an oral dose is absorbed. EBR is thought to have low permeability and plasma exposure is slightly decreased when it is taken with food. As for GZR, taking it with agents that increase gastric pH had no major effect on plasma levels.

EBR is extensively bound (>99.9%) to plasma proteins with no measurable effect of renal or hepatic impairment on free drug. The estimated Vd is large, with a POPPK-predicted Vc ~30% higher in HCV-infected patients than in healthy subjects. The estimated apparent oral clearance CL/F for a typical HCV-infected patient is ~30 L/h, which is comparable to that in healthy male subjects (32.4 L/h). With a blood:plasma ratio of 0.62, total blood clearance of EBR may be ~60% higher than plasma clearance.

EBR is eliminated mainly into faeces as parent drug (~75%) as well as oxidative metabolites formed via CYP3A. There were no circulating metabolites detected in plasma. Since oxidative metabolites in faeces represented ~19% of the dose it appears that metabolism and excretion of parent drug contribute to the elimination of EBR to a similar extent. The apparent $t_{1/2}$ in HCV-infected patients is ~24 h, which is slightly longer than in healthy subjects, and gives an accumulation ratio ~1.9-fold.

Time to steady state was ~5 days in HCV-infected patients although C_{trough} values from Phase 2 and 3 studies indicated that stable concentrations were achieved within one to two weeks in cirrhotic and non-cirrhotic HCV-infected patients. EBR PK appears to be linear and time-independent at least up to 100 mg QD. Data from the 800 mg dose used in the TQT study showed that PK was less than dose proportional at > 100 mg, consistent with low solubility limiting its absorption.

The mean steady-state EBR plasma concentration-time profiles were comparable between non-cirrhotic HCV-infected patients and healthy subjects. The POPPK model also showed that HCV status was a significant covariate only on V_c. This finding, which contrasts with that for GZR, is thought to reflect the fact that EBR disposition does not depend on OATP1B uptake so that EBR PK is not so sensitive to the effects of reduced functional liver mass and OATP1B transporter abundance in HCV-infected patients. The POPPK analysis showed no statistically significant influence of cirrhosis or Child-Pugh B status on EBR PK. The between-subject variability was ~37-48% for AUC and ~34-61% for C_{max} in HCV-infected patients whilst within-subject variability was ~27% for AUC and ~35% for C_{max}.

Applicant's bounds applied to PK data for concluding no important effect on safety or efficacy

As described in the next section (Pharmacodynamics), it was not agreed that the applicant's lower bounds of 0.4 for GZR and 0.5 for EBR were conservative for the purposes of concluding on effects on efficacy. What is more, exposure to GZR may be reduced as HCV is treated, with unpredictable improvements in hepatic function affecting disposition. The upper bound of 5.0 for GZR was based on the relationship between GZR AUC and Late ALT/AST Elevation Events (see further under Pharmacodynamics and Safety). The upper bound of 2.0 for EBR was based on safety in sub-populations of the Phase 2 and 3 studies with higher than average EBR exposures. These were considered to be acceptable cut-off values.

It is not practical to tailor the dose of EBR/GZR to the individual when there will be only a FDC tablet of 100/50 mg marketed and when there are no clear grounds on which TDM could be based. It was concluded that avoidance of co-administration with certain types of medications for the few months it now takes to treat HCV should be advised so as to provide a secure basis for HCV eradication and to protect patient safety. A simple and pragmatic approach was considered necessary (see further below under interactions).

Drug interactions

In clinical studies in HCV-infected patients drugs specifically prohibited were:

- Drugs known to be hepatotoxic, including but not limited to etifoxine, isoniazid, nitrofurantoin
- Herbal supplements
- Strong and moderate CYP 3A/P-gp inducers, including but not limited to: nafcillin, rifampicin, carbamazepine, phenytoin, phenobarbital, bosentan, modafinil, St. John's Wort
- OATP inhibitors, including but not limited to ciclosporin, gemfibrozil, eltrombopag, lapatinib
- Certain HIV medications, including but not limited to efavirenz, etravirine, all HIV PI and PI/r
- HMG-CoA reductase inhibitors simvastatin, fluvastatin, rosuvastatin > 10 mg, atorvastatin > 10 mg
- Systemic corticosteroids ≥ 10 mg prednisone per day

Use of 200 mg GZR in many DDI studies was appropriate to give similar exposures as achieved with 100 mg in non-Asian HCV-infected patients without cirrhosis or CKD. Co-administration of single doses

of potential victims on day 11 of EBR/GZR dosing ensured that approximate steady state had been reached.

Co-administration of EBR/GZR with the following has been contraindicated during the procedure:

- Inhibitors of OATP1B1/3
- Inducers of CYP3A4 or P-gp

Co-administration of EBR/GZR with the following has been “not recommended”:

Strong inhibitors of CYP3A4 or P-gp

In summary

The applicant conducted a comprehensive range of PK studies and applied POPPK and PBPK modelling. The revised proposals regarding implications of PK data for the SmPC are acceptable.

Pharmacodynamics

The final proposal was for treatment of GT1a, GT1b and 4.

The median GZR EC50 values against chimeric replicons encoding NS3/4A sequences from clinical isolates were from 0.2 nM. The highest value was 5.85 nM for genotype 3 (2.1 7.6 nM; N=6) (i.e. ~30-fold range). The EBR median EC50 values against chimeric replicons encoding NS5A sequences from clinical isolates were from 0.005 nM for GT1a to 0.02 nM for GT3a (0.01 0.33 nM; N=9) (i.e. ~60-fold range). Therefore GT3 seems to be generally less susceptible than other genotypes. In contrast, GT4 isolates appear to be very susceptible.

Not only are GZR and EBR less active against GT3 compared to GT1 in vitro but also the two monotherapy studies in HCV-infected male subjects indicated notable differences in GZR and EBR activity between GT1 and GT3. For 100 mg GZR the drop in viral load was much less for GT3 compared to GT1a and 1b and there was a rapid recovery post-treatment. For 50 mg EBR there appeared to be some leeway in dose for GT1a and 1b but for GT3 the marked difference between 10 mg (3/5 did not respond) and 50 mg suggested that there could be little leeway for any reduction in exposure.

Furthermore, in Phase 2 EBR/GZR with RBV for 12 or 18 weeks performed poorly against GT3 in TN and non-cirrhotic patients (SVR12 rates in the FAS were 9/20 [45%] and 12/21 [57%]). These results pointed to a concern that pairing SOF with EBR/GZR may mean that efficacy is heavily driven by SOF and the strategy could result in selection of resistant HCV during wider use.

Across genotypes various NS3 substitutions at positions 43, 56, 77, 163, 168 and 178 can have very marked effects on GZR activity when they occur alone with further reductions when they occur together. NS3 substitutions especially at A156 and at D168 confer reduced susceptibility to GZR and to other NS3/4A protease inhibitors. For EBR NS5A various substitutions reduce activity by 6- to 2000 fold with further reductions when they occur together. EBR activity was reduced >5-fold by M/L28T/A, Q/R30E/H/R/G/K/L/D, L31M/V/F, H58D and Y93C/H/N. Some NS5A substitutions confer resistance to EBR and other NS5A inhibitors.

Taking into account that most failures were in GT1a-infected patients, virologic failure during or after EBR/GZR was frequently associated with NS3 and/or NS5A RAVS. The persistence of NS5A RAVs at FUW24 was a concern and was taken into account when concluding on the genotype-specific posology.

Exposure-efficacy analyses – applicant's lower acceptance bounds applied to PK

The applicant's proposed lower bound of 0.4 for GZR was derived from the AUCSS at 50 mg (0.68 $\mu\text{M}\cdot\text{hr}$) relative to 100 mg (1.77 $\mu\text{M}\cdot\text{hr}$) in a pooled non-cirrhotic, non-Japanese, non-CKD population. The applicant considered that the derived ratio of 0.4, supported by viral dynamics modelling, was conservative for identifying a boundary at which there would be no loss of efficacy.

The applicant's proposed lower bound of 0.5 for EBR was derived from the AUC with 25 mg EBR when dosed in combination with 100 mg GZR. This is proposed to be a conservative threshold taking into account the similar efficacy observed for 50 mg and 20 mg doses in Phase 2. Patients with HCV resistant to NS5A inhibitors have lower SVR12 rates and more sensitivity to changes in EBR exposure.

Exposure-efficacy analyses showed that:

- GZR AUC0-24 and Ctrough were not associated with SVR12, suggesting that exposures at the 100 mg dose were on the maximal response plateau of the E-R curve for efficacy against GT1 and 4.
- EBR AUC0-24 and EBR Ctrough were both associated with SVR12 in GT1 and 4.

AUC0-24 was a slightly better predictor of SVR12 than Ctrough. Significant covariates were treatment duration, baseline log10 HCV RNA and presence or absence of baseline resistance (of any degree) to NS5A inhibitors. In general, SVR12 rates were lower for short treatment durations (8 weeks), higher baseline log10 HCV RNA and in patients who had baseline resistance to NS5A inhibitors, especially if infected with GT1a. Hence, low SVR12 rates were primarily associated with GT1a, >800,000 IU/mL and NS5A resistance associated with > 5-fold shift in EBR activity (~5% TN GT1a).

The applicant concluded, based heavily on GT1 data, that the viral response to EBR/GZR 100/50 mg is relatively insensitive to variations in exposure at the recommended doses and regimens. These analyses were concluded to be useful but they did not adequately support the lower bounds of the applicant's interpretive criteria for effects of intrinsic and extrinsic factors on GZR and EBR GMRs. In addition, there was concern that combinations of factors would result in drops in exposures giving low GMRs for both GZR and EBR simultaneously.

Exposure-safety analyses – applicant's upper acceptance bounds applied to PK

In Phase 2 TN non-cirrhotic and cirrhotic patients with GT1 GZR doses of 400 mg or 800 mg, each with PR, was associated with ALT/AST >5 x ULN after TW4 following prior on-treatment normalisation between TW2 and TW4. The GZR AUC0-24 was particularly well correlated with these Late ALT/AST Elevation Events. In the applicant's defined hepatic safety pool of 2405 patients who received at least 8 weeks EBR/GZR at the proposed dose there were 25 (1%) with such an event.

For GZR 100 mg QD the actual and predicted GM steady-state exposures in the applicant's reference population of HCV-infected patients were similar. A 5-fold increase in GZR AUC0-24 relative to the reference population corresponded to a predicted population Late ALT/AST Elevation Event rate of ~2%, on which basis the applicant considers that the upper margin applied to interpretation of GMRs (5-fold) is justified. An event rate of 5% was predicted at a GM AUC0-24 ~ 23.7 $\mu\text{M}\cdot\text{h}$, which represents an exposure margin ~14-fold the GM with 100 mg doses in the reference population and ~11-fold that in a broader population.

Higher than average GZR and/or EBR exposures are predicted in patients on strong/moderate CYP3A inhibitors, females, Asians, elderly, patients with mild hepatic impairment and compensated cirrhosis or renal impairment/severe CKD. The GZR fold-exposures in some of these subgroups are modest yet the rates of late ALT elevations were:

- Higher in females (1.7%) vs. males (0.2%).

- Higher in Asians (2.4%) and blacks (0.9%) compared to whites (0.5%).
- Higher in those aged >65 years (1.6%) vs. <65 years (0.7%).
- Higher when BMI was <25 kg/m² (1.1%) vs. >25 kg/m² (0.5%).

It can be anticipated that the risk increases when multiple factors are combined.

Although late ALT elevation events are of concern, most patients completed treatment and there was resolution in all cases during or after treatment. For other AEs the patterns between subgroups predicted and not predicted to have higher than average exposures did not appear to be markedly different between patients who received EBR/GZR and those who received placebo (two studies had a placebo control).

The SmPC includes contraindications related to GZR exposures >5-fold the reference population. Warnings and/or statements that co-administration is not recommended were introduced regarding factors that increase exposure to ~3-5-fold. Amendments were also made to better manage the influence of factors that reduce exposure to GZR and/or EBR.

EBR 50 mg daily had no dose-related safety findings and Phase 1 studies using higher EBR doses indicated no limiting toxicity. Therefore the applicant's upper bound of 2-fold for EBR appeared to be acceptable. In any event, the contraindications and warnings applied based on GZR exposures cover any concerns that could be raised from the effects on EBR exposures.

2.4.5. Conclusions on clinical pharmacology

The applicant conducted a comprehensive range of PK studies and applied POPPK and PBPK modelling. The implications of PK data are reflected in the SmPC.

2.5. Clinical efficacy

The major clinical efficacy studies also defined as core studies in support the marketing authorisation application are summarised in Table 28.

Table 22. Overview of clinical efficacy studies

Phase	Trial	GT	Regimen	Population(s)	Control	N	Data at NDA Submission
2	035	1, 3	8, 12, or 18 weeks $\pm$ RBV	TN ($\pm$ cirrhosis) Prior PR null-responders ($\pm$ cirrhosis) HIV co-infected (TN, non-cirrhotic)	None	573	SVR ₂₄ for GT1 SVR ₁₂ for GT3
2	047	2, 4, 5, 6	GT2: GZR + EBR + R; GZR + R For GT4, 5, 6: 12 weeks $\pm$ RBV	TN (non-cirrhotic)	None	98	SVR ₂₄
2	048	1	12 weeks + RBV	Prior DAA/PR failures ($\pm$ cirrhosis)	None	79	SVR ₁₂
2	074	1, 3	4, 6, 8, or 12 weeks + SOF	TN ($\pm$ cirrhosis)	None	143	SVR ₁₂
2/3	052	1	12 weeks, no RBV (ITG vs. DTG)	CKD Stages 4-5, including dialysis	Placebo (DTG)	235 (122 ITG/PK) (113 DTG)	SVR ₁₂ on ITG/PK
3	060	1, 4, 6	12 weeks, no RBV (ITG vs. DTG)	Treatment naïve ($\pm$ cirrhosis)	Placebo (DTG)	421 (316 ITG) (105 DTG)	SVR ₁₂ on ITG
3	061	1, 4, 6	12 weeks, no RBV	HIV co-infected (TN, $\pm$ cirrhosis)	None	218	SVR ₁₂
3	068	1, 4, 6	12 or 16 weeks $\pm$ RBV	PR PTF, $\pm$ cirrhosis, $\pm$ HIV co-infection	None	420	SVR ₁₂

2.5.1. Main core studies

Study MK5172-P035-V01 C-WORTHY – Study Title: A Phase II Randomized Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of MK-5172 and MK-8742 $\pm$ Ribavirin (RBV) in Subjects with Chronic Hepatitis C Virus Infection

Study MK5172-P047 C-SCAPE – Study Title: A Phase II Clinical Trial to Evaluate the Efficacy and Safety of a Combination Regimen of MK-5172 with/without MK-8742 and/or Ribavirin (RBV) in Treatment-naïve Subjects with Chronic Hepatitis C Genotype 2, 4, 5 and 6 Infection

Study MK5172-P048-V01 C-SALVAGE – Study Title: A Phase II Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of MK-5172 + EBR + Ribavirin (R) in Subjects with Chronic Hepatitis C Virus Infection Who Failed Prior Direct Acting Antiviral Therapy

Study MK5172-P074-V01 C-SWIFT – Study Title: A Phase II Open-Label Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of EBR/GZR, and SOF in Treatment-Naïve Subjects with Chronic HCV GT1 and GT3 Infection

Study MK5172-P052-V01 C-SURFER – Study Title: A Phase II/III Randomized Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of GZR and EBR in Subjects with Chronic Hepatitis C Virus Infection and Chronic Kidney Disease

Study MK5172-P060-V01 C-EDGE – Study Title: A Phase III Randomized Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of Grazoprevir (GZR)/Elbasvir (EBR) in Treatment-Naïve Subjects with Chronic HCV GT1, GT4, and GT6 Infection

Study MK5172-P061-V01 C-EDGE CO-INFECTION – Study Title: A Phase III Open-Label Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of EBR/GZR in Treatment-Naïve Subjects with Chronic HCV Genotype 1, 4 or 6 (GT1, GT4, and GT6) Infection who are Co-Infected with HIV

Study MK5172-P068-V01 C-EDGE – Treatment Experienced – Study Title: A Phase III Randomized Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of EBR/GZR in Subjects who have Failed Prior Treatment with Pegylated Interferon and Ribavirin (P/R) with Chronic HCV GT1, GT4, and GT6 Infection

During the procedure additional efficacy data were provided as follows:

- SVR24 results were reported from studies in which SVR12 data were previously reported. In several cases the SVR12 data were also revised to include data not previously available but resulted in very minor changes.
- SVR12 and SVR24 results were reported from the deferred treatment groups (DTG) of studies 052 and 060 and were compared to those reported for ITG.
- SVR12 data were reported from study 062, a Phase 3 study in 301 HCV-infected patients on opiate substitution therapy (OST).

Common features

The common features of these studies were:

- GZR and EBR were taken without regard to food.
- Ribavirin (RBV) dosing (where used) was weight-based and was taken BID with food using SmPC-recommended dose adjustment if needed.
- Genotyping of HCV was commonly based on the line probe assay (LiPA) in Phase 2 and Abbott RealTime HCV Genotype II test for Phase 3. Sequencing part of the NS5B region, followed by either BLAST (basic local alignment search tool) analysis or phylogenetic analysis. The latter process (NS5B mini-amplicon sequencing) was used for confirmatory genotyping. Testing was at centralised laboratories and discordances were reported in CSRs. GT6 cannot be accurately genotyped by LiPA so NS5B sequencing was performed for all cases.
- Plasma HCV RNA levels were measured using the Roche COBAS™ Taqman™ HCV Test v2.0 (HPS), with LLOQ of 25 IU/mL and LOD of 9.3 IU/mL in Phase 2 and version with version with LLOQ and LOD of 15 IU/mL applied for Phase 3. The eligibility cut-off was > 10,000 IU/mL.
- SVR12 was defined as HCV RNA <25 IU/mL (either TD[u] or TND) 12 weeks post all therapy.
- Those with HCV RNA ≥ 25 IU/mL in Phase 2 or >15 IU/mL in Phase 3 (TD[q]) at TW4 confirmed within 2 weeks were discontinued.
- The HCV NS3 and NS5A genes were amplified using RT-PCR followed by population sequencing. The limit of variant detection in the population was >25% of the viral population.

Absence of cirrhosis could be based on:

Liver biopsy within prior 2 years showing METAVIR or equivalent Stage 0 [F0] to 2 [F2])

- Fibroscan within 12 months pre-enrolment with a result of ≤12.5 kPa

- FibroTest (FibroSure®) score of ≤ 0.48 and AST platelet ratio index (APRI) ≤ 1

Presence of cirrhosis could be based on:

- Liver biopsy within 2 years pre-enrolment showing cirrhosis (METAVIR or equivalent F4)
- Fibroscan within 12 months pre-enrolment with a result of > 12.5 kPa
- FibroTest (FibroSure®) score of > 0.75 and APRI > 2

Design features

Other design features were:

- In study 060 patients were HCV TN, non-cirrhotic or cirrhotic and infected with GT1, GT4 or GT6. Patients with decompensated liver disease (manifested by the presence or history of ascites, oesophageal or gastric variceal bleeding, hepatic encephalopathy or other signs or symptoms of advanced liver disease) and cirrhotic patients with a CPT score > 6 were excluded.
- In study 061 patients were HCV TN and co-infected with HCV GT1, GT4 or GT6. About 20% were to have compensated cirrhosis.
- In study 068 patients were HCV TE, non-cirrhotic or cirrhotic (compensated) and infected with GT1, GT4 or GT6. They were null responders, partial responders or relapsers.

Histories of alcohol abuse, malignancy or HBV co-infection were exclusion criteria in all studies. Co-infection with HIV was an exclusion criterion in study 060.

Efficacy endpoints

SVR4 = Sustained Virologic Response 4 weeks after the end of all study therapy based on HCV RNA $<$ LLOQ and either detected but not quantifiable [TD (u)] or not detectable [TND] 4 weeks after the end of all study therapy

SVR12 = As above at 12 weeks after the end of all study therapy

SVR24 = As above at 24 weeks after the end of all study therapy

Lack of efficacy was categorised as:

Non-response: HCV RNA detected at end of treatment without HCV RNA $<$ LLOQ on treatment

Rebound: > 1 log₁₀ IU/mL increase in HCV RNA from nadir while on treatment and confirmed from a separate blood draw within 2 weeks

Virologic breakthrough: confirmed (as above) HCV RNA $\geq$ LLOQ [TD (q)] after being $<$ LLOQ previously while on treatment

Relapse: confirmed (as above) HCV RNA $\geq$ LLOQ [TD (q)] following end of all study therapy, after becoming undetectable (TND) at end of treatment

Sample size

- Study 060 planned to randomise ~ 400 patients with ~ 300 in the immediate treatment arm. Assuming a response rate of at least 85% in the immediate treatment arm, the study had over

99% power to demonstrate that the SVR12 rate was superior to the reference rate of 73% at an overall one-sided 2.5% alpha-level. The calculation was based on SAS PROC POWER based on a ztest using the normal approximation to the binomial distribution.

- Study 061 planned to enrol ~ 200 patients without stratification but enrolment was managed to ensure that ~20% had compensated cirrhosis. Assuming a true response rate of at least 85%, the study had over 99% power to demonstrate that the SVR12 rate is superior to the historical reference rate of 70% at an overall one-sided 2.5% alpha-level.
- Study 068 planned to enrol ~ 400 patients to provide ~ 100 per treatment group. There was 99% power to demonstrate that the SVR12 rate in at least one of the arms was superior to 58% at an overall 0.05 alpha level. The power calculation was based on the assumption of an underlying response rate of at least 80%.
- Study 060 was double blind up to 4 weeks post-treatment, at which time the placebo group received open-label EBR/GZR. Randomisation (ratio 3:1) was stratified by cirrhosis and HCV GT.
- Study 061 was open label and not randomised.
- Study 068 was open-label except that the investigators and patients were blinded to the assigned treatment duration through TW12. Patients were randomised (1:1:1:1) to receive EBR/GZR for 12 weeks $\pm$ RBV or for 16 weeks $\pm$ RBV. Randomisation was stratified by cirrhotic/non-cirrhotic and by prior PR treatment response. Enrolment was managed to ensure the majority had GT1.

Analysis populations

The Full Analysis Set (FAS; all treated) was the primary population for the analysis of efficacy. The Per-Protocol (PP) population was used for a sensitivity analysis of SVR12 and for analyses of secondary efficacy endpoints.

Analysis for efficacy endpoints

There were 3 types of missing data handled by different approaches:

1. Intermittent missing: If a missing data point was immediately preceded and followed by non-missing HCV RNA outcomes, the missing value was imputed as the worst outcome of the two.
2. Non-intermittent missing related to the study drug: patients with missing values due to premature study discontinuations for treatment-related reasons were considered as treatment failures.
3. Non-intermittent missing unrelated to the study drug: missing data due to premature study discontinuations with reasons unrelated to treatment (such as loss to follow-up, protocol violation, withdrawal of consent, administrative reasons) were imputed as M=F in the primary analysis of SVR12 rates. For the HCV RNA results, data following this type of withdrawal from study were excluded from the analysis. Sensitivity analyses using M=F were also planned.

There were 3 types of missing data handled by different approaches:

- Intermittent missing: If a missing data point was immediately preceded and followed by non-missing HCV RNA outcomes, the missing value was imputed as the worst outcome of the two.

- Non-intermittent missing related to the study drug: patients with missing values due to premature study discontinuations for treatment-related reasons were considered as treatment failures.
- Non-intermittent missing unrelated to the study drug: missing data due to premature study discontinuations with reasons unrelated to treatment (such as loss to follow-up, protocol violation, withdrawal of consent, administrative reasons) were imputed as M=F in the primary analysis of SVR12 rates. For the HCV RNA results, data following this type of withdrawal from study were excluded from the analysis. Sensitivity analyses using M=F were also planned.

Results

In each study the treatment and study completion rates were very high. Generally the demographics were balanced between groups in the studies that included a placebo control. The results for study 062 were very similar to those for previously reported studies, indicating that adherence was high in the opiate-dependent population.

Summary of cirrhotic patients

The Phase 2/3 studies with EBR/GZR 50/100 mg were confined to non-cirrhotic patients and 402 Child Pugh A compensated cirrhotic patients. The majority had GT1a (54.5%; 37.8% had GT1b) and 58% had failed prior peg-IFN/ribavirin $\pm$ 1st generation PIs. Regarding features of these patients with Child Pugh A compensated cirrhosis:

- Thrombocytopenia: 25.1% had a platelet count below 100,000 / μ L. In the GT1a and GT1b populations the proportions with < 100,000 platelets/ μ L were 19.6% and 30.3%, respectively.
- Hypoalbuminaemia: 6.2% (6.9% GT1a and 5.9% GT1b) had albumin <3.5 g/dL at baseline (<3.0 g/dL was an exclusion criterion).
- INR: 24.9% (21.0% GT1a and 27.6% GT1b) had an INR >ULN at baseline but none was >1.7.
- Severity of Fibrosis: the most common method for diagnosis of cirrhosis was Fibroscan (64% of cirrhotics). Among those with an entry Fibroscan, 36.1% had a score > 25.0 kilopascals (31.7% GT1a and 36.4% GT1b).

Summary of efficacy for GT1 and GT4

Efficacy data are presented below by GT and potential prognostic parameters for the pooled Resistance Analysis Population (RAP), which includes patients from Phase 2 or 3 trials who achieved SVR12 or met criteria for virologic failure and for whom baseline sequencing data was available. The RAP does not include any subject who discontinued the study for reasons other than virologic failure.

GT1a - Tables 29 and 30 show that SVR12 rates were similar for non-cirrhotic (94.7% for 12 weeks EBR/GZR) and cirrhotic patients (95.4% for 12 weeks EBR/GZR) and 100% for all patients who received 16 or 18 weeks of EBR/GZR with RBV.

Table 23. SVR12 of Non-Cirrhotic GT1a subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	397	376	94.7%	(92.5, 96.4)	129	124	96.1%	(92.0, 98.5)	41	38	92.7%	(82.2, 98.0)	44	44	100.0%	(93.4, 100.0)
Baseline HCV RNA																
≤800,000 IU/mL	117	116	99.1%	(96.0, 100.0)	28	27	96.4%	(84.2, 99.8)	5	5	100.0%	(54.9, 100.0)	4	4	100.0%	(47.3, 100.0)
>800,000 IU/mL	280	260	92.9%	(89.8, 95.2)	101	97	96.0%	(91.2, 98.6)	36	33	91.7%	(79.9, 97.7)	40	40	100.0%	(92.8, 100.0)
≤2,000,000 IU/mL	213	204	95.8%	(92.7, 97.8)	60	59	98.3%	(92.3, 99.9)	13	12	92.3%	(68.4, 99.6)	18	18	100.0%	(84.7, 100.0)
>2,000,000 IU/mL	184	172	93.5%	(89.7, 96.2)	69	65	94.2%	(87.2, 98.0)	28	26	92.9%	(79.2, 98.7)	26	26	100.0%	(89.1, 100.0)
Baseline NS5A RAVs																
Absent	373	364	97.6%	(95.8, 98.7)	121	119	98.3%	(94.9, 99.7)	37	37	100.0%	(92.2, 100.0)	41	41	100.0%	(93.0, 100.0)
Present	24	12	50.0%	(31.9, 68.1)	8	5	62.5%	(28.9, 88.9)	4	1	25.0%	(1.3, 75.1)	3	3	100.0%	(36.8, 100.0)
IL-28B Genotype																
CC	113	108	95.6%	(90.9, 98.2)	25	23	92.0%	(76.9, 98.6)	7	7	100.0%	(65.2, 100.0)	8	8	100.0%	(68.8, 100.0)
non-CC	282	266	94.3%	(91.5, 96.4)	103	100	97.1%	(92.6, 99.2)	34	31	91.2%	(78.8, 97.6)	36	36	100.0%	(92.0, 100.0)
Unknown	2	2	100.0%	(22.4, 100.0)	1	1	100.0%	(5.0, 100.0)	0	0	--	--	0	0	--	--

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Table 24. SVR12 of Cirrhotic GT1a subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	109	104	95.4%	(90.6, 98.2)	52	47	90.4%	(80.8, 96.1)	47	44	93.6%	(84.3, 98.2)	52	52	100.0%	(94.4, 100.0)
Baseline HCV RNA																
≤800,000 IU/mL	19	19	100.0%	(85.4, 100.0)	9	9	100.0%	(71.7, 100.0)	5	4	80.0%	(34.3, 99.0)	11	11	100.0%	(76.2, 100.0)
>800,000 IU/mL	90	85	94.4%	(88.7, 97.8)	43	38	88.4%	(77.1, 95.3)	42	40	95.2%	(85.8, 99.2)	41	41	100.0%	(93.0, 100.0)
≤2,000,000 IU/mL	53	51	96.2%	(88.6, 99.3)	18	17	94.4%	(76.2, 99.7)	11	10	90.9%	(63.6, 99.5)	22	22	100.0%	(87.3, 100.0)
>2,000,000 IU/mL	56	53	94.6%	(86.7, 98.5)	34	30	88.2%	(75.1, 95.9)	36	34	94.4%	(83.5, 99.0)	30	30	100.0%	(90.5, 100.0)
Baseline NS5A RAVs																
Absent	102	100	98.0%	(94.0, 99.7)	46	46	100.0%	(93.7, 100.0)	44	42	95.5%	(86.4, 99.2)	48	48	100.0%	(94.0, 100.0)
Present	7	4	57.1%	(22.5, 87.1)	6	1	16.7%	(0.9, 58.2)	3	2	66.7%	(13.5, 98.3)	4	4	100.0%	(47.3, 100.0)
IL-28B Genotype																
CC	32	31	96.9%	(86.0, 99.8)	10	9	90.0%	(60.6, 99.5)	11	11	100.0%	(76.2, 100.0)	6	6	100.0%	(60.7, 100.0)
non-CC	77	73	94.8%	(88.5, 98.2)	41	37	90.2%	(79.1, 96.6)	36	33	91.7%	(79.9, 97.7)	46	46	100.0%	(93.7, 100.0)
Unknown	0	0	--	--	1	1	100.0%	(5.0, 100.0)	0	0	--	--	0	0	--	--

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Tables 31 and 32 provide similar tabulations of relapse rates, which accounted for the great majority of virologic failures and therefore reflect SVR12 rates.

Table 25. Relapse rates of Non-Cirrhotic GT1a subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	397	19	4.8%	(3.2, 6.9)	129	5	3.9%	(1.5, 8.0)	41	2	4.9%	(0.9, 14.6)	44	0	0.0%	(0.0, 6.6)
Baseline HCV RNA																
≤800,000 IU/mL	117	1	0.9%	(0.0, 4.0)	28	1	3.6%	(0.2, 15.9)	5	0	0.0%	(0.0, 45.1)	4	0	0.0%	(0.0, 52.7)
>800,000 IU/mL	280	18	6.4%	(4.2, 9.4)	101	4	4.0%	(1.4, 8.8)	36	2	5.6%	(1.0, 16.5)	40	0	0.0%	(0.0, 7.2)
≤2,000,000 IU/mL	213	9	4.2%	(2.2, 7.3)	60	1	1.7%	(0.1, 7.7)	13	1	7.7%	(0.4, 31.6)	18	0	0.0%	(0.0, 15.3)
>2,000,000 IU/mL	184	10	5.4%	(3.0, 9.0)	69	4	5.8%	(2.0, 12.8)	28	1	3.6%	(0.2, 15.9)	26	0	0.0%	(0.0, 10.9)
Baseline NS5A RAVs																
Absent	373	7	1.9%	(0.9, 3.5)	121	2	1.7%	(0.3, 5.1)	37	0	0.0%	(0.0, 7.8)	41	0	0.0%	(0.0, 7.0)
Present	24	12	50.0%	(31.9, 68.1)	8	3	37.5%	(11.1, 71.1)	4	2	50.0%	(9.8, 90.2)	3	0	0.0%	(0.0, 63.2)
IL-28B Genotype																
CC	113	5	4.4%	(1.8, 9.1)	25	2	8.0%	(1.4, 23.1)	7	0	0.0%	(0.0, 34.8)	8	0	0.0%	(0.0, 31.2)
non-CC	282	14	5.0%	(3.0, 7.7)	103	3	2.9%	(0.8, 7.4)	34	2	5.9%	(1.1, 17.4)	36	0	0.0%	(0.0, 8.0)
Unknown	2	0	0.0%	(0.0, 77.6)	1	0	0.0%	(0.0, 95.0)	0	0	--	--	0	0	--	--

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects who met relapse criteria and the percentage calculated as (n/N)*100.

Table 26. Relapse rates of Cirrhotic GT1a subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	109	4	3.7%	(1.3, 8.2)	52	5	9.6%	(3.9, 19.2)	47	3	6.4%	(1.8, 15.7)	52	0	0.0%	(0.0, 5.6)
Baseline HCV RNA																
≤800,000 IU/mL	19	0	0.0%	(0.0, 14.6)	9	0	0.0%	(0.0, 28.3)	5	1	20.0%	(1.0, 65.7)	11	0	0.0%	(0.0, 23.8)
>800,000 IU/mL	90	4	4.4%	(1.5, 9.9)	43	5	11.6%	(4.7, 22.9)	42	2	4.8%	(0.9, 14.2)	41	0	0.0%	(0.0, 7.0)
≤2,000,000 IU/mL	53	1	1.9%	(0.1, 8.6)	18	1	5.6%	(0.3, 23.8)	11	1	9.1%	(0.5, 36.4)	22	0	0.0%	(0.0, 12.7)
>2,000,000 IU/mL	56	3	5.4%	(1.5, 13.3)	34	4	11.8%	(4.1, 24.9)	36	2	5.6%	(1.0, 16.5)	30	0	0.0%	(0.0, 9.5)
Baseline NS5A RAVs																
Absent	102	2	2.0%	(0.4, 6.0)	46	0	0.0%	(0.0, 6.3)	44	2	4.5%	(0.8, 13.6)	48	0	0.0%	(0.0, 6.1)
Present	7	2	28.6%	(5.3, 65.9)	6	5	83.3%	(41.8, 99.2)	3	1	33.3%	(1.7, 86.5)	4	0	0.0%	(0.0, 52.7)
IL-28B Genotype																
CC	32	0	0.0%	(0.0, 8.9)	10	1	10.0%	(0.5, 39.4)	11	0	0.0%	(0.0, 23.8)	6	0	0.0%	(0.0, 39.3)
non-CC	77	4	5.2%	(1.8, 11.5)	41	4	9.8%	(3.4, 21.0)	36	3	8.3%	(2.3, 21.2)	46	0	0.0%	(0.0, 6.3)
Unknown	0	0	--	--	1	0	0.0%	(0.0, 95.0)	0	0	--	--	0	0	--	--

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects who met relapse criteria and the percentage calculated as (n/N)*100.

For 12 weeks EBR/GZR alone the factor that had the largest impact on efficacy was the presence of baseline NS5A RAVs. The impact of baseline viral load was most apparent using a viral load cut-off of 800,000 IU/mL. Among all GT1a-infected subjects with baseline viral HCV RNA $\leq$ 800,000 IU/mL, one non-cirrhotic and no cirrhotic patients failed to achieve SVR12.

Logistic regression showed an interaction between baseline viral load and the presence of NS5A RAVs on efficacy such that loads $>800,000$ IU/mL had an impact on SVR12 only in the presence of baseline NS5A RAVs which conferred >5 -fold resistance to EBR. The applicant pointed out that it is not practical to base treatment recommendations on NS5A RAV status at baseline. Currently, there are no commercial assays available for HCV resistance testing in Europe. In academic centres able to test for NS5A RAVs the methodologies and interpretation of results are not standardised. The reported prevalence of RAVs and magnitude of impact of baseline NS5A RAVs on efficacy varies according to the methodology used to detect RAVs (population sequencing vs. next generation sequencing or NGS), the sensitivity threshold applied and the specific NS5A substitutions included in the RAV analysis.

For example, using population sequencing, $<10\%$ of patients in the EBR/GZR trials were found to harbour specific NS5A variants at baseline that either reduce the activity of EBR in vitro or were observed in virologic failures. In this small number, the presence of these specific RAVs at baseline had an impact on the efficacy of 12 weeks EBR/GZR.

When the same population was analysed using NGS with a 1% sensitivity threshold and a broader definition of "RAVs" more than a third of patients had baseline "RAVs" but $>90\%$ achieved SVR12.

Until a consensus is reached on the detection and interpretation of baseline RAVs, deriving guidance parameters based on NS5A resistance testing is not a feasible approach. In addition, in the pooled efficacy population baseline NS5A RAVs that conferred >5 -fold resistance to EBR were found in 5.3% TN and 7.8% TE GT1a-infected patients of which $\sim 50\%$ achieved SRV12. Therefore baseline testing for RAVs would only predict about half of the failures.

Baseline viral load $\leq 800,000$ IU/mL appeared to be a useful parameter to determine which GT1a-infected subjects should be treated with a 12 week EBR/GZR regimen. This sub-group represented 25% of the GT1a-infected population. The SVR12 rate for 12 weeks EBR/GZR was 93% for those with $>800,000$ IU/mL. If this viral load cut-off was used for determining which patients receive 16 weeks EBR/GZR + RBV $\sim 70\%$ would be over-treated.

Additional analyses were conducted to further explore the effects of baseline viral load and cirrhotic status using the RAP. High baseline viral load resulted in numerically lower SVR12 rates among GT1a-infected patients who received 12 weeks EBR/GZR in each of the viral load strata. No virologic failures occurred in those who received 16 or 18 weeks of EBR/GZR with RBV. To further examine the impact of baseline HCV RNA a receiver operating characteristic (ROC) curve was created. Of the 506 GT1a-infected patients in the RAP, 480 (94.9%) achieved SVR12. The threshold (cut-off) that maximised the diagnostic performance (using Youden Index) was 1.17 million for baseline HCV RNA. This threshold had high specificity (92.3%) but low sensitivity (36.0%), with a high positive predictive value (98.9%) and low negative predictive value (7.3%). The accuracy of this threshold was very low at 38.9%. Of the 331 with baseline viral load >1.17 million, 307 (93%) achieved SVR12, which corresponds to over-treating 61% (307/506) of the entire population.

In addition, data on GT1b is shown in Tables 35-38 as shown for GT1a.

Table 27. SVR12 of GT1a subjects (Treatment Naïve and Treatment Experienced) by Treatment Regimen and Various BL HCV RNA thresholds (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Baseline Viral Load																
<=800,000 IU/mL	136	135	99.3%	(96.6, 100.0)	37	36	97.3%	(87.8, 99.9)	10	9	90.0%	(60.6, 99.5)	15	15	100.0%	(81.9, 100.0)
>800,000 IU/mL	370	345	93.2%	(90.7, 95.3)	144	135	93.8%	(89.4, 96.7)	78	73	93.6%	(87.0, 97.4)	81	81	100.0%	(96.4, 100.0)
<=1,000,000 IU/mL	156	154	98.7%	(96.0, 99.8)	45	44	97.8%	(89.9, 99.9)	12	11	91.7%	(66.1, 99.6)	19	19	100.0%	(85.4, 100.0)
>1,000,000 IU/mL	350	326	93.1%	(90.5, 95.2)	136	127	93.4%	(88.7, 96.5)	76	71	93.4%	(86.7, 97.4)	77	77	100.0%	(96.2, 100.0)
<=2,000,000 IU/mL	266	255	95.9%	(93.3, 97.7)	78	76	97.4%	(92.2, 99.5)	24	22	91.7%	(76.0, 98.5)	40	40	100.0%	(92.8, 100.0)
>2,000,000 IU/mL	240	225	93.8%	(90.5, 96.1)	103	95	92.2%	(86.4, 96.1)	64	60	93.8%	(86.3, 97.8)	56	56	100.0%	(94.8, 100.0)
<=4,000,000 IU/mL	380	364	95.8%	(93.7, 97.3)	120	117	97.5%	(93.7, 99.3)	45	42	93.3%	(83.7, 98.2)	63	63	100.0%	(95.4, 100.0)
>4,000,000 IU/mL	126	116	92.1%	(86.9, 95.6)	61	54	88.5%	(79.5, 94.5)	43	40	93.0%	(82.9, 98.1)	33	33	100.0%	(91.3, 100.0)
<=6,000,000 IU/mL	433	412	95.2%	(93.1, 96.7)	144	138	95.8%	(91.9, 98.2)	61	57	93.4%	(85.6, 97.7)	75	75	100.0%	(96.1, 100.0)
>6,000,000 IU/mL	73	68	93.2%	(86.1, 97.3)	37	33	89.2%	(77.0, 96.2)	27	25	92.6%	(78.5, 98.7)	21	21	100.0%	(86.7, 100.0)
<=8,000,000 IU/mL	458	436	95.2%	(93.2, 96.7)	152	145	95.4%	(91.5, 97.8)	69	64	92.8%	(85.4, 97.1)	83	83	100.0%	(96.5, 100.0)
>8,000,000 IU/mL	48	44	91.7%	(81.9, 97.1)	29	26	89.7%	(75.4, 97.1)	19	18	94.7%	(77.4, 99.7)	13	13	100.0%	(79.4, 100.0)
<=10,000,000 IU/mL	470	447	95.1%	(93.1, 96.6)	161	153	95.0%	(91.2, 97.5)	76	70	92.1%	(85.0, 96.5)	86	86	100.0%	(96.6, 100.0)
>10,000,000 IU/mL	36	33	91.7%	(79.9, 97.7)	20	18	90.0%	(71.7, 98.2)	12	12	100.0%	(77.9, 100.0)	10	10	100.0%	(74.1, 100.0)

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Table 28. SVR12 of GT1a subjects (Treatment Naïve and Treatment Experienced) by Treatment Regimen, Various BL HCV RNA thresholds, and Various Subgroups (Resistance Analysis Population)

Subgroup			GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
			N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Baseline Viral Load	Cirrhosis Status	Baseline NS5A RAVs																
≤800,000 IU/mL	Non-cirrhotic	Absent	114	113	99.1%	(95.9, 100.0)	26	25	96.2%	(83.0, 99.8)	4	4	100.0%	(47.3, 100.0)	4	4	100.0%	(47.3, 100.0)
		Present	3	3	100.0%	(36.8, 100.0)	2	2	100.0%	(22.4, 100.0)	1	1	100.0%	(5.0, 100.0)	0	0	--	--
	Cirrhotic	Absent	19	19	100.0%	(85.4, 100.0)	9	9	100.0%	(71.7, 100.0)	5	4	80.0%	(34.3, 99.0)	11	11	100.0%	(76.2, 100.0)
		Present	0	0	--	--	0	0	--	--	0	0	--	--	0	0	--	--
>800,000 IU/mL	Non-cirrhotic	Absent	259	251	96.9%	(94.5, 98.5)	95	94	98.9%	(95.1, 100.0)	33	33	100.0%	(91.3, 100.0)	37	37	100.0%	(92.2, 100.0)
		Present	21	9	42.9%	(24.5, 62.8)	6	3	50.0%	(15.3, 84.7)	3	0	0.0%	(0.0, 63.2)	3	3	100.0%	(36.8, 100.0)
	Cirrhotic	Absent	83	81	97.6%	(92.6, 99.6)	37	37	100.0%	(92.2, 100.0)	39	38	97.4%	(88.4, 99.9)	37	37	100.0%	(92.2, 100.0)
		Present	7	4	57.1%	(22.5, 87.1)	6	1	16.7%	(0.9, 58.2)	3	2	66.7%	(13.5, 98.3)	4	4	100.0%	(47.3, 100.0)
≤1,000,000 IU/mL	Non-cirrhotic	Absent	125	124	99.2%	(96.3, 100.0)	33	32	97.0%	(86.4, 99.8)	5	5	100.0%	(54.9, 100.0)	6	6	100.0%	(60.7, 100.0)
		Present	5	4	80.0%	(34.3, 99.0)	2	2	100.0%	(22.4, 100.0)	1	1	100.0%	(5.0, 100.0)	0	0	--	--
	Cirrhotic	Absent	26	26	100.0%	(89.1, 100.0)	10	10	100.0%	(74.1, 100.0)	6	5	83.3%	(41.8, 99.2)	13	13	100.0%	(79.4, 100.0)
		Present	0	0	--	--	0	0	--	--	0	0	--	--	0	0	--	--
>1,000,000 IU/mL	Non-cirrhotic	Absent	248	240	96.8%	(94.3, 98.4)	88	87	98.9%	(94.7, 99.9)	32	32	100.0%	(91.1, 100.0)	35	35	100.0%	(91.8, 100.0)
		Present	19	8	42.1%	(23.0, 63.2)	6	3	50.0%	(15.3, 84.7)	3	0	0.0%	(0.0, 63.2)	3	3	100.0%	(36.8, 100.0)
	Cirrhotic	Absent	76	74	97.4%	(92.0, 99.5)	36	36	100.0%	(92.0, 100.0)	38	37	97.4%	(88.1, 99.9)	35	35	100.0%	(91.8, 100.0)
		Present	7	4	57.1%	(22.5, 87.1)	6	1	16.7%	(0.9, 58.2)	3	2	66.7%	(13.5, 98.3)	4	4	100.0%	(47.3, 100.0)
≤2,000,000 IU/mL	Non-cirrhotic	Absent	203	198	97.5%	(94.9, 99.0)	58	57	98.3%	(92.1, 99.9)	11	11	100.0%	(76.2, 100.0)	17	17	100.0%	(83.8, 100.0)
		Present	10	6	60.0%	(30.4, 85.0)	2	2	100.0%	(22.4, 100.0)	2	1	50.0%	(2.5, 97.5)	1	1	100.0%	(5.0, 100.0)
	Cirrhotic	Absent	50	50	100.0%	(94.2, 100.0)	17	17	100.0%	(83.8, 100.0)	11	10	90.9%	(63.6, 99.5)	20	20	100.0%	(86.1, 100.0)
		Present	3	1	33.3%	(1.7, 86.5)	1	0	0.0%	(0.0, 95.0)	0	0	--	--	2	2	100.0%	(22.4, 100.0)
>2,000,000 IU/mL	Non-cirrhotic	Absent	170	166	97.6%	(94.7, 99.2)	63	62	98.4%	(92.7, 99.9)	26	26	100.0%	(89.1, 100.0)	24	24	100.0%	(88.3, 100.0)
		Present	14	6	42.9%	(20.6, 67.5)	6	3	50.0%	(15.3, 84.7)	2	0	0.0%	(0.0, 77.6)	2	2	100.0%	(22.4, 100.0)
	Cirrhotic	Absent	52	50	96.2%	(88.4, 99.3)	29	29	100.0%	(90.2, 100.0)	33	32	97.0%	(86.4, 99.8)	28	28	100.0%	(89.9, 100.0)
		Present	4	3	75.0%	(24.9, 98.7)	5	1	20.0%	(1.0, 65.7)	3	2	66.7%	(13.5, 98.3)	2	2	100.0%	(22.4, 100.0)
≤4,000,000 IU/mL	Non-cirrhotic	Absent	285	279	97.9%	(95.9, 99.1)	81	80	98.8%	(94.3, 99.9)	22	22	100.0%	(87.3, 100.0)	27	27	100.0%	(89.5, 100.0)
		Present	16	8	50.0%	(27.9, 72.1)	5	5	100.0%	(54.9, 100.0)	2	1	50.0%	(2.5, 97.5)	2	2	100.0%	(22.4, 100.0)
	Cirrhotic	Absent	75	75	100.0%	(96.1, 100.0)	32	32	100.0%	(91.1, 10.0)	19	18	94.7%	(77.4, 99.7)	32	32	100.0%	(91.1, 100.0)
		Present	4	2	50.0%	(9.8, 90.2)	2	0	0.0%	(0.0, 77.6)	2	1	50.0%	(2.5, 97.5)	2	2	100.0%	(22.4, 100.0)

Subgroup			GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
			N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Baseline Viral Load	Cirrhosis Status	Baseline NS5A RAVs																
>4,000,000 IU/mL	Non-cirrhotic	Absent	88	85	96.6%	(91.4, 99.1)	40	39	97.5%	(88.7, 99.9)	15	15	100.0%	(81.9, 100.0)	14	14	100.0%	(80.7, 100.0)
		Present	8	4	50.0%	(19.3, 80.7)	3	0	0.0%	(0.0, 63.2)	2	0	0.0%	(0.0, 77.6)	1	1	100.0%	(5.0, 100.0)
	Cirrhotic	Absent	27	25	92.6%	(78.5, 98.7)	14	14	100.0%	(80.7, 100.0)	25	24	96.0%	(82.4, 99.8)	16	16	100.0%	(82.9, 100.0)
		Present	3	2	66.7%	(13.5, 98.3)	4	1	25.0%	(1.3, 75.1)	1	1	100.0%	(5.0, 100.0)	2	2	100.0%	(22.4, 100.0)
≤6,000,000 IU/mL	Non-cirrhotic	Absent	322	314	97.5%	(95.6, 98.8)	97	95	97.9%	(93.7, 99.6)	25	25	100.0%	(88.7, 100.0)	32	32	100.0%	(91.1, 100.0)
		Present	19	9	47.4%	(27.4, 68.0)	5	5	100.0%	(54.9, 100.0)	2	1	50.0%	(2.5, 97.5)	3	3	100.0%	(36.8, 100.0)
	Cirrhotic	Absent	86	86	100.0%	(96.6, 100.0)	38	38	100.0%	(92.4, 100.0)	2	1	50.0%	(2.5, 97.5)	38	38	100.0%	(92.4, 100.0)
		Present	6	3	50.0%	(15.3, 84.7)	4	0	0.0%	(0.0, 52.7)	32	30	93.8%	(81.6, 98.9)	2	2	100.0%	(22.4, 100.0)
>6,000,000 IU/mL	Non-cirrhotic	Absent	51	50	98.0%	(91.0, 99.9)	24	24	100.0%	(88.3, 100.0)	12	12	100.0%	(77.9, 100.0)	9	9	100.0%	(71.7, 100.0)
		Present	5	3	60.0%	(18.9, 92.4)	3	0	0.0%	(0.0, 63.2)	2	0	0.0%	(0.0, 77.6)	0	0	--	--
	Cirrhotic	Absent	16	14	87.5%	(65.6, 97.7)	8	8	100.0%	(68.8, 100.0)	12	12	100.0%	(77.9, 100.0)	10	10	100.0%	(74.1, 100.0)
		Present	1	1	100.0%	(5.0, 100.0)	2	1	50.0%	(2.5, 97.5)	1	1	100.0%	(5.0, 100.0)	2	2	100.0%	(22.4, 100.0)
≤8,000,000 IU/mL	Non-cirrhotic	Absent	340	332	97.6%	(95.8, 98.8)	102	100	98.0%	(94.0, 99.7)	27	27	100.0%	(89.5, 100.0)	35	35	100.0%	(91.8, 100.0)
		Present	21	10	47.6%	(28.6, 67.2)	5	5	100.0%	(54.9, 100.0)	3	1	33.3%	(1.7, 86.5)	3	3	100.0%	(36.8, 100.0)
	Cirrhotic	Absent	90	90	100.0%	(96.7, 100.0)	40	40	100.0%	(92.8, 100.0)	36	34	94.4%	(83.5, 99.0)	43	43	100.0%	(93.3, 100.0)
		Present	7	4	57.1%	(22.5, 87.1)	5	0	0.0%	(0.0, 45.1)	3	2	66.7%	(13.5, 98.3)	2	2	100.0%	(22.4, 100.0)
>8,000,000 IU/mL	Non-cirrhotic	Absent	33	32	97.0%	(86.4, 99.8)	19	19	100.0%	(85.4, 100.0)	10	10	100.0%	(74.1, 100.0)	6	6	100.0%	(60.7, 100.0)
		Present	3	2	66.7%	(13.5, 98.3)	3	0	0.0%	(0.0, 63.2)	1	0	0.0%	(0.0, 95.0)	0	0	--	--
	Cirrhotic	Absent	12	10	83.3%	(56.2, 97.0)	6	6	100.0%	(60.7, 100.0)	8	8	100.0%	(68.8, 100.0)	5	5	100.0%	(54.9, 100.0)
		Present	0	0	--	--	1	1	100.0%	(5.0, 100.0)	0	0	--	--	2	2	100.0%	(22.4, 100.0)
≤10,000,000 IU/mL	Non-cirrhotic	Absent	345	337	97.7%	(95.9, 98.8)	107	105	98.1%	(94.2, 99.7)	30	30	100.0%	(90.5, 100.0)	36	36	100.0%	(92.0, 100.0)
		Present	21	10	47.6%	(28.6, 67.2)	6	5	83.3%	(41.8, 99.2)	4	1	25.0%	(1.3, 75.1)	3	3	100.0%	(36.8, 100.0)
	Cirrhotic	Absent	97	96	99.0%	(95.2, 100.0)	42	42	100.0%	(93.1, 100.0)	39	37	94.9%	(84.7, 99.1)	45	45	100.0%	(93.6, 100.0)
		Present	7	4	57.1%	(22.5, 87.1)	6	1	16.7%	(0.9, 58.2)	3	2	66.7%	(13.5, 98.3)	2	2	100.0%	(22.4, 100.0)
>10,000,000 IU/mL	Non-cirrhotic	Absent	28	27	96.4%	(84.2, 99.8)	14	14	100.0%	(80.7, 100.0)	7	7	100.0%	(65.2, 100.0)	5	5	100.0%	(54.9, 100.0)
		Present	3	2	66.7%	(13.5, 98.3)	2	0	0.0%	(0.0, 77.6)	0	0	--	--	0	0	--	--
	Cirrhotic	Absent	5	4	80.0%	(34.3, 99.0)	4	4	100.0%	(47.3, 100.0)	5	5	100.0%	(54.9, 100.0)	3	3	100.0%	(36.8, 100.0)
		Present	0	0	--	--	0	0	--	--	0	0	--	--	2	2	100.0%	(22.4, 100.0)

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Table 29. SVR12 of Non-Cirrhotic GT1b subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	233	229	98.3%	(96.1, 99.4)	78	76	97.4%	(92.2, 99.5)	41	40	97.6%	(88.9, 99.9)	37	37	100.0%	(92.2, 100.0)
Baseline HCV RNA																
≤800,000 IU/mL	87	86	98.9%	(94.7, 99.9)	24	23	95.8%	(81.7, 99.8)	10	10	100.0%	(74.1, 100.0)	10	10	100.0%	(74.1, 100.0)
>800,000 IU/mL	146	143	97.9%	(94.8, 99.4)	54	53	98.1%	(91.5, 99.9)	31	30	96.8%	(85.6, 99.8)	27	27	100.0%	(89.5, 100.0)
≤2,000,000 IU/mL	146	145	99.3%	(96.8, 100.0)	37	36	97.3%	(87.8, 99.9)	18	18	100.0%	(84.7, 100.0)	14	14	100.0%	(80.7, 100.0)
>2,000,000 IU/mL	87	84	96.6%	(91.3, 99.1)	41	40	97.6%	(88.9, 99.9)	23	22	95.7%	(81.0, 99.8)	23	23	100.0%	(87.8, 100.0)
Baseline NS5A RAVs																
Absent	191	190	99.5%	(97.5, 100.0)	63	61	96.8%	(90.3, 99.4)	31	31	100.0%	(90.8, 100.0)	26	26	100.0%	(89.1, 100.0)
Present	42	39	92.9%	(82.6, 98.0)	15	15	100.0%	(81.9, 100.0)	10	9	90.0%	(60.6, 99.5)	11	11	100.0%	(76.2, 100.0)
IL-28B Genotype																
CC	58	57	98.3%	(92.1, 99.9)	9	9	100.0%	(71.7, 100.0)	11	10	90.9%	(63.6, 99.5)	4	4	100.0%	(47.3, 100.0)
Non-CC	174	171	98.3%	(95.6, 99.5)	69	67	97.1%	(91.2, 99.5)	30	30	100.0%	(90.5, 100.0)	33	33	100.0%	(91.3, 100.0)
Unknown	1	1	100.0%	(5.0, 100.0)	0	0	--	--	0	0	--	--	0	0	--	--

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Table 30. SVR12 of Cirrhotic GT1b subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	66	66	100.0%	(95.6, 100.0)	52	50	96.2%	(88.4, 99.3)	28	28	100.0%	(89.9, 100.0)	21	21	100.0%	(86.7, 100.0)
Baseline HCV RNA																
≤800,000 IU/mL	20	20	100.0%	(86.1, 100.0)	13	12	92.3%	(68.4, 99.6)	1	1	100.0%	(5.0, 100.0)	7	7	100.0%	(65.2, 100.0)
>800,000 IU/mL	46	46	100.0%	(93.7, 100.0)	39	38	97.4%	(88.4, 99.9)	27	27	100.0%	(89.5, 100.0)	14	14	100.0%	(80.7, 100.0)
≤2,000,000 IU/mL	37	37	100.0%	(92.2, 100.0)	36	34	94.4%	(83.5, 99.0)	11	11	100.0%	(76.2, 100.0)	11	11	100.0%	(76.2, 100.0)
>2,000,000 IU/mL	29	29	100.0%	(90.2, 100.0)	16	16	100.0%	(82.9, 100.0)	17	17	100.0%	(83.8, 100.0)	10	10	100.0%	(74.1, 100.0)
Baseline NS5A RAVs																
Absent	57	57	100.0%	(94.9, 100.0)	41	41	100.0%	(93.0, 100.0)	22	22	100.0%	(87.3, 100.0)	18	18	100.0%	(84.7, 100.0)
Present	9	9	100.0%	(71.7, 100.0)	11	9	81.8%	(53.0, 96.7)	6	6	100.0%	(60.7, 100.0)	3	3	100.0%	(36.8, 100.0)
IL-28B Genotype																
CC	26	26	100.0%	(89.1, 100.0)	7	7	100.0%	(65.2, 100.0)	7	7	100.0%	(65.2, 100.0)	4	4	100.0%	(47.3, 100.0)
Non-CC	40	40	100.0%	(92.8, 100.0)	45	43	95.6%	(86.7, 99.2)	21	21	100.0%	(86.7, 100.0)	17	17	100.0%	(83.8, 100.0)

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Table 31. Relapse rates of Non-Cirrhotic GT1b subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	233	4	1.7%	(0.6, 3.9)	78	1	1.3%	(0.1, 5.9)	41	1	2.4%	(0.1, 11.1)	37	0	0.0%	(0.0, 7.8)
Baseline HCV RNA																
≤800,000 IU/mL	87	1	1.1%	(0.1, 5.3)	24	1	4.2%	(0.2, 18.3)	10	0	0.0%	(0.0, 25.9)	10	0	0.0%	(0.0, 25.9)
>800,000 IU/mL	146	3	2.1%	(0.6, 5.2)	54	0	0.0%	(0.0, 5.4)	31	1	3.2%	(0.2, 14.4)	27	0	0.0%	(0.0, 10.5)
≤2,000,000 IU/mL	146	1	0.7%	(0.0, 3.2)	37	1	2.7%	(0.1, 12.2)	18	0	0.0%	(0.0, 15.3)	14	0	0.0%	(0.0, 19.3)
>2,000,000 IU/mL	87	3	3.4%	(1.0, 8.7)	41	0	0.0%	(0.0, 7.0)	23	1	4.3%	(0.2, 19.0)	23	0	0.0%	(0.0, 12.2)
Baseline NS5A RAVs																
Absent	191	1	0.5%	(0.0, 2.5)	63	1	1.6%	(0.1, 7.3)	31	0	0.0%	(0.0, 9.2)	26	0	0.0%	(0.0, 10.9)
Present	42	3	7.1%	(2.0, 17.4)	15	0	0.0%	(0.0, 18.1)	10	1	10.0%	(0.5, 39.4)	11	0	0.0%	(0.0, 23.8)
IL-28B Genotype																
CC	58	1	1.7%	(0.1, 7.9)	9	0	0.0%	(0.0, 28.3)	11	1	9.1%	(0.5, 36.4)	4	0	0.0%	(0.0, 52.7)
Non-CC	174	3	1.7%	(0.5, 4.4)	69	1	1.4%	(0.1, 6.7)	30	0	0.0%	(0.0, 9.5)	33	0	0.0%	(0.0, 8.7)
Unknown	1	0	0.0%	(0.0, 95.0)	0	0	--	--	0	0	--	--	0	0	--	--

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects who met relapse criteria and the percentage calculated as (n/N)*100.

Table 32. Relapse rates of Cirrhotic GT1b subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks				GZR 100 mg with EBR 50 mg + RBV for 12 Weeks				GZR 100 mg with EBR 50 mg for 16/18 Weeks				GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks			
	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†	N	n	%	90% CI†
Total	66	0	0.0%	(0.0, 4.4)	52	2	3.8%	(0.7, 11.6)	28	0	0.0%	(0.0, 10.1)	21	0	0.0%	(0.0, 13.3)
Baseline HCV RNA																
≤800,000 IU/mL	20	0	0.0%	(0.0, 13.9)	13	1	7.7%	(0.4, 31.6)	1	0	0.0%	(0.0, 95.0)	7	0	0.0%	(0.0, 34.8)
>800,000 IU/mL	46	0	0.0%	(0.0, 6.3)	39	1	2.6%	(0.1, 11.6)	27	0	0.0%	(0.0, 10.5)	14	0	0.0%	(0.0, 19.3)
≤2,000,000 IU/mL	37	0	0.0%	(0.0, 7.8)	36	2	5.6%	(1.0, 16.5)	11	0	0.0%	(0.0, 23.8)	11	0	0.0%	(0.0, 23.8)
>2,000,000 IU/mL	29	0	0.0%	(0.0, 9.8)	16	0	0.0%	(0.0, 17.1)	17	0	0.0%	(0.0, 16.2)	10	0	0.0%	(0.0, 25.9)
Baseline NS5A RAVs																
Absent	57	0	0.0%	(0.0, 5.1)	41	0	0.0%	(0.0, 7.0)	22	0	0.0%	(0.0, 12.7)	18	0	0.0%	(0.0, 15.3)
Present	9	0	0.0%	(0.0, 28.3)	11	2	18.2%	(3.3, 47.0)	6	0	0.0%	(0.0, 39.3)	3	0	0.0%	(0.0, 63.2)
IL-28B Genotype																
CC	26	0	0.0%	(0.0, 10.9)	7	0	0.0%	(0.0, 34.8)	7	0	0.0%	(0.0, 34.8)	4	0	0.0%	(0.0, 52.7)
Non-CC	40	0	0.0%	(0.0, 7.2)	45	2	4.4%	(0.8, 13.3)	21	0	0.0%	(0.0, 13.3)	17	0	0.0%	(0.0, 16.2)

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects who met relapse criteria and the percentage calculated as (n/N)*100.

The applicant concluded that 12 weeks EBR/GZR is appropriate for all GT1b-infected patients.

GT4 - 12 weeks of EBR/GZR resulted in two patients failing to achieve SVR12 whereas all patients who received 16 or 18 weeks of EBR/GZR achieved SVR12. The applicant concluded that these data plus the virological data supported the use of the same posology for GT4 and GT1a-infected patients.

Table 33. SVR12 of Non-Cirrhotic GT4 subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks			GZR 100 mg with EBR 50 mg + RBV for 12 Weeks			GZR 100 mg with EBR 50 mg for 16/18 Weeks			GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks		
	N	n (%)	90% CI†	N	n (%)	90% CI†	N	n (%)	90% CI†	N	n (%)	90% CI†
Total	52	51 (98.1)	(91.2, 99.9)	19	19 (100.0)	(85.4, 100.0)	3	2 (66.7)	(13.5, 98.3)	4	4 (100.0)	(47.3, 100.0)
Baseline HCV RNA												
<=800,000 IU/mL	21	21 (100.0)	(86.7, 100.0)	7	7 (100.0)	(65.2, 100.0)	0	0 ()		1	1 (100.0)	(5.0, 100.0)
>800,000 IU/mL	31	30 (96.8)	(85.6, 99.8)	12	12 (100.0)	(77.9, 100.0)	3	2 (66.7)	(13.5, 98.3)	3	3 (100.0)	(36.8, 100.0)
<=2,000,000 IU/mL	34	34 (100.0)	(91.6, 100.0)	12	12 (100.0)	(77.9, 100.0)	1	1 (100.0)	(5.0, 100.0)	3	3 (100.0)	(36.8, 100.0)
>2,000,000 IU/mL	18	17 (94.4)	(76.2, 99.7)	7	7 (100.0)	(65.2, 100.0)	2	1 (50.0)	(2.5, 97.5)	1	1 (100.0)	(5.0, 100.0)
Baseline NS5A RAV Status												
Did Not Have Baseline NS5A RAVs	30	29 (96.7)	(85.1, 99.8)	11	11 (100.0)	(76.2, 100.0)	0	0 ()		2	2 (100.0)	(22.4, 100.0)
Had Baseline NS5A RAVs	22	22 (100.0)	(87.3, 100.0)	8	8 (100.0)	(68.8, 100.0)	3	2 (66.7)	(13.5, 98.3)	2	2 (100.0)	(22.4, 100.0)
IL28B Genotype												
CC	15	15 (100.0)	(81.9, 100.0)	1	1 (100.0)	(5.0, 100.0)	1	1 (100.0)	(5.0, 100.0)	2	2 (100.0)	(22.4, 100.0)
Non-CC	37	36 (97.3)	(87.8, 99.9)	18	18 (100.0)	(84.7, 100.0)	2	1 (50.0)	(2.5, 97.5)	2	2 (100.0)	(22.4, 100.0)

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Table 34. SVR12 of Cirrhotic GT4 subjects (Treatment-Naïve and Treatment-Experienced) by Treatment Regimen and Various Subgroups (Resistance Analysis Population)

Subgroup	GZR 100 mg with EBR 50 mg for 12 Weeks			GZR 100 mg with EBR 50 mg + RBV for 12 Weeks			GZR 100 mg with EBR 50 mg for 16/18 Weeks			GZR 100 mg with EBR 50 mg + RBV for 16/18 Weeks		
	N	n (%)	90% CI†	N	n (%)	90% CI†	N	n (%)	90% CI†	N	n (%)	90% CI†
Total	11	10 (90.9)	(63.6, 99.5)	5	4 (80.0)	(34.3, 99.0)	2	1 (50.0)	(2.5, 97.5)	4	4 (100.0)	(47.3, 100.0)
Baseline HCV RNA												
<=800,000 IU/mL	6	6 (100.0)	(60.7, 100.0)	0	0 ()		1	1 (100.0)	(5.0, 100.0)	2	2 (100.0)	(22.4, 100.0)
>800,000 IU/mL	5	4 (80.0)	(34.3, 99.0)	5	4 (80.0)	(34.3, 99.0)	1	0 (0.0)	(0.0, 95.0)	2	2 (100.0)	(22.4, 100.0)
<=2,000,000 IU/mL	9	9 (100.0)	(71.7, 100.0)	3	3 (100.0)	(36.8, 100.0)	2	1 (50.0)	(2.5, 97.5)	3	3 (100.0)	(36.8, 100.0)
>2,000,000 IU/mL	2	1 (50.0)	(2.5, 97.5)	2	1 (50.0)	(2.5, 97.5)	0	0 ()		1	1 (100.0)	(5.0, 100.0)
Baseline NS5A RAV Status												
Did Not Have Baseline NS5A RAVs	9	8 (88.9)	(57.1, 99.4)	4	4 (100.0)	(47.3, 100.0)	1	1 (100.0)	(5.0, 100.0)	2	2 (100.0)	(22.4, 100.0)
Had Baseline NS5A RAVs	2	2 (100.0)	(22.4, 100.0)	1	0 (0.0)	(0.0, 95.0)	1	0 (0.0)	(0.0, 95.0)	2	2 (100.0)	(22.4, 100.0)
IL28B Genotype												
CC	3	3 (100.0)	(36.8, 100.0)	0	0 ()		0	0 ()		0	0 ()	
Non-CC	8	7 (87.5)	(52.9, 99.4)	5	4 (80.0)	(34.3, 99.0)	2	1 (50.0)	(2.5, 97.5)	4	4 (100.0)	(47.3, 100.0)

†Confidence Interval based on Clopper-Pearson method.

N = Number of subjects included in the subgroup analysis.

n (%) = Number of subjects with undetectable (TND) or unquantifiable (TD(u)) HCV RNA at the Follow-Up Week 12 visit and the percentage calculated as (n/N)*100. A missing HCV RNA result is imputed to TND or TD(u) if TND or TD(u) at both preceding and subsequent visits.

Baseline and treatment emergent RAVs

Study 060

The baseline prevalence of GT1 NS3 RAVs commonly associated with resistance to first generation PIs was 39.6% (86 GT1a and 25 GT1b). RAVs associated with >5-fold lower susceptibility to GZR occurred in 1.1% (3/280), all of which were D168E in GT1b and two were in cirrhotic patients. There was no association between baseline GT1 NS3 RAVs and virologic failure. The Q80K mutation was detected in 61/151 (40.4%) GT1a and in 2/129 (1.6%) GT1b but there was no association with treatment response. Baseline variants commonly associated with resistance to first generation PIs occurred in 7/18 (38.9%) with GT4 and all achieved SVR12.

Based on in-vitro GT1a replicon data, >5-fold reduced susceptibility to EBR occurred with M/L28T/A, Q/R30E/H/R/G/K/L/D, L31M/V/F, H58D and Y93C/H. In GT1 37/284 (13.0%) had one or more baseline RAVs at these loci and SVR12 was achieved in 28/37 (75.7%) vs. 245/247 (99.2%) without RAVs. For RAVs causing ≤5-fold shifts 10/11 achieved SVR12 compared to 18/26 with RAVs causing >5-fold shifts (Q30R, L31M and Y93C/H/S). For the 19/37 with GT1a SVR12 was achieved in only 11/19 (57.9%) but for the 18/37 with GT1b only one did not achieve SVR12.

In the FAS population with baseline viral load > 800,000 IU/mL, SVR12 was achieved in 183/189 (96.8%) without vs. 22/33 (66.7%) with NS5A RAVs at baseline. For GT1a the respective rates were 94/98 (95.9%) vs. 7/16 (43.7%) but for GT1b rates were 80/81 (98.8%) vs. 11/12 (91.7%). Among the 13 RAP patients (10 GT1a, one GT1b) 6 GT1a failures had treatment-emergent NS3 RAVs associated with a >5-fold decrease in GZR susceptibility. Nine of the 10 GT1a failures and the GT1b failure had treatment-emergent NS5A RAVs at failure and all were associated with a >5-fold decrease in EBR susceptibility.

Study 061

The baseline prevalence of GT1 NS3 RAVs commonly associated with resistance to first generation PIs was 40.7%. One GT1b virus had a RAV associated with >5-fold lower susceptibility to GZR (D168E). There was no association between baseline GT1 NS3 RAVs and virologic failure.

In GT1 15/183 (8%) had one or more baseline RAVs and 9 (5%) had RAVs associated with >5-fold loss of EBR activity (12% in GT1b vs. 7% GT1a). The most common were M28V, Q30H/L/R, L31M, and Y93C/H/S. Rates were similar in cirrhotics and non-cirrhotics. SVR12 was achieved in 13/15 (86.7%; 8/10 GT1a and 5/5 GT1b) vs. 164/168 (97.6%) without RAVs. For RAVs causing >5-fold shifts there was an impact on SVR12 rates for GT1a but not GT1b.

Study 068

The baseline prevalence of GT1 NS3 RAVs commonly associated with resistance to first generation PIs was 33.2% (123/370) and one GT1b virus had a RAV associated with >5-fold lower susceptibility to GZR (D168E) but the patient achieved SVR12. There was no clear association between baseline GT1 NS3 RAVs and virologic failure.

There were 50 patients (13.5%) infected with GT1 harbouring one or more baseline NS5A RAVs of which 40 (10.8%) had RAVs associated with > 5-fold loss of EBR activity (12% of GT1b vs. 9% of GT1a). In the absence of NS5A RAVs the SVR12 rate was 99.4% but among those with RAVs associated with > 5-fold reduction in susceptibility the rate was 28/40 (70% overall; 52% for GT1a and 89% for GT1b). For GT1a (but not GT1b) there was an effect of adding RBV to the latter cases such that SVR12 rates were 7/10 with RBV and 4/11 without RBV (pooled across duration groups) as

well as an effect of duration (10/18 after 12 weeks but 18/22 after 16 weeks therapy; pooled across RBV groups).

Nine of the 13 RAP patients (11 GT1a, 2 GT1b) had treatment-emergent NS3 RAVs and no patient had WT for both NS3 and NS5A at failure. There were 7/8 failures treated without RBV who had treatment-emergent NS3 RAVs vs. 2/5 who received RBV. Eleven of 13 RAP patients (6 null responders) had NS5A treatment-emergent RAVs and 7/13 had Q30H/R. All 13 had RAVs associated with >5-fold reduced susceptibility at failure.

Study 048 (all GT1 failures on prior DAA/PR)

Of the 79 patients enrolled 30 had GT1a and 49 had GT1b. The mean time since prior treatment failure was similar for GT1a-infected and GT1b-infected patients (592 vs. 535 days). NS3 RAVs conferring either < or > a 5 fold reduction in GZR activity occurred in 45.6% (36/79) but only 4/78 (5.1%) had variants associated with a >5-fold shift in GZR activity and 3 achieved SVR12. Two of the 3 patients who failed to achieve SVR12 had baseline variants associated with ≤ 5-fold reduced susceptibility to GZR and one had the R155T variant associated with a >5-fold reduction in GZR activity. Two had NS5A variants that confer >5-fold reduced activity of EBR.

Efficacy against GT3

The data come only from study 074, which evaluated 4, 6, 8 or 12 weeks treatment with EBR/GZR (100/50 mg) and SOF (400 mg) in TN patients with GT1 or GT3, including compensated cirrhosis.

In GT3 non-cirrhotic patients 8 or 12 weeks treatment was highly efficacious, as was 12 weeks treatment in those with cirrhosis.

Table 35. Analysis of the proportion of subjects with Sustained Viral Response (HCV-RNA <15 IU/mL) 12 Weeks after End of study therapy (SVR12) Per protocol Population Subjects with Genotype 3

Treatment	N	N (%)	95 % Confidence Interval
GT3 NC: GZR 100 mg +EBR 50 mg + Sofosbuvir 400 mg for 8 Weeks	15	14 (93.3)	(68.1, 99.8)
GT3 NC: GZR 100 mg +EBR 50 mg + Sofosbuvir 400 mg for 12 Weeks	14	14 (100)	(76.8, 100.0)
GT3 C: GZR 100 mg +EBR 50 mg + Sofosbuvir 400 mg for 12 Weeks	11	10 (90.9)	(58.7, 99.8)

Subgroup	GT3 NC: GZR 100 mg + EBR 50 mg + Sofosbuvir 400 mg for 8 Weeks			GT3 NC: GZR 100 mg + EBR 50 mg + Sofosbuvir 400 mg for 12 Weeks			GT3 C: GZR 100 mg + EBR 50 mg + Sofosbuvir 400 mg for 12 Weeks			Total		
	N	n (%)	95% CI [†]	N	n (%)	95% CI [†]	N	n (%)	95% CI [†]	N	n (%)	95% CI [†]
Gender												
Male	11	10 (90.9)	(58.7, 99.8)	8	8 (100.0)	(63.1, 100.0)	9	8 (88.9)	(51.8, 99.7)	28	26 (92.9)	(76.5, 99.1)
Female	4	4 (100.0)	(39.8, 100.0)	6	6 (100.0)	(54.1, 100.0)	2	2 (100.0)	(15.8, 100.0)	12	12 (100.0)	(73.5, 100.0)
IL28B Genotype												
CC	6	6 (100.0)	(54.1, 100.0)	3	3 (100.0)	(29.2, 100.0)	5	4 (80.0)	(28.4, 99.5)	14	13 (92.9)	(66.1, 99.8)
Non-CC	9	8 (88.9)	(51.8, 99.7)	11	11 (100.0)	(71.5, 100.0)	6	6 (100.0)	(54.1, 100.0)	26	25 (96.2)	(80.4, 99.9)
HCV Genotype												
3	15	14 (93.3)	(68.1, 99.8)	14	14 (100.0)	(76.8, 100.0)	11	10 (90.9)	(58.7, 99.8)	40	38 (95.0)	(83.1, 99.4)
Baseline HCV RNA												
≤800,000 IU/mL	8	8 (100.0)	(63.1, 100.0)	8	8 (100.0)	(63.1, 100.0)	4	4 (100.0)	(39.8, 100.0)	20	20 (100.0)	(83.2, 100.0)
>800,000 IU/mL	7	6 (85.7)	(42.1, 99.6)	6	6 (100.0)	(54.1, 100.0)	7	6 (85.7)	(42.1, 99.6)	20	18 (90.0)	(68.3, 98.8)
≤2,000,000 IU/mL	9	9 (100.0)	(66.4, 100.0)	11	11 (100.0)	(71.5, 100.0)	7	7 (100.0)	(59.0, 100.0)	27	27 (100.0)	(87.2, 100.0)
>2,000,000 IU/mL	6	5 (83.3)	(35.9, 99.6)	3	3 (100.0)	(29.2, 100.0)	4	3 (75.0)	(19.4, 99.4)	13	11 (84.6)	(54.6, 98.1)
≤10,000,000 IU/mL	14	13 (92.9)	(66.1, 99.8)	13	13 (100.0)	(75.3, 100.0)	11	10 (90.9)	(58.7, 99.8)	38	36 (94.7)	(82.3, 99.4)
>10,000,000 IU/mL	1	1 (100.0)	(2.5, 100.0)	1	1 (100.0)	(2.5, 100.0)	0	0 ()		2	2 (100.0)	(15.8, 100.0)
Hepatic Fibrosis Status												
Non-Cirrhotic	15	14 (93.3)	(68.1, 99.8)	14	14 (100.0)	(76.8, 100.0)	0	0 ()		29	28 (96.6)	(82.2, 99.9)
Cirrhotic	0	0 ()		0	0 ()		11	10 (90.9)	(58.7, 99.8)	11	10 (90.9)	(58.7, 99.8)

Summary of main studies

Tables 42-44 summarise the efficacy results from the main studies supporting the present application.

Table 36. SVR in genotype 1b†-infected subjects¶

Baseline Characteristics	SVR
	EBR with GZR for 12 weeks (N = 312)
Overall SVR	96% (301/312)
Outcome for subjects without SVR	
On-treatment virologic failure*	0% (0/312)
Relapse	1% (4/312)
Other‡	2% (7/312)
SVR by cirrhosis status	
Non-cirrhotic	95% (232/243)
Cirrhotic	100% (69/69)

†Includes four subjects infected with genotype 1 subtypes other than 1a or 1b.

¶Includes subjects from C-EDGE TN, C-EDGE COINFECTION, C-EDGE TE, C-WORTHY and C-SURFER.

*Includes subjects with virologic breakthrough.

‡Other includes subjects who discontinued due to adverse event, lost to follow-up, or subject withdrawal.

Table 37. SVR in genotype 1a-infected subjects

Baseline Characteristics	SVR	
	EBR with GZR 12 Weeks N = 519	EBR with GZR + RBV 16 Weeks N = 58
Overall SVR	93% (483/519)	95% (55/58)
Outcome for subjects without SVR		
On-treatment virologic failure*	1% (3/519)	0% (0/58)
Relapse	4% (23/519)	0% (0/58)
Other‡	2% (10/519)	5% (3/58)
SVR by cirrhosis status		
Non-cirrhotic	93% (379/408)	92% (33/36)
Cirrhotic	94% (104/111)	100% (22/22)
SVR by presence of baseline NS5A resistance-associated polymorphisms†, §		
Absent	97% (464/476)	100% (51/51)
Present	53% (16/30)	100% (4/4)
SVR by baseline HCV RNA		
<=800,000 IU/mL	98% (135/138)	100% (9/9)
>800,000 IU/mL	91% (348/381)	94% (46/49)

Includes subjects from C-EDGE TN, C-EDGE COINFECTION, C-EDGE TE, C-WORTHY and C-SURFER.

*Includes subjects with virologic breakthrough.

‡Other includes subjects who discontinued due to adverse event, lost to follow-up, or subject withdrawal.

†Includes subjects with baseline sequencing data and who either achieved SVR12 or met criteria for virologic failure.

Table 38. SVR in genotype 4-infected subjects¶

Baseline Characteristics	SVR	
	EBR with GZR 12 Weeks N = 65	EBR with GZR + RBV 16 Weeks N = 8
Overall SVR	94% (61/65)	100% (8/8)
Outcome for subjects without SVR		
On-treatment virologic failure*	0% (0/65)	0% (0/8)
Relapse†	3% (2/65)	0% (0/8)
Other‡	3% (2/65)	0% (0/8)
SVR by cirrhosis status		
Non-cirrhotic§	96% (51/53)	100% (4/4)
Cirrhotic	83% (10/12)	100% (4/4)
SVR by baseline HCV RNA		
<=800,000 IU/mL‡	93% (27/29)	100% (3/3)
>800,000 IU/mL‡	94% (34/36)	100% (5/5)

¶Includes subjects from C-EDGE TN, C-EDGE COINFECTION, C-EDGE TE and C-SCAPE.

*Includes subjects with virologic breakthrough. Both relapsers had baseline HCV RNA >800,000 IU/mL

‡Both subjects who failed to achieve SVR for reasons other than virologic failure had baseline HCV RNA <=800,000 IU/mL.

2.5.2. Discussion on clinical efficacy

Two of the applicant's designated core studies were placebo-controlled (052 and 060). The others were uncontrolled, which was an acceptable approach at the time these studies were initiated due to lack of approved IFN-free regimens and anticipated severe difficulties of enrolment into DAA+PR controlled studies. HCV-RNA data were not generated in the placebo groups, which is acceptable since the spontaneous resolution rate of HCV is negligible.

The asymptotic Wald test was planned and asymptotic (Wald) 95% CI were to be calculated when the protocols were written. Since very few patients did not achieve SVR12, in which case the asymptotic method might produce unreliable inferences, the 2-sided 1-sample exact test was used instead and the Clopper-Pearson method was then used to construct the 95% confidence intervals for the SVR12 rate. This change in the method of analysis after seeing the results can be accepted in this case because it led the data being analysed with an exact method that is generally more conservative than the asymptotic method. Furthermore the statistical assessor has repeated the analysis with the originally planned method and the results are practically the same, varying by less than 1% in all studies.

The FAS population served as the primary population for the analysis of efficacy except that 052 used the mFAS population, which gave results almost identical to the FAS. Missing SVR12 results were usually imputed as clinical failures, which is the preferred option.

The different studies had different thresholds for success intended to show that EBR/GZR-containing regimens provided SVR12 rates superior to those described using available treatments. Some of these criteria may be questionable but in reality this does not matter since the applicant's claims are based on studies in which the observed SVR12 rates and lower bounds of the 95% CI are all very high. Since

there were no randomised active-controlled studies it is not appropriate to comment on how EBR/GZR may compare to recently-approved IFN-free regimens.

Study 068 used an exact one-sided test for a binomial proportion to test whether at least one of the treatment arms had an SVR12 rate of at least 58%. For each test, a one-sided alpha-level of 0.0125 was used to control for multiplicity. The use of a one-sided alpha of 0.0125 does not adequately control the type I error at 2.5% one-sided. Nevertheless if 2.5% was to be divided by the 4 comparisons, this would give a significance level of 0.00625 and all the p-values lie below this. Hence the results can be considered statistically significant and a response rate as low as 58% has been excluded.

Pooling across treatment durations, the difference in SVR12 rates between patients who received RBV and those who did not was 3.3% (95% CI -1.3%, 8.2%), which supports the conclusion that addition of RBV does not substantially increase SVR12 rates. Pooling regimens with and without RBV for a given treatment duration, the difference in SVR12 rates for 12 vs. 16 weeks treatment was 1.5% (95% CI -3.2%, 6.3%), which supports a conclusion that 16 weeks did not substantially increase SVR12 rates. However, as acknowledged by the applicant, those who received 16 weeks with RBV had the highest SVR12 rate. See below for further discussion of the posology.

Genotyping

The LiPA Assay from Versant (2.0) and the Abbott HCV Genotype test were performed at a central laboratory in Phase 2 a Phase 3 programmes respectively while the NS5B mini-amplicon was used for confirmatory typing. The latter targets a different region of the virus so that discordance between the two is possible. In addition, there have been instances in the trials of patients with GT2 based on LiPA who were subsequently identified as GT1b by NS5B mini-amplicon followed by either BLAST (basic local alignment search tool) analysis or phylogenetic analysis. These patients were thought to have recombinant virus, i.e. with a sequence similar to GT2 at the 5'UTR but a sequence at the NS5B region that is homologous to GT1.

HCV polymorphisms

Baseline substitutions that impacted on SVR12 rates in GT1, 3 and 4 were confined to NS5A. Baseline NS5A polymorphisms in GT1a that confer >5 fold reductions in EBR activity in vitro were identified in <10% of TN and TE patients but their presence reduced SVR12 from 98% and 99% to 55% and 50% in respective subgroups. NS5A RAVs that result in > 5-fold shift in EBR activity were associated with a greater reduction in SVR12 vs. those associated with < 5-fold shift. Also, the presence of baseline NS5A resistance had a greater impact on SVR12 for GT1a compared to the other genotypes.

In patients with GT1, 4 and 6 resistance analyses were conducted for 54 virologic failures for which sequence data were available (7 with on treatment virologic failure, 47 with post treatment relapse). Treatment-emergent substitutions were detected in both HCV drug targets in 23/37 (62%) genotype 1a, 1/8 (13%) genotype 1b, 2/5 (40%) genotype 4 and 1/4 (25%) genotype 6 subjects.

Proposed posology by patient sub-group

The CHMP considered as not appropriate or desirable to approve a new treatment for HCV with posology that is based on the prior response to PR or to a first generation PI with PR, which was the basis of the applicant's initial proposal for defining regimens.

Compensated cirrhosis (CP-A only) does not impact on the efficacy of EBR/GZR after controlling for presence of NS5A RAVs and baseline viral load. In reality, this likely reflects the fact that a minority of the cirrhotic patients treated were at the more severe end of the spectrum.

The most reliable regimen for all patients, regardless of GT, NS5A variants or viral load, was 16 weeks EBR/GZR with RBV. Nevertheless, there was reluctance to impose this regimen on all patients suitable for treatment with EBR/GZR if a shorter regimen of EBR/GZR alone appeared to be very reliable for a specific patient sub-population.

GT1a and GT4

The evidence supported the use of the same regimens for GT1a and GT4.

The data suggested two regimens for GT1a, regardless of cirrhotic status, but specifying in section 4.2 that the recommendations apply only to patients with compensated cirrhosis (CP-A only):

EBR/GZR for 12 weeks for all patients with baseline loads $\leq$ 800,000; there seems to be no appreciable benefit for extending treatment to 16 weeks or adding RBV in these patients

EBR/GZR + RBV for 16 weeks to be considered but not mandated for all other patients, i.e. all with > 800,000 IU/mL (GT1a and GT4) and/or NS5A RAVs (GT1a)

This position takes into account the data on persistence of NS5A RAVs in failures and the potential for cross-resistance with currently approved NS5A inhibitors. The applicant accepted this posology during the procedure.

GT1b

It was agreed that 12 weeks EBR/GZR could be the regimen for GT1b regardless of other factors. It was acknowledged that there will be a few patients with NS5A RAVs who would have benefitted from addition of RBV and treatment for 16 weeks. However, there was slightly less concern about RAV persistence in GT1b failures and there seemed to be a lesser effect of viral load.

GT3

The applicant's proposed posology for 12 weeks EBR/GZR+SOF to treat GT3 regardless of cirrhosis was based on the 96.6% (28/29) SVR12 rate (pooling 8 and 12 weeks regimens) in non-cirrhotics and the 91% rate (10/11) in cirrhotic patients. However, there was concern regarding the in-vitro data, the monotherapy efficacy data and, especially, the prior poor results (high relapse rate) when EBR/GZR plus RBV was given for 12 weeks to GT3 patients. Based on these CHMP recommendations, the applicant withdrew the proposed posology for GT3 during the procedure.

Other issues

In Study 052 a heterogeneous group of patients infected with GT1 and with eGFR < 30 mL/min/1.73m² (majority on HD) received EBR/GZR 50/100 mg QD for 12 weeks. About half had GT1a and HCV RNA > 800,000 IU/mL, 6.0% had cirrhosis and most were TN (80.4%). SVR12 was achieved in 115/116 (99.1%; 95.3, 100). These data suggest that EBR/GZR regimens selected on the basis of viral and host factors to be determined can be applied to patients with or without CKD.

2.5.3. Conclusions on the clinical efficacy

EBR/GZR demonstrated in the various regimens proposed by sub-population is associated with high SVR12 rates. The lack of comparison with active control was considered acceptable since there is negligible spontaneous resolution of chronic HCV infection and there were no approved DAA-only containing regimens at the time that the programme was initiated. The approach to the statistical analyses of these uncontrolled efficacy data was acceptable. The different studies had different thresholds for success intended to show that EBR/GZR-containing regimens provided SVR12 rates

superior to those described using available treatments. Although EBR/GZR is clearly effective, basing regimen selection on treatment history was not a useful or appropriate approach. The SmPC includes the regimen selection based on virological factors. The data support use of 12 weeks EBR/GZR for GT1b infections and for GT1a or GT4 infections when the baseline viral load is <800,000 IU/mL. This regimen may also be used for GT1a when none of a list of selected NS5A RAVs is present (if testing is done). To safeguard response rates in case of baseline RAVs in GT1a viruses and to minimise the risk of selecting for resistance 16 weeks plus RBV may be considered for GT1a and GT4 when baseline viral load is >800,000 IU/mL or, for GT1a, when selected NS5A RAVs have been detected. These regimens appear to be suitable for patients who have compensated cirrhosis (CP-A only), are HIV co-infected or have CKD.

2.6. Clinical safety

Patient exposure

There were 1,234 subjects enrolled and dosed with either EBR or GZR in Phase I studies and 3163 HCV-infected patients received at least one dose of a GZR-containing regimen or placebo in the Phase 2/3 studies. Additional safety data were provided during the procedure from the ongoing studies.

Table 39. EBR/GZR Exposure Data

Protocol	Overall Subject Enrollment	Subjects who Received GZR Alone	Subjects who Received GZR with EBR	Subjects who Received Placebo	Subjects who Received Control Study Medication and No GZR or EBR	Subjects who Participated in Completed Trials or Arms of Trials who Received Dosing with GZR Alone	Subjects who Participated in Completed Trials or Arms of Trials who Received Dosing with GZR and EBR	Subjects who Participated in Completed Trials or Arms of Trials who Received Dosing with Placebo	Subjects who Participated in Completed Trials or Arms of Trials who Received Dosing with Control Study Medication	Subjects in whom Dosing was Ongoing
003	368	302			66	302			66	
038	87	87				87				
039	26	26				26				
047	98	30	68			30	68			
035	573		573				573			
048	79		79				79			
058	62		62				62			
059	40		40				40			
074	143		143				143			
052	235		122	113			122	113		
060	421		316	105			316	105		
061	218		218				218			
068	420		420				420			
062	301		201	100						301
065	92		61	31						92
Totals	3163	445	2303	349	66	445	2041	218	66	393

Adverse events

The protocol 060/061/068 pool

The overall AE profile of EBR/GZR (no RBV) was comparable to that of placebo while EBR/GZR (+ RBV) was associated with higher rates of AEs and drug-related AEs vs. EBR/GZR (no RBV). With EBR/GZR

($\pm$ RBV) the most frequently reported (in $>10\%$) AEs were fatigue (17.0%) and headache (16.4%) with rates that were slightly lower without RBV. In the placebo group the most frequently reported AEs were headache (18.1%) and fatigue (17.1%). For EBR/GZR (no RBV) the AE rates by SOC were comparable between cirrhotic and non-cirrhotic patients and were also comparable with rates in cirrhotic patients who received placebo. For EBR/GZR (+ RBV) rates were generally higher in cirrhotic vs. non-cirrhotic patients but there was no clear difference in the distributions of particular AEs by SOC.

Table 40. Adverse Event Summary Treatment Phase and First 14 Follow-Up Days
PN060/PN061/PN068 12-Week Safety Population Pool

	GZR 100 mg with EBR 50 mg		GZR 100 mg with EBR 50 mg + RBV		All GZR 100 mg with EBR 50 mg +/- RBV		Placebo	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in population	639		104		743		105	
with one or more adverse events	448	(70.1)	85	(81.7)	533	(71.7)	72	(68.6)
with no adverse event	191	(29.9)	19	(18.3)	210	(28.3)	33	(31.4)
with drug-related [†] adverse events	230	(36.0)	67	(64.4)	297	(40.0)	41	(39.0)
with serious adverse events	15	(2.3)	3	(2.9)	18	(2.4)	3	(2.9)
with serious drug-related adverse events	0	(0.0)	1	(1.0)	1	(0.1)	0	(0.0)
who died	2	(0.3)	0	(0.0)	2	(0.3)	0	(0.0)
discontinued [‡] due to an adverse event	4	(0.6)	1	(1.0)	5	(0.7)	1	(1.0)
discontinued due to a drug-related adverse event	2	(0.3)	1	(1.0)	3	(0.4)	1	(1.0)
discontinued due to a serious adverse event	1	(0.2)	0	(0.0)	1	(0.1)	0	(0.0)
discontinued due to a serious drug-related adverse event	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

[†] Determined by the investigator to be related to the drug.

[‡] Study medication withdrawn.

GZR 100 mg with EBR 50 mg includes GZR 100 mg + EBR 50 mg and GZR/EBR 100 mg/ 50 mg.

Includes all subjects in the PN060/PN061/PN068 12-Week Safety Population Pool who received 12 weeks of EBR/GZR FDC from the following studies: P060 (immediate treatment arm only), P061 and P068 (12-week arms only). The placebo subjects included in the display are from P060 (deferred treatment arm).

EBR/GZR (no RBV) and EBR/GZR (+ RBV) gave similar overall AE profiles in HCV mono-infected vs. HCV/HIV co-infected patients although total AE rates were lower in the HCV mono-infected. Rates by SOC were generally comparable between those with and without HIV co-infection and regardless of RBV but very few co-infected patients (5) received EBR/GZR (+ RBV).

Drug-related AEs were similar between EBR/GZR (no RBV) vs. placebo with higher rates in the EBR/GZR (+RBV) group, in particular for anaemia, nausea, fatigue, dyspnoea and pruritus. The majority of drug-related AEs were of mild or moderate severity. Severe drug-related AEs were rare with no clear differences in the incidence of severe drug-related AEs by SOC. With EBR/GZR (+ RBV) rates of drug-related AEs overall were higher for cirrhotic vs. non-cirrhotic patients, especially for asthenia, fatigue and pruritus. In addition, cirrhotic patients who received EBR/GZR (+ RBV) had higher rates of anaemia, fatigue, dyspnoea, gastrointestinal complaints and pruritus vs. EBR/GZR (no RBV). Rates of drug-related AEs overall and by SOC were comparable among HCV/HIV co-infected and HCV mono-infected subjects who received EBR/GZR (no RBV). No placebo group patient had HIV.

Table 41. Subjects With Drug-Related Adverse Events (Incidence $\geq$ 5% in One or More Treatment Groups) Treatment Phase and First 14 Follow-Up Days PN060/PN061/PN068 12-Week Safety Population Pool

	GZR 100 mg with EBR 50 mg		GZR 100 mg with EBR 50 mg + RBV		All GZR 100 mg with EBR 50 mg +/- RBV		Placebo	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in population	639		104		743		105	
with one or more drug-related adverse events	230	(36.0)	67	(64.4)	297	(40.0)	41	(39.0)
with no drug-related adverse events	409	(64.0)	37	(35.6)	446	(60.0)	64	(61.0)
Blood and lymphatic system disorders	1	(0.2)	13	(12.5)	14	(1.9)	0	(0.0)
Anaemia	0	(0.0)	12	(11.5)	12	(1.6)	0	(0.0)
Gastrointestinal disorders	93	(14.6)	24	(23.1)	117	(15.7)	18	(17.1)
Nausea	31	(4.9)	14	(13.5)	45	(6.1)	5	(4.8)
General disorders and administration site conditions	84	(13.1)	31	(29.8)	115	(15.5)	12	(11.4)
Asthenia	17	(2.7)	8	(7.7)	25	(3.4)	2	(1.9)
Fatigue	68	(10.6)	23	(22.1)	91	(12.2)	10	(9.5)
Metabolism and nutrition disorders	14	(2.2)	6	(5.8)	20	(2.7)	1	(1.0)
Decreased appetite	10	(1.6)	6	(5.8)	16	(2.2)	0	(0.0)
Musculoskeletal and connective tissue disorders	24	(3.8)	7	(6.7)	31	(4.2)	3	(2.9)
Nervous system disorders	80	(12.5)	26	(25.0)	106	(14.3)	15	(14.3)
Dizziness	11	(1.7)	7	(6.7)	18	(2.4)	4	(3.8)
Headache	61	(9.5)	16	(15.4)	77	(10.4)	9	(8.6)
Psychiatric disorders	48	(7.5)	18	(17.3)	66	(8.9)	9	(8.6)
Insomnia	14	(2.2)	9	(8.7)	23	(3.1)	3	(2.9)
Respiratory, thoracic and mediastinal disorders	6	(0.9)	15	(14.4)	21	(2.8)	2	(1.9)
Cough	3	(0.5)	7	(6.7)	10	(1.3)	1	(1.0)
Dyspnoea	2	(0.3)	8	(7.7)	10	(1.3)	1	(1.0)
Skin and subcutaneous tissue disorders	32	(5.0)	17	(16.3)	49	(6.6)	9	(8.6)
Pruritus	7	(1.1)	10	(9.6)	17	(2.3)	7	(6.7)

Every subject is counted a single time for each applicable row and column.

A system organ class or specific adverse event appears on this report only if its incidence in one or more of the columns meets the incidence criterion in the report title, after rounding.

GZR 100 mg with EBR 50 mg includes GZR 100 mg + EBR 50 mg and EBR/GZR 500 mg/ 100 mg.

Includes all subjects in the PN060/PN061/PN068 12-Week Safety Population Pool who received 12 weeks of EBR/GZR FDC from the following studies: P060 (immediate treatment arm only), P061 and P068 (12-week arms only). The placebo subjects included in the display are from P060 (deferred treatment arm).

In the ISP

The overall AE profile of GZR with EBR (no RBV) was comparable to that of placebo.

Table 42. Adverse Event Summary Treatment Phase and First 14 Follow-Up Days Integrated Safety Population Pool

	GZR 100 mg with EBR 50 mg		GZR 100 mg with EBR 50 mg + RBV		All GZR 100 mg with EBR 50 mg +/- RBV		Placebo	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in population	1,033		657		1,690		105	
with one or more adverse events	738	(71.4)	549	(83.6)	1,287	(76.2)	72	(68.6)
with no adverse event	295	(28.6)	108	(16.4)	403	(23.8)	33	(31.4)
with drug-related [†] adverse events	414	(40.1)	444	(67.6)	858	(50.8)	41	(39.0)
with serious adverse events	25	(2.4)	17	(2.6)	42	(2.5)	3	(2.9)
with serious drug-related adverse events	1	(0.1)	3	(0.5)	4	(0.2)	0	(0.0)
who died	2	(0.2)	1	(0.2)	3	(0.2)	0	(0.0)
discontinued [‡] due to an adverse event	5	(0.5)	11	(1.7)	16	(0.9)	1	(1.0)
discontinued due to a drug-related adverse event	3	(0.3)	5	(0.8)	8	(0.5)	1	(1.0)
discontinued due to a serious adverse event	1	(0.1)	2	(0.3)	3	(0.2)	0	(0.0)
discontinued due to a serious drug-related adverse event	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

For EBR/GZR (+ RBV) the total rates of AEs were slightly higher for 16-week (89.6%) and 18-week (91.9%) regimens vs. the 12-week regimen (80.5%) and the corresponding rates for drug-related AEs were 76.4% and 79.1% vs. 62.5%. Overall, the most frequently reported AEs were fatigue (20.9%), headache (19.1%) and nausea (10.8%). The majority was of mild intensity. There were 25/1690 (1.5%) with one or more AEs in liver function analyses (15/1033 [1.5%] without and RBV 10/657 [1.5%] with RBV). ALT and AST increased both occurred in 17/1690 [1.0%]. Also, 28/1690 (1.7%) had one or more AEs concerning hepatic and hepatobiliary disorders (10/1033 [1.0%] without and 18/657 [2.7%] with RBV) of which hyperbilirubinaemia occurred more often with RBV (9/657 [1.4%] vs. 2/1033 [0.2%]). For EBR/GZR (no RBV) rates of AEs by SOC were comparable in cirrhotic and non-cirrhotic patients and the only marked difference was that nausea was less common in cirrhotic patients (9.2% vs. 4.2%). Drug-related AEs were reported in 50.8% of all patients in the ISP but rates were 68% for EBR/GZR (with RBV) compared to 39-40% for EBR/GZR (no RBV) and placebo.

Table 43. Subjects With Drug-Related Adverse Events (Incidence $\geq$ 5% in One or More Treatment Groups) Treatment Phase and First 14 Follow-Up Days Integrated Safety Population Pool

	GZR 100 mg with EBR 50 mg		GZR 100 mg with EBR 50 mg + RBV		All GZR 100 mg with EBR 50 mg +/- RBV		Placebo	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in population	1,033		657		1,690		105	
with one or more drug-related adverse events	414	(40.1)	444	(67.6)	858	(50.8)	41	(39.0)
with no drug-related adverse events	619	(59.9)	213	(32.4)	832	(49.2)	64	(61.0)
Blood and lymphatic system disorders	3	(0.3)	68	(10.4)	71	(4.2)	0	(0.0)
Anaemia	0	(0.0)	60	(9.1)	60	(3.6)	0	(0.0)
Gastrointestinal disorders	161	(15.6)	170	(25.9)	331	(19.6)	18	(17.1)
Nausea	51	(4.9)	83	(12.6)	134	(7.9)	5	(4.8)
General disorders and administration site conditions	172	(16.7)	228	(34.7)	400	(23.7)	12	(11.4)
Asthenia	48	(4.6)	61	(9.3)	109	(6.4)	2	(1.9)
Fatigue	124	(12.0)	162	(24.7)	286	(16.9)	10	(9.5)
Investigations	22	(2.1)	36	(5.5)	58	(3.4)	1	(1.0)
Musculoskeletal and connective tissue disorders	64	(6.2)	56	(8.5)	120	(7.1)	3	(2.9)
Nervous system disorders	152	(14.7)	153	(23.3)	305	(18.0)	15	(14.3)
Headache	119	(11.5)	107	(16.3)	226	(13.4)	9	(8.6)
Psychiatric disorders	84	(8.1)	114	(17.4)	198	(11.7)	9	(8.6)
Insomnia	26	(2.5)	58	(8.8)	84	(5.0)	3	(2.9)
Respiratory, thoracic and mediastinal disorders	15	(1.5)	89	(13.5)	104	(6.2)	2	(1.9)
Dyspnoea	4	(0.4)	42	(6.4)	46	(2.7)	1	(1.0)
Skin and subcutaneous tissue disorders	52	(5.0)	128	(19.5)	180	(10.7)	9	(8.6)
Skin and subcutaneous tissue disorders	52	(5.0)	128	(19.5)	180	(10.7)	9	(8.6)
Pruritus	14	(1.4)	58	(8.8)	72	(4.3)	7	(6.7)
Rash	7	(0.7)	45	(6.8)	52	(3.1)	0	(0.0)

Serious adverse event/deaths/other significant events

Among all who received EBR/GZR, drug-related hepatic and hepatobiliary disorders were reported in 18/1690 (1.1%) patients (2/1033 [0.2%; both had hepatic pain] without and 16/657 [2.4%] with RBV). Of the latter 16, 9 had hyperbilirubinaemia, 3 had jaundice, 3 had ocular icterus and 3 had hepatic pain. AEs in liver function analysis were reported in 12/1690 (0.7%) patients (5/1033 [0.5%] without and 7/657 [1.1%] with RBV). With EBR/GZR alone 5 (0.5%) had ALT increased and 3 (0.3%) had AST increased. With RBV addition 6 (0.9%) had blood bilirubin increased and single patients had blood bilirubin unconjugated increased or transaminases increased.

SAEs

In the pooled studies 060/061/068

For EBR/GZR (no RBV) there were 15/639 (2.3%; none drug-related) patients who had non-fatal SAEs compared to 3/104 (2.9%; one drug-related) for EBR/GZR (+ RBV) and 3/105 (2.9%; none drug-related) for placebo. The EBR/GZR patient with a drug-related SAE had two SAEs of abdominal pain and a transient ischemic attack (TIA), which occurred at TW6 and TW11, respectively. She completed 12 weeks of therapy, her TIA resolved and she achieved SVR12 but the investigator considered the TIA to be drug-related. There was no clustering of SAEs in non-cirrhotic vs. cirrhotic patients or in mono-infected vs. HIV co-infected patients.

In the ISP

Most (34/39) SAEs occurred in only one patient each, the exceptions being abdominal pain (3, one received RBV) and urinary tract infection (2, both received RBV). There was no clear relationship of non-fatal SAEs by SOC with treatment duration, cirrhosis status or HCV/HIV co-infection status. One patient who received EBR/GZR (no RBV) had drug-related SAEs compared to 3 who received EBR/GZR (+RBV), of which 2 were AEs associated with overdose, and none in the placebo group.

The three SAEs additional to the one (TIA) described above that were considered by investigators to be drug-related included:

- A patient treated with EBR/GZR x 18 weeks with abdominal pain for which no cause was identified. She completed treatment and achieved SVR12.
- A patient with HIV treated with EBR/GZR + RBV x 12 weeks with asthenia while inadvertently taking EBR/GZR BID for 2 weeks. The asthenia worsened to severe intensity after the dose error was corrected but it later improved and resolved.
- A patient treated with EBR/GZR + RBV x 16 weeks with anaemia associated with an accidental overdose (1000 mg/day instead of 800 mg/day). Her haemoglobin fell from > 15 to 10.9 g/dL and recovered after her RBV dose was corrected. She achieved SVR12.

Deaths

There were no deaths in Phase 1 or in Phase 2 dose-finding studies. Two patients in the pooled studies 060/061/068 died. One patient had a malignant ventricular arrhythmia and was found dead at home at 2 weeks post-EOT with autopsy-documented coronary disease. The other had a strangulated hernia at TW2 and died 2 days after a laparoscopic appendectomy. The conclusion from autopsy was death due to complications of gastroesophageal strangulation due to a hiatal hernia. In the ISP there were three deaths. The additional death concerned a motor vehicle accident but the patient was not driving at the time.

Adverse events leading to discontinuation

In the pooled studies 060/061/068

Four of 639 (0.6%) treated with EBR/GZR (no RBV) discontinued due to an AE, including two discontinuations due to protocol-specified criteria (ALT or AST increased) that met the criteria for both Late ALT/AST Elevation Events and hepatic laboratory ECI criteria. The other two discontinued due to AEs of anxiety and palpitations or of ascites (patient died of peritonitis, sepsis and progressive renal failure 22 days after study medication discontinuation). One patient treated with EBR/GZR (+ RBV) due to an AE (affect lability) and one patient who received placebo discontinued due to a rash. Among

all patients in the 12-Week Safety Population Pool who received EBR/GZR (with or without RBV) the rate was 5/743 (0.7%) for discontinuations due to AEs.

In the ISP

Sixteen of 1690 (0.9%) patients discontinued due to an AE of which 3 discontinued due Late ALT/AST Elevation Events. Of the rest, discontinuations were due to AEs including anxiety (2), palpitations (2) and dyspnoea (2) or due to SAEs of ascites, atrial fibrillation and gastrointestinal inflammation (1 each; none drug-related). In 8/16 the AEs were considered to be drug-related (3 discontinued RBV only).

Laboratory findings

In the pooled studies 060/061/068

ALT and AST

For EBR/GZR (no RBV) worsening ALT Grade 1.1-2.5 x from baseline occurred in 22/639 (3.4%) while 2/639 (0.3%) reached >2.5-5.0 x baseline and 5/639 (0.8%) reached >5.0-fold. Grade 3 and Grade 4 elevations that were worse than baseline occurred in 10 patients (5 each; 0.8%).

For EBR/GZR (+ RBV) worsening ALT Grade from baseline occurred in only one patient with an increase >2.5-5.0 x baseline.

The placebo group showed the common pattern of elevated ALT at baseline and worsening Grade from baseline, which occurred in 60/105 (57.1%).

For EBR/GZR (no RBV) the mean ALT was >ULN at baseline (77.0 IU/L) and decreased by -48.3 IU/L by TW2. The decreases in ALT were maintained through follow-up week 4 (mean change -53.0 IU/L). For EBR/GZR (+ RBV) the baseline mean ALT (77.3 IU/L) decreased by -50 IU/L by TW2 and decreases were maintained through FW4 (mean change -56.4 IU/L). A similar pattern applied to the AST data.

Table 44. Subjects With Laboratory Findings That Met Predetermined Criteria Treatment Phase and First 14 Follow-Up Days ALT, AST and Total Bilirubin PN060/PN061/PN068 12-Week Safety Population Pool

Test Name (Unit)	Criterion [†]	GZR 100 mg with EBR 50 mg		GZR 100 mg with EBR 50 mg + RBV		Placebo	
		(N = 639)		(N = 104)		(N = 105)	
		n/m	(%)	n/m	(%)	n/m	(%)
CHEMISTRY							
Alanine Aminotransferase (IU/L)	Grade 1: 1.25 - 2.5 x ULN	16/639	(2.5)	1/104	(1.0)	26/105	(24.8)
	Grade 2: 2.6 - 5.0 x ULN	6/639	(0.9)	0/104	(0.0)	20/105	(19.0)
	Grade 3: 5.1 - 10.0 x ULN	5/639	(0.8)	1/104	(1.0)	9/105	(8.6)
	Grade 4: >10.0 x ULN	5/639	(0.8)	0/104	(0.0)	0/105	(0.0)
	1.1 - 2.5 x Baseline	22/639	(3.4)	0/104	(0.0)	58/105	(55.2)
	>2.5 - 5.0 x Baseline	2/639	(0.3)	1/104	(1.0)	2/105	(1.9)
	>5.0 x Baseline	5/639	(0.8)	0/104	(0.0)	0/105	(0.0)
Aspartate Aminotransferase (IU/L)	Grade 1: 1.25 - 2.5 x ULN	15/639	(2.3)	1/104	(1.0)	26/105	(24.8)
	Grade 2: 2.6 - 5.0 x ULN	10/639	(1.6)	2/104	(1.9)	18/105	(17.1)
	Grade 3: 5.1 - 10.0 x ULN	2/639	(0.3)	0/104	(0.0)	2/105	(1.9)
	Grade 4: >10.0 x ULN	2/639	(0.3)	0/104	(0.0)	1/105	(1.0)
	1.1 - 2.5 x Baseline	30/639	(4.7)	2/104	(1.9)	49/105	(46.7)
	>2.5 - 5.0 x Baseline	7/639	(1.1)	2/104	(1.9)	2/105	(1.9)
	>5.0 x Baseline	2/639	(0.3)	0/104	(0.0)	0/105	(0.0)
Bilirubin (mg/dL)	Grade 1: 1.1 - 1.5 x ULN	37/639	(5.8)	26/104	(25.0)	4/105	(3.8)
	Grade 2: 1.6 - 2.5 x ULN	15/639	(2.3)	13/104	(12.5)	4/105	(3.8)
	Grade 3: 2.6 - 5.0 x ULN	2/639	(0.3)	5/104	(4.8)	0/105	(0.0)
	Grade 4: >5.0 x ULN	0/639	(0.0)	0/104	(0.0)	0/105	(0.0)
	>2.5 - 5.0 x Baseline	12/639	(1.9)	21/104	(20.2)	0/105	(0.0)
	>5.0 -10.0 x Baseline	2/639	(0.3)	5/104	(4.8)	0/105	(0.0)
Bilirubin (mg/dL)	>10.0 x Baseline	0/639	(0.0)	0/104	(0.0)	0/105	(0.0)

Total Bilirubin

The majority had total bilirubin levels WNL during treatment. Elevations were more frequent with EBR/GZR (+ RBV), usually in the first two weeks of therapy, and spontaneously resolved. Total bilirubin elevations were generally accompanied by declining ALT levels. No patient who received EBR/GZR ($\pm$ RBV) and one who received placebo had ALT/AST $>3\times$ ULN concomitant with total bilirubin $>2\times$ ULN. Worsening of Grade from baseline occurred in 14/639 (2.2%) who received EBR/GZR (no RBV) and 26/104 (25%) who received additional RBV. In the latter, the time course was consistent with effects of hepatic bilirubin transporter saturation secondary to RBV-induced haemolysis. Grade 1 and Grade 2 elevations were more frequent among cirrhotic patients treated with EBR/GZR (+ RBV) but there was no such difference observed for EBR/GZR (no RBV).

In the ISP (= Integrated Safety Pool - includes all subjects who received EBR (50 mg) and GZR (100 mg) as single entities or FDC)

The majority had elevated ALT and AST at baseline. Following initiation of EBR/GZR (+/- RBV), ALT/AST levels usually declined in parallel with the decrease in HCV RNA. Grade 3 or 4 ALT/AST elevations were uncommon, mostly transient and few were associated with other hepatic laboratory evaluations or abdominal symptoms. The rates of Grade 3 elevations were similar to the rates for those treated with placebo but rates of Grade 4 ALT/AST elevations were higher vs. placebo.

Among all patients who received GZR with EBR ($\pm$ RBV), 26/1690 (1.5%) had Grade 3 or Grade 4 ALT elevations (25 occurring on treatment). Thirteen of these 26 experienced ALT $>5\times$ ULN in the setting of a Late ALT/AST Elevation Event (see further below). The majority of initial ALT $>5\times$ ULN values occurred after TW4. In 9/26 they occurred at TW1 and in the others between TW6 and TW12. One patient who received RBV experienced ALT $>3\times$ ULN concomitant with total bilirubin $>2\times$ ULN in the first two weeks of therapy. At the time of the total bilirubin elevation the ALT was declining. AST worsened by 2 or more Grades in 0.8%.

Three patients had ALT or AST that increased >500 IU/L from baseline during treatment until the last day of follow up (up to FW24). All three received EBR/GZR (no RBV) of which two had a Late ALT/AST Elevation Event and discontinued study medication and one had a hepatic laboratory event of clinical interest (ECI).

Table 45. Subjects With Laboratory Findings That Met Predetermined Criteria Treatment Phase and First 14 Follow-Up Days ALT, AST and Total Bilirubin Integrated Safety Population Pool

Test Name (Unit)	Criterion [†]	GZR 100 mg with EBR 50 mg		GZR 100 mg with EBR 50 mg + RBV		Placebo	
		(N = 1033)		(N = 657)		(N = 105)	
		n/m	(%)	n/m	(%)	n/m	(%)
CHEMISTRY							
Alanine Aminotransferase (IU/L)	Grade 1: 1.25 - 2.5 x ULN	27/1033	(2.6)	12/656	(1.8)	26/105	(24.8)
	Grade 2: 2.6 - 5.0 x ULN	10/1033	(1.0)	5/656	(0.8)	20/105	(19.0)
	Grade 3: 5.1 - 10.0 x ULN	11/1033	(1.1)	3/656	(0.5)	9/105	(8.6)
	Grade 4: >10.0 x ULN	6/1033	(0.6)	1/656	(0.2)	0/105	(0.0)
	1.1 - 2.5 x Baseline	41/1033	(4.0)	15/656	(2.3)	58/105	(55.2)
	>2.5 - 5.0 x Baseline	3/1033	(0.3)	3/656	(0.5)	2/105	(1.9)
	>5.0 x Baseline	8/1033	(0.8)	0/656	(0.0)	0/105	(0.0)
Aspartate Aminotransferase (IU/L)	Grade 1: 1.25 - 2.5 x ULN	28/1033	(2.7)	10/656	(1.5)	26/105	(24.8)
	Grade 2: 2.6 - 5.0 x ULN	14/1033	(1.4)	6/656	(0.9)	18/105	(17.1)
	Grade 3: 5.1 - 10.0 x ULN	6/1033	(0.6)	1/656	(0.2)	2/105	(1.9)
	Grade 4: >10.0 x ULN	3/1033	(0.3)	0/656	(0.0)	1/105	(1.0)
	1.1 - 2.5 x Baseline	53/1033	(5.1)	20/656	(3.0)	49/105	(46.7)
	>2.5 - 5.0 x Baseline	8/1033	(0.8)	3/656	(0.5)	2/105	(1.9)
	>5.0 x Baseline	4/1033	(0.4)	0/656	(0.0)	0/105	(0.0)
Bilirubin (mg/dL)	Grade 1: 1.1 - 1.5 x ULN	58/1033	(5.6)	146/656	(22.3)	4/105	(3.8)
	Grade 2: 1.6 - 2.5 x ULN	24/1033	(2.3)	98/656	(14.9)	4/105	(3.8)
	Grade 3: 2.6 - 5.0 x ULN	3/1033	(0.3)	37/656	(5.6)	0/105	(0.0)
	Grade 4: >5.0 x ULN	0/1033	(0.0)	2/656	(0.3)	0/105	(0.0)
	>2.5 - 5.0 x Baseline	21/1033	(2.0)	162/656	(24.7)	0/105	(0.0)
	>5.0 -10.0 x Baseline	2/1033	(0.2)	29/656	(4.4)	0/105	(0.0)
	>10.0 x Baseline	0/1033	(0.0)	1/656	(0.2)	0/105	(0.0)

Worsening ALT or AST Grade from baseline occurred slightly more frequently with longer durations of treatment but rates of Grade 1-4 elevations did not appear to differ according to treatment duration. Overall, there were no clear differences in worsening of ALT or AST Grade according to cirrhosis status, regardless of RBV co-administration.

Late ALT/AST Elevation Events (>5xULN)

Late ALT/AST Elevation Events are considered by the applicant to best describe GZR hepatic safety based on:

The rate of Late ALT/AST Elevation Events increased with GZR >100 mg when given with PR.

The majority of those who discontinued study medication for protocol-defined hepatic laboratory discontinuation criteria had Late ALT/AST Elevation Events.

In the two placebo-controlled studies (060 and 052) Late ALT/AST Elevation Events were observed in patients who received EBR/GZR but not in the placebo group. In contrast, hepatic laboratory ECI and discontinuations due to hepatic laboratory abnormalities occurred in both treatment groups.

Late ALT/AST Elevation Events had the following features:

Approximately half occurred at or after TW8 and the majority by TW10 but one-quarter were detected at TW12. All resolved on treatment or post-treatment. Most were transient, resolving within 4 weeks and with mean duration of 2 weeks.

Most were not associated with meaningful changes in other LFTs or with abdominal symptoms.

- In a minority there was a potential alternative non-study drug aetiology identified.
- Late ALT/AST Elevation Events occurred in <1% of total patients who received GZR 100 mg QD.
- Rates varied by intrinsic factors. Rates were higher in females (1.7% vs. males 0.2%), in Asians (2.4%) vs. blacks (0.9%) and whites (0.5%), in those aged >65 years (1.6%) vs. <65 years (0.7%) and in those with BMI <25 kg/m² (1.1%) vs. >25 kg/m² (0.5%). There was no difference by cirrhosis status.
- 23/25 patients with Late ALT/AST Elevation Events had ALT elevations >5x ULN and 2 had an AST elevation >5x ULN. The latter 2 were considered to have increased AST due to skeletal muscle breakdown, rather than hepatic injury.
- No patient who received GZR 100 mg had concomitant ALT/AST >3x ULN and total bilirubin >2x ULN that was consistent with hepatocellular injury.

Of the 25 patients (any dose of GZR) who had Late ALT/AST Elevation (>5X ULN) Events:

- All achieved resolution to <5X ULN of which 14 (56.0%) did so before or at EOT. All achieved resolution to baseline ALT/AST levels and 6/25 (24.0%) did so before/at EOT.
- 23/25 (92%) achieved return to normal ($\leq$ ULN) ALT/AST levels of which 5 did so before (3) or at (2) EOT and 18 did so after EOT.
- Thus 2/25 did not return to normal ALT/AST levels by 24 weeks post-therapy or at last documented laboratory follow-up. One of these two patients had ALT baseline = 102 IU/L, peak = 391 IU/L (TW8), final = 62 IU/L (follow-up week [FW] 24), with ULN = 40 IU/L; AST

baseline = 60 IU/L, peak = 259 IU/L (TW8), final = 42 IU/L (FW24), with ULN = 43 IU/L. The other had ALT baseline = 45 IU/L, peak = 170 IU/L (TW12), final = 35 IU/L (FW4), with ULN = 33 IU/L; AST baseline = 53 IU/L, peak = 133 IU/L (TW12), final = 45 IU/L (FW4), with ULN = 36 IU/L.

Late ALT/AST Elevation Events >5xULN by PK

In 13 Phase 2 and 3 studies 2279 patients had available GZR PK data for analysis, of which 2236 had both PK and safety lab data for the evaluation of Late ALT/AST Elevation Events. This population includes 22/25 patients with Late ALT/AST Elevation Events, of which 14 (14/2004, 0.7%) received GZR 100 mg QD, 4 (4/41, 9.8%) 400 mg QD and 4 (4/36, 11.1%) 800 mg. There were no placebo group cases.

The GZR steady state AUC₀₋₂₄, C_{max} and C₂ were well correlated with Late ALT/AST Elevation Events. AUC₀₋₂₄ appeared to be slightly more predictive than the other parameters. After dividing the population that received GZR 100 mg into two subgroups according to factors identified to increase AUCs, the predicted event rates were consistent with the observed rates. These exposure-response analyses suggested that a 5-fold increase in GZR AUC₀₋₂₄ relative to the reference population corresponded to a predicted Late ALT/AST Elevation Event rate of ~2% vs. 0.5% in the reference population. The predicted ALT/AST Elevation Event rate reached 5% when at a GM GZR AUC₀₋₂₄ ~ 23.7 µM·hr, which is ~14-fold the value for 100 mg GZR in the reference population. Since GZR AUC₀₋₂₄ increases in a greater than dose proportional manner, a 5-fold increase vs. the reference population equates with exposure after a 200 mg GZR dose and a 14-fold increase equates with exposure after doses between 200 and 400 mg.

Combinations of factors were considered using the POPPK models. The highest GZR exposures were predicted to occur in female Asian patients with cirrhosis with GMR [90% CI] of 3.63 [3.16, 4.17] vs. the “reference” population of male white patients with no cirrhosis. Adding in additional effects of low weight (e.g. 53 kg) and increased age (e.g. 67 years) gave an estimated fold increase in GZR of 4.37 [3.91, 4.89].

If extrinsic factor effects were also to be considered in patients who have a combination of intrinsic factors, the use of moderate or strong inhibitors of CYP3A/P-gp may further increase GZR AUC. Based on the POPPK model the additional effect of moderate inhibitors of CYP3A/P-gp is estimated to result in a 32% increase in GZR AUC vs. those not using such agents who are not elderly, low-weight, Asian, cirrhotic and female. Combining this 32% increase with the estimated increase that results from the combination of all intrinsic factors leads to an estimated increase in GZR exposure of slightly greater than 5-fold and less than 6-fold vs. the reference population. This level of exposure is predicted to result in <3% probability of a Late ALT/AST Elevation Event of >5X ULN.

In the POPPK dataset there were 89 (3%) patients who took moderate CYP3A inhibitors with GZR or GZR and EBR in Phase 2 and 3 studies. This population included some patients with intrinsic factors associated with increased GZR exposure, including 6 Asians, 4/6 were female and 2/6 aged > 65 years. Co-administration of GZR and EBR with moderate CYP3A inhibitors did not appear to increase the incidence of AEs known to be associated with GZR and EBR.

The effect of strong inhibitors of CYP3A/P-gp may be larger given the 3-fold increase in GZR AUC that was observed in healthy subjects following co-administration with ketoconazole. There are no clinical data to describe the effect of co-administration in HCV-infected patients. It is possible that a patient with all of the intrinsic factor effects associated with increases in GZR exposure who also uses a strong inhibitor of CYP3A/P-gp may have a ~10-fold increase in GZR AUC compared to the reference population, which has a predicted ~3.8% probability of a Late ALT/AST Elevation Event of >5X ULN.

Late ALT/AST elevations to >2-5xULN

There were 52/2405 (2.2%) patients in the Hepatic Safety Population (HSP - includes all subjects who received GZR (any dose). This population includes a variety of regimens and GZR doses) who had an on-treatment late ALT/AST elevation to >2X to 5X ULN. In the placebo groups the rates for such elevations, reflecting the course of HCV without treatment, were 1.8% in study 052 and 3.8% in study 060. Overall 30/52 (57.7%) had only ALT elevations, 5 (9.6%) had only AST elevations and 17 (32.7%) had both ALT/AST (>2X to 5X ULN) elevations.

- 46/52 (88.5%) were aged 18-64 years and 6 (11.5%) were $\geq$ 65 years
- 30 (57.7%) were male
- 42 (80.8%) were White, 5 (9.6%) were Black and 3 (5.8%) were Asian
- 26 (50.0%) received 100 mg GZR (22 without IFN)

Furthermore, Late ALT/AST (>2X to 5X ULN) Elevation Events were observed to be >2X higher in:

- Asian males (3/109 [2.8%]) than in Asian females (0/103 [0%])
- Black females (3/120 [2.5%]) than in Black males (2/210 [1.0%])
- Non-Hispanics (48/2143 [2.2%]) vs. Hispanics
- Non-cirrhotics (45/1842 [2.4%]) vs. cirrhotics (7/562 [1.2%])
- PR TN (44/1710 [2.6%]) vs. TE (8/695 [1.2%]) Those who
- Those who received IFN (29/389 [7.5%]) vs. no IFN (23/2016 [1.1%]); confounded by GZR dose
- Those who received moderate CYP3A4 inhibitors (4/63 [6.3%]) vs. no such agents (48/2342 [2.0%]); confounded by the small number of events and concomitant GZR dose
- Those who received strong CYP3A4 inhibitors (52/2393 [2.2%]) vs. no such agents (0/12)

Of the 52, 51 (98.1%) completed study medication. Late ALT/AST Elevations (>2X to 5X ULN) were noted at or prior to TW8 in 29 (56.8%) and at or prior to TW12 in 48 (94.1%). Individual logistic regression models were developed to assess the impact of these factors and of GZR dose, age, gender, race and Asian race.

In one set of such analyses, GZR dose, age, gender, race (white, black or African- American, Asian or other) or Asian race (Asian or not-Asian) were analysed; only GZR dose was associated in a statistically significant manner with Late ALT/AST Elevation (>2X to 5X ULN) Events.

Additional logistic regression analyses demonstrated that only IFN use (which is highly confounded with the effects of GZR dose) and concomitant use of moderate CYP3A4 inhibitors (confounded by small subject numbers [n=4] and administration of >100 mg GZR doses in 3/4 cases) were statistically significantly ($p<0.05$) associated with Late ALT/AST Elevation (>2X to 5X ULN) Events. The use of strong CYP3A4 inhibitors was not associated with a higher frequency of these laboratory abnormalities.

The association of a limited number of clinical factors with an increased rate of such ALT/AST elevations were likely due to chance and/or confounding factors. The rate was similar to that observed in the placebo groups in two studies. In contrast, elevations to >5xULN were not observed in these placebo groups. Hence the definition of late elevations based on >5xULN in patients with normal baseline levels was discriminative for the effect of GZR.

Of the 52 patients with Late ALT/AST Elevation (>2X to 5X) ULN Events:

- 51 (98.1%) had resolution to $\leq$ 2X ULN ALT/AST levels of which 42 did so before (39) or at EOT
- 51 (98.1%) had resolution to $\leq$ baseline ALT/AST levels of which 40 did so before (35) or at EOT
- 49 (94.2%) had resolution to $\leq$ ULN ALT/AST levels of which 34 did so before (32) or at EOT

Safety in special populations

Safety data for the 234 patients aged $\geq$ 65 years were assessed in 5-year brackets. The largest age subset (N = 78) was aged 65 - 69 years.

Table 46. Adverse Event Summary by Age Category Treatment Phase and First 14 Follow-Up Days Integrated Safety Population Pool - Age 65 Years and Above

	GZR 100 mg with EBR 50 mg				GZR 100 mg with EBR 50 mg + RBV			
	65 to 69	70 to 74	75 to 79	80 and above	65 to 69	70 to 74	75 to 79	80 and above
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Subjects in population	78	28	10	2	54	9	6	0
with one or more adverse events	56 (71.8)	18 (64.3)	7 (70.0)	2 (100.0)	45 (83.3)	7 (77.8)	6 (100.0)	0
with no adverse event	22 (28.2)	10 (35.7)	3 (30.0)	0 (0.0)	9 (16.7)	2 (22.2)	0 (0.0)	0
with drug-related [†] adverse events	34 (43.6)	4 (14.3)	2 (20.0)	1 (50.0)	35 (64.8)	7 (77.8)	6 (100.0)	0
with serious adverse events	2 (2.6)	0 (0.0)	1 (10.0)	0 (0.0)	3 (5.6)	0 (0.0)	0 (0.0)	0
with serious drug-related adverse events	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
who died	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
discontinued [‡] due to an adverse event	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.6)	0 (0.0)	0 (0.0)	0
discontinued due to a drug-related adverse event	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.7)	0 (0.0)	0 (0.0)	0
discontinued due to a serious adverse event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
discontinued due to a serious drug-related adverse event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0

	All GZR 100 mg with EBR 50 mg +/- RBV				Placebo			
	65 to 69	70 to 74	75 to 79	80 and above	65 to 69	70 to 74	75 to 79	80 and above
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Subjects in population	132	37	16	2	14	2	2	0
with one or more adverse events	101 (76.5)	25 (67.6)	13 (81.3)	2 (100.0)	9 (64.3)	2 (100.0)	1 (50.0)	0
with no adverse event	31 (23.5)	12 (32.4)	3 (18.8)	0 (0.0)	5 (35.7)	0 (0.0)	1 (50.0)	0
with drug-related [†] adverse events	69 (52.3)	11 (29.7)	8 (50.0)	1 (50.0)	7 (50.0)	2 (100.0)	0 (0.0)	0
with serious adverse events	5 (3.8)	0 (0.0)	1 (6.3)	0 (0.0)	1 (7.1)	0 (0.0)	1 (50.0)	0
with serious drug-related adverse events	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
who died	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
discontinued [‡] due to an adverse event	4 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
discontinued due to a drug-related adverse event	3 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
discontinued due to a serious adverse event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
discontinued due to a serious drug-related adverse event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0

There were no deaths in this subset. Discontinuations occurred in 3 patients in the 65-69 years bracket. Rates for AEs, drug-related AEs and SAEs were similar to placebo across the age brackets. Late elevations in ALT occurred in 3 patients aged 65-69 (1) and 70-74 (2) years, all of whom took EBR/GZR without RBV.

Study 052 was confined to patients with stage 4 or 5 chronic kidney disease (CKD). The applicant reported safety data from the ITG and DTG groups during the procedure. Overall and within each SOC the rates of AEs (including by PT) were similar or lower in the DTG group.

Table 47. Adverse event summary treatment period and first 14 follow-up days- all subjects treated protocol 052

	Intensive PK arm: GZR 100mg + EBR 50mg for 12 Weeks		Immediate treatment arm: GZR 100mg + EBR 50mg for 12 Weeks		Deferred treatment arm: GZR Placebo + EBR Placebo for 12 Weeks		Deferred treatment arm: GZR/EBR for 12 Weeks	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in population	11		111		113		102	
with one or more adverse events	9	(81.8)	84	(75.7)	95	(84.1)	61	(59.8)
with no adverse event	2	(18.2)	27	(24.3)	18	(15.9)	41	(40.2)
with drug-related [†] adverse events	4	(36.4)	38	(34.2)	39	(34.5)	24	(23.5)
with serious adverse events	0	(0.0)	16	(14.4)	19	(16.8)	12	(11.8)
with serious drug-related adverse events	0	(0.0)	0	(0.0)	1	(0.9)	1	(1.0)
who died	0	(0.0)	1	(0.9)	3	(2.7)	0	(0.0)
discontinued [‡] due to an adverse event	0	(0.0)	0	(0.0)	5	(4.4)	3	(2.9)
discontinued due to a drug-related adverse event	0	(0.0)	0	(0.0)	3	(2.7)	1	(1.0)
discontinued due to a serious adverse event	0	(0.0)	0	(0.0)	3	(2.7)	2	(2.0)
discontinued due to a serious drug-related adverse event	0	(0.0)	0	(0.0)	1	(0.9)	1	(1.0)

A non-cirrhotic, 53 year old African American male with GT1b infection who was not on HD discontinued due to interstitial nephritis. He had prior biopsy-proven membranous glomerulonephropathy, cryoglobulinaemia, nephrolithiasis and hypertension. At baseline the creatinine was 2.0 mg/dL and was 2.5 mg/dL at TW12 on placebo. Four weeks after starting EBR/GZR he was hospitalised with renal stones and therapy was discontinued at ~ week 6. Subsequent creatinine values were between 2.7 and 5.3 mg/dL. The last recorded value was 2.8 mg/dL. Renal biopsy initially showed interstitial nephritis and repeat renal biopsy showed membranous glomerulopathy.

A 43 year old male Hispanic patient with GT1a and cirrhosis had a Hepatic EC1. ALT at end of placebo was 72 U/L. At TW8 of EBR/GZR ALT was 182 U/L (vs. baseline 55 U/L) but at TW12 it was 56 U/L and at FU4 it was 43 U/L. The patient had marked elevations of alkaline phosphatase, GGT and eosinophil counts as well as mild elevation of ALT, AST and bilirubin during placebo and EBR/GZR.

Safety related to drug-drug interactions and other interactions

Use of CYP3A4 inhibitors

In DDI studies CYP3A/P-gp inhibitors increased AUCs of GZR and EBR by ~ 3.3- and ~1.8-fold, respectively. Use of weak, moderate and strong CYP3A inhibitors was permitted in Phase 2/3 studies.

In the POPPK dataset there were 89 (3%) and 16 (0.6%) patients who took moderate and strong CYP3A inhibitors, respectively, with GZR or EBR/GZR. POPPK could not estimate the effect of strong CYP3A inhibitors but moderate CYP3A inhibitors were estimated to give GZR and EBR AUCs ~20% and 30% higher. In the ISP 35/1690 patients took concomitant moderate CYP3A4 inhibitors with EBR/GZR (with or without RBV) and there was an increased rate of SAEs in these patients 6/35 (17.1%) vs. those who did not take moderate CYP3A4 inhibitors (36/1655 [2.2%]). These differences were not present in the subset that took EBR/GZR (no RBV). The 6 who took moderate CYP3A4 inhibitors and had SAEs did not appear to have EBR/GZR-related SAEs (skin ulcer, atrial fibrillation, gastrointestinal inflammation, colitis, infectious colitis and tibia fracture). Only 9 ISP patients took strong CYP3A4 inhibitors.

Use of statins

On co-administration with GZR+EBR, the rosuvastatin AUC and C_{max} were increased by 2.3- and 5.5-fold, respectively, and atorvastatin AUC and C_{max} were increased by 2- and 4-fold, respectively. Concomitant use of 10 mg QD of these statins was permitted in the Phase 2 and 3 studies. In the ISP 34/1690 took any statin for at least 7 consecutive days with EBR/GZR (with or without RBV). Based on these very limited numbers, spread across a range of different statins, there was no clear difference in safety profile vs. those who did not take statins.

Oral contraceptives including ethinyl oestradiol (EE)

In separate DDI studies GZR and EBR had no clinically relevant effects on the pharmacokinetics of EE and LNG after a single dose of Nordette (ethinyl estradiol [EE] 0.03 mg/levonorgestrel [LNG] 0.15 mg). Plasma PK of GZR or EBR were not assessed but no DDI was anticipated. Contraceptive medications were allowed in the Phase 2 and 3 studies. For patients in the ISP pool with available PK estimates from POPPK modeling, the median GZR AUC for those who took OCPs concomitantly (N=35) was slightly lower than for those who did not (N=614).

For the 35 female patients who took OCPs with EBR/GZR (with or without RBV) there were no clear differences in frequencies of AEs, drug-related AEs, SAEs or discontinuations vs. those women not taking OCPs. There were no meaningful differences in the incidence of AEs by SOC between the two groups. There were also no clear differences for any of the laboratory parameters evaluated, including ALT, AST, total bilirubin, alkaline phosphatase or INR. None of the 35 women had Grade 2, 3 or 4 elevations in ALT, AST, total bilirubin or alkaline phosphatase. None of the 25 patients who experienced a late ALT/AST elevation (>5x ULN) were on any OCPs, including EE.

2.6.1. Discussion on clinical safety

The safety of EBR/GZR, with or without RBV, has been evaluated in a large and diverse population. The total size of the safety database is acceptable and it adequately covers patients with compensated cirrhosis (CP-A scores only).

Leaving aside the hepatic events, EBR/GZR alone appears to have a benign safety profile. In particular it is notable that very few patients throughout the programme have failed to complete their assigned treatment periods. Addition of RBV confers the additional safety problems well known to be associated with this agent but even with RBV few patients discontinued due to AEs.

Based on data from the two placebo-controlled Phase 2/3 studies several of the commonest AEs (such as fatigue, headache and nausea) have occurred at similar rates in EBR/GZR and placebo groups. The safety profile of EBR/GZR did show some differences according to baseline factors such as age, gender, race/ethnicity, cirrhosis, HCV/HIV co-infection or CKD but in the pooled data from 060/061/068 the

patterns of differences between subgroups were similar to those observed in the placebo group. Extending the duration of treatment from 12 to 16 weeks did not lead to additional AEs or higher rates of AEs.

Following the safety data obtained in the Phase 2 study 003, the applicant instituted a special hepatic monitoring programme. The focus was on Late ALT/AST Elevation Events (5xULN), as defined by the applicant, which were clearly related to GZR exposures. At the 100 mg dose, taking into account multiple forbidden concomitant medications in clinical trials, these events occurred in <1% of patients treated with GZR 100 mg. In the pooled analysis of studies 060/061/068 7/743 patients (~0.9%) had Late ALT/AST Elevation Events and in the ISP 13/1690 (0.8%) had Late ALT/AST Elevation Events. At this GZR dose (100 mg) no patient had concomitant ALT/AST >3x ULN and total bilirubin >2x ULN that was consistent with hepatocellular injury. Late ALT/AST Elevation Events generally occurred at or after TW8. Most have been short-lived and all so far have resolved. They were not accompanied by abnormalities of other tests of hepatic function or by liver-related symptoms.

These Late ALT/AST Elevation Events may not per se lead to permanent hepatic damage but there is currently no clear explanation as to why they occur when they do. Also, the rate of these Late ALT/AST Elevation Events was increased by intrinsic factors that increase GZR exposures. In the Phase 2/3 studies there were several forbidden concomitant medications known to increase GZR exposures. During the procedure the applicant updated the SmPC to better reflect the risks by describing rates GZR exposures by intrinsic factors, contraindicating use with OATP1B inhibitors (which could also potentially affect efficacy since they limit hepatic uptake of GZR) and adding adequate statements that use with CYP3A/P-gp inhibitors is not recommended. There remains a possibility that GZR exposures could be substantially increased when a combination of intrinsic and extrinsic factors tending to increase GZR exposure occurs in an individual patient when the occurrence of each factor alone would not necessarily cause concern. However, the final revised SmPC adequately reflects this risk.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

The safety profile of EBR/GZR 50/100 mg is generally benign. Increases in transaminases relatively late during treatment period are associated with higher than usual GZR plasma exposures. The underlying mechanism of these events is not understood at present but so far they have occurred at low rates and have resolved. This risk is considered to be manageable.

2.7. Risk Management Plan

Safety concerns

Important identified risks	Late ALT Elevation Hyperbilirubinemia Drug resistance development Drug interactions with • CYP3A inducers • OATP1B inhibitors • strong CYP3A inhibitors • Fixed Dose Combination of elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate or alafenamide • atorvastatin, rosuvastatin, lovastatin, simvastatin, fluvastatin • tacrolimus
Important potential risks	None
Missing information	Exposure in pediatric patients Exposure in pregnant or lactating women Exposure in HCV-infected patients with moderate or severe hepatic impairment (Child-Pugh B or C) Exposure in liver transplant patients Exposure in patients with HBV/HCV co-infection

Pharmacovigilance plan

Study / Activity	Objectives	Safety Concerns Addressed	Status	Date for Submission of Interim / Final Reports (target dates)
Protocol 017: A Long-Term Follow-up Study to Evaluate the Durability of Virologic Response and/or Viral Resistance Patterns	Protocol 017 is an open-label study of 3-year follow-up for subjects enrolled in GZR and EBR trials. The objective of the study is to assess persistence of	Information on the long-term follow-up of resistance development to GZR and/or EBR	Ongoing	Interim Report 3Q 2016 Final Report 2Q 2021

Study / Activity	Objectives	Safety Concerns Addressed	Status	Date for Submission of Interim / Final Reports (target dates)
of Subjects With Chronic Hepatitis C Who Have Been previously Treated with MK-5172 in a Prior Clinical Trial (Category 3)	efficacy in subjects who had achieved SVR ₁₂ ; and to assess reversion of RAVs to wild-type virus in subjects who were virologic failures and had developed RAVs to GZR and/or EBR.			
A Phase 1 biocomparison study to compare the adult FDC (MK-5172/MK-8742) with the MK-5172 and MK-8742 single entity age-appropriate formulations (when co-administered)	This is a relative bioavailability study which will be conducted in healthy adults to compare the pharmacokinetics of the adult MK-5172 and MK-8742 single entity formulations with the corresponding age-appropriate formulations prior to the use of the age-appropriate formulation(s) in pediatric studies	PK of age-appropriate formulations	Planned	2Q 2017

Study / Activity	Objectives	Safety Concerns Addressed	Status	Date for Submission of Interim / Final Reports (target dates)
Protocol 079: A three part combined Phase 1 PK and Phase 3 clinical treatment study in pediatric subjects infected with HCV infection, ages 3 to 18 years	Part A will establish PK, safety and efficacy in G1, 4 and 6 treatment naïve, non-cirrhotic HCV-infected children Part B will establish PK, safety and efficacy in G1, 4 and 6 infected subjects who are (i.) treatment naïve with compensated cirrhosis or (ii.) failed prior interferon or pegylated-interferon with RBV therapy with or without compensated cirrhosis Part C will follow any subject enrolled in Parts A or B for 3 years	Safety of EBR/GZR in children and adolescents	Planned	3Q 2020 for Parts A and B. 3Q 2023 for Part C

The Applicant's proposal to address the safety concerns listed above within the above pharmacovigilance plan is considered acceptable.

Risk minimisation measures

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
<u>Important Identified Risk:</u> Late ALT Elevation	Listed under SmPC Sections 4.4 Special warnings and precautions for use and 4.8 Undesirable Effects	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
<u>Important Identified Risk:</u> Hyperbilirubinemia	Listed under SmPC Section 4.8 Undesirable Effects	None
<u>Important Identified Risk:</u> Drug Resistance	Listed under SmPC Section 5.1 Pharmacodynamic properties	None
<u>Important Identified Risk:</u> Drug Interactions with CYP3A inducers	Listed under SmPC, Sections 4.3 Contraindications, 4.4 Special warnings and precautions for use, 4.5 Interaction with other medicinal products and other forms of interaction, and 5.2 Pharmacokinetic properties Package leaflet – Section 2. What you need to know before you take ZEPATIER	None
<u>Important Identified Risk:</u> Drug Interactions with OATP1B inhibitors (see cobicistat containing regimens)	Listed under SmPC Sections 4.3 Contraindications, 4.4 Special warnings and precautions for use and 4.5 Interaction with other medicinal products and other forms of interaction Package leaflet – Section 2. What you need to know before you take ZEPATIER	None
<u>Important Identified Risk:</u> Drug Interactions with strong CYP3A inhibitors	Listed under SmPC Sections 4.4 Special warnings and precautions for use, 4.5 Interaction with other medicinal products and other forms of interaction, and 5.2 Pharmacokinetic properties Package leaflet – Section 2. What you need to know before you take ZEPATIER	None
<u>Important Identified Risk:</u> Drug Interactions with Fixed Dose Combination of elvitegravir, cobicistat, emtricitabine, and tenofovir	Listed under SmPC Sections 4.3 Contraindications, 4.4 Special warnings and precautions for use and 4.5 Interaction with other medicinal products and other forms	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
disoproxil fumarate or alafenamide	of interaction Package leaflet – Section 2. What you need to know before you take ZEPATIER	
<u>Important Identified Risk:</u> Drug Interaction with atorvastatin, rosuvastatin, lovastatin, simvastatin, fluvastatin	Listed under SmPC Sections 4.4 Special warnings and precautions for use and 4.5 Interaction with other medicinal products and other forms of interaction Package leaflet – Section 2. What you need to know before you take ZEPATIER	None
<u>Important Identified Risk:</u> Drug Interaction with tacrolimus	Listed under SmPC Section 4.5 Interaction with other medicinal products and other forms of interaction	None
<u>Missing Information:</u> Exposure in pediatric patients	Listed under SmPC Sections 4.2 Posology and method of administration, 4.4 Special warnings and precautions for use, 4.5 Interaction with other medicinal products and other forms of interaction, 4.8 Undesirable effects, 5.1 Pharmacodynamic properties, and 5.2 Pharmacokinetic properties. Package leaflet – Section 2. What you need to know before you take ZEPATIER	None
<u>Missing Information:</u> Exposure in pregnant or lactating women	Listed under SmPC Sections 4.6 Fertility, pregnancy and lactation, 5.3 Preclinical safety data Package leaflet - Section 2. What you need to know before you take ZEPATIER	None
<u>Missing Information:</u> Exposure in HCV-infected patients with moderate or severe hepatic impairment (Child-Pugh B or C)	Listed under SmPC Sections 4.2 Posology and method of administration, 4.3 Contraindications, 4.8 Undesirable effects, and 5.2 Pharmacokinetic properties	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
	Package leaflet - Section 2. What you need to know before you take ZEPATIER	
<u>Missing information:</u> Exposure in liver transplant patients	Listed under SmPC Section 4.2 Posology and method of administration	None
<u>Missing Information:</u> Exposure in patients with HBV/HCV co-infection	Listed under SmPC Section 4.4 Special warnings and precautions for use Package leaflet - Section 2. What you need to know before you take ZEPATIER	None

The applicant's proposal for routine risk minimisation measures is considered sufficient to address these safety concerns.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.3 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use*.

2.9.2. Labelling exemptions

A request to omit EXP and Lot from the wallet labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The wallet has a window through which EXP and Lot are visible on the blister. Therefore, it was felt unnecessary to repeat this information on the wallet itself as it would appear through a window in the inner carton (the blister is glued within the inner wallet).

The particulars to be omitted as per the QRD Group decision described above will however be included in the Annexes published with the EPAR on EMA website, and translated in all languages but will appear in grey-shaded to show that they will not be included on the printed materials.

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

Due to the low production volumes, the QRD Group accepted the request for English only blister label (INN in English or Latin); however, the QRD Group requested that the invented name must also be added. The following information has been agreed to be displayed on the blister label: Invented name, INN (English or Latin), EXP, Lot and 2D code.

In addition, because of space constraints, the QRD Group accepted the translation exemption request of EXP and Lot on the outer carton.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zepatier (elbasvir / grazoprevir) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

Benefits

Beneficial effects

The clinical programme has demonstrated that EBR/GZR in the various regimens proposed by sub-population is associated with high SVR12 rates. The fact that there is no comparison with active controlled groups was acceptable at the time that the programme was initiated since there is negligible spontaneous resolution of chronic HCV infection and there were no approved DAA-only containing regimens. The approach to the statistical analyses of these uncontrolled efficacy data was acceptable. The different studies had different thresholds for success intended to show that EBR/GZR -containing regimens provided SVR12 rates superior to those described using available treatments. Some of these criteria may be questionable but in reality this does not matter since the applicant's final claims are based on studies in which the observed SVR12 rates and lower bounds of the 95% CI are all very high.

Although EBR/GZR is clearly effective, basing regimen selection on treatment history was not a useful or appropriate approach. The SmPC includes the regimen selection based on virological factors. The data support use of 12 weeks EBR/GZR for GT1b infections and for GT1a or GT4 infections when the

baseline viral load is <800,000 IU/mL. This regimen may also be used for GT1a when none of a list of selected NS5A RAVs is present (if testing is done). To safeguard response rates in case of baseline RAVs in GT1a viruses and to minimise the risk of selecting for resistance 16 weeks plus RBV may be considered for GT1a and GT4 when baseline viral load is >800,000 IU/mL or, for GT1a, when selected NS5A RAVs have been detected. These regimens appear to be suitable for patients who have compensated cirrhosis (CP-A only), are HIV co-infected or have CKD.

Uncertainty in the knowledge about the beneficial effects

Risks

There is very considerable potential for cross-resistance between each of GZR and EBR and other licensed agents in their respective classes but there are some exceptions. In addition, at least some further generation molecules in each class may not be affected by RAVs associated with markedly reduced susceptibility to GZR or EBR.

So far there are few data available on the persistence of resistance-associated substitutions after failure of EBR/GZR but the available data indicate that treatment-emergent NS5A resistance-associated substitutions have generally persisted for a long period whereas the majority of NS3 resistance associated substitutions have not.

There remain some concerns regarding the efficacy of EBR/GZR when plasma exposures are reduced to 40-50% of those in a reference HCV-infected patient. It is not possible to fully concur with the applicant that the viral response to EBR/GZR 100/50 mg is relatively insensitive to variations in exposure at the recommended doses and regimens. However, the concern can be somewhat be mitigated by that fact that the use of Zepatier with inducers of CYP3A or P-gp is contraindicated.

Unfavourable effects

The safety of EBR/GZR, with or without RBV, has been evaluated in a large and diverse population. The total size of the safety database is acceptable. Leaving aside the hepatic events, EBR/GZR alone appears to have a benign safety profile. Addition of RBV confers the additional safety problems well known to be associated with this agent.

Following the safety data obtained in study 003 the applicant instituted a special hepatic monitoring programme. The focus has been on Late ALT/AST Elevation Events, which were clearly related to GZR exposures. At the 100 mg dose, taking into account multiple forbidden concomitant medications in clinical trials, these events occurred in <1% of patients treated with GZR 100 mg. In the pooled analysis of the three Phase 3 studies (060/061/068) seven out of 743 patients (~0.9%) had Late ALT/AST Elevation Events and in the ISP 13/1690 (0.8%) had Late ALT/AST Elevation Events. At this GZR dose (100 mg) no patient had concomitant ALT/AST >3x ULN and total bilirubin >2x ULN that was consistent with hepatocellular injury.

Uncertainty in the knowledge about the unfavourable effects

In the applicant's defined hepatic safety pool of 2405 patients who received at least 8 weeks GZR there were 25 (1%) patients with a Late ALT/AST Elevation Event. These late events, which followed a period of normalisation on treatment, were not the only elevations in AST/ALT observed. There were 53 patients with late elevations by 2-5-fold, while 36 patients had such an event and/or had a hepatic laboratory events of clinical interest (ECI) and/or discontinued treatment because they met the pre-defined hepatic safety criteria.

Late ALT/AST Elevation Events have generally occurred at or after TW8. They were not accompanied by abnormalities of other tests of hepatic function or by liver-related symptoms. Most have been short-lived and all so far have resolved. The rate of these Late ALT/AST Elevation Events was higher in patients with intrinsic factors that increase GZR exposures. Thus far the applicant predicts a rate of <2% for any separate factor but a rate of 3.8% is predicted as possible for combinations of several intrinsic and extrinsic factors in the same patient. The mechanism behind such events, which occur after resolution of baseline abnormalities and while still on treatment, is unknown.

The question then arises how important such elevations are to patient management and safety. Firstly, it appears that 20/25 such patients completed their assigned treatment. Secondly, there was resolution in all cases. Nevertheless, the rates that may be observed during routine use, when multiple factors that increase GZR exposures may co-exist despite contraindications and warnings in the SmPC, could be higher. Also, as more cases accrue it may be that not all fully resolve. As a consequence, in addition to the adequate wording in the SmPC, these issues will closely be followed in the post-authorisation.

Effects Table for EBR /GZR in treatment of HCV

Effect	Short Description	SVR12 rates	Comparator	Uncertainties / Strength of evidence
Favourable effects				
SVR12 (clinical cure based on virological endpoint) in patients with GT1 and 4	Proportion of patients with SVR12 when treated with the applicant's recommended regimens	>90% in TN and TE; cirrhotic and non-cirrhotic	N/A Effectively no spontaneous resolution	- Responses in GT1b are not identical to those with GT1a; responses in GT1a more impacted by baseline NS5A RAVs 12 weeks EBR/GZR alone can be used for GT1b. This regimen could also be used for GT1a and GT4 when baseline viral load is <800,000 IU/mL.
SVR12 (clinical cure based on virological endpoint) in patients with NS5A RAVs	NS5A RAVs (some conferring < or > 5-fold shifts in EBR activity in vitro) have been associated with reduction in SVR12 rates	SVR12 reduced to 50-55%	"	- Impact was on responses in GT1a patients - Need to consider 16 weeks EBR/GZR+RBV when baseline viral load is >800,000 IU/mL or certain baseline NS5A RAVs detected
SVR12 (clinical cure based on virological endpoint) in patients with GT3	" When combined with SOF	14/14 non-cirrhotic and 10/11 cirrhotic patients	"	- In-vitro and monotherapy data indicate lower activity of GZR and EBR vs. GT3 - Poor SVR12 for EBR/GZR + RBV - Use for GT3 is not supported.
SVR12 (clinical cure based on virological endpoint) in patients with CrCL < 30 mL/min	For TE patients the applicant has a footnote stating that EBR/GZR can be given without RBV when CrCL is < 30 mL/min	No data to support this use	"	- Omitting RBV from the regimen in patients for whom it is recommended cannot be supported - SOF cannot be used for GT3 in patients with CrCL<30 mL/min
Unfavourable effects				
Late ALT/AST Elevation Events	ALT > 5-fold ULN after on-treatment normalisation	Overall < 1% but higher rates in various	None with placebo	- Mechanism unknown - Unknown risk when pre-disposing factors combine in a single patient

Effect	Short Description	SVR12 rates	Comparator	Uncertainties / Strength of evidence
		sub-groups		Low rate may not apply when EBR/GZR is used with multiple medications
Other AEs	Rates overall and for most PTs very similar to or lower than for placebo			There were only two studies with a placebo group and most data come from one study so comparisons are to some extent limited by denominators

Benefit-risk balance

Importance of favourable and unfavourable effects

EBR/GZR provides another DAA-only (IFN-free) regimen for treatment of HCV infections. The SVR12 rates have been very high. The final proposed posology is based on virological factors and it is acceptable.

Very few patients have discontinued treatment during clinical trials, indicating that in general adverse events have not prompted cessation of therapy. The factors that may lead to increased rates of hepatic events have been identified and to some extent controlled for in the SmPC but in routine use combinations of factors may lead to higher rates than reported from trials. RMP monitoring is important.

Benefit-risk balance

Discussion on the benefit-risk balance

EBR/GZR provides another DAA-only (IFN-free) regimen for treatment of HCV infections. The SVR12 rates have been very high. Efficacy data support use of 12 weeks EBR/GZR for GT1b infections and for GT1a or GT4 infections when the baseline viral load is <800,000 IU/mL. This regimen may also be used for GT1a when none of selected NS5A RAVs are present. In case of baseline RAVs in GT1a viruses 16 weeks plus RBV may be considered for GT1a and for GT4/ GT1a when baseline viral load is >800,000 IU/mL.

The treatment regimen of 12 weeks EBR/GZR is suitable for patients who have compensated cirrhosis (CP-A only), HIV co-infection or have CKD.

The safety profile of EBR/GZR is benign. Late ALT/AST Elevation Events have been observed which so far seems to be manageable as have a short duration, have resolved and were not accompanied by abnormalities of other tests of hepatic function or by liver-related symptoms.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Zepatier in the treatment of chronic hepatitis C is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that elbasvir and grazoprevir are qualified as new active substances.