26 May 2016
EMA/399285/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Epclusa

International non-proprietary name: sofosbuvir / velpatasvir

Procedure No. EMEA/H/C/004210/0000

Note

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

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

An agency of the European Union

© European Medicines Agency, 2016. Reproduction is authorised provided the source is acknowledged.

Table of contents

1. Background information on the procedure 7
1.1. Submission of the dossier 7
1.2. Steps taken for the assessment of the product 8

2. Scientific discussion 9
2.1. Introduction 9
2.2. Quality aspects 10
2.2.1. Introduction 10
2.2.2. Active substance 10
2.2.3. Finished medicinal product 15
2.2.4. Discussion on chemical, and pharmaceutical aspects 18
2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects 18
2.2.6. Recommendation for future quality development 18
2.3. Non-clinical aspects 19
2.3.1. Introduction 19
2.3.2. Pharmacology 20
2.3.3. Pharmacokinetics 25
2.3.4. Toxicology 37
2.3.5. Ecotoxicity/environmental risk assessment 47
2.3.6. Discussion on non-clinical aspects 50
2.3.7. Conclusion on the non-clinical aspects 50
2.4. Clinical aspects 51
2.4.1. Introduction 51
2.4.2. Pharmacokinetics 53
2.4.3. Discussion on clinical pharmacology 66
2.4.4. Conclusions on clinical pharmacology 70
2.5. Clinical efficacy 70
2.5.1. Dose response studies 71
2.5.2. Main studies 73
2.5.3. Discussion on clinical efficacy 87
2.5.4. Conclusions on the clinical efficacy 89
2.6. Clinical safety 89
2.6.1. Discussion on clinical safety 101
2.6.2. Conclusions on the clinical safety 102
2.7. Risk Management Plan 102
2.8. Pharmacovigilance 108
2.9. Product information 108
2.9.1. User consultation 108
2.9.2. Additional monitoring 108

3. Benefit-Risk Balance..... 108

4. Recommendations..... 111

List of abbreviations

ALT	alanine aminotransferase
APRI	AST to Platelet Ratio Index
ARV	antiretroviral
AST	aspartate aminotransferase
ATV	atazanavir
AUC	area under the plasma/serum concentration vs. time curve
AUCtau	area under the plasma/serum concentration vs. time curve over the dosing interval
CatA	cathepsin A
CL/F	apparent oral clearance after administration of the drug:
Cmax	maximum observed plasma/serum concentration of drug
Ctau	observed drug concentration at the end of the dosing interval
BCRP	breast cancer resistance protein
BCS	Biopharmaceutics Classification System
BSEP	bile salt export pump
CLcr	creatinine clearance
COBI,	cobicistat (Tybost)
CPT	Child-Pugh-Turcotte
CsA	cyclosporine (cyclosporin A)
DAA	direct-acting antiviral
DCV	daclatasvir (BMS-790052)
DDI	drug-drug interaction
DILI	drug-induced liver injury
DRV	darunavir
DSC	Differential Scanning Calorimetry
DTG	dolutegravir
EC	European Commission
EC50	concentration of a compound inhibiting virus replication by 50%
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
eGFR	estimated glomerular filtration rate
EFV	efavirenz
EU	European Union
EVG	elvitegravir (Vitekta)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (coformulated; Stribild)
FDC	fixed-dose combination
FTC,	emtricitabine
FTC/RPV/TDF	emtricitabine/rilpivirine/tenofovir disoproxil fumarate (coformulated; Complera/Eviplera)
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada)
GC	Gas Chromatography
GT	genotype
GVS	Gravimetric vapour sorption

H2RA	H2-receptor antagonist
HBV	hepatitis B virus
HCV	hepatitis C virus
HDPE	high-density polyethylene
HIV, HIV-1	human immunodeficiency virus, type 1
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPLC	High performance liquid chromatography
IC50	concentration that results in 50% inhibition
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
IFN	interferon
IFN-α	interferon-alpha
IR	Infrared
Ka	apparent first-order absorption rate constant
LDV	ledipasvir
LDV/SOF	ledipasvir/sofosbuvir (coformulated; Harvoni)
LLOQ	lower limit of quantitation
LPV	lopinavir
MATE1	multidrug and toxin extrusion 1
MELD	model for end-stage liver disease
MRP2	multidrug resistance-associated protein 2
NMR	Nuclear Magnetic Resonance
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NOR	Normal Operating Range
NS (3/4A/5A/5B)	nonstructural protein (3/4A/5A/5B)
NTCP	sodium-taurocholate cotransporter protein
OAT	organic anion transporter
OATP	organic anion transporter polypeptide
OCT	organic cation transporter
PAR	Proven Acceptable Range
PDE	Permitted Daily Exposure
Peg-IFN	pegylated interferon
Ph. Eur.	European Pharmacopoeia
P-gp	P-glycoprotein
PMI	Potentially mutagenic impurities
PPI	proton pump inhibitor
/r	boosted with ritonavir
QbD	Quality by design
RAL	raltegravir

RAV	resistance-associated variant
RBV	ribavirin
RH	Relative Humidity
ROI	Residue on ignition
RPV	rilpivirine
RTV	ritonavir
SmPC	Summary of Product Characteristics
SOF	sofosbuvir (GS-7977; Sovaldi)
SOF/VEL	sofosbuvir/velpatasvir (coformulated)
SVR, SVRxx	sustained virologic response, sustained virologic response, sustained virologic response at "xx" weeks following completion of all treatment
TAF	tenofovir alafenamide
TDF	tenofovir disoproxil fumarate
TE	treatment experienced
TFV	tenofovir
TN	treatment naïve
TSE	Transmissible Spongiform Encephalopathy
UGT1A1	uridine disphosphate glucuronosyltransferase 1A1
ULN	upper limit of normal range
UPLC	ultra-high performance liquid chromatography
UV	Ultraviolet
VEL	velpatasvir (GS-5816)

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Gilead Sciences International Ltd submitted on 14 November 2015 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Epclusa, 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 23 April 2015.

The applicant applied for the following indication: treatment of chronic hepatitis C virus (HCV) infection in adults.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that velpatasvir was considered to be a new active substance.

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 tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0099/2015 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0099/2015 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.

Applicant's requests for consideration

New active Substance status

The applicant requested the active substance velpatasvir contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

Scientific Advice/Protocol Assistance

The applicant did not seek scientific advice at the CHMP.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Filip Josephson Co-Rapporteur: Alar Irs

- The application was received by the EMA on 14 November 2015.
- Accelerated Assessment procedure was agreed-upon by CHMP on 22 October 2015.
- The procedure started on 4 December 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 22 February 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 23 February 2016. 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.
- PRAC assessment overview, adopted by PRAC on 17 March 2016.
- During the meeting on 1 April 2016, 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 1 April 2016.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 25 April 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 11 May 2016.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 19 May 2016.
- 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 Epclusa.

2. Scientific discussion

2.1. Introduction

Hepatitis C virus (HCV) infection is a major European public health challenge, with a prevalence of 0.4-3.5% in different EU member states. It is the most common single cause of liver transplantation in the Union.

Hepatitis C virus has significant genetic (RNA sequence) variability and is classified on this basis into at least 6 genotypes. There is a significant geographical variation in the distribution of HCV genotypes. In North America and Europe, genotype 1 HCV infection predominates. In Asia, genotype 3 HCV infection is most prevalent followed by genotype 1 HCV infection. North Africa and the Middle East have high genotype 4 HCV infection prevalence. Genotype 5 HCV infection is primarily found in southern Africa while genotype 6 HCV infection is most prevalent in Southeast Asia. HCV genotype does not clearly impact the rate of disease progression. Treatment response, however, with available regimens, differs between genotypes.

During the last few years there has been a transformation in the treatment of HCV infection with the development of direct-acting antivirals (DAAs) targeting viral proteins essential to viral replication. Recently approved DAA-based treatment regimens are generally well tolerated and result in high sustained virologic response (SVR) at 12 weeks following completion of all treatment (SVR12) rates across most, but not all, patient populations. Patients with certain genotypes, notably genotype 3, remain hard to treat, in particular in combination with negative predictors of cure e.g. cirrhosis and pre-treatment resistance-associated variants. The only recommended IFN-free alternative for the treatment of genotype 2-infection includes RBV (all patients) and does not yield fully optimal results in those hardest to cure (with negative predictive factors including cirrhosis).

There are currently limited recommended treatment options for patients with decompensated liver disease. In the EU, SOF+RBV is recommended across all HCV genotypes for the treatment of patients awaiting liver transplant with the treatment duration guided by the assessment of benefit – risk. Also in the EU, LDV/SOF+RBV and SOF + DVC + RBV are recommended regimens for the treatment of patients with genotype 1 or 4 HCV infection with decompensated cirrhosis who are either pre- or post-liver transplant.

Despite the rapid development of new therapies, including interferon-free regimens, there remains an unmet medical need for certain groups of European patients with hepatitis C virus infection, in particular for those with genotype 2 and 3 and severe liver disease.

Sofosbuvir is a nucleotide prodrug that potently inhibits genotype 1 to 6 HCV RNA replicons in vitro and has demonstrated high sustained virologic response (SVR) rates when administered with RBV to subjects with chronic genotype 2 and 3 HCV infection and with pegylated interferon + ribavirin (PEG+RBV) to subjects with chronic genotype 1, 2, 3, 4, and 6 HCV infection.

Velpatasvir is a novel HCV nonstructural protein 5A (NS5A) inhibitor that is being developed in combination with sofosbuvir and other direct acting antivirals for the treatment of HCV infection.

Due to the additive antiviral interaction and lack of cross-resistance observed in vitro between Sofosbuvir and Velpatasvir, the administration of these 2 drugs as a film-coated tablet is expected to provide significant antiviral activity and a favourable resistance profile.

The medicinal product containing sofosbuvir and velpatasvir together as an oral fixed-dose combination in immediate-release film-coated tablet (400/100 mg) is indicated for the treatment of chronic hepatitis C virus (HCV) infection in adults.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as film coated tablets containing 400 mg sofosbuvir and 100 mg velpatasvir as active substances in a fixed dose combination.

Other ingredients are:

For the tablet core: copovidone, microcrystalline cellulose, croscarmellose sodium, magnesium stearate.

For the film coating: polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, iron oxide red.

The product is available in high density polyethylene (HDPE) bottle with a polypropylene child-resistant closure as described in section 6.5 of the SmPC.

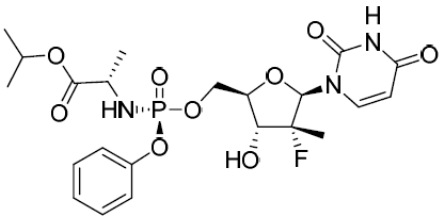
2.2.2. Active substance

Sofosbuvir

General information

Sofosbuvir is the active substance of the already-authorised products Sovaldi and Harvoni. Information on its quality is essentially the same as in the Sovaldi and Harvoni dossiers.

The chemical name of sofosbuvir is (*S*)-isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphorylamino)propanoate corresponding to the molecular formula $C_{22}H_{29}FN_3O_9P$ and has a relative molecular mass of 529 g/mol. It has the following structure:



The structure of Sofosbuvir was unambiguously confirmed by ^{1}H, ^{13}C, ^{31}P and ^{19}F NMR, UV spectroscopy, IR spectroscopy, mass spectrometry, elemental analysis and single crystal X-ray crystallography.

Sofosbuvir is a white to off-white non-hygroscopic crystalline solid, slightly soluble in water (pH 1.2-7.7), freely soluble in ethanol and acetone, soluble in 2-propanol, and insoluble in heptane.

Sofosbuvir is chiral and possesses 6 stereogenic centres. Enantiomeric purity is controlled in synthesis intermediates by chiral HPLC. Sofosbuvir exibits polymorphism. Eight polymorphic forms of sofosbuvir have been observed and the manufacturing process consistently produces sofosbuvir as the most thermodynamically stable polymorphic form that may contain a small amount of a metastable form that

was determined to be pharmaceutically equivalent. Therefore it was considered acceptable not to control the presence of the metastable form in the active substance as per ICH Q6A (decision tree #4). Other polymorphic forms are excluded by the manufacturing process and their absence is confirmed by DSC.

Manufacture, characterisation and process controls

Sofosbuvir is synthesized in six synthetic steps using three well-defined starting materials with acceptable specifications. During the procedure the applicant also introduced an alternative manufacturing process in addition to the previous one to reduce chlorinated solvent use and improve process efficiency. The alternative process was approved for Solvadi and Harvoni products (procedures EMEA/H/C/002798/WS0904/0027/G and EMEA/H/C/003850/WS0904/0027/G, approval date 7 April 2016). After recrystallisation, the active substance is then sieved or milled to afford material of a suitable particle size for formulation. GMP manufacturing for Sofosbuvir occurs at multiple manufacturers.

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. 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 based on the manufacturing experience to date.

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

Sofosbuvir is packaged in double-lined polyethylene bags closed with plastic or wire ties. The bags are held in high-density polyethylene drums (or other suitable secondary container) with lids of appropriate size and fitted with a tamper-evident security seal. The polyethylene used complies with EC requirements.

Specification

The active substance specification includes tests for appearance, identity (IR, HPLC), clarity of solution, assay (HPLC), impurities (HPLC), residual solvents and volatile organic impurities (GC), metals (ICP-OES), particle size (Ph. Eur.), and polymorphic form (DSC – Ph. Eur.).

Rationale for the absence of tests for water content (non-hygroscopic) and microbiological testing (low water content and water activity, isolation from organic solvent) was considered justified. Residue on ignition testing is not suitable as the active substance contains phosphorous: this test is replaced with a combination of clarity of solution test and ICP for elemental impurities. The applicant committed to review the acceptance limits for two residual solvents when sufficient commercial scale data is obtained from batches manufactured at the recently approved manufacturing site and from batches manufactured using the alternative process.

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 assay has been presented.

Batch analysis data on 45 batches of the active substance ranging from laboratory through pilot to commercial scale, and used for development, stability, toxicology, clinical studies, and validation were provided. 3 of the 45 batches were manufactured according to the alternative manufacturing process. Assessment focussed primarily on later pilot commercial scale batches used for development, stability and validation. The results were within the specifications and consistent from batch to batch.

Stability

Stability data were provided on two pilot scale batches of active substance from two of the three proposed sources in a container closure system representative of that intended for the market. For those batches, stability data are on-going and results were provided for up to one month under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines. The absence of stability data for the third active substance source was considered acceptable due to the fact that this manufacturer was approved for Sovaldi and Harvoni products (EMEA/H/C/002798/WS0841/0026/G and EMEA/H/C/003850/WS0841/0018/G approved in December 2015).

Stability data were also provided for 6 batches of active substance from sources other than the ones proposed for this application. Results are available for 12 or 18 months under long term conditions at 25 ºC / 60% RH and for up to 6 months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines. Those stability data were considered to be also representative for all the proposed active substance sources.

Photostability testing following ICH guideline Q1B was performed on 1 batch. Stressed studies were carried out on a single batch between -20 and 50 ºC for up to 4 weeks. Forced degradation was carried out under acidic (0.1 M HCl), alkaline (10 mM Na_2CO_3) and oxidative (3% H_2O_2) conditions and at 105 ºC.

The parameters tested were appearance, assay, impurity content, water content, and polymorphic form. The analytical methods used were the same as for release, except for water content, measured by DVS, and were stability indicating.

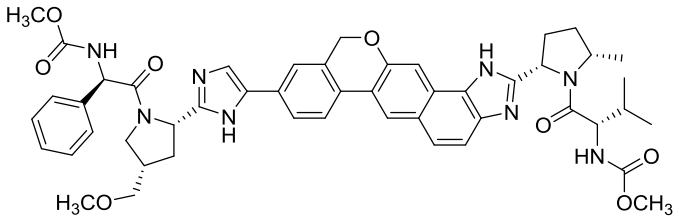
Sofosbuvir was shown to be stable under long-term, accelerated and stressed conditions and is not sensitive to light. Forced degradation revealed that the active substance may degrade *via* oxidation or hydrolysis in solution, but remains stable in the solid state even up to 105 ºC after 1 week.

The stability results indicate that the active substance manufactured by the proposed suppliers is sufficiently stable. The stability results justify the proposed 24 months retest period in the proposed container.

Velpatasvir

General information

The chemical name of velpatasvir is Methyl {(1*R*)-2-[(2*S*,4*S*)-2-(5-{2-[(2*S*,5*S*)-1-{(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}-5-methylpyrrolidin-2-yl]-1,11-dihydro[2]benzopyrano[4',3':6,7]naphtho[1,2-*d*]imidazol-9-yl}-1*H*-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl}carbamate corresponding to the molecular formula $C_{49}H_{54}N_8O_8$. It has a relative molecular mass of 883.0 g/mol and the following structure:



The structure of velpatasvir was unambiguously confirmed by ^1H and ^{13}C NMR, UV spectroscopy, IR spectroscopy, mass spectrometry, elemental analysis and single crystal X-ray crystallography.

The active substance is a white to tan or yellow hygroscopic solid. Only one solid form is known to date. Velpatasvir belongs to Biopharmaceutics Classification System (BCS) Class 4 (low solubility relative to dose and low permeability) and exhibits pH-dependent solubility; it is soluble at pH 1.2, sparingly soluble at pH 2 and practically insoluble at pH > 5.

Velpatasvir exhibits stereoisomerism due to the presence of six chiral centres and is produced as a single stereoisomer. The stereoisomers are controlled either as specified impurities in velpatasvir or by the specifications of active substance intermediates or by process design.

Velpatasvir is considered to be a new active substance.

Manufacture, characterisation and process controls

The active substance is synthesized by two manufacturers in seven main steps using four well defined starting materials with acceptable specifications.

Re-work and re-process procedures are described and are considered acceptable.

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 applicant has applied QbD principles in the development of the velpatasvir active substance manufacturing process. The active substance critical quality attributes were identified.

The preferred conditions for the manufacture of velpatasvir were selected through traditional univariate experimentation. Upon selection of the preferred conditions, additional studies were performed to establish proven acceptable ranges (PARs) for all important process parameters. These broad PARs support the more narrow normal operating ranges (NORs) used to describe the manufacturing process. The ranges included in manufacturing process description have been studied by univariate experiments. The applicant states that within the narrow ranges defined in the manufacturing description, at each velpatasvir manufacturer, individualised set points are selected to tailor the process to their specific equipment and that they do not constitute a design space. The applicant clarified that design-of-experiments (DoE) studies were used to increase the understanding of potential multivariable interactions in the process and not to establish a design space for velpatasvir manufacture. A risk assessment was conducted to identify critical process parameters (CPPs). 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. Impurity genesis, fate and purge studies are described and support the specifications for the starting materials, intermediates and active substance velpatasvir. Potentially mutagenic impurities (PMIs) that may arise from the synthesis of velpatasvir and may be present in the active substance have been identified. Control strategies for these PMIs were established with understanding of fate and purge and associated process controls. These strategies assure that the levels of these PMIs in the active substance are controlled below 30% of the concentration limit calculated based on the threshold of toxicological concern.

Velpatasvir is packaged in double polyethylene bags closed with plastic or wire ties; each double bag is contained in a polyethylene-lined aluminium foil pouch; the outer polyethylene-lined foil bag is heat sealed; the foil bags are placed in a high density polyethylene drum (or other suitable secondary

containment) fitted with a lid. The polyethylene used for the bags complies with the Ph. Eur. requirements.

Specification

The active substance specification includes tests for: appearance, identity (IR, HPLC), clarity of solution, assay (HPLC), impurities (HPLC, UPLC), residual solvents and organic volatile impurities (GC), water content (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.

Residue on ignition (ROI) is not included as an active substance release test because the velpatasvir manufacturing process and the required clarity of solution test provide adequate control for insoluble impurities including inorganic impurities. The absence of ROI test was considered acceptable.

In view of the content of elemental impurities observed, the absence of control of elemental impurities was considered acceptable.

Active substance particle size is not critical for tablet processability, stability, content uniformity, dissolution or appearance taking into account the finished product manufacture. Therefore the absence of active substance particle size control was considered acceptable.

Microbiological examination is not included in the specification for velpatasvir for the following reasons: the relationship between water content and water activity of velpatasvir is well understood, the acceptance limit for water content in velpatasvir ensures microbiological growth is not supported, the active substance container closure system minimizes increase in water content over time, and data are available to show low bioburden of the active substance at release and on stability. The absence of microbiological control was considered acceptable.

The absence of control of PMIs was considered acceptable in view of the control strategy implemented by the active substance manufacturer.

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 assay and impurities testing has been presented.

Batch analysis data are provided for 22 batches of velpatasvir ranging from laboratory through pilot to production scale used for non-clinical, clinical and stability studies. All studies have been conducted with the same form of the active substance. The results are within the specifications and consistent from batch to batch.

Stability

Stability data were provided on seven batches of active substance stored in a container closure system representative of that intended for the market for up to twelve months under long term conditions at 30 ºC / 75% RH and for six months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines. The sizes of the stability batches were from pilot to production scale and the batches were from the proposed manufacturers.

The following parameters were tested*:* appearance, assay, impurity content, and water content at each scheduled time point. Microbiological examination is conducted at the initial and annual time points for samples stored at the long-term storage condition in accordance with USP <61> and <62> or Ph. Eur.

2.6.12. Currently the analytical methods used are the same as for release and for stability studies and are stability indicative. However over the course of clinical development, improvements were made to the analytical methods used to monitor critical quality attributes and stability of the drug substance. A bridging study was conducted by analysing three batches of velpatasvir using the test parameters of the clinical method and the intended commercial method. It has been demonstrated that data obtained using the clinical methods are valid and comparable to data obtained using the intended commercial method.

No significant changes were observed in any of the monitored parameters through the 12 months of storage at long term conditions and 6 months of storage conditions at accelerated conditions compared to the initial values.

Photostability testing following the ICH guideline Q1B was performed on one batch. Results showed that velpatasvir is photolabile. Results on stress conditions (-20°C during four weeks and 50°C/ambient conditions during two weeks) were also provided on one batch. Data confirmed that velpatasvir will remain stable at both extreme temperatures.

The stability results indicate that the active substance manufactured by the proposed suppliers is sufficiently stable. The stability results justify the proposed retest period of 24 months when stored in the proposed container.

2.2.3. Finished medicinal product

Description of the product and Pharmaceutical development

The finished product is a an immediate-release fixed-dose combination (FDC) tablet containing 400 mg sofosbuvir (SOF) and 100 mg velpatasvir (VEL).

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards except for the film coating material, Opadry II Pink tested according to an in house standard and except for the colorant iron oxide red contained in opadry II pink that complies with EU Regulation 231/2012 standard. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

The compatibility of velpatasvir or sofosbuvir with typical formulation excipients was demonstrated as well as the compatibility of velpatasvir with sofosbuvir.

Velpatasvir is a BCS Class 4 compound with pH-dependent solubility in the physiological pH range. As such, food and gastrointestinal pH may influence its dissolution properties and biopharmaceutical performance. Approaches to formulation of velpatasvir were thus focused on maximising dissolution and mitigating potential food and gastrointestinal pH effects while maintaining velpatasvir chemical and physical stability.

Three formulation and manufacturing process approaches were selected based on prior experience and expert knowledge, and evaluated for incorporating velpatasvir in single-agent tablets. The formulation and manufacturing process were selected based on the results of studies performed to compare the dissolution properties, in vivo pharmacokinetic performance and chemical stability of the different formulation and manufacturing process approaches.

A VEL single-agent tablet formulation was developed to support Phase 1 and Phase 2 clinical trials. All clinical studies have used amorphous velpatasvir free base and the same solid form of velpatasvir and finished product intermediate formulation. The solid form of velpatasvir remains unchanged in the

finished product intermediate. Velpatasvir remains amorphous in the finished product intermediate with no known ability to crystallise. A film coating material was used to inhibit photodegradation.

A fixed-dose combination tablet (SOF/VEL tablet) was developed prior to initiating Phase 3 clinical trials.

Sofosbuvir is a BCS Class 3 compound with low apparent permeability and high solubility. A precedent for the formulation of sofosbuvir in an immediate release solid oral dosage form has been established through its use in commercial products, including Sovaldi and Harvoni.

SOF/VEL FDC tablets of 400/100 mg strength and 400/25 mg were developed. Opadry II Pink was used as film coating material. SOF/VEL FDC tablets of 400/100 mg strength and 400/25 mg strength demonstrated similar pharmacokinetic performance to the respective co administered single-agent tablets in relative bioavailability (BA) study, with no need for dose adjustment. The selection of the 100 mg dose for velpatasvir was supported by Phase 2 efficacy studies. The 400/100 mg strength SOF/VEL tablet formulation was used in all Phase 3 clinical trials and is the same as that intended for marketing.

The quantitative composition of the 400/100 mg strength batch used for relative bioavailability study and the batch used in the Phase 3 clinical study is identical. The *in vitro* dissolution profile comparisons provided demonstrated that the 400/100 mg strength batch used for relative bioavailability study exhibited comparable sofosbuvir and velpatasvir dissolution performance to representative batches of Sovaldi (400 mg strength) and VEL single-agent tablets (100 mg strength).

The *in vitro* dissolution profile comparisons provided also demonstrated that primary stability batches and all clinical batches of SOF/VEL 400/100 mg tablets showed comparable dissolution profiles to that observed for the 400/100 mg strength batch used for relative bioavailability study.

A detailed discussion of the dissolution method development has been provided. The dissolution method was considered acceptable. The discriminatory power of the dissolution method has been demonstrated.

The physical stability, chemical stability, and/or dissolution properties of the finished product intermediate were evaluated. The results justified the limits selected for the specifications or the in-process control.

The applicant has applied QbD principles in the development of the finished product manufacturing process. The finished product critical quality attributes have been identified.

An initial risk assessment was conducted based on expert knowledge and VEL single-agent tablet and SOF/VEL tablet process. The results of this initial risk assessment supported the evaluation of certain unit operations and process parameters in development studies to define the control strategy for the commercial manufacturing process. The applicant has presented an enhanced approach (QbD) development of the manufacturing process and as a result proposed both a target and normal operating ranges in the manufacturing process description. The applicant stated that no design space is claimed and committed to run the process at the target parameters. The applicant intends to operate within the NORs proposed for all unit operations. This approach was endorsed. It is expected that movement outside of the proposed normal operating ranges will be considered a change to the manufacturing process and would initiate a quality investigation and potentially regulatory post approval change process.

The primary packaging is a HDPE bottle with a polypropylene child resistant closure. 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

The manufacturing process of the finished product intermediate is performed by two manufacturers and consists of four main steps: feed solution preparation, drying, secondary drying, and packaging. The manufacturing process of the finished product is performed by two manufacturers and consists of four main steps: powder processing (dispensing, blending, dry granulation), tablet compression, film coating and packaging. The process is considered to be a standard manufacturing process.

The in-process controls are adequate for this type of manufacturing process and pharmaceutical form. Holding times and finished product intermediate shelf-life were justified. The process will be validated prior to commercial distribution of SOF/VEL tablets on a minimum of three consecutive production scale batches according to the process validation scheme provided. This was considered acceptable.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance, identification (HPLC, UV), water content (Ph. Eur.), assay of each active substance (HPLC), sofosbuvir degradation product content (HPLC), velpatasvir degradation product content (HPLC), uniformity of dosage units (Ph. Eur.), dissolution (Ph. Eur.), microbiological examination (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 assay and impurities testing has been presented.

Batch analysis results are provided for 13 batches using all proposed active substance sources and all proposed manufacturers. Batch analysis results confirmed the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data were provided for six pilot scale batches of finished product from both manufacturers stored under long term conditions for up to eighteen months at 25 ºC / 60% RH or 30 ºC / 75% RH and for up to six months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines. The batches of medicinal product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, assay, degradation product content, dissolution, water content. Microbiological examination is performed annually on samples stored at 30 °C/75% RH. The analytical procedures used are the same as the one used at release and are stability indicating.

In addition, one pilot batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Stability studies were also conducted at -20 °C and 60 °C/ambient humidity for 4 days on one pilot batch.

No significant changes observed in any quality attribute for up to eighteen months at the long-term and six months at accelerated storage conditions. Photostability results demonstrated that the finished product is not sensitive to light. No significant change was observed stability studies conducted at -20 °C and 60 °C/ambient humidity for 4 days.

Supportive stability data were also provided for five laboratory scale finished product batches manufactured with finished product intermediate stored in bulk from five to sixteen month prior tablet manufacturing. The batches were stored for up to 24 month at 25 ºC / 60% RH and 30 ºC / 75% RH and

for up to 6 months at 40 ºC / 75% RH. Based on the stability data available including the 6 month stability data at 25 ºC / 60% RH, 30 ºC / 75% RH and 40 ºC / 75% RH provided for the laboratory scale finished product batch manufactured with finished product intermediate stored in bulk for 16 months prior to tablet manufacturing, the applicant proposal to define the start of shelf life for the finished product as the date when finished product intermediate and sofosbuvir are combined with excipients was accepted. The holding time of sixteen months for finished product intermediate was accepted as well.

Based on available stability data, the proposed finished product shelf-life of 24 months without any storage conditions as stated in the SmPC (section 6.3) is acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used. The magnesium stearate used to manufacture SOF/VEL tablets is obtained exclusively from vegetable sources.

2.2.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The applicant has applied QbD principles in the development of velpatasvir active substance and in the development of the finished product and their manufacturing processes. However, design spaces were not claimed for either. 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.

At the time of the CHMP opinion, there was a minor unresolved quality issue related to the limits for two residual solvents in the sofosbuvir active substance specifications 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.

2.2.6. Recommendation 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 point for investigation:

- to review the acceptance limits for two residual solvents in the sofosbuvir active substance specifications when sufficient commercial scale data is obtained from batches manufactured at a recently approved manufacturing site and from batches manufactured using the alternative process.

2.3. Non-clinical aspects

2.3.1. Introduction

SOF/VEL is a fixed combination of sofosbuvir (approved NS5B polymerase inhibitor) and velpatasvir, a new NS5A-inhibitor. HCV NS5A is a multifunctional protein with key functions in HCV replication, virus assembly, and the modulation of cellular signaling pathways (Sheel and Rice, Nature Medicine, 2013).

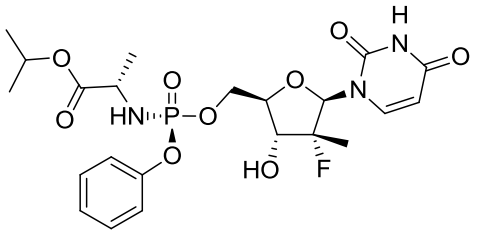
Sofosbuvir is a HCV nonstructural protein (NS)5B polymerase nucleotide inhibitor that demonstrates potent in vitro inhibition of HCV replicon ribonucleic acid (RNA) replication. Sofosbuvir has been approved for use once daily for the treatment of chronic HCV infection in adults. Sofosbuvir is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine analogue triphosphate form (GS 461203) within the hepatocyte. GS-461203 is incorporated by the HCV NS5B polymerase during HCV RNA replication, and acts to inhibit RNA replication via chain termination. SOF exhibits broad genotypic coverage in genotypes 1 to 6 replicon assays.

Velpatasvir is a HCV NS5A inhibitor that has displayed potent in vitro inhibition of HCV RNA replication across genotypes 1 to 6 in replicon cell lines. The non-structural protein 5A (NS5A) protein of HCV is an essential viral protein that plays roles in both viral RNA replication and the assembly of HCV virions. Experimental data support the conclusion that VEL targets NS5A as its mode of action.

The combination of SOF and VEL is suggested to exhibit an additive antiviral activity and not to obtain any cross resistance.

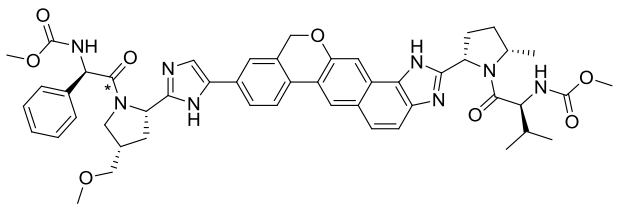
Physical chemistry

Sofosbuvir

Structure of the active substance	
Molecular weight.	529.45 g/mol
Solubility in water (mg/mL @ 37 °C)	pH 2 (HCl) 2.0 pH 4.5 (Acetate buffer) 2.1 pH 6.8 (Phosphate buffer) 1.9 pH 7.7 (Unbuffered) 2.2
Pka.	9.3
Distribution coefficient.	log P = 1.62 (in n-octanol/0.15M KCl)
Solubility in other solvents (mg/mL @ ambient temp.)	Methanol 675 Acetone 313 Acetonitrile 235 Ethanol 204 2-Propanol 45 Ethyl acetate 23
Stability.	Sofosbuvir is stable at both long-term and accelerated conditions.
Possible chirality and its consequences.	Sofosbuvir has six stereocenters and is chirally pure.
Polymorphism.	Eight solid forms of sofosbuvir have been isolated in laboratory studies. Sofosbuvir Form II is a unsolvated polymorph and the designated commercial drug substance.

Hygroscopicity.	Non-hygroscopic

Velpatasvir

Structure of the active substance Site of labelling marked with an asterisk (see structure).	
Molecular weight.	883.0 g/mol
Solubility in water (mg/mL, @ 25°C)	Water, pH 1.2a > 36 Water, pH 2.0a 3.6 Sodium acetate buffer, pH 5.0 < 0.1 Phosphate buffer, pH 6.8 < 0.1 FeSSIF, pH 5.0b 0.1 FaSSIF, pH 6.5c < 0.1
Pka.	pKa,1 = 3.2 (weak base) pKa,2 = 4.6 (weak base)
Distribution coefficient.	Log D 6.31 (pH 8)
Solubility in other solvents (mg/mL, @ 25°C)	Acetonitrile > 36 Acetone > 350 Dichloromethane > 36 Ethanol > 350 Ethyl acetate > 36 Methanol > 36 2-Propanol 9.5 Toluene 13.3
Stability.	Velpatasvir remain stable at temperatures of -20 °C for 4 weeks and at 50 °C for up to 2 weeks. Velpatasvir is sensitive to light.
Possible chirality and its consequences.	Velpatasvir contains six stereocenters and is produced as a single stereoisomer. There are eight potential diastereomers
Hygroscopicity.	Hygroscopic.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Sofosbuvir is a HCV NS5B polymerase nucleotide inhibitor that displays potent inhibition of HCV RNA replication in vitro. In human hepatocytes, SOF is converted to an active uridine triphosphate form (GS-461203) that has been shown to directly inhibit NS5B polymerase activity in a biochemical assay, at IC50 values ranging from 0.7 to 2.6 μM. Sofosbuvir demonstrates activity against stable genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a HCV replicons at EC50 values of 0.014 to 0.11 μM (PC-PSI-7977-09-0006, PC-PSI-7977-09-0012, and PC-334-2005).

In vitro resistance selection experiments demonstrated that the NS5B S282T mutation was the primary SOF in vitro resistance mutation (PC-334-2006, PC-334-2010, and PC-PSI-7977-09-0008).

Velpatasvir (GS-5816) inhibits HCV replication by interfering with the HCV NS5A protein. The enzymatic function of NS5A has no known, therefore, it is not possible biochemically confirm NS5A inhibition by VEL.

Hepatitis C virus replicon studies have shown that VEL has antiviral activity against HCV genotypes 1 to 6 with mean EC50 values ranging from 0.002 to 0.13 nM (PC-281-2024). No cytotoxicity was observed at the highest concentrations tested (concentration that results in 50% cytotoxicity > 44,444 nM).

The mean EC50 value of VEL against chimeric replicons encoding NS5A sequences from clinical isolates was 0.029 nM for genotype 1 (range [Min, Max]: 0.005, 0.5 nM; N = 57), 0.027 nM for genotype 2 (range: 0.0003, 0.36 nM; N = 37), 14.8 nM for genotype 3 (range: 0.002, 319.1 nM; N = 40), 0.005 nM for genotype 4 (range: 0.001, 0.014 nM; N = 31); 0.007 nM for genotype 5 (range: 0.001, 0.019 nM; N = 35); and 0.11 nM for genotype 6 (range: 0.0005, 2.6 nM; N = 49).

In vitro resistance selection experiments demonstrated that depending of genotype (1a, 1b, 2a, 3a, 4a, 5a, and 6a) several NS5A mutations significantly increase VEL resistance (PC-281-2027). Phenotypic analysis of a broad panel of NS5A resistance-associated substitutions in HCV genotype 1 to 6 replicons demonstrated that VEL high potency against a wide range of NS5A RAVs and has an improved resistance barrier compared with first generation NS5A inhibitors (PC-281-2013, PC-281-2023, and PC-281-2030).

Sofosbuvir/Velpatasvir: In vitro, the combination of SOF and VEL exhibited additive antiviral activity. No antiviral antagonism was observed, and no significant change in cell viability was observed in combination studies of SOF and VEL (PC-334-2014). In vitro studies demonstrated no cross-resistance between SOF and VEL when tested individually against HCV mutations resistant to other classes of HCV inhibitors. The NS5B S282T mutant replicon, which conferred low-level reduced susceptibility to SOF, was susceptible to VEL (Table 1). Similarly, SOF was fully active against a panel of NS5A mutants that showed a reduced susceptibility to VEL. Furthermore, double-class mutants (NS5B S282T + NS5A resistance-associated variants [RAVs]) displayed a significant reduced replication capacity compared to wild type or single NS5A RAVs in the replicon.

Table 1 Antiviral Activity of SOF and VEL Against Wild Type and S282T Mutant of Genotype 1b

	SOF			VEL		
	EC_{50} (nM)[a]		Fold Change[b]	EC_{50} (nM)[a]		Fold Change[b]
Replicon	Wild type	S282T		Wild-type	S282T	
Genotype 1b	21.5	189.2	8.8	0.004	0.004	1

a EC50 indicated average for 2 or more independent experiments.

b Fold change from the corresponding wild type.

Secondary pharmacodynamic studies

Sofosbuvir

In Vitro Cytotoxicity

The cytotoxicity of SOF was evaluated in multiple cell lines. In 2 human hepatocarcinoma cell lines, Huh-7 and HepG2, the SOF concentration that resulted in 50% cytotoxicity (CC50) was 95.9 µM and 90.6 µM, respectively. In human pancreatic adenocarcinoma cells (BxPC3), 2 human T cell leukemia cell lines (CEM and MT4), and human metastatic prostate carcinoma cells (PC-3), the CC50 values were greater than the respective highest concentration of SOF tested (89–200 µM) (PC-PSI-7977-09-0004 and PC-334-2005). In studies using GS-9851 (the diasteromer mixture of SOF and GS-491241), the CC50 values for GS-9851 were > 50 µM (the highest concentration tested) in human erythroid and myeloid bone marrow progenitor cells (PC-PSI-7851-08-0022).

Mitochondrial Toxicity

Sofosbuvir showed no mitochondrial toxicity in cell-based assays measuring mitochondrial DNA (mtDNA) depletion or selective cytochrome c oxidase protein depletion. Sofosbuvir did not alter mtDNA levels at concentrations of 50 µM and 100 µM in HepG2 and CEM cells, respectively (PC-334-2012, PC-PSI-7977-09-0007). The mitochondrial biogenesis studies demonstrated that SOF had no effect on

cytochrome c oxidase expression in PC-3 cells at the highest concentration tested (100 µM) (PC-334-2015). Similarly, GS-9851 showed no inhibition of cytochrome c oxidase expression at 100 µM in both HepG2 and PC-3 cell lines (PC-334-2015). The IC_{50} value for the active metabolite GS-461203, when tested against the mitochondrial RNA polymerase and mitochondrial DNA polymerase gamma, was greater than the highest concentrations evaluated (200 µM and 500 µM, respectively) (PC-334-2013, PC-PSI-7851-09-0015).

Activity Against Host Polymerases

The GS-461203, was tested in vitro for activity against host DNA and RNA polymerases. The IC_{50} was greater than the highest concentration tested (200 µM) against human DNA polymerases alpha and beta (PC-334-2013, PC-PSI-7851-08-0029). The IC_{50} values were also greater than the highest concentration tested (200 µM) against the human RNA polymerase II (PC-334-2013, PC-PSI-7851-09-0015).

In Vitro Receptor Binding Potencies

The effects of GS-9851 and the major metabolite GS-331007 were evaluated to determine the potential for off-target activity against a panel of receptors, enzymes, and ion channels. At 10 µM, GS-9851 and GS-331007 did not show greater than 50% inhibition or induction of any target (PC-PSI-7851-09-0004, PC-334-2026).

Velpatasvir

Activity against other viruses

The pharmacologic activity of VEL against other viruses is described in detail in the clinical part of this submission. Briefly, VEL was tested for antiviral activity against bovine viral diarrhea virus (BVDV), RSV, HBV, HIV-1, HRV, influenza A and B, and a panel of flaviviruses (including West Nile virus, yellow fever virus, dengue virus, and banzai virus). In contrast to its antiviral activity against HCV, VEL is reported to show no selective antiviral activity against BVDV (a related flavivirus) or against any of the other viruses tested at the highest concentration tested or the highest concentration without cytotoxicity.

In Vitro Cytotoxicity

The in vitro cytotoxicity of VEL was evaluated in 2 hepatic cell lines Huh-7 and HepG2, the prostate carcinoma cell line PC-3, the T-lymphoblastoid cell line MT-4, and normal lung-derived MRC-5 cells. Following 5 days of continuous compound exposure, CC50 values for VEL were > 44,444 nM in 4 of the 5 cell lines tested, and 4028 nM in PC-3 cells. These values conferred a selectivity index of 270,000 to > 3,000,000 for genotype 1 HCV and VEL is thus concluded to show low cellular cytotoxicity.

In Vitro Receptor Binding Potencies

The effects of VEL on a standard panel of receptors, enzymes, and ion channels were evaluated to determine the potential for off-target activity. At 10 µM VEL (dispensed from a 10 mM stock solution in DMSO), there were no significant responses on any target and only a weak to moderate effect (between 25-50%) indicated in the melatonin receptor MT1 ((ML1A) (h) agonist radioligand assay; using [^{125}I]2-iodomelatonin) with a 38% inhibition of binding at 10µM.

SOF/VEL

In Vitro Cytotoxicity

No significant cell cytotoxicity was observed with either SOF or VEL as individual agents. In vitro studies of the combination SOF and VEL were performed in genotype 1b, 2a, 3a, and 4a replicon cell lines. Cytotoxicity was quantified by cell viability at a SOF top concentration of 320 nM in combination with VEL

at a top concentration of 0.064 nM. No significant change in cell viability was observed. In addition, SOF is unlikely to produce adverse effects related to the inhibition of human RNA and DNA polymerases, and there is no indication of mitochondrial toxicity associated with SOF. In conclusion, based on the low potential for off-target activity by SOF and VEL, no additional pharmacodynamics studies are considered necessary with the SOF/VEL combination.

Safety pharmacology programme

The nonclinical safety pharmacology profiles of SOF (dosed as GS-9851) and VEL were independently characterized. Study designs and parameters evaluated are consistent with accepted principles and practices as outlined in the ICH S7A Guideline (Safety Pharmacology Studies for Human Pharmaceuticals). All studies were conducted in accordance with GLP regulations.

In Vitro Studies

The effect of GS-9851 on hERG inhibition assays

Organ Systems Evaluated	Test System	Method of Administration	Dose (µM)	No. per Group	Noteworthy Findings	GLP[a]	Gilead Study Number
Cardiovascular (hERG Inhibition) with GS-9851	HEK293 Cells	In Vitro	10, 300	3 cells per concentration	0.6% and 12.7% inhibition at 10 and 300 µM, respectively IC_{50} > 300 µM	Yes	SA-PSI-7851-08-009[b]
Cardiovascular (hERG Inhibition) with Metabolite GS-566500	HEK293 Cells	In Vitro	10, 100, 300	3 cells per concentration	-0.1%, 1%, and 4.6% inhibition at 10, 100, and 300 µM, respectively IC_{50} > 300 µM	No	PC-PSI-7851-08-0023
Cardiovascular (hERG Inhibition) with Metabolite GS-606965	HEK293 Cells	In Vitro	3, 10, 100	3 cells at 3 and 100 µM, 5 cells at 10 µM	0.2%, 4.3%, and 3.7% inhibition at 3, 10, and 100 µM, respectively IC_{50} > 100 µM	No	PC-PSI-7851-08-0028
Cardiovascular (hERG Inhibition) with Metabolite GS-331007	HEK293 Cells	In Vitro	10, 100	3 cells per concentration	0.8% and 0.6% inhibition at 10 and 100 µM, respectively IC_{50} > 100 µM	No	PC-PSI-7851-09-0001

GLP = Good Laboratory Practice; HEK293 = human embryonic kidney cells; hERG = human ether-a-go-go related gene

Conversions: Sofosbuvir (SOF, GS-7977, PSI-7977) and GS-9851: 1 µM = 0.529 µg/mL; GS-566500: 1 µM = 0.411 µg/mL; GS-606965: 1 µM = 0.340 µg/mL; GS-331007: 1 µM = 0.260 µg/mL

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

b. Study dosed with the diastereomer mixture GS-9851.

In hERG inhibition assays in vitro, the IC_{50} of GS-9851 and the metabolites GS-566500, GS-606965, and GS-331007 could not be calculated as there was minimal inhibition of the hERG current in vitro at concentrations up to 159 (300 µM), 123 (300 µM), 34 (100 µM), and 26 (100 µM) µg/mL, respectively, the highest concentrations tested (SA-PSI-7851-08-009, PC-PSI-7851-08-0023, PC-PSI-7851-08-0028, PC-PSI-7851-09-0001).

In Vivo Studies

The effect of GS-9851 on the CNS, cardiovascular, and respiratory systems

Organ Systems Evaluated	Species/Strain	Method of Administration	Dose (mg/kg)	Sex and No. per Group	Noteworthy Findings	GLP[a]	Gilead Study Number
Central Nervous System	Rat/Sprague Dawley	Oral Gavage	0, 100, 300, 1000	5/sex/group	None. NOEL 1000 mg/kg	Yes	SA-PSI-7851-08-006
Cardiovascular System	Dog/Beagle	Oral by Capsule	0, 100, 300, 1000	3/sex; Latin square design	None. NOAEL 1000 mg/kg	Yes	SA-PSI-7851-08-007
Respiratory System	Rat/Sprague Dawley	Oral Gavage	0, 100, 300, 1000	5/sex/group	None. NOEL 1000 mg/kg	Yes	SA-PSI-7851-08-008

GLP = Good Laboratory Practice; HEK293 = human embryonic kidney cells; hERG = human ether-a-go-go related gene

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

There were no findings in any study suggesting a low potential for clinically relevant adverse neurological, cardiovascular, or respiratory effects. There were no adverse effects in Sprague-Dawley rats on the CNS, cardiovascular system or on respiratory function at doses up to 1000 mg/kg (SA-PSI-7851-08-006, SA-PSI-7851-08-007, SA-PSI-7851-08-008).

Velpatasvir

In Vitro Studies

The effect of VEL on hERG inhibition assays

Organ Systems Evaluated	Test System	Method of Administration	Dose (µM)	No. per Group	Noteworthy Findings	GLP[a]	Gilead Study Number
Cardiovascular (hERG Inhibition)	HEK293 Cells	In Vitro	3, 6.5	3 cells per concentration	IC_{50} > 6.5 µM	Yes	PC-281-2006

GLP = Good Laboratory Practice; HEK293 = human embryonic kidney cells; hERG = human ether-a-go-go related gene

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

In Vivo Studies

Safety pharmacology studies performed with velpatasvir (GS-5816)

Type of study/Report No	Dose/Group	Noteworthy Findings
Cardiovascular		
Cardiovascular Safety Pharmacology Evaluation In Telemetry-Instrumented Conscious Dogs / PC-281-2003 / GLP	4 Male Beagle dogs 0, 5, 20, and 100 mg/kg Single dose via oral gavage using a Latin square design on Days 1, 8, 15, and 22 GS-5816 (lot no: 5126-180-38)	No GS-5816-related mortality, morbidity, or effects on clinical observations, body temperature, body weight or food consumption were noted. (All dogs were transferred back to the stock colony.) Mean plasma levels for GS-5816 at 4.5 hours post dose were 149, 730 and 1267 ng/mL for dogs administered 5, 20 and 100 mg/kg (ranges: 113-214, 647-810, and 799-1590 ng/mL, respectively) GS-5816 had no effect on qualitative or quantitative ECG parameters or on hemodynamic data. (Minor hemodynamic effects were considered incidental.) **Conclusion**: VEL did not have any cardiovascular effects in dogs at the doses tested ≤ 100 mg/kg (mean plasma conc. ≤ 1267 ng/mL as compared to clinical Cmax of 259 ng/mL)
Effect of GS-5816 on cloned hERG in HEK cells / PC-281-2006 / GLP	HEK293 cells stably transfected with hERG cDNA n=3 (Ctrl: n=4) 0, 3, 6.5 µM @33-35ºC GS-5816 was insoluble in the assay vehicle at higher concentrations. Samples for homogeneity and concentration determination were collected from the DMSO stock solutions and from the dosing formulations.	GS-5816 concentrations were within ±10.0% of nominal concentrations (one 6.5µM replicate measured 89.3% of nominal). Precipitate was noted in the 6.5 µM formulation in HB-PS + 0.3% DMSO, indicating that the actual hERG exposure concentration may have been less than nominal. GS-5816 inhibited hERG current by (Mean ± SEM) 0.7 ± 0.3% at 3 µM (n = 3) and 0.9 ± 1.0% at 6.5 µM (n = 3) versus 0.7 ± 0.7% (n = 4) in control. Positive control (60 nM terfenadine) inhibited hERG potassium current by 80.7 ± 1.8% (n = 2). **Conclusion**: IC50 predicted to be > 6.5 µM (5.7 µg/mL as compared to clinical Cmax of 0.26 µg/mL)
CNS		
CNS evaluation in rat: Modified Irwin battery of assessments (including home cage, hand-held, open-field, and elicited response observations).	8 male rats (SD)/group 0, 20, 60, or 200 mg/kg	All rats survived until scheduled euthanasia on Day 2. No effects related to GS-5816 were observed for clinical signs or body temperature at any dose level. No neurological effects related to GS-5816 were

/ PC-281-2004 / GLP		evident at any time point. **Conclusion**: GS-5816 did not give any effects on neurological function in rats. NOAEL for neurological function in rats is 200 mg/kg.
Respiratory		
Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of GS-5816 in rats / PC-281-2005 / GLP	8 male rats (SD)/group 0, 20, 60, or 200 mg/kg	No morbidity or signs of toxicity were observed at any dose level. GS-5816 did not affect any of the respiratory parameters tested (respiration rate, tidal volume, or minute Volume). **Conclusion**: GS-5816 did not give any effects on respiratory function in rats. NOAEL in rats is 200 mg/kg.

2.3.3. Pharmacokinetics

Pharmacokinetic studies

The absorption, distribution, metabolism, and excretion profiles of SOF and VEL have been evaluated in nonclinical studies in human extracts and cells in vitro as well as in in vivo studies in animals. Nonclinical evaluation of the PK of SOF/VEL is primarily based on studies conducted with the individual agents.

Methods of analysis

Sofosbuvir, Velpatasvir

The high performance liquid chromatography (HPLC) and HPLC coupled to tandem mass spectrometry (LC-MS/MS) methods were used to examine SOF, VEL and their metabolites in culture media, plasma, bile and urine samples during PK studies. These methods did not strictly conform to GLP guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision. All bioanalytical methods for toxicokinetic analyses supporting the GLP safety studies with SOF and VEL were validated. The LC-MS/MS method was used to determine the PK parameters in the toxicokinetics studies. Validation parameters included selectivity, sensitivity, linearity, recovery, carryover, intra- and inter-assay precision and accuracy, sample collection stability, stock solution stability, injection medium integrity, short-term matrix stability, freeze-thaw matrix stability, long-term matrix stability, and dilution integrity, re-injection reproducibility.

Following radiochemicals were used: [^{3}H]GS-9851, [^{14}C]SOF, [^{14}C]VEL. The [^{3}H] and [^{14}C]-derived radioactivities in biological samples, including cell culture media, blood, plasma, excised tissues, excreta, and effluent from the HPLC, were determined by liquid scintillation counting.

TRIzol was used to isolate RNA, which was analyzed by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to assess the effect of SOF and VEL on CYPs mRNA levels.

Velpatasvir

Analysis of VEL in plasma, bile, and urine samples from PK studies in CD-1 mice, Sprague Dawley rats, New Zealand white (NZW) rabbits, beagle dogs, and cynomolgus monkey utilized methods based on LC/MS/MS that were not fully validated under the conditions of GLP. These methods were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra assay accuracy and precision.

Analytical methods and validation reports for methods supporting GLP TK studies

Type of Study	Test System	Analytical Method	LLOQ (ng/mL) & Recovery (%)	Study Number
Partial Method Validation	K_2 EDTA Mouse Plasma	LC/MS/MS	2.00 97.7*)/103.1**)	BA-281-2007 (8298450)
Method Validation	K_2 EDTA Rat Plasma	LC/MS/MS	2.00 93.0*)/95.5**)	BA-281-2002 (8259478)
Partial Method Validation	K_2 EDTA Rabbit Plasma	LC/MS/MS	2.00 91.0*)/100.0**)	BA-281-2004 (8271133)
Method Validation	K_2 EDTA Dog Plasma	LC/MS/MS	2.00 89.2*)/97.4**)	BA-281-2003 (8259479)

K2EDTA = potassium ethylenediaminetetraacetic acid; LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; VEL = velpatasvir

*) overall recoveries for GS-5816 **) recovery of internal standard

Validation parameters included selectivity, sensitivity, linearity, recovery, carryover, intra- and inter assay precision and accuracy, sample collection stability, stock solution stability, injection medium integrity, short-term matrix stability, freeze-thaw matrix stability, long-term matrix stability, and dilution integrity, re injection reproducibility.

Reproducibility

The results of incurred sample reproducibility analyses that were conducted during the rat and dog toxicology studies confirmed the repeatability of the methods. Only one (23.5%) of thirty-six reanalysed samples from rats (TX-281-2003) and nine (30.6, 25.2, 26.3, 30.4, 23.3, 24.6, 31.6, 40.5, 20.4 %) of fifty-three samples from dogs (TX-281-2004) showed a difference >20%. Thus 97% of samples in the rat study and 83% from the study in dogs where within 20.0% of each other and met the acceptance criteria: "The incurred sample reproducibility (ISR) analysis was considered acceptable if at least two-thirds (rounded up) of the repeat results and original results were within 20.0% of each other."

Absorption

Sofosbuvir

In vitro absorption studies

Sofosbuvir was stable when incubated in simulated gastric and intestinal fluids (PC-PSI-7851-08-0012, PC-PSI-7977-09-0003). Sofosbuvir showed partially saturable efflux transport with low forward permeability that increased with concentration when incubated at different concentrations with Caco-2 cell monolayers (AD-334-2003).

Single and multiple dose in vivo studies

In rodents, administration of GS-9851 resulted in the rapid appearance of GS-331007 in plasma and liver. GS-9851 was not detected in mice or rats immediately following administration (as early as 15 minutes), suggesting rapid conversion to GS-331007 by plasma esterase.

The pharmacologically active metabolite, GS-461203, was efficiently formed in rat liver but was not detected in mice administered lower doses (PC-PSI-7851-08-0017, PC-PSI-7851-08-0019). GS-461203 was detected in livers of mice in the micronucleus study following oral administration at higher doses (SA-PSI-7851-08-0005). In dogs, SOF was well absorbed (F_a = 39.7%) and highly extracted by the liver (74% of the absorbed dose), resulting in high (C_{max} = 47.5 µM following administration at 5 mg/kg) and persistent ($t_{1/2}$ = 17.8 h) liver levels of GS-461203 (AD-334-2011, AD-334-2012).

Table 2: Plasma PK of GS-9851 Treatment to in the SD Rat, CD-1 Mice, Beagle Dog, and Cynomolgus Monkey

Study ID	Species	N	Dose (mg/kg)	Route	Analyte	Cmax (ng/mL)	Tmax (h)	AUC (ng•h/mL)
PC-PSI-7851-08-0017	Rat	Male 21	50	Oral	GS-331007	415	4.00	3131
PC-PSI-7851-08-0019	Mice	Male 21	50	Oral	GS-331007	2774	1.00	24453
PC-PSI-7851-08-0018	Dog	Male 1	50	Oral	GS-331007	2604	6.00	31785
PC-PSI-7851-08-0018	Monkey	Male 1	50	Oral	GS-331007	348.5	2.00	7379

The toxicokinetic profiles of SOF, GS-9851, and select metabolites were examined in plasma following oral administration to mice, rats, rabbits, and dogs in toxicology studies. The C_{max} and AUC of monitored compounds generally increased proportionally with dose. No marked sex differences in exposure were observed (typically < 2-fold) and no evidence for accumulation was observed following repeat dosing.

The plasma and liver PK of select metabolites were studied after 4 days of once daily administration of GS-9851 to a single dog or monkey. The predominant metabolites GS-331007 and GS-566500 were observed in the livers of both species. The pharmacologically active triphosphate, GS-461203, was efficiently formed in dog liver but was not detected in the monkey (PC-PSI-7851-08-0018).

Velpatasvir

In vivo absorption of VEL has been assessed in mice, Sprague-Dawley rats, NZW rabbits, beagle dogs, and Cynomolgus monkeys following intravenous and oral administration. In vitro permeability studies have been carried out in Caco-2 cell monolayers. However, according to the applicant permeability values of VEL could not be reliably obtained due to low recovery of the compound and poor reproducibility (no data shown).

Systemic clearance (CL) of VEL was low in all species tested and was less than 30% of hepatic blood flow. Velpatasvir was well distributed, with Vss values ranging from 1.4 to 1.6 L/kg.

Table 3: Mean plasma pharmacokinetic parameters for velpatasvir following 30-Minute intravenous infusion of velpatasvir to rats, rabbits, dogs, and monkeys

Species (dose*)	**$AUC_{(0-\infty)}$ (nM•h)**	**CL (L/h/kg)**	**V_{ss} (L/kg)**	**$t_{½}$ (h)**
Sprague-Dawley Rat (1mg/kg)	1320 ± 240	0.94 ± 0.19	1.61 ± 0.31	2.36 ± 0.26
New Zealand White Rabbit (5 mg/kg)	11800 ± 2560	0.44 ± 0.09	1.55 ± 0.22	5.05 ± 1.14
Beagle Dog (0.25 mg/kg)	1010 ± 187	0.25 ± 0.04	1.46 ± 0.43	5.51 ± 0.46
Cynomolgus Monkey (0.5 mg/kg)	2080 ± 705	0.30 ± 0.09	1.58 ± 0.62	4.18 ± 3.65

*) via a 30-min infusion. Data presented as mean ± SD, n=3 (1 nM VEL = 0.883 ng/mL)

After oral administration of velpatasvir in solution absorption Cmax in plasma was reached at 1.0, 1.3, and 3.3 hours in the rat, dog, and monkey, respectively. The oral bioavailability (%F) of VEL ranged from 25% to 30% in these species, when administered in solution at the dose levels studied.

Table 4: Mean plasma pharmacokinetic parameters for velpatasvir following oral administration of velpatasvir in solution to Sprague-Dawley rats, Beagle dogs, and Cynomolgus monkeys

Species	Dose (mg/kg)	T_{max} (h)	C_{max} (nM)	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (nM•h)	%F
Sprague-Dawley Rat	2.0	1.0 ± 0.0	116 ± 53	2.33 ± 0.38	709 ± 478	27.7 ± 18.7
Beagle Dog	0.5	1.3 ± 0.6	71.3 ± 27.2	9.14 ± 5.11	585 ± 343	25.0 ± 12.9
Cynomolgus Monkey	1.0	3.3 ± 1.2	157 ± 25	5.49 ± 0.20	1280 ± 123	29.7 ± 2.8

Formulation contained 5% ethanol, 55% polyethylene glycol 400 and 40% citrate buffer (pH 2.2).

Data presented as mean ± SD, n=3 (1 nM VEL = 0.883 ng/mL)

Formulation Development

Velpatasvir has low intrinsic (unionized) aqueous solubility (approximately 3 µg/mL at pH 8.2), and its low solubility limited the exposure that could be obtained during toxicology studies. Less than proportional increases in exposure were observed following oral administration in organic solution or aqueous suspension at higher doses in mice, rats, rabbits, and dogs. In order to optimize the formulation for toxicology studies the PK of VEL, following oral administration in various formulations, was assessed in rats and rabbits (AD-281-2020, AD-281-2035, and AD-281-2036).

In rat the formulations included: A) solution of 15% Solutol-HS-15, 45% propylene glycol, and 40% water, pH adjusted to 2.0 with HCl; B) suspension of 0.2% hydroxypropyl methylcellulose, 0.2% Tween 20, and 0.9% benzyl alcohol in water; and C) solution of 100% Capmul MCM, NF. The plasma exposure to VEL was slightly higher when dosed with formulation A compared with the other 2 formulations and this formulation was selected for subsequent toxicology studies.

In female NZW rabbits the formulations evaluated included: A) solution of 100% oleic acid; B) solution of 60% propylene glycol and 40% Solutol HS-15; and C) suspension of 0.5% hydroxypropyl methylcellulose, 0.1% Tween 20, 0.9% benzyl alcohol and 98.5% water. The plasma exposure to VEL in rabbits was the highest when dosed as an aqueous suspension (Formulation C), and this formulation was selected for subsequent toxicology studies. In addition, the highest exposure to VEL obtained when single ascending oral doses of VEL were administered to female NZW rabbits in a solution of 25% Solutol HS-15 and 75% propylene glycol (AD-281-2036) was shown to be comparable to that achieved in the aqueous suspension (Formulation C).

Sofosbuvir/Velpatasvir

The effect of VEL on the bidirectional permeability of SOF was assessed in vitro using Caco 2 cell monolayers (AD-334-2002). The apical to basolateral (forward) permeability of SOF (incubated at 10 µM) was increased from 0.25×10^{-6} cm/s to 0.66×10^{-6} cm/s and the basolateral to apical (reverse) permeability decreased from 10.9×10^{-6} cm/s to 7.39×10^{-6} cm/s, which resulted in a decreased efflux ratio of SOF from 43.6 to 11.2 in the presence of 1 µM VEL.

Distribution

Protein Binding

Sofosbuvir

Protein binding of SOF was low (< 70%) and concentration independent in dog and human plasma. Sofosbuvir was not stable in mouse, rat, and rabbit plasma, and plasma binding in those matrices was not determined. Protein binding of GS 331007 was minimal in mouse, rat, rabbit, dog, and human plasma (< 10%, PC PSI 7977 11 0001).

Velpatasvir

Plasma protein binding of VEL was determined for CD-1 mice, Sprague-Dawley rats, beagle dogs, Rhesus monkeys, Cynomolgus monkeys, and humans in vitro by equilibrium dialysis at a VEL concentration of 2 µM in plasma (AD-281-2037 and AD-281-2001). The protein binding of VEL in human plasma was also determined in vitro by equilibrium dialysis at different VEL plasma concentrations ranging from 0.1 to 2 µM (AD-281-2029). Velpatasvir was highly protein bound in plasma from all species (> 99.5% bound) (Table 5). There was no notable change in VEL protein binding from 0.1 to 2 µM VEL in human plasma (C_{max} observed in the clinic: 259 ng/mL, or 0.293 µM).

Table 5: Protein binding of velpatasvir in plasma from different species

Species	Unbound (%)	Bound (%)
CD-1 Mouse	< 0.1	> 99.9
Sprague-Dawley Rat	0.22 ± 0.03	99.78 ± 0.03
Beagle Dog	0.19 ± 0.02	99.81 ± 0.02
Cynomolgus Monkey	0.41 ± 0.07	99.59 ± 0.07
Rhesus Monkey	0.28 ± 0.01	99.72 ± 0.01
Human	0.30 ± 0.02	99.70 ± 0.02

Initial incubation concentration was 2 µM.

Data presented as mean ± SD from 3 determinations

Tissue Distribution

Sofosbuvir

After oral administration of [^{14}C]SOF to pigmented and non-pigmented rats, drug-related material was rapidly absorbed and widely distributed to tissues (SA-PSI-7977-09-0005). The highest concentrations of radioactivity in tissues were found in the alimentary canal, lymphatic system, and excretory system. The lowest concentrations of total radioactivity were observed in the CNS, bone, eye lens, and white adipose. Elimination of radioactivity from tissues was nearly complete at the last time point (144 or 168 hours post dose), with most tissue concentrations decreasing to below limit of quantification by 48 hours post dose. There was no evidence for a specific association of SOF or its metabolites with melanin in rats.

Velpatasvir

Velpatasvir is well distributed with a volume of distribution from 1.4 to 1.6 L/kg in the rat, dog, and monkey (AD-281-2002, AD-281-2003, AD-281-2004).

After oral administration of [^{14}C]VEL to mice and to pigmented and non-pigmented rats, drug-related material was quickly distributed to most tissues, especially the liver (AD-281-2021 and AD-281-2018). Although VEL likely binds to melanin, it is quickly removed from pigmented skin. Low levels of radioactivity were transiently detected in mouse brain and in mouse and rat testes, suggesting that

[^{14}C]VEL-derived radioactivity poorly crossed the blood:brain barrier in mice and the blood:testis barrier in mice and rats, which is consistent with VEL being a substrate of efflux transporters.

Sofosbuvir/Velpatasvir

No nonclinical distribution studies have been done with the combination of SOF and VEL.

Distribution in Pregnant or Nursing Animals

Sofosbuvir

[^{14}C]Sofosbuvir-derived radioactivity was absorbed and widely distributed to tissues of pregnant, nonpregnant, and postpartum female rats after a single oral dose (SA-PSI-7977-11-0008). Drug-derived radioactivity was inefficiently transferred through the placenta and was found in amniotic fluid and absorbed into fetuses. Low levels of drug-derived radioactivity were quantifiable in the milk collected from postpartum females. Relatively low amounts of drug-derived radioactivity were transferred into nursing pups. Tissue distribution in the nursing pups was limited with detectable levels in the liver and GI contents only (SA-PSI-7977-11-0008).

Velpatasvir

[^{14}C]Velpatasvir-derived radioactivity was absorbed and widely distributed to maternal tissues after a single oral dose of [^{14}C]VEL to pregnant rats on Gestation Day 13 or 18 (AD-281-2031). [^{14}C]VEL-derived radioactivity did not cross the placenta, and no radioactivity was detected in fetal blood or whole fetuses.

Toxicokinetic parameters for VEL were determined in pregnant mice (TX-281-2032), rats (TX-281-2009, TX-281-2013), and rabbits (TX-281-2010, TX-281-2014). Exposure in pregnant animals was generally similar to that in non-pregnant animals.

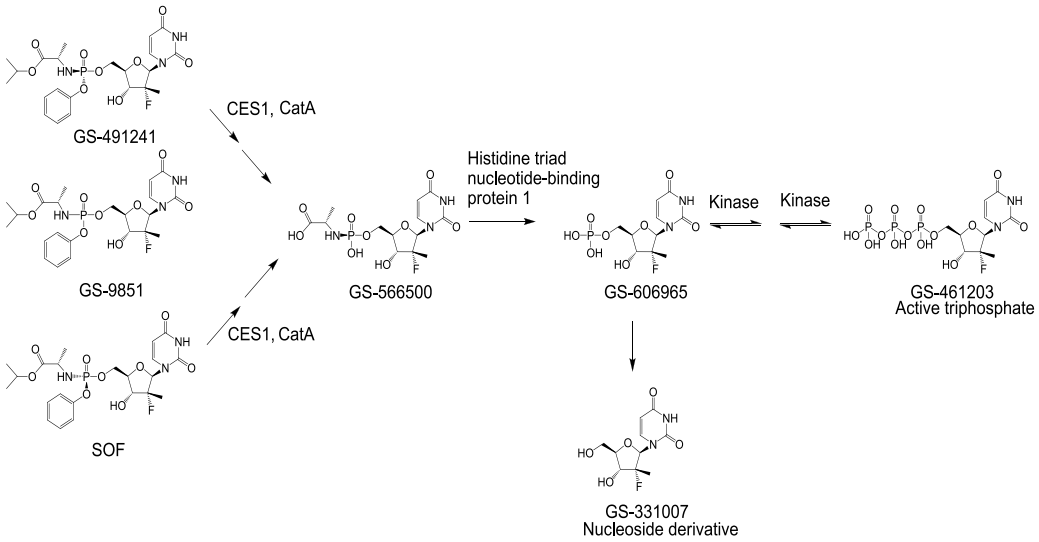
The plasma exposure of VEL was detected in neonates and increased greater than proportionally with the increase in the maternal dose level (TX-281-2027). Maternal plasma exposure (AUC) to VEL was > 20-fold higher than in pups.

Metabolism

Sofosbuvir

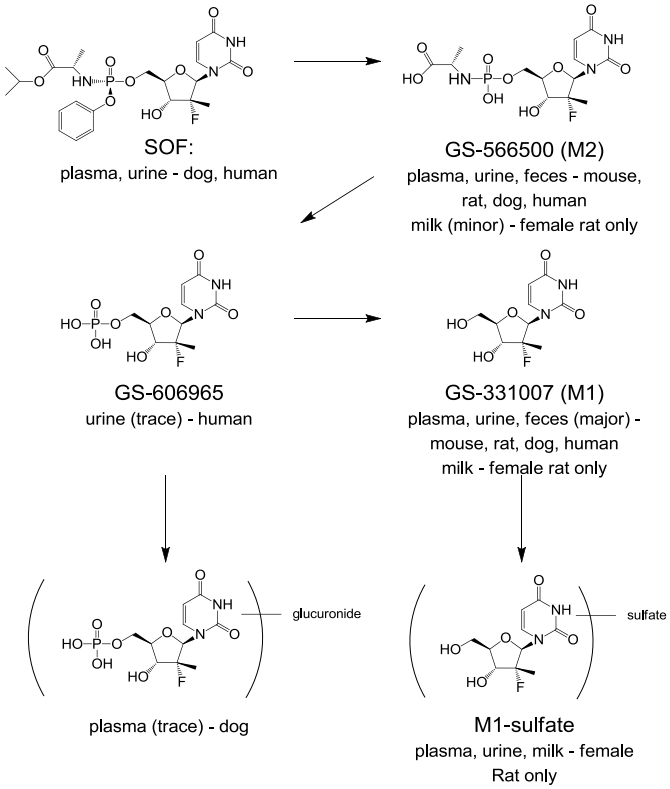
Sofosbuvir is a nucleotide prodrug and requires activation in hepatocytes to form the pharmacologically active triphosphate metabolite, GS 461203. GS-7977 was efficiently metabolized to its active triphosphate analogue metabolite, GS-461203, in primary hepatocytes from human following a 2-hour pulse incubation and there was no apparent gender difference in maximal triphosphate formed (AD-334-2017). The first step in the intracellular activation of SOF is the hydrolytic cleavage of the isopropyl ester by estareses such as cathepsin A (CatA) and carboxylesterase 1 (CES1), but was not cleaved by CES2. Ester hydrolysis results in the release of isopropanol and a metastable intermediate that chemically degrades to release phenol and the intermediate metabolite GS 566500. Subsequent cleavage of the phosphoramidate linkage catalyzed by the histidine triad nucleotide binding protein 1 (HINT1) results in release of the endogenous amino acid alanine and GS 606965 (AD-334-2018). Two sequential phosphorylation steps, catalyzed by the nucleotide kinases uridine monophosphate-cytidine monophosphate (UMP-CMP) kinase and nucleoside diphosphate kinase (NDPK), result in formation of the pharmacologically active triphosphate metabolite GS 461203 (AD-334-2019).

Figure 1: Intracellular metabolic pathways of GS 9851, GS 491241, and Sofosbuvir



SOF is stable in plasma from non-rodent species and highly unstable in hepatic subcellular fractions. The presence of high plasma esterase activity in some rodent species results in rapid metabolism in mouse and rat plasma and blood. Isomeric conversion between SOF and GS 491241 was not detected in rat, dog, and human plasma and human urine. The primary route of intracellular SOF metabolism is hydrolytic cleavage of the isopropyl ester by CatA and CES1. Pathways involving CYP isozymes, flavin containing monooxygenase (FMO) enzymes, or uridine diphosphate glucuronosyltransferases (UGTs) are not likely to be important considerations in the disposition of SOF based on studies completed with SOF and its metabolites, GS 566500, GS 606965, and GS 331007 in human liver microsomes incubated under different conditions. GS-7977 and GS-331007 were not substrates for recombinant CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 (AD-334-2015).

Figure 2: Proposed biotransformation pathways of Sofosbuvir



Velpatasvir

The rate of in vitro metabolism of VEL was low in mouse, monkey and human hepatic microsomes (predicted hepatic Cl <0.17 – 0.98 L/h/kg). Similarly, its metabolic turnover rate was low and could not be determined in cryopreserved human hepatocytes. There was no significant metabolic turnover of VEL by CYP1A2, CYP2C9, CYP2C19, or CYP2D6 and slow metabolic turnover of VEL by CYP2B6, CYP2C8, and CYP3A4 was observed.

Table 6: Rates of metabolism of VEL by major human cytochrome P450:s

Compound	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
Velpatasvir (% Positive Control)	< 0.12 (< 0.8%)	0.13 (6.6%)	1.26 (5.5%)	< 0.47 (< 2.2%)	< 0.12 (< 12%)	< 0.23 (< 1.0%)	2.09 (18%)

(AD 281-2007)

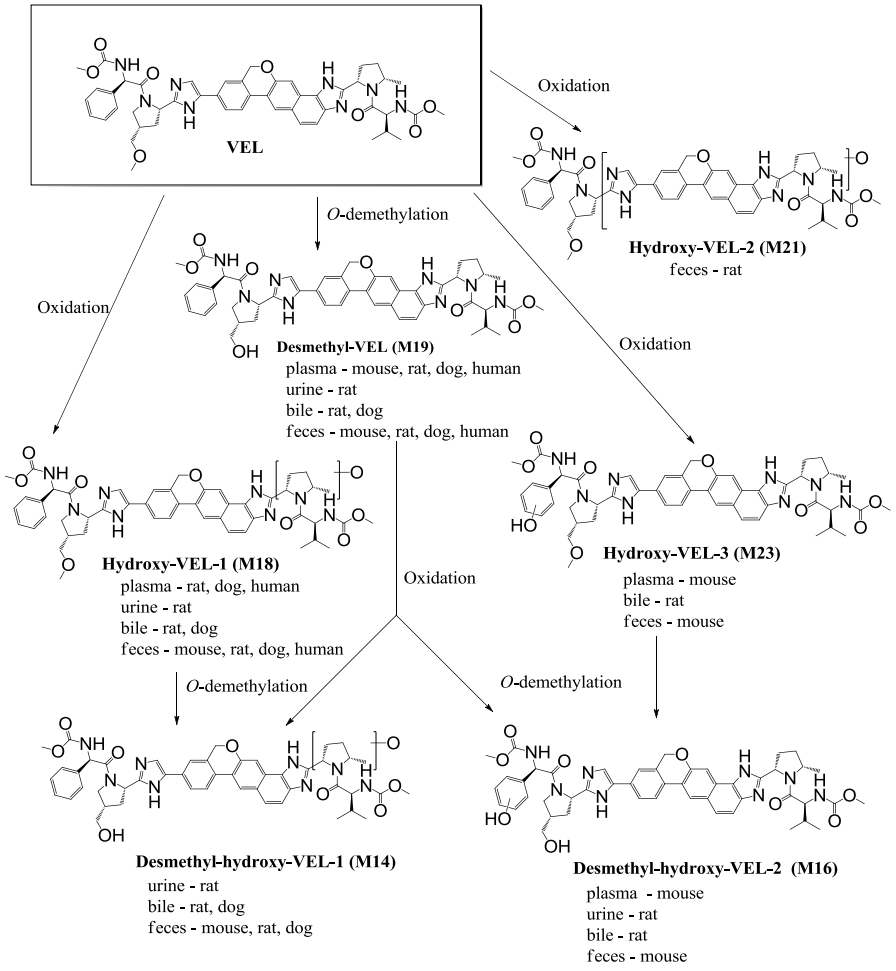
Unchanged VEL was the most abundant circulating component. Of the total radioactivity exposure in plasma, unchanged VEL accounted for approximately 71% in CD-1 mice, 92% in rasH2 mice, 83% in rats, 81% in dogs, and approximately 98.9% in human subjects. The O-demethyl-metabolite, desmethyl-VEL (M19), was identified in the plasma of all species, accounting for 7.1%, 8.3%, 12.4%, and 0.7% of the total radioactivity exposure in plasma in CD-1 mice, rats, dogs, and human subjects, respectively. The monohydroxylated metabolite, hydroxyl-VEL-1 (M18), was identified in rat, dog, and human plasma, accounting for 1.2%, 3.1%, and 0.4% of the total radioactivity exposure in plasma in these species, respectively. The monohydroxylated metabolite, hydroxyl-VEL-3 (M23), and the O-demethyl-hydroxylated metabolite, desmethyl-hydroxy-VEL-2 (M16), were identified in CD-1 mouse plasma, respectively accounting for 17% and 2.7% of the total radioactivity exposure in plasma.

Table 7: Major metabolites in plasma following oral administration of VEL (% Total Plasma AUC)

Compound	CD-1 Mouse	rasH2 Mouse	Sprague-Dawley Rat	Beagle Dog	Human
M1	ND	ND	0.18	ND	ND
M16	2.70	ND	ND	ND	ND
M18	ND	ND	1.24	3.10	0.4
M19	7.10	ND	8.27	12.4	0.7
M23	17.0	ND	ND	ND	ND
Velpatasvir	70.8	92.0	82.7	81.0	98.9
Total	97.6	92.0	92.4	96.5	100

AUC = area under the plasma concentration-time curve from time zero to 12 hours post dose in CD-1 mice and rats, from time zero to 8 hours post dose in rasH2 mice and dogs, and from time zero to 24 hours postdose in human subjects; ND = not detected

Figure 3: Proposed biotransformation pathways of velpatasvir



Sofosbuvir/Velpatasvir

The effect of VEL on the formation of GS 461203 in primary human hepatocytes after incubation with SOF was assessed in vitro (AD 334 2010). When 10 µM SOF was incubated with hepatocytes for 2 hours, in the absence or presence of 10 µM VEL, the GS 461203 concentrations were 69.8 and 49.5 pmol/million cells, respectively. These results suggest that VEL does not markedly affect the intracellular activation of SOF.

Excretion

Sofosbuvir

Sofosbuvir is excreted in urine with urinary recovery of administered radiolabeled material accounting for 65.6%, 72%, and 81%, in mice, rats and dogs, respectively. In bile duct cannulated rats, 6% of the dose was eliminated in bile.

Velpatasvir

Most of the [^{14}C]VEL-derived radioactivity was rapidly excreted after oral administration in all 3 species investigated. By 168 hours after oral administration, a mean of 95.9%, 96.9%, and 93.6% of the administered radioactivity was excreted in feces from CD-1 mice, rats, and dogs, respectively. Less than 0.27% of the administered radioactivity was excreted in urine from these animals.

In BDC rats, a mean of 83.1%, 13.7%, and 0.248% of the administered radioactivity dose was excreted in feces, bile, and urine, respectively. In BDC dogs, a mean of 71.2%, 18.7%, and 0.245% of the administered radioactivity was excreted in feces, bile, and urine, respectively. The mean overall recovery of radioactivity after oral dosing, was 96.5% in CD-1 mice, 97.1% in both bile duct-intact and BDC rats, 94.9% in bile duct-intact dogs, and 91.1% in BDC dogs.

The biliary and urinary excretion of VEL was also examined in BDC Sprague-Dawley rats following a single 30-minute intravenous infusion at 2 mg/kg (AD-281-2005). An average of 16% of the total dose was recovered as unchanged parent drug in rat bile. Only a trace amount of VEL was detected in rat urine.

Sofosbuvir/Velpatasvir

No nonclinical excretion studies have been done with the combination of SOF and VEL.

Excretion into Breast Milk

Sofosbuvir

Excretion of SOF in breast milk was studied in postpartum female rats after a single oral dose (SA-PSI-7977-11-0008). Low levels of drug-derived radioactivity were quantifiable in the milk. The nucleoside metabolite GS-331007 was the predominant metabolite observed in the milk at a milk:plasma concentration ratio of 0.1 at 1 hour post dose.

Velpatasvir

After oral dosing of [^{14}C]VEL to lactating rats, [^{14}C]VEL-derived radioactivity was transferred into milk with a T_{max} of 4 hours and was not detectable by 24 hours post dose (AD-281-2031). The mean milk:plasma exposure (AUC) ratio was 1.74.

Pharmacokinetic drug interactions

Sofosbuvir

Cytochrome P450 and UGT1A1 Inhibition

Sofosbuvir did not inhibit the activity of CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 in vitro (IC_{50} > 100 µM).

GS-331007 did not inhibit any of the CYP enzymes tested (IC_{50} > 300 µM). No evidence for mechanism-based inhibition of CYP3A by SOF was observed (PC-PSI-7977-09-0011, AD-334-2020).

No inhibition of CYP1A2, 2C19, 2C9, 2C8, 2D6, and 3A4 was observed by other SOF metabolites GS-607596, GS-606965, GS-566500, and GS-461203 at concentrations up to 100 μM (PC-PSI-7851-09-0009).

Sofosbuvir showed weak dose dependent inhibition (IC_{50} = 198 μM) of human UGT1A1 while no inhibition was observed by GS-331007 and GS-606965 (IC_{50} > 300 μM; AD-334-2022).

Table 8: Human UGT1A1 Inhibition Potential of Sofosbuvir

Compound	Role	IC_{50} (μM)
SOF	Nucleotide prodrug	198
GS-606965	Nucleoside monophosphate metabolite	> 300
GS-331007	Nucleoside metabolite	> 300
Atazanavir	Clinically relevant positive control	0.52

GS-331007 = nucleoside analog; GS-607596 = nucleoside analog diphosphate; LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; SOF = sofosbuvir; UGT = uridine diphosphate glucuronosyltransferase

Intracellular Activation

The effects of CYP inhibitors and anti-HCV agents on the formation of the pharmacologically active triphosphate GS-461203 following incubation of SOF were determined in primary human hepatocytes (AD-334-2010, PC-PSI-7977-11-0002). Incubation with the HCV inhibitors VEL, ledipasvir, daclatasvir, tegobuvir, vedroprevir, or GS-9669, or the CYP inhibitors ritonavir or ketoconazole, did not markedly affect the formation of GS-461203 (less than 30% change).

Assessment of Induction Liability

Sofosbuvir caused little or no induction of CYP mRNA or activities when assessed in cultured human hepatocytes from 3 separate donors treated once daily for 3 consecutive days (PC-PSI-7977-10-0005). Small increases in CYP2B6 activity and CYP2B6 and CYP3A4 mRNA levels observed at the highest concentration tested (100 μM) were less than 15% of those caused by the positive controls.

Interaction with Transporters

The absorptive permeability of SOF through Caco-2 monolayers was increased by CsA and anti-HCV agents due to inhibition of the efflux transport of SOF (AD-334-2002). Modest decreases (approximately 2-fold) in efflux were observed with LDV, tegobuvir, and GS-9669; moderate effects of approximately 4-fold were observed with VEL.

Sofosbuvir is a substrate for BCRP and P-gp (PC-PSI-7977-11-0006). No evidence for SOF transport by the basolaterally expressed hepatic transporters, including organic cation transporter 1 (OCT1) or organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1, OATP1B3) was observed in vitro (AD-334-2004, PC-PSI-7977-11-0007).

No evidence for GS-331007 transport by P-gp or BCRP was obtained in vitro (PC-PSI-7977-11-0006). GS-331007 was also not a substrate for renal transporters, including organic anion transporter 1 and 3 (OAT1, OAT3), OCT2, and multidrug and toxin extrusion 1 (MATE1) (AD-334-2005, AD-334-2021).

Sofosbuvir and GS-331007 were not inhibitors of the transporters P-gp, BCRP, multidrug resistance related protein (MRP) 2, bile salt export pump (BSEP), OATP1B1, OATP1B3, and OCT1 (8215026, AD-334-2004, AD-334-2016, AD-334-2021, PC-PSI-7977-11-0006, and PC-PSI-7977-11-0007). GS-331007 was also not an inhibitor of the renal transporters OAT1, OAT3, OCT2, and MATE1.

Velpatasvir

Cytochrome P450 and UGT1A1 Inhibition

Velpatasvir did not inhibit the activities of the tested human enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 (IC_{50} > 25 µM; AD-281-2008). VEL had an inhibitory effect on the activity of human UGT1A1 in vitro (IC_{50} = 1.56 µM, AD-281-2016).

Table 9: UGT1A1 Inhibition Potential of Velpatasvir

Enzyme	Activity	Calculated IC_{50} (µM)	
		Control Inhibitor[a]	VEL
UGT1A1	Estradiol 3-Glucuronidation	1.99	1.56

LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; UDP = uridine 5'-diphosphate; UGT = UDP glucuronosyl transferase; VEL = velpatasvir (GS-5816)
a Control Inhibitor: silybin (0-100 µM)

Assessment of Induction Liability

Velpatasvir caused little or no induction of CYP mRNA or activities when assessed in cultured human hepatocytes from 3 different donors (AD-281-2025). Small increases in CYP2B6 and 3A4 activity and mRNA levels observed at the highest concentration tested of 10 µM were less than 20% of those caused by the positive controls. There was no concentration-dependent mRNA increase for CYP2C9, P-gp, or UGT1A1.

Interaction with Transporters

Velpatasvir is a substrate of P-gp and BCRP, and inducers may decrease its absorption. There was no clear in vitro evidence to show that VEL is a substrate of OATP1B1, OATP1B3, or OCT1 (AD-281-2011, AD-281-2026).

The IC_{50} for VEL exceeded the C_{max} unbound to plasma protein (2.93 nM) by greater than 500-fold for P-gp, MRP2, NTCP, OATP1B1, OATP1A2, OATP2B1, OCT1, OCT2, OAT1, OAT3, and MATE1, thus indicating no potential for systemic drug interactions mediated by these transporters. The IC_{50} for OATP1B1 (0.26 µM) and BCRP (0.30 µM) exceeded the VEL unbound C_{max} by greater than 80-fold, illustrating minimal potential for systemic drug-drug interactions mediated by these transporters.

Velpatasvir also showed some potential to inhibit the hepatic uptake transporters OATP1B1 (IC_{50} = 1.5 µM) and OATP1B3 (IC_{50} = 0.26 µM) during first pass based on an estimated unbound hepatic inlet concentration of 0.305 µM (assuming plasma protein binding of 1%).

Sofosbuvir/Velpatasvir

Cytochrome P450 and UGT1A1 Inhibition

Velpatasvir is subject to oxidative metabolism by CYP3A4, CYP2B6, and CYP2C8, while no evidence for oxidative metabolism of SOF has been observed. VEL exposure was decreased by a CYP inducer and increased by inhibitors of these metabolizing enzymes during clinical studies (GS-US-281-0115). The UGT1A1 substrate dolutegravir was unaffected by coadministration with SOF/VEL in a clinical drug-drug interaction study (GS-US-342-1167).

Intracellular Activation

Coincubation of SOF and VEL with primary human hepatocytes in vitro decreased formation of the pharmacologically active triphosphate metabolite of SOF, GS-461203 (AD-334-2010).

Additive to minor synergistic antiviral activity was observed for the combination of SOF and VEL in HCV genotype 1a, 2a, 3a, and 4a replicon systems in primary human hepatocytes (PC-334-2004 and PC-334-2014).

Interaction with Transporters

Sofosbuvir and VEL are substrates of intestinal efflux transporters and their intestinal absorption may be increased by coadministration with inhibitors of intestinal efflux transporters or reduced by inducers. Sofosbuvir is a substrate but not an inhibitor of P-gp and BCRP. VEL is a substrate of P-gp and BCRP. VEL has the potential to inhibit intestinal P-gp, BCRP, and OATP2B1 at concentrations achievable during absorption. The permeability of SOF across Caco-2 cell monolayers in vitro is increased in the presence of transport inhibitors including VEL (AD-334-2002), which is consistent with the increase in SOF plasma exposure observed following coadministration of SOF with VEL (GS-US-281-0101).

2.3.4. Toxicology

Sofosbuvir

Sofosbuvir has been analysed in a single-dose oral toxicity study in rats; repeat-dose oral toxicity studies in mice (up to 3 months), rats (up to 6 months) and dogs (up to 9 months), genotoxicity tests both in vitro and in vivo; a full developmental and reproductive toxicity program, and two-year oral carcinogenicity studies in mice and rats.

Velpatasvir

Repeat dose toxicity, genotoxicity, reproductive toxicity, local tolerance of VEL, as well as phototoxicity and the potential for sensitization to velpatasvir have been characterized in in vitro and in vivo studies. Most in vivo studies utilized oral administration as this is the clinical route of administration.

Velpatasvir has low intrinsic aqueous solubility (0.003 mg/mL at pH 8.2), and low solubility in both fasted and fed state simulated intestinal fluids (0.028 and 0.21 mg/mL, respectively). In order to maximize oral exposure in the nonclinical toxicology studies, 4 formulations were evaluated in rats and rabbits, and 2 formulations were evaluated in dogs. Results demonstrated that a 60% organic formulation [45% (v/v) propylene glycol and 15% (v/v) Kolliphor® HS 15 in reverse osmosis [RO] water, pH 2.0 ± 0.1] was optimal in rats and dogs and the high dose levels administered in the GLP toxicity studies (200 mg/kg and 100 mg/kg in rats and dogs, respectively) are considered the maximal achievable doses via oral administration. Administration of velpatasvir in aqueous suspension (0.5% w/v hydroxypropyl methylcellulose [HPMC], 0.1% v/v Tween 20 and 0.9% v/v benzyl alcohol in RO water) produced the highest exposure in rabbits. In mice, aqueous vehicle suspensions (0.2% w/v HPMC, 0.2% v/v Tween 20, and 99.6% v/v deionized water) provided increase in exposure up to 1000 mg/kg.

Table 10: Velpatasvir Toxicology Program

Study Type and Duration	Route of Administration	Species	Compound Administered
Repeat Dose Toxicity			
5 Days	Oral	Rat	VEL
2 weeks	Oral	Rat, Dog	VEL
4 weeks	Oral	Mouse	VEL
13 weeks	Oral	Rat[a], Dog[a]	VEL
26 weeks	Oral	Rat	VEL
39 weeks	Oral	Dog	VEL
Genotoxicity			
In vitro reverse mutation assay	In vitro	Bacteria	VEL
In vitro chromosome aberration assay	In vitro	Human lymphocytes	VEL

In vivo micronucleus assay	Oral	Rat	VEL
Carcinogenicity (ongoing)	Oral	Mouse, Rat	VEL
Developmental and Reproductive Toxicity			
Fertility and early embryonic development	Oral	Rat	VEL
Embryo-fetal development	Oral	Mouse, Rat, Rabbit	VEL
Prenatal and postnatal development, including maternal function	Oral	Rat	VEL
Local Tolerance			
Eye irritation	Topical/ex vivo	Bovine	VEL
Skin irritation	Topical	Rabbit	VEL
Other Studies			
Sensitization	Topical	Mouse	VEL
Phototoxicity	In vitro, In vivo	Mouse 3T3, Rat	VEL
In vitro reverse mutation assay	In vitro	Bacteria	GS-604527[b]
Impurities – qualification study	Oral	Rat	VEL

a 13-week interim sacrifice

b Impurity

Single dose toxicity

Sofosbuvir

Sofosbuvir (administered as GS-9851) has minimal toxicity after oral dosing to rats. The lethal dose is greater than 1800 mg/kg (SA-PSI-7851-09-0001). At 1800 mg/kg, the mean GS-331007 Cmax was 15.0 (males) and 15.2 (females) µg/mL and AUC last was 205 (males) and 176 (females) µg•h/mL.

Table 11: Single-Dose Toxicity in Rat

Study ID	Species/ Sex/Number/ Group	Dose/Route	Approx. lethal dose / observed max non-lethal dose	Major findings
SA-PSI-7851-09-0001	Rat SD/ 12/sex toxicol, 30/sex TK	GS-9851: 50, 300, 1800/ oral gavage	Not obtained/ 1800 mg/kg	No mortality, clinical signs of toxicity, body weight, macroscopic pathology, no organ weight differences.

Velpatasvir

No formal single dose toxicity studies with VEL have been conducted. Single doses up to 600 mg/kg in rats and 200 mg/kg in dogs were well tolerated in PK studies (AD 281 2014 and AD-281-2013).

Repeat dose toxicity

Sofosbuvir

The repeat dose toxicity studies have been conducted in mice, rats, and dogs. The potential target organs identified with SOF were liver (dog) and gastrointestinal (GI) tract (dog). Slight (< 10%) haematological changes in red cell indices/erythropoiesis (dog) were also noted.

Table 12: Pivotal Toxicity Studies in Mice and Rats

Study ID/GLP	Species/Sex/ Number/Group	Dose (mg/kg/day) /Route	Duration	NOAEL (mg/kg/day)	Major findings
SA-PSI-GLP7977-09-0008 / YES	CD-1 mice/42/ sex/group	SOF - 0, 100, 300, 1000/ oral gavage	13 weeks	M: 100 F: 300	Mortality 0: 2M, 100: 5M 2F, 300: 4M 1F 1000: 3F, ↓ body weight change (M)
SA-PSI-7851-08-001/ YES	Rat CD IGS/10/sex/dose 3/sex/dose (recovery)	GS-9851: 0, 30, 250, 2000 /Oral gavage	7 days	250	Mortality 2000: 3M 6F. Multifocal cardiac myofiber degeneration (M/F), watery diarrhea (M/F)
SA-PSI-7851-09-0003 /YES	Rat CD IGS /10/sex/dose, 5/sex/dose (recovery)	GS-9851: 0, 20, 100, 500; Oral gavage	28 days	500	Body weight ↓ 100: M, 500: M/ F. Albumin ↑ F 100 and 500. Cholesterol ↑ F 100 and 500.
SA-PSI-7977-09-0007 /YES	Rat SD/9/sex/dose, 5/sex/dose (recovery)	SOF - 0, 20, 100, 500; Oral gavage	90 days + 4 weeks recovery	500	No noteworthy findings
SA-PSI-7977-10-0004/ YES	Rat/15/sex/dose 5/sex/dose (recovey)	SOF - 0, 20, 100, 500; Oral gavage	6 months + 4 week recovery	500	Mortality 0: 2M, 20: 2F, 100: 2M 1F, 500: 1M. Gluc ↑ 500: F TSH ↓ 100: M

M = Male; F = Female

Table 13: Pivotal Toxicity Studies in Beagle Dog

Study ID /GLP	Species/Sex/ Number/Group	Dose (mg/kg/day) /Route	Duration	NOAEL (mg/kg/ day)	Major findings
SA-PSI-7851 -08-002/ YES	Beagle dog/4/sex/dose 1/sex/dose (recovery)	GS-9851 0, 30, 150, 1500; Oral via capsule	7-days + 14 days recovery	150	1500: Body weight change ↓ (M/F), GI irritation, neutophils increase (M), serum ALP ↑(M/F), AST and Bilirub ↑ (M), QTc interval ↑ M, liver weight ↑ (M/F)
SA-PSI-7851 -09-0002 /YES	Beagle dog /3/sex/dose 2/sex/dose (recovery)	GS-9851 0, 20, 100, 500; Oral via capsule	28 days + 14 days recovery	100	500 M/F: GI irritation, Red cell indices (erythropoies) and body weight ↓.
SA-PSI-7977 -09-0006 /YES	Beagle dog /6/sex/dose 2/sex/dose (recovery)	SOF 0, 20, 100, 500; Oral via capsule	90 days + 4 weeks recovery	100	500: ↓ body weight in 2 M; ↓ erythroid precursors, in bone marrow cytology; minor thyroid and parathyroid weight ↑ (M); black foci on stomach mucosa in 1 M.
SA-PSI-7977 -10-0003 /YES	Beagle Dog/6/sex/dose	SOF 0, 20, 100, 500; Oral via capsule	39 weeks + 4 weeks recovery	100	500: Sacrificed moribund 1 M, ALP ↑ F, spleen abs weight ↑ M. ↑ soft stool, emesis M/F. Necropsy: reddened intestinal mucosa, gelatinous red intestinal contents. Microscopic examination: hemorrhage within the jejunum mucosa, coagulated blood on top of the mucosa of distal intestinal tract and moderated vacuolation of the myocardium.

M = Male; F = Female

The exposures based on plasma GS-331007 AUC values at the NOAEL doses in the longest duration studies were approximately 2- and 12-fold (mice; males and females, respectively), 5-fold (rats, sexes combined), and 6-fold (dogs, sexes combined) higher than the systemic exposure in subjects treated once daily with SOF/VEL FDC.

Velpatasvir

Table 14: Pivotal Toxicity Studies in Mice, Rats, and Dogs

Study ID /GLP	Species/Sex/Number/ Group	Dose/Route	Duration	NOAEL mg/kg/day	Major findings
TX-281-2028 /YES	CByB6F1-Tg(HRAS) 2Jic Mice/ 36/ sex/group	VEL 0, 100, 300, 1500; Oral gavage	4 weeks	1500	1500: ↓ white blood cell, abs. neutrophil and abs. lymphocyte count
TX-281-2003 /YES	Rat SD/9/sex/dose, 5/sex/dose (recovery)	VEL 0, 20, 60, 200; Oral gavage	2 weeks, 1 week recovery	200	No noteworthy findings
TX-281-2008 /YES	Beagle dog/9/sex/dose 5/sex/dose (recovery)	VEL 0, 5, 20, 100; Oral gavage	13 weeks, 4 week recovery	100	Mortality 100: 1M; fibrinogen, and globulin concentration ↓, albumin ↑ (F), vomitus (M/F)

M = Male; F = Female

The exposures based on plasma VEL AUC values at the NOAEL doses in the longest duration studies were approximately 74-fold (mice), 5-fold (rats), and 10-fold (dogs) higher than the systemic exposure in subjects treated once daily with SOF/VEL FDC.

Sofosbuvir/Velpatasvir

No repeat dose studies with SOF/VEL have been conducted.

Genotoxicity

Sofosbuvir

Table 15: Genotoxicity Studies Conducted with GS-9851

Type of test/study ID/GLP	Test system	Concentration /Concentration range/ Metabolising system	Results
Ames test/ SA-PSI-7851-08-003/ Yes	*S. typhimurium* and *E. coli*	1.5, 5, 15, 50, 150, 500, 1500, 5000/1.5-5000 µg/plate +/- S9	Negative
In Vitro Chrom Aber Test/ SA-PSI-7851-08-004/ Yes	Human periph lymph; 4- and 20-hours	313, 1250, 2500, 5000/ 313-5000 (µg/mL) +/- S9	Negative
In Vivo Micronucleus Test/ SA-PSI-7851-08-005/ Yes	Mouse, micronuclei in bone marrow	0, 500, 1000, 2000 mg/kg	Negative

Velpatasvir

Table 16: Genotoxicity Studies Conducted with VEL

Type of test/study ID/GLP	Test system	Concentrations/ Concentration range/ Metabolising system	Results Positive/negative/ equivocal
Ames test TX-281-2005 Yes	*S. typhimurium* and *E. coli*	1.5, 5, 15, 50, 150, 500, 1500, 5000/1.5-5000 µg/plate +/- S9	Negative
In vitro chromosome aberration test TX-281-2006 Yes	Human peripheral lymphocytes	1250, 2500, 5000/ 313-5000 (µg/mL) +/- S9	Negative
In vivo micronucleus test TX-281-2003 Yes	SD Rat, micronuclei in bone marrow	0, 84, 172, 245, 350, 500 mg/kg	Negative

Carcinogenicity

Long-term studies

Sofosbuvir

The carcinogenicity potential of SOF was evaluated in 2-year carcinogenicity studies in mice (TX-334-2002) and rats (TX-334-2001). Sofosbuvir was not considered carcinogenic at doses up to 200/600 (males/females) mg/kg/day in mice and 750 mg/kg/day in rats. Exposure margins (based on

GS-331007) at these doses were 3/15 (male/female in mice) and 8 times (rats, sexes combined) above SOF clinical exposure following SOF/VEL FDC administration.

Velpatasvir

A 6-month rasH2 transgenic mouse study (Study TX-281-2043) and a 2-year rat carcinogenicity study (Study TX-281-2030) with VEL are ongoing.

Reproduction Toxicity

Sofosbuvir

Table 17: Developmental and Reproductive Toxicity Studies in Rats and Rabbits

Study type/ Study ID / GLP	Species; Number per group	Route / dose (mg/kg/ day)	Dosing period	Major findings	NOEL mg/kg day/ GS-331007 AUClast (ng·h/ml)
Male fertility/ SA-PSI-7977-10-0005/Yes	Rat; 22	Oral gavage/ SOF: 0, 20, 100, 500	28 days prior to mating	A shortened mean precoital interval (≥ 100 mg/kg/day)	M,F 500/ 55
Female fertility/ SA-PSI-7977-10-0005/Yes	Rat; 22	Oral gavage/ SOF: 0, 20, 100, 500	14 days prior to mating, GD 6-18	No effects on intrauterine growth, survival, or external, visceral, and skeletal fetal morphology	F 500 / 72.1 on GD 18
Embryo-fetal development/ SA-PSI-7977-10-0008/Yes	Rat; 24	Oral gavage/ SOF: 0, 20, 100, 500	13 days GD 6-18	No effect on reproductive function in either sex, no effect on embryo-fetal development	M F: 500/ 72.1 on GD 18
Embryo-fetal development/ SA-PSI-7977-11-0006/Yes	Rabbit; 20	Oral gavage/ SOF: 0, 30, 90, 300	14 days GD 6-19	No effects on intrauterine growth, survival, or external, visceral, and skeletal fetal morphology.	M F: 300/ 120 on GD 19
Pre & postnatal/ TX-334-2003/Yes	Rat; F0 Females: 25, F1 Litters: 25, F1 Males: 25, F1 Females: 25	Oral gavage/ SOF: 0, 50, 250, 500	GD 6-LD 20 or GD 6-24 rats that did not deliver a litter	GS-331007 exposure on lactation Day 10 was 12-fold higher than mean human exposure at 400 mg. GS-331007 milk:plasma ratio of 0.1 at 1 h postdose.	F0 F: 500/83.3 F1 M: 500/1.50 F1 F: 500/1.48

The exposures based on GS-331007 plasma AUC values at the NOEL doses in the fertility and rat embryo-foetal development studies were approximately 4-fold (sexes combined, based on Day 24 AUC from 28-day repeat dose rat study [SA-PSI-7851-09-0003]) and 5-fold higher, respectively, than the AUC in subjects treated once daily with SOF at 400 mg. In the rabbit embryo-foetal development study, SOF and GS-331007 plasma AUC values at the NOEL were 7- and 14-fold higher, respectively, than the AUC in subjects treated once daily with SOF/VEL.

In the pre/postnatal study, the maternal NOAEL for general toxicity and the NOEL for reproduction in the dams and viability and growth of the offspring were 500 mg/kg/day (GS-331007 exposure on lactation Day 10 was approximately 6-fold higher than mean human exposure at 400 mg).

Velpatasvir

Table 18: Developmental and Reproductive Toxicity Studies in Mice, Rats, and Rabbits

Study ID /GLP	Species/Sex/Number/ Group	Dose/Route	Duration	NOAEL mg/kg/day	Major findings
TX-281-2028 /YES	CByB6F1-Tg(HRAS)2Jic Mice/ 36/ sex/group	VEL 0, 100, 300, 1500; Oral gavage	4 weeks	1500	1500: ↓ white blood cell, abs. neutrophil and abs. lymphocyte count
TX-281-2003 /YES	Rat SD/9/sex/dose, 5/sex/dose (recovery)	VEL 0, 20, 60, 200; Oral gavage	2 weeks, 1 week recovery	200	No noteworthy findings
TX-281-2008 /YES	Beagle dog/9/sex/dose 5/sex/dose (recovery)	VEL 0, 5, 20, 100; Oral gavage	13 weeks, 4 week recovery	100	Mortality 100: 1M; fibrinogen, and globulin concentration ↓, albumin ↑ (F), vomitus (M/F)

The NOEL for fertility and early embryonic development in rats is 200 mg/kg/day. When compared to the mean AUC following administration of the SOF/VEL FDC, the margin of exposure for VEL at the NOEL is approximately 6-fold (sexes combined; based on VEL exposure on Day 14 of the 2-week rat study.

At the developmental NOAELs, VEL exposures in the mouse, rat, and rabbit were approximately 31-, 6- and 0.7-fold compared with the clinical exposure of SOF/VEL FDC.

In the rat pre- and postnatal study, VEL at doses up to 200 mg/kg/day had no maternal effects, and no effects on behaviour, reproduction, or development of the offspring. Velpatasvir maternal exposure (LD 10 AUC_{last} 13.9 µg•h/mL) at the maternal and F_1 offspring NOEL in the pre- and postnatal development study was approximately 5-fold higher than the mean clinical exposure with the SOF/VEL FDC.

Sofosbuvir/Velpatasvir

There are no reproductive or developmental studies with SOF/VEL FDC.

Toxicokinetic data

Table 19: Pivotal Toxicokinetic Studies in Mice and Rats

Study ID	Daily Dose (mg/kg/day)	GS-331007 AUClast(ng.h/ml) at the NOAEL		Animal:Human GS-331007 AUC at the NOAEL	
		♂	♀	♂	♀
SA-PSI-7977-09-0008	SOF – 100 (M), 300 (F); Oral gavage	23.7	161	3	22
SA-PSI-7851-08-001	GS-9851 - 250; Oral gavage	41.4	20.9	6	3
SA-PSI-7977-10-0004	SOF - 500; Oral gavage	66.5	65.5	9	9

Table 20: Pivotal Toxicokinetic Studies in Beagle Dog

Study ID	Daily Dose (mg/kg/day)	GS-331007 AUC (ng.h/ml) at the NOAEL		Animal:Human GS-331007 AUC at the NOAEL	
		♂	♀	♂	♀
SA-PSI-7851-08-002	GS-9851 - 150; oral via capsule	120	91.6	17	13
SA-PSI-7977-10-0003	SOF 100; oral via capsule	76.3	104	11	14

Exposure to VEL generally increased with the increase in dose level from 100 to 1500 mg/kg/day. The increases in peak concentration Cmax and AUC0-24 were less-than-dose proportional between 100 and 1500 mg/kg/day. Sex-based differences were less than 2-fold in VEL Cmax and AUC0-24 values. No accumulation of VEL was observed after multiple doses in mice.

Table 21: Mean Toxicokinetic Parameters of Velpatasvir in the 4-Week Oral Gavage Study in Mice

VEL (mg/kg/day)	Sex	AUC_{0-24} (ng·h/mL)		C_{max} (ng/mL)	
		Day 1	Day 26	Day 1	Day 26
100	Male	69200	83300	8240	8560
	Female	79500	64900	10800	8020
300	Male	122000	86700	12300	7720
	Female	105000	82300	11800	11700
1500	Male	146000	170000	13400	14200
	Female	261000	269000	18300	18300

Exposure to VEL increased with increasing dose level from 20 to 200 mg/kg/day. The increases in Cmax and AUC0-t were less than dose proportional between 20 and 200 mg/kg/day. Sex-based differences in VEL Cmax and AUC0-t values were less than 2-fold. No notable accumulation of VEL was observed after once daily dosing for 2 weeks in rats.

Table 22: Mean Toxicokinetic Parameters of Velpatasvir in the 2-Week Oral Gavage Study in Rats

VEL (mg/kg/day)	Sex	AUC_{0-t} (ng·h/mL)[a]		C_{max} (ng/mL)	
		Day 1	Day 14	Day 1	Day 14
20	M	5263	5569	597	800
	F	4522	3193[b]	608	578
60	M	10371	14529	914	1323
	F	9770	8364	967	1018
200	M	17285	21396	1167	1607
	F	15852	12865	1154	1041

F = female; M = male
a last time point = 24 h
b last time point = 12 h

Exposure to VEL increased with the increase in dose level from 5 to 100 mg/kg/day. Increases in Cmax and AUC0-t were generally dose-proportional between 5 and 20 mg/kg/day and less-than-dose proportional between 20 and 100 mg/kg/day. Sex-based differences were less than 2-fold in VEL mean Cmax and AUC0-t values. No accumulation of VEL was observed after multiple dosing.

Table 23: Mean Toxicokinetic Parameters of Velpatasvir in the 39-Week Oral Gavage Study in Dogs

VEL (mg/kg/day)	Sex	C_{max} (ng/mL)			$AUC_{(0-t)}$ (ng·h/mL)[a]		
		Day 1	Week 13	Week 39	Day 1	Week 13	Week 39
5	M	335	303	273	1880[b]	1500[b]	1500
	F	231	326	356	1250[c]	1720	1950
20	M	946	929	859	8610	8700	6380
	F	1170	1050	1050	10900	10500	7030
100	M	2370	2090	1940	32800	28600	25500
	F	2040	2060	2170	25300	31100	29800

F = female; M = male
a last time point = 24 h
b last time point = 22.3 h
c last time point = 17.1 h

Local Tolerance

Sofosbuvir

The local tolerance in the GI tract was conducted during the repeat dose oral toxicity studies with SOF. Sofosbuvir-related emesis and soft stools/diarrhoea was observed more frequently at doses ≥ 100 mg/kg/day in the dog studies as compared with controls. The soft stools were also observed in the rat

studies. These effects may partly be due to the vehicle administered, as similar findings were observed in vehicle control animals.

Sofosbuvir was classified as a non-irritant to skin (TX-334-2009), and was predicted to be a non-severe irritant to eyes (TX-334-2008). In the quantitative whole body radiography study using pigmented and non-pigmented rats, SOF and its major metabolites did not accumulate in dermal or ocular tissues (SA-PSI-7977-09-0005).

Velpatasvir

Evaluation of local tolerance in the GI tract conducted during the chronic repeat dose oral studies in the rat and dog did not show any notable effects on the GI tract.

Velpatasvir was classified as a nonirritant to skin (TX-281-2040), and was not considered a severe irritant to eyes (TX-281-2039).

Sofosbuvir/Velpatasvir

No local tolerance studies were conducted for the SOF/VEL FDC.

Other toxicity studies

Antigenicity

Antigenicity studies with SOF or VEL have not been conducted based on their lack of antigenic properties. Sofosbuvir (TX-334-2010) and VEL (TX-281-2041) showed no potential for sensitization in local lymph node assays in mice.

Immunotoxicity

No specific immunotoxicity studies were conducted with SOF or VEL.

Dependence

No specific studies on dependency of SOF or VEL were conducted. Tissue distribution studies using radiolabeled SOF in rat and dog or VEL in mouse and rat indicated that very low concentrations of radioactivity at C_{max} were observed in the CNS.

Metabolites

No specific studies with SOF metabolites were conducted. There are no unique human metabolites with SOF and the predominant metabolites were similar across species. The predominant metabolites of SOF, GS-566500 and GS-331007, were adequately evaluated in repeat dose studies in mouse, rat, and dog; in embryo-fetal development toxicity studies; and in a prenatal and postnatal developmental toxicity study. There are no major or unique human metabolites with VEL. Velpatasvir is excreted primarily as the parent compound in the bile in all species.

Studies on impurities

The impurities and degradation products related to SOF and VEL have been identified in batches of the active pharmaceutical ingredient (API) or drug product. From the SOF/VEL FDC starting materials, process intermediates and actual impurities and potential impurities were described, predicted potential mutagenicity for 1 VEL starting material, 3 starting material impurities, 1 process intermediate, and 3 process impurities. Testing during development and process validation demonstrates control of these impurities to levels that are below the threshold of toxicological concern.

Sofosbuvir

Two repeat dose studies were conducted in rats to determine if there were unexpected toxic effects from SOF-related process impurities (SA-PSI-7977-11-0003 [14 days] and TX-334-2007 [28 days]). No adverse treatment-related effects were observed and there were no differences in findings in animals treated with lots containing SOF-related process impurities to those observed in previous studies, or to a comparator lot.

Velpatasvir

GS-604527, a starting material in the manufacturing process of VEL, was positive in TA98 and TA100 in the absence and presence of S9 in the bacterial reverse mutation assay (TX-281-2033).

A 2-week oral gavage toxicity study was conducted in rats to determine the toxicity potential of VEL-related process impurities (TX-281-2042). No adverse treatment-related effects were observed and there were no differences in findings in animals treated with lots containing VEL-related process impurities to those observed in previous studies, or to a comparator lot.

Sofosbuvir/Velpatasvir

The combination of SOF and VEL in the FDC did not introduce new impurities or degradation products. Toxicity studies with the single agents were considered sufficient to qualify observed impurities and degradation products and no additional qualification studies were necessary.

Other Toxicity Studies

Sofosbuvir

Sofosbuvir and GS-9851 demonstrated no evidence of mitochondrial toxicity (PC-334-2012, PC-PSI-7977-09-0007, PC-PSI-7851-08-0009, PC-334-2015).

Fourteen-day oral bridging toxicity studies comparing SOF with the diastereomer mixture GS-9851 at 500 mg/kg/day in both rats (SA-PSI-7977-09-0001) and dogs (SA-PSI-7977-09-0002) did not reveal any toxicity or exposure differences between the 2 compounds.

Sofosbuvir does not absorb light within the range of 290 to 700 nm and there are no nonclinical or clinical findings indicative of phototoxicity with SOF.

Velpatasvir

Velpatasvir was positive in the Balb/c 3T3 neutral red uptake phototoxicity assay (TX-281-2015).

Velpatasvir at doses up to 200 mg/kg/day (5-fold exposure margin versus clinical C_{max}) did not produce any reactions indicative of phototoxicity (TX-281-2016).

2.3.5. Ecotoxicity/environmental risk assessment

Sofosbuvir is already approved in the EU and no additional assessment of the submitted ERA for SOF was required. The ERA is based on the major metabolite of sofosbuvir (GS-331007) which is concluded not to be expected to pose a risk to the environment.

Velpatasvir accumulates in sediment in a persistent manner and additional evaluation is required on the effects of VEL on sediment-dwelling organisms. The applicant has committed to providing an updated ERA report once the final report for the bioaccumulation in sediment-dwelling benthic oligochaetes study has

been completed. It is not possible to conclude on the PBT / vPvB status of Velpatasvir until the results of the bioaccumulation in sediment-dwelling benthic oligochaetes study (OECD 315) are available**.**

Summary of main study results for Sofosbuvir

Substance (INN/Invented Name): 2'-Deoxy-2'-fluoro-2'-C-methyluridine (GS-331007, environmentally relevant pharmaceutical residue of **Sofosbuvir**					
CAS-number (if available): 863329-66-2					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	- 1.28 (pH = 4) -0.417 (pH = 7) -0.576 (pH = 9)			Potential PBT: No
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log K_{ow}	-1.28…-0.417			not B
	BCF	*not assessed*			-
Persistence	DT50 or ready biodegradability	*$DT_{50,water}$: 51-56 days (dissipation)*			P
Toxicity	NOEC or CMR	*NOEC = 26 000 µg/L* *2-year carcinogenicity study is ongoing*			*not T* *result has no relevance as not B*
PBT-statement :	The compound is not considered as PBT nor vPvB				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
PEC $_{surfacewater}$, Default: Phase II refinement based on sales projections at local scale	2,0 10.4	µg/L			> 0.01 threshold: Yes
Other concerns (e.g. chemical class)	-	-			No
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106 and OPPTS 835.1110	Soil: K_{oc}=17.1, 18.0, 31.2 L/kg Sludge: K_d =12.8, 32.9 L/kg			-
Ready Biodegradability Test	OECD 301	*not provided*			OECD 308 test performed
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50,\ water}$ =51-56 days (dissipation) $DT_{50,\ sediment}$ = *NA* $DT_{50,\ whole\ system}$ = 60-66 d (dissipation) $DT_{50,\ whole\ system}$ = > 100 d (degradation) % shifting to sediment => 10 % from day 3			One significant transformation product formed
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/ *Pseudokirchneriella subcapitata*	OECD 201	NOEC	≥ 94 000 61 000	µg/L	0-72 h, growth rate 0-72 h, inhibition
Daphnia sp. Reproduction Test	OECD 211	NOEC	26 000	µg/L	21 day, reporduction
Fish, Early Life Stage Toxicity Test/*Pimephales promelas*	OECD 210	NOEC	≥ 10 000	µg/L	-
Activated Sludge, Respiration Inhibition Test	OECD 209	EC	≥ 1 000 000	µg/L	as EC_{10}
Phase IIb Studies					
Bioaccumulation	OECD 305	BCF	-	L/kg	%lipids: -
Aerobic and anaerobic transformation in soil	OECD 307	DT50 %CO_2	-		-
Soil Micro organisms: Nitrogen Transformation Test	OECD 216	%effect	-	mg/kg	-

Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC	-	mg/kg	-
Earthworm, Acute Toxicity Tests	OECD 207	NOEC	-	mg/kg	-
Collembola, Reproduction Test	ISO 11267	NOEC	-	mg/kg	-
Sediment dwelling organism / *Chironomus riparius*	OECD 218	NOEC	20	mg/kg	

Summary of main study results for velpatasvir

Substance (INN/Invented Name): Velpatasvir					
CAS-number (if available): 1377049-84-7					
PBT screening		Result			**Conclusion**
Bioaccumulation potential log K_{ow}	OECD123	log D = 6.31 (at pH8)			Potential PBT (Y)
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log K_{ow}	log D = 6.31 (at pH8)			Possibly B
	BCF				?
Persistence	DT_{50} or ready biodegradability				P
Toxicity	NOEC or CMR				T
PBT-statement :	Inconclusive until data obtained for bioaccumulation in sediment-dwelling benthic oligochaetes (OECD 315)				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
PEC_{SW} , default or refined (i.e. max HCV prevalence from ECDC)	Default : 0.50 Refined : 2.60	µg/L			> 0.01 ug/L threshold (Y)
Other concerns (e.g. chemical class)					(N)
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106	*K_d soil: 294-3062 L/kg* *K_d soil mean = 1009 L/kg* *K_{oc} soil > 10 000 L/kg* *K_d sludge : 1355-4387 L/kg* *K_d sludge mean = 2672 L/kg* *K_{oc} sludge: 3673 – 11 544 L/kg* *K_{oc} sludge mean = 7137 L/kg*			List all values
Ready Biodegradability Test	OECD 301	Not readily biodegradable			
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50,\ water}$ = 1.3-1.7 d $DT_{50,\ sediment}$ = 109-136 d $DT_{50,\ whole\ system}$ = 62.4 d % shifting to sediment = >10% AR at D14.			
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC	≥ 49	µg/L	*Pseudokirchneriella subcapitata*
Daphnia sp. Reproduction Test	OECD 211	NOEC	6.56	µg/L	*D. magna*
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC	≥ 200	µg/L	Fathead minnow
Activated Sludge, Respiration Inhibition Test	OECD 209	EC	≥ 105	mg/L	
Phase IIb Studies					
Bioaccumulation	OECD 305	BCF	NA	L/kg	%lipids:
Aerobic and anaerobic transformation in soil	OECD 307	DT50 $\%CO_2$	NA		for all 4 soils
Soil Microorganisms: Nitrogen Transformation Test	OECD 216	%effect	NA	mg/kg	
Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC	NA	mg/kg	
Earthworm, Acute Toxicity Tests	OECD 207	NOEC	NA	mg/k	

				g	
Collembola, Reproduction Test	ISO 11267	NOEC	NA	mg/kg	
Sediment dwelling organism	OECD 218	NOEC	4143 (norm)	mg/kg	*Chironomus riparius*

2.3.6. Discussion on non-clinical aspects

Both SOF and VEL have been shown to inhibit HCV genotypes 1-6 replicons. In vitro combination of SOF and VEL exhibited an additive and antiviral activity and also an overlapping activity against resistant mutants. No antiviral antagonism was observed, and no significant change in cell viability or in vitro cross-resistance has been seen.

Sofosbuvir and velpatasvir show low potential for off-target activity and no further studies with the combination have therefore been performed or are considered to be needed.

Due to the differences in metabolism and elimination pathways of SOF and VEL the administration of the combination does not affect the PK profile of each other in the liver or systemic circulation. However, a higher plasma exposure to SOF is seen after administration of the combination as compared to when given alone which is consistent with the identified in vitro interaction via the intestinal efflux transporters P-gp and BCRP with SOF being a substrate and VEL both a substrate and inhibitor of these transporters.

Sofosbuvir seemed overall well tolerated in general toxicity studies of up to 9 months in rat and dog. In toxicity studies at high doses effects were noted in the gastrointestinal tract, liver and the haematological system. Reproductive toxicity was studied in rat and rabbit and while no relevant potential for adverse reproductive effects was evident, the high dose likely was suboptimal in these studies. Velpatasvir also seemed to be well tolerated and no target tissues were identified in any of the repeat dose toxicological studies performed up to 26 week in rat and 39 week in dog. No reproductive toxicity was seen in rat or mouse. However, it has not convincingly been able to show that velpatasvir may not have a teratogenic potential in the rabbits. This information is reflected in sections 4.6 and 5.3 of the SmPC.

Studies in vitro and in vivo for genotoxic potential were negative and consistent with a low mutagenic potential of both sofosbuvir and velpatasvir. Long-term carcinogenicity studies in mouse and rat showed no carcinogenic potential for sofosbuvir. Carcinogenicity studies with velpatasvir are ongoing.

Sofosbuvir and velpatasvir thus does not have any overlapping toxicological profiles and there is no toxicological concern with this combination based on available non-clinical data. No toxicological studies have been performed with the combination of sofosbuvir and velpatasvir and none are considered to be needed.

The toxicological qualification of specified impurities and residual solvents is considered to be sufficient for both compounds.

2.3.7. Conclusion on the non-clinical aspects

The review of non-clinical data available for sofosbuvir and velpatasvir overall indicates no major issues for concern for the combination of these two substances.

The applicant will provide the VEL carcinogenicity study reports as soon as finalised (reports are projected to be available in Q4 2016 (mouse), and Q4 2017 (rat)). In addition the applicant committed to provide an updated ERA report once the final report for the bioaccumulation in sediment-dwelling benthic oligochaetes study has been completed (Q2 2017).

2.4. Clinical aspects

2.4.1. Introduction

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.

This application concerns once daily oral fixed-dose combination for Sofosbuvir (SOF)/Velpatasvir (VEL) tablet (400 mg/100 mg).

SOF single agent: There were 18 clinical pharmacology studies conducted with SOF and/or GS-9851 (as monotherapy or in combination with Peg-IFN and/or RBV). Those have already been presented in the Sovaldi MAA dossier and are not reiterated here.

VEL single agent: There were 9 clinical pharmacology studies submitted, conducted with VEL as single agent (Table 24).

SOF/VEL FDC: There were 12 clinical pharmacology studies conducted with SOF/VEL informing on the clinical pharmacology in Table 25.

A number of in vitro studies in human biomaterial were performed to evaluate metabolism, protein binding, and drug-drug interaction potential of velpatasvir.

Table 24: Overview of clinical studies with velpatasvir as a Single-Agent Tablet or in Combination with Other Compounds)

Study No.	Study Description	VEL			Dosage Form of Co-administered or Control Drugs
		Dosage Form	Dose (mg)	n[a]	
GS-US-281-0101	Phase 1 study to evaluate the safety and PK of VEL, the effect of food on VEL PK, and the PK interactions between VEL and SOF and its metabolites in healthy subjects	5-mg tablet 50-mg tablet	5, 50 100 150 450	102	placebo tablet SOF 400-mg tablet
GS-US-281-1054	Phase 1 study to evaluate the effect of VEL on QT/QTc interval in healthy subjects	50-mg tablet	100 500	49	VEL placebo-to-match tablet moxifloxacin 400-mg tablet
GS-US-281-1055	Phase 1 study to evaluate the PK, metabolism, and excretion of VEL in healthy subjects	1.45 mg [14C]VEL and 98.55 mg capsule	100	8	Not applicable
GS-US-281-0102	Phase 1b study to evaluate the safety, PK, and antiviral activity of VEL in subjects with HCV infection	5-mg tablet 50-mg tablet	5, 25 50, 100 150	70	placebo tablet
GS-US-281-1056	Phase 1 study evaluate the single-dose PK and safety of VEL in subjects with severe renal impairment and matched healthy control subjects	50-mg tablet	100	19	Not applicable

Study No.	Study Description	VEL			Dosage Form of Co-administered or Control Drugs
		Dosage Form	Dose (mg)	n[a]	
GS-US-281-0112	Phase 1 study to evaluate the single-dose PK and safety of VEL in subjects with normal hepatic function, moderate hepatic impairment, and severe hepatic impairment	50-mg tablet	100	33	Not applicable
GS-US-281-0115	Phase 1 study to evaluate the potential drug-drug interaction between VEL and probe drugs in healthy subjects	50-mg tablet	100	75	pravastatin 40-mg tablet rosuvastatin 10-mg tablet digoxin 0.25-mg tablet rifampin 300-mg capsule ketoconazole 200-mg tablet cyclosporine 100-mg capsule
GS-US-281-0119	Phase 1 study to evaluate the potential drug-drug interaction between VEL and a representative H2RA or PPI in healthy subjects	50-mg tablet	100	24	omeprazole 20-mg capsule famotidine 20-mg tablet
GS-US-281-1058	Phase 1 study to evaluate the potential drug-drug interaction between VEL and a representative hormonal contraceptive in healthy female subjects	50-mg tablet	100	15	Ortho Tri-Cyclen® Lo norgestimate 0.180/ 0.215/0.250-mg/ethinyl estradiol 0.025-mg tablet

[a] Number of subjects who received at least 1 dose of VEL

Table 25: Clinical Pharmacology Studies Containing SOF/VEL or SOF+VEL

Study Number	Study Description	SOF/VEL or SOF+VEL		Dosage Form of Co-administered or Control Drugs
		Dose (mg)	n[a]	
GS-US-342-0104	Phase 1 study to evaluate bioavailability of SOF/VEL FDC tablets relative to individual tablet formulations and the effect of food the PK of SOF/VEL FDC tablets in healthy subjects	SOF/VEL 400/25 SOF/VEL 400/100	82	SOF 400-mg tablet VEL 25-mg tablet VEL 100-mg tablet
GS-US-342-1167	Phase 1 study to evaluate the potential drug-drug interaction between SOF/VEL and HIV ARVs in healthy subjects	SOF/VEL 400/100	102	FTC/RPV/TDF 200/25/300-mg EFV/FTC/TDF 600/200/300-mg TDF tablet DTG 50-mg tablet E/C/F/TAF 150/150/200/10-mg
GS-US-342-1326	Phase 1 study to evaluate the potential drug-drug interaction between SOF/VEL and HIV ARVs in healthy subjects	SOF/VEL 400/100	135	E/C/F/TDF 150/150/200/300-mg tablet DRV 800-mg tablet RTV 100-mg tablet FTC/TDF 200/300-mg tablet ATV 300-mg capsule LPV/r 200/50-mg tablet RAL 400-mg tablet
GS-US-342-1346	Phase 1 study to evaluate the potential drug-drug between SOF/VEL and a representative H2RA or PPI in healthy subjects	SOF/VEL 400/100	60	omeprazole 20-mg capsule famotidine 40-mg tablet
GS-US-342-1709	Phase 1 study to evaluate the potential drug-drug between SOF/VEL and a representative PPI and food in healthy subjects	SOF/VEL 400/100	120	omeprazole 20-mg capsule omeprazole 40-mg capsule

2.4.2. Pharmacokinetics

Clinical pharmacology studies

This application concerns once daily oral fixed-dose combination for Sofosbuvir (SOF)/Velpatasvir (VEL) tablet (400 mg/100 mg).

SOF single agent: There were 18 clinical pharmacology studies conducted with SOF and/or GS-9851 (as monotherapy or in combination with Peg-IFN and/or RBV). Those have already been presented in the Sovaldi MAA dossier and are not reiterated here.

VEL single agent: There were 9 clinical pharmacology studies submitted, conducted with VEL as single agent (Table 26).

SOF/VEL FDC: There were 12 clinical pharmacology studies conducted with SOF/VEL informing on the clinical pharmacology in Table 27 (phase II and III studies are listed Clinical Efficacy Section).

A number of in vitro studies in human biomaterial were performed to evaluate metabolism, protein binding, and drug-drug interaction potential of velpatasvir.

Table 26: Overview of clinical VEL studies with reference to Clinical Pharmacology (as a Single-Agent Tablet or in Combination with Other Compounds)

Study No.	Study Description	VEL			Dosage Form of Co-administered or Control Drugs
		Dosage Form	Dose (mg)	n[a]	
GS-US-281-0101	Phase 1 study to evaluate the safety and PK of VEL, the effect of food on VEL PK, and the PK interactions between VEL and SOF and its metabolites in healthy subjects	5-mg tablet 50-mg tablet	5, 50 100 150 450	102	placebo tablet SOF 400-mg tablet
GS-US-281-1054	Phase 1 study to evaluate the effect of VEL on QT/QTc interval in healthy subjects	50-mg tablet	100 500	49	VEL placebo-to-match tablet moxifloxacin 400-mg tablet
GS-US-281-1055	Phase 1 study to evaluate the PK, metabolism, and excretion of VEL in healthy subjects	1.45 mg [14C]VEL and 98.55 mg capsule	100	8	Not applicable
GS-US-281-0102	Phase 1b study to evaluate the safety, PK, and antiviral activity of VEL in subjects with HCV infection	5-mg tablet 50-mg tablet	5, 25 50, 100 150	70	placebo tablet
GS-US-281-1056	Phase 1 study evaluate the single-dose PK and safety of VEL in subjects with severe renal impairment and matched healthy control subjects	50-mg tablet	100	19	Not applicable
GS-US-281-0112	Phase 1 study to evaluate the single-dose PK and safety of VEL in subjects with normal hepatic function, moderate hepatic impairment, and severe hepatic impairment	50-mg tablet	100	33	Not applicable
GS-US-281-0115	Phase 1 study to evaluate the potential drug-drug interaction between VEL and probe drugs in healthy subjects	50-mg tablet	100	75	pravastatin 40-mg tablet rosuvastatin 10-mg tablet digoxin 0.25-mg tablet rifampin 300-mg capsule ketoconazole 200-mg tablet cyclosporine 100-mg capsule
GS-US-281-0119	Phase 1 study to evaluate the potential drug-drug interaction between VEL and a representative H2RA or PPI in healthy subjects	50-mg tablet	100	24	omeprazole 20-mg capsule famotidine 20-mg tablet

Study No.	Study Description	VEL			Dosage Form of Co-administered or Control Drugs
		Dosage Form	Dose (mg)	n[a]	
GS-US-281-1058	Phase 1 study to evaluate the potential drug-drug interaction between VEL and a representative hormonal contraceptive in healthy female subjects	50-mg tablet	100	15	Ortho Tri-Cyclen® Lo norgestimate 0.180/ 0.215/0.250-mg/ethinyl estradiol 0.025-mg tablet

a Number of subjects who received at least 1 dose of VEL

Table 27: Clinical Studies Containing SOF/VEL or SOF+VEL Contributing Information to the Summary of Clinical Pharmacology

Study Number	Study Description	SOF/VEL or SOF+VEL		Dosage Form of Co-administered or Control Drugs
		Dose (mg)	n[a]	
GS-US-342-0104	Phase 1 study to evaluate bioavailability of SOF/VEL FDC tablets relative to individual tablet formulations and the effect of food the PK of SOF/VEL FDC tablets in healthy subjects	SOF/VEL 400/25 SOF/VEL 400/100	82	SOF 400-mg tablet VEL 25-mg tablet VEL 100-mg tablet
GS-US-342-1167	Phase 1 study to evaluate the potential drug-drug interaction between SOF/VEL and HIV ARVs in healthy subjects	SOF/VEL 400/100	102	FTC/RPV/TDF 200/25/300-mg EFV/FTC/TDF 600/200/300-mg TDF tablet DTG 50-mg tablet E/C/F/TAF 150/150/200/10-mg
GS-US-342-1326	Phase 1 study to evaluate the potential drug-drug interaction between SOF/VEL and HIV ARVs in healthy subjects	SOF/VEL 400/100	135	E/C/F/TDF 150/150/200/300-mg tablet DRV 800-mg tablet RTV 100-mg tablet FTC/TDF 200/300-mg tablet ATV 300-mg capsule LPV/r 200/50-mg tablet RAL 400-mg tablet
GS-US-342-1346	Phase 1 study to evaluate the potential drug-drug between SOF/VEL and a representative H2RA or PPI in healthy subjects	SOF/VEL 400/100	60	omeprazole 20-mg capsule famotidine 40-mg tablet
GS-US-342-1709	Phase 1 study to evaluate the potential drug-drug between SOF/VEL and a representative PPI and food in healthy subjects	SOF/VEL 400/100	120	omeprazole 20-mg capsule omeprazole 40-mg capsule

Velpatasvir is a new chemical entity and the PK evaluation aimed at characterisation of the disposition of the compound and its interaction potential to support dosing recommendations and to predict patient populations and clinical situations in which pharmacokinetics may be different from that in the average patient population evaluated in the clinical development programme. Velpatasvir has a wide safety margin. The clinical pharmacology has been investigated in healthy volunteers and HCV patients.

Sofosbuvir is an authorised medicinal product (Sovaldi′ 2013 and is contained in the fixed dose combination of Harvoni [sofosbuvir/Ledipasvir], 2014). All relevant data along with new data obtained with the fixed dose combination with velpatasvir is described in this assessment report.

Analytical methods

The bioanalytical methods for the measurement of VEL, SOF and SOF metabolites (GS-566500 and GS-331007) concentrations in human plasma were based on deuterium and/or ^{13}C labelled internal standards and LC/MS/MS. The sample preparation for VEL was liquid-liquid extraction and for SOF and metabolites sample preparation was protein precipitation extraction. All methods were validated. The SOF intracellular active metabolite GS-461203 has not been possible to measure in vivo.

Pharmacokinetic data analysis

Standard statistical methods and non-compartmental methods were used to characterize the pharmacokinetics. Population PK analysis was characterised by non-linear mixed effects modelling including data from healthy subjects in phase I studies and patients in phase II/III studies.

Formulations

All phase III studies and a majority of the DDI studies were performed with the intended commercial fixed dose combination (FDC) formulation, SOF/VEL (400 mg/100 mg). The SOF/VEL FDC tablets at 400mg/100mg showed similar AUC_{inf} and C_{max} for SOF, GS-566500, GS-331007 and VEL as compared to co-administered SOF and VEL as mono components. The point estimates for AUC_{inf} and C_{max} (%GLSM ratio) for the FDC tablet were within 90-107%.

Absorption

Sofosbuvir

For sofosbuvir, following a single dose of the SOF/VEL FDC tablet in fasted state, the t_{max} was 1 and 3 h for SOF and GS-331007, respectively.

In vitro studies show that SOF is subject to marked efflux, mediated by P-gp and/or BCRP. Co-administration of a single dose CsA increased the exposure to sofosbuvir 4.5-fold. The bioavailability of drug related material is at least 50%, although the absolute value is unknown.

Velpatasvir

Velpatasvir was relatively rapidly absorbed with a t_{max} of 3 h after administration of the SOF/VEL FDC tablet in healthy volunteers. The solubility is pH dependent (41 mM at pH 2 and <0.11 mM at pH 5) and in FaSSIF and FeSSIF the solubility was <11 µM and 11 µM, respectively. In vitro, VEL was shown to be a BRCP and P-gp substrate. Co-administration of a single dose of 600 mg Cyclosporin A (CsA) increased the exposure to velpatasvir approximately 2-fold. The absolute bioavailability of VEL has not been determined in humans.

SOF/VEL

Co-administration of velpatasvir with sofosbuvir has an effect on exposure of sofosbuvir (approx. 2-fold increase). The half-life of SOF and metabolites were unaffected and the effect on exposure is likely due to intestinal inhibition P-gp and/or BCRP caused by velpatasvir. Exposure of metabolite GS-566500 was also increased nearly 2-fold, while GS-331007 was in general unaffected. There was no relevant change of velpatasvir steady state PK parameters when co-administrated with single dose SOF.

Food effect

Sofosbuvir/velpatasvir

The effect of food on PK after a single-dose of SOF/VEL FDC tablet was investigated in healthy volunteers. Administration with food increased AUC of SOF by 60% and 78% and slightly decreased C_{max} by 5% and 12% after a moderate and high fat meal, respectively. The AUC of GS-331017 was not affected with or without food, while C_{max} decreased 25% and 37%, respectively. The plasma exposure achieved for VEL upon administration of SOF/VEL after a moderate and high fat meal was an increase of 34% and 21%

(AUC) and 31% and 5% (C_{max}), respectively. The influence of food on velpatasvir and sofosbuvir exposure was limited and thus SOF/VEL can be administered without regard to food.

Distribution

Sofosbuvir

The unbound plasma fraction of SOF was approximately 15% ex vivo and seems to be independent of concentration. No effect of renal impairment was seen on degree of binding. GS-331007 is minimally bound to plasma proteins. The mean whole blood-to-plasma concentration ratio was approximately 0.71. In the Pop-PK analysis Vc/F for SOF was estimated to 197 L.

Velpatasvir

Velpatasvir is highly protein bound in plasma (in vitro, fraction unbound 0.3-0.5%) and independent of concentration (0.1–2 µM). In severe renally impaired subjects the unbound fraction was similar to subjects with normal renal function (~0.3%). Unbound plasma concentration in subjects with hepatic impairment (HI) was determined in vitro and was found to be similar (0.2-0.3%) in plasma from in subjects with mild, moderate or severe HI compared to subjects with normal liver function. The mean whole blood-to-plasma concentration ratio ranged from 0.52 to 0.67. In the population PK analysis the estimated apparent oral volume of distribution of the central compartment (V_c/F) for VEL was 392 L in a typical subject (male HCV patient with normal or Child-Pugh-Turcotte A (CPT A) hepatic function).

Elimination

From the popPK analysis the estimated CL/F in a typical HCV patient (male with normal/CPT A hepatic function) was 47 L/hr and 352 L/h, and $t_{½}$ was 19 h and 0.39 h, for velpatasvir and sofosbuvir, respectively.

Excretion

Sofosbuvir

Following a single 400 mg oral dose of [14C]-sofosbuvir, mean total recovery of the dose was greater than 92%, consisting of approximately 80%, 14%, and 2.5% recovered in urine, faeces, and expired air, respectively. The majority of the sofosbuvir dose recovered in urine was GS-331007 (78%) while 3.5% was recovered as sofosbuvir. This data indicate that renal clearance is the major elimination pathway for GS-331007 with a large part actively secreted. While subject to active tubular secretion, GS 331007 is not a substrate for renal transporters including organic anion transporter (OAT) 1 or 3, OCT2, MRP2, P gp, BCRP or MATE1. Sofosbuvir is not a substrate for hepatic uptake transporters, OATP1B1 or 1B3, and OCT1.

Velpatasvir

In the human mass balance study following a single dose of 100 mg the mean cumulative urinary and fecal recovery of [^{14}C]-radioactivity was 95% with relative recovery of 0.4% in urine and 94% in faeces. Velpatasvir was the major compound found in faeces accounting for on average 77% of the administered dose. Unchanged parent drug was <0.1% of dose in urine. Velpatasvir is a substrate for hepatic uptake transporter organic anion transporting polypeptide (OATP) 1B and efflux transporters P-gp and BCRP, but not a substrate for organic cat-ion transporter (OCT) 1.

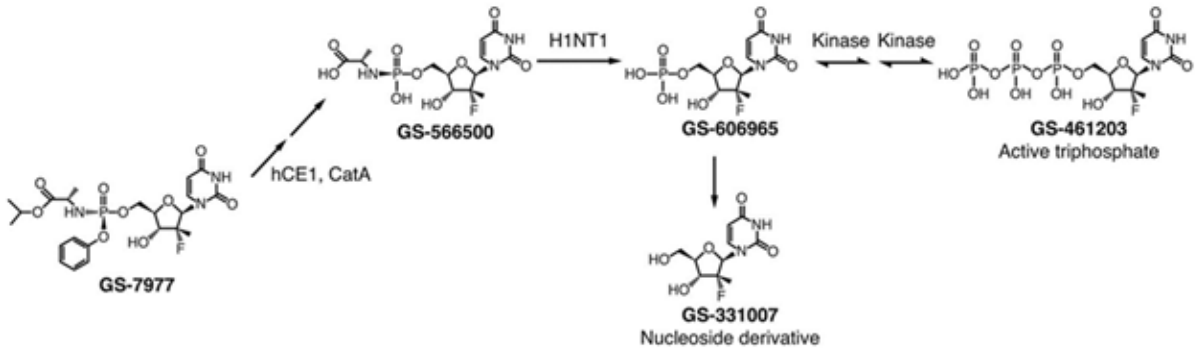
Metabolism

Sofosbuvir

Sofosbuvir is subject to extensive first-pass metabolism in the intestine and in the liver. The active metabolite GS-461203 is formed through several metabolic steps (Figure 4). In vitro, SOF is rapidly hydrolysed by CatA and CES1 to form GS-566500 which is further metabolised to eventually form the active triphosphate nucleoside analogue GS-461203. Sofosbuvir and GS-331007 are not substrates of UGT1A1 or CYP3A4, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 enzymes.

In plasma, GS-331007 constituted the majority (90%) of measured radioactivity. The intermediate metabolite GS-566500 has a t_{max} of 1 h and has a half-life of 2 h. The major metabolite GS-331007 peaks at 2 h and has a half-life of 26 h. GS-331007 and GS-566500 are not active metabolites.

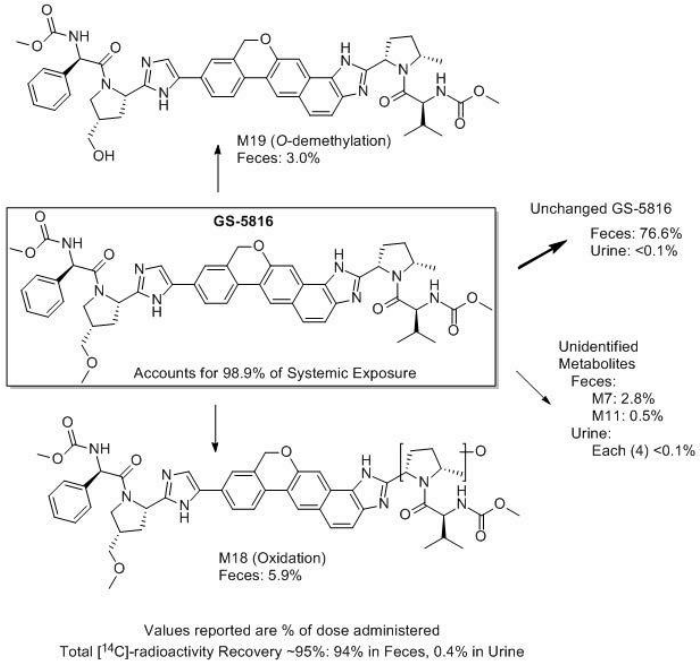
Figure 4: Intracellular metabolism pathway of SOF (GS-7977)



Velpatasvir

Velpatasvir was relatively stable when incubated in human microsomes or hepatocytes. In the human ADME study most (~99%) of the total radioactivity in the AUC-pooled plasma samples was attributed to parent compound with 0.4% and 0.5% attributed to metabolites hydroxy-GS-5816-1 (M18) and desmethyl-GS-5816 (M19). In faeces, parent compound on average accounting for 77% of the administered dose, followed by M18 and M19, accounting for 6% and 3% of the dose up to 144 hours post-dose, respectively (Figure 5). In addition 2 unknown metabolites M7 and M11 were detected in feces each accounted for 2.8% and 0.5% of the dose, respectively.

Figure 5: Proposed major biotransformation and excretion Pathways of velpatasvir (GS-5816) in humans



Dose proportionality and time dependency

Velpatasvir exhibited nonlinear PK after single dose across the dose range of 5 to 450 mg. Increase in exposure was larger than dose-proportional from 5 to 50 mg and increased less than in proportion to dose at doses from 50 mg to 450 mg in healthy subjects. In HCV patients, after multiple dosing there was no clear systematic deviations from dose proportionality for velpatasvir in the range 25 to 150 mg. There was no evidence of time-dependent PK for VEL over a 3 day period.

For SOF no indication of non-linearity was observed in the range 200-1200 mg. There was no evidence of time-dependent PK for SOF over a 7 day period.

Inter-individual variability

Inter-individual variability of the VEL PK was determined to 51% for CL/F, 69% for V_c/F, 51% for V_p and 54% for K_a (CV%) from the population PK analysis.

Population PK analysis

The population PK of velpatasvir was described by a 2-compartment model with first order absorption, first order elimination from the central compartment and an absorption lag time. Female gender and hepatic function were statistically significant covariates on CL/F and Vc/F. Females had lower CL/F and V/F compared to males. HCV infected subjects with CPT-B or C had higher CL/F and V/F compared to subjects with normal or CPT-A hepatic function. Food had an effect on F1, K_a and T_{lag}. However, no covariate had a clinically meaningful impact on velpatasvir exposure. Other tested covariates, including age, weight, race, renal function, (compensated) cirrhosis, genotypes, IL28B status did not show any statistically significant impact on the PK of velpatasvir.

For sofosbuvir a one-compartment model with first order absorption, first order elimination from the central compartment and an absorption lag time was used for description of plasma PK. Female gender and hepatic function were statistically significant covariates on CL/F. Females had lower CL/F compared to males and HCV infected subjects with CPT-B or C had lower CL/F compared to subjects with normal or CPT-A hepatic function. Food had an effect on K_a. However, no covariate had a clinically meaningful impact on sofosbuvir exposure. Other tested covariates, such as age, weight, race, renal function, (compensated) cirrhosis, genotypes, IL28B status did not show any statistically significant impact on the PK of sofosbuvir.

Pharmacokinetics in target population

Sofosbuvir

The CL/F for SOF was 352 L/h for a typical subject (fasting male HCV infected patient without hepatic impairment or CPT-A), typical Vc/F was 197 L and t½ was 0.4 h. For the once daily regimen of SOF/VEL FDC, model predicted steady state AUC was 1135 ng·hr/mL for a typical subject. The model predicted AUC values for the population (5 to 95%tile) were 731 to 2510 ng·hr/mL.

Velpatasvir

Based on the population PK analysis, velpatasvir CL/F and half-life in a typical subject (fasting male HCV-infected with normal or hepatic function CPT-A) was determined to be 46.5 L/h and 18.8 h, respectively. The model predicted steady state exposure to velpatasvir after once daily regimen of SOF/VEL (400 mg/100 mg), was 2149 ng·hr/mL for a typical subject. The model predicted AUC values for the population (5 to 95%tile) were 1066 to 6307 ng·hr/mL (-50% to 193% different from typical value).

Special Populations

Hepatic impairment

Sofosbuvir

The pharmacokinetics of sofosbuvir were studied following 7 day dosing of 400 mg sofosbuvir in HCV infected patients with moderate and severe hepatic impairment (CPT Class B and C). Relative to patients with normal hepatic function, the sofosbuvir AUC_{0-24} was 126% and 143% higher in moderate and severe hepatic impairment, while the GS 331007 AUC0 24 was 18% and 9% higher, respectively.

Velpatasvir

Single dose (100 mg) PK of VEL was investigated in subjects with moderate (Child-Pugh-Turcotte-B, CPT-B) and severe (CPT-C) hepatic impairment. The plasma exposure (AUC) was marginally affected while Cmax was lowered in subjects with both moderate and severe hepatic impairment compared to subjects with normal liver function. The VEL half-life was slightly increased in subjects with HI compared to subjects with normal liver function.

Renal impairment

Sofosbuvir

The pharmacokinetics of sofosbuvir were studied in HCV negative patients with mild (eGFR ≥ 50 and <80 mL/min/1.73 m2), moderate (eGFR ≥ 30 and <50 mL/min/1.73 m2), severe renal impairment (eGFR <30

mL/min/1.73 m2) and patients with ESRD requiring haemodialysis following a single 400 mg dose of sofosbuvir. Relative to patients with normal renal function (eGFR >80 mL/min/1.73 m2), the sofosbuvir AUC was 61%, 107% and 171% higher in mild, moderate and severe renal impairment, while the GS 331007 AUC was 55%, 88% and 451% higher, respectively. In patients with ESRD, sofosbuvir AUC was 28% higher when sofosbuvir was dosed 1 h before haemodialysis compared with 60% higher when dosed 1 hour after haemodialysis, respectively. The AUC0-inf of GS-331007 in patients with ESRD administered with sofosbuvir 1 hour before or 1 h after haemodialysis was at least 10-fold and 20-fold higher, respectively. GS 331007 is efficiently removed by haemodialysis with an extraction coefficient of approximately 53%. Following a single 400 mg dose of sofosbuvir, a 4 h haemodialysis removed 18% of administered dose.

Velpatasvir

The pharmacokinetics of velpatasvir was studied with a single dose of 100 mg in HCV negative subjects with severe renal impairment median (CrCL<30 ml/min by Cockcroft-Gault). The exposure (AUC) of VEL was approximately 50% higher, with similar C_{max}, in the subjects with severe renal impairment as compared to subjects with normal renal function.

Age, sex, race, body weight

Female subjects had a lower CL/F of VEL and SOF compared to male subjects, resulting in an approximately 50% and 20% higher exposure, respectively, compared to male subjects.

The Population PK analyses of SOF, GS-331007 and VEL did not suggest a significant effect of race (described as White, Black, Asian and Other) on the kinetics of either compound.

Body weight (range 40 – 182 kg) did not have a clinically significant effect on VEL or SOF exposure according to the population pharmacokinetic analysis.

No formal PK study in elderly patients has been conducted. However, the impact of age (range 18 – 82 years) on the PK of SOF and VEL has been evaluated as a covariate in the population PK analyses. No clinically significant effect on VEL exposure was observed in subjects ≥75 years administered velpatasvir, however the number of subjects was limited (n=14).

The safety and efficacy of SOF/VEL in children and adolescents aged <18 years have not yet been established. The SOF/VEL FDC is not indicated in patients less than 18 years.

Drug-Drug Interactions

Effects of other medical products on the pharmacokinetics of velpatasvir and SOF/VEL

Velpatasvir is relatively metabolically stable in incubations in human microsomes and hepatocytes and the majority of the administered oral radioactive dose was found as parent compound in faeces. The major elimination pathway(s) for VEL is not fully understood, however most likely biliary excretion is a major pathway. In vitro, VEL was determined to be a substrate for P-gp and BCRP and indicated to be an OATP1B3 substrate, but not an OCT1 or OATP1B1 substrate.

Velpatasvir was administered with both single and repeated dosing of rifampicin. The duration of the repeat dosing was a bit short (7 days) for full effect of induction, but the resulting decrease in exposure was 70-80% decrease.

The effect of ketoconazole (P-gp and CYP3A4 inhibitor) was limited on the exposure of VEL (AUC increased 70%) and administration of single dose rifampicin (OATP1B1/3 inhibitor as single dose) resulted in 47% increase of AUC. In addition, velpatasvir exposure was approx. 2-fold when co-administered with CsA (an inhibitor of multiple transporters).

The solubility of VEL is pH dependent and therefore medical products that increase gastric pH are expected to decrease plasma concentration of VEL. When SOF/VEL was combined with famotidine simultaneously or staggered (VEL dosed 12 hours after famotidine) the decrease in exposure was approximately 10-20% for both SOF and VEL. Co-administration of SOF/VEL with 20 mg omeprazole simultaneously in the fasting state, the decrease of SOF and VEL exposure (AUC) was 29% and 46%, respectively, and staggered (given 12 hours after omeprazole), 44% and 55% respectively.

SOF/VEL (400/100 mg) has been studied in several combinations of antiretroviral treatments. Summaries of the effect of HIV ARV regimens on the SOF/VEL PK in healthy subjects are given in Table 28. For the combinations EVG/COBI/FTC/TDF, RAL+ FTC/TDF, FTC/RPV/TDF, E/C/F/TAF and DTG there was no or slight increase of the VEL exposure. For the combination with ATV+RTV+ FTC/TDF the increase was substantial.

When SOF/VEL was administered with EFV/FTC/TDF the exposure of VEL decreased substantially (50-60%). Both VEL and SOF exposure decreased up to 30-40% when co-administered with DRV+RTV+ FTC/TDF and LPV/r+ FTC/TDF (Table 28).

Table 28: Summary of PK parameters of VEL, SOF and metabolites and evaluated antiretroviral treatment when SOF/VEL were administered alone compared with administration of SOF/VEL + ARVs

	SOF/VEL+ARV / SOF/VEL									
	SOF PK Parameters			**GS-566500 PK Parameters**		**GS-331007 PK Parameters**		**VEL PK Parameters**		
ARVs	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	C_{tau}	AUC	C_{max}	C_{tau}
EFV/FTC/TDF	↔	↑38%	↔	↔	↔	↔	↔	↓53%	↓47%	↓57%
FTC/RPV/TDF	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
DTG	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
EVG/COBI/FTC/TAF	↑37%	↔	↔	↔	↑48%	↔	↑58%	↑50%	↑30%	↑60%
EVG/COBI/ FTC/TDF	↔	↔	↔	↔	↔	↔	↑45%	↔	↔	↑37%
DRV+RTV+ FTC/TDF	↓28%	↓38%	↔	↔	↔	↔	↔	↔	↓24%	↔
ATV+RTV+ FTC/TDF	↔	↔	↔	↔	↔	↔	↑42%	↑142%	↑55%	↑301%
LPV/r+ FTC/TDF	↓29%	↓41%	↔	↔	↔	↔	↔	↔	↓30%	↑63%
RAL+ FTC/TDF	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔

90% CIs of the %GLSM ratios were within (↔), extended above (↑), or extended below (↓) the predetermined alteration boundaries of 70% to 143%

Effects of velpatasvir and SOF/VEL on the pharmacokinetics of other medical products

Velpatasvir showed no direct inhibition on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 or UGT1A1. Based in vitro data, no relevant induction signal was observed for AhR (CYP1A2) and CAR (CYP2B6). An in vitro induction signal was detected for PXR (CYP3A4), however based on clinical data interactions due to induction of PXR is considered low.

In vitro studies showed that VEL was not an inhibitor of drug transporters BSEP, MATE1, MRP2, OAT1, OAT3, OATP1A2, OATP2B1, OCT1, OCT2, or NTCP (sodium taurocholate cotransporter protein) at clinically relevant concentrations. In vitro, VEL showed a concentration-dependent inhibition of OATP1B1, OATP1B3, P-gp and BCRP with IC_{50} of 1.5, 0.26, 20.6 and 0.30 µM, respectively. These results indicate that a clinically relevant interaction between VEL and OATP1B1 and 1B3 and intestinal inhibition of P-gp and BCRP by VEL substrates cannot be excluded. The inhibitory potential of VEL on OATP1B1/3, P-gp and BCRP has been investigated in clinical DDI studies.

There was a limited effect on the cyclosporine (CYP3A4 substrate) exposure (slightly decreased) following co-administration after multiple dosing with VEL relative to CsA administered alone.

For pravastatin (OATP1B1 and MRP2 and possibly CYP3A substrate) the in vivo exposure (AUC) was approximately 1.4-fold following co-administration with VEL, relative to pravastatin administration alone. Rosuvastatin (OATP1B1/3 and BCRP substrate) exposure was 2.7-fold following co-administration with VEL, relative to rosuvastatin administration alone. The median half-life for both pravastatin and rosuvastatin was similar with and without velpatasvir.

Digoxin (P-gp probe) exposure increased following co-administration with VEL, relative to digoxin administration alone.

It can be concluded that VEL is an in vivo P-gp, BCRP and OATP1B1/3 inhibitor.

The effect of VEL on the PK of a representative hormonal contraceptive (OC) medication (norgestimate /ethinyl estradiol) was studied. Similar systemic exposures of norelgestromin and norgestrel were achieved following OC co-administration with and without VEL (exposure decreased 3% to 10% point

estimates). An approximate 39% increase in ethinyl estradiol Cmax and decrease in Ctau (approximately 17% point estimate 90% CI 65, 106) were observed with no change in AUCtau when VEL was co-administered with OC compared with OC alone.

Table 29 Effect of VEL on the PK of Co-administered Drugs in Healthy Subjects

Change in PK Parameter	GS-US-281-0101[a]		GS-US-281-1058[b]		
			Norgestimate/Ethinyl Estradiol		
	SOF	GS-331007	ethinyl estradiol	norgestrel	norelgestromin[c]
AUC_{inf} or AUC_{tau}	↑138%	↔	↔	↔	↔
C_{max}	↑81%	↓36%	↑39%	↔	↔
C_{tau}	ND	ND	↓17%	↔	↔

GS-US-281-0115[b]				
	Digoxin	Pravastatin	Rosuvastatin	Cyclosporine A
AUC_{inf}	↑34%	↑35%	↑169%	↓12%
C_{max}	↑88%	↑28%	↑161%	↔
C_{tau}	ND	ND	ND	ND

a VEL dose = 150 mg b VEL dose = 100 mg c pharmacologically active metabolite of norgestimate

ND = not determined

90% CIs of the GLSM ratio were within (↔), extended above (↑), or extended below (↓) the predetermined lack of PK alteration boundaries of 70% to 143% except for digoxin and CsA (80% to 125%).

SOF/VEL has been studied in several combinations of antiretroviral treatments. Summaries of the effect of SOF/VEL 400/100 mg on the PK of HIV ARV regimens in healthy subjects are given in Table 30. The exposure of tenofovir (TFV) increased in all cohorts with the highest increase with the EFV/FTC/TDF combination.

Sofosbuvir

Effects of other medical products on the pharmacokinetics of sofosbuvir

Renal secretion is involved in the elimination of GS-566500 and GS-331007. The transporter(s) involved are unknown, but it has been shown that GS-331007 is not a substrate for renal transporters OAT1 or 3, OCT2, MRP2, P-gp, BCRP or MATE1. Sofosbuvir is a substrate of P-gp and BCRP, but not a substrate for hepatic uptake transporters, OATP1B1, OATP1B1B3, or OCT1.

A 600 mg single dose of CsA had a large effect on SOF exposure with a 4.5-fold increase. However, the exposure to GS-331007 was not statistically different. Tacrolimus did not affect exposure to SOF or its metabolites.

The in vivo effect on SOF exposure of the strong P-gp inducer rifampicin was significant. Medicinal products that are potent P gp inducers (e.g. rifampicin, rifabutin, St. John's wort, carbamazepine, phenobarbital and phenytoin) are contraindicated with sofosbuvir (Sovaldi®, Post Approval Measurement EMEA/H/C/002798/II/0018). In addition, medicinal products that are moderate P gp inducers (e.g. oxcarbazepine and modafinil) are not recommended to be co-administered with sofosbuvir.

Effects of sofosbuvir on the pharmacokinetics of other medical products

Sofosbuvir and its metabolite, GS-331007 did not show detectable in vitro inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6, CYP3A or UGT1A1.

Induction of CYP3A4 and CYP2B6 was observed in vitro. An in vivo DDI study with oral contraceptives co-administered with sofosbuvir for 7 days did not show any sign of reduced exposure.

Sofosbuvir and GS 331007 are not inhibitors of drug transporters P-gp, BCRP, MRP2, BSEP, OATP1B1, OATP1B3 and OCT1. GS 331007 is not an inhibitor of OAT1, OCT2, and MATE1.

DDI studies have been performed in healthy volunteers and patients to evaluate effect of SOF on the PK of methadone, CsA and tacrolimus. Further, the effect of these medications on the PK of SOF and its metabolites has been evaluated. Methadone exposure was unaffected by SOF as were exposures to CsA and tacrolimus, although C_{max} for tacrolimus was decreased by almost 30%.

Table 30: Summary of PK parameters of antiretroviral treatments when ARVs were administered alone compared with administration of SOF/VEL + ARVs

GS-US-342-1167

Change in PK Parameter	EFV/FTC/TDF			FTC/RPV/TDF				E/C/F/TAF				
	EFV	FTC	TFV	RPV	FTC	TFV	DTG	EVG	COBI	FTC	TFV	TAF
AUC_{tau}	↔	↔	↑81%	↔	↔	↑40%	↔	↔	↔	↔	↔	↔
C_{max}	↔	↔	↑77%	↔	↔	↑44%	↔	↔	↔	↔	↔	↓20%
C_{tau}	↔	↔	↑121%	↔	↔	↑84%	↔	↔	↑103%	↔	↔	NC

GS-US-342-1326

	EVG/COBI/FTC/TDF				DRV/r+FTC/TDF				ATV/r+FTC/TDF				LPV/r+ FTC/TDF				RAL+FTC/TDF		
	EVG	COBI	FTC	TFV	DRV	RTV	FTC	TFV	ATV	RTV	FTC	TFV	LPV	RTV	FTC	TFV	RAL	FTC	TFV
AUC_{tau}	↔	↔	↔	↔	↔	↔	↔	↑39%	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑40%
C_{max}	↔	↔	↔	↑36%	↔	↔	↔	↑55%	↔	↔	↔	↑55%	↔	↔	↔	↑42%	↔	↔	↑46%
C_{tau}	↔	↑71%	↔	↑45%	↔	↔	↔	↑52%	↑39%	↑29%	↔	↑39%	↔	↔	↔	↔	↓21%	↔	↑70%

ATV = atazanavir; COBI = cobicistat; DRV = darunavir; DTG = dolutegravir; E/C/F/TAF = elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide fumarate (coformulated); EFV = efavirenz; EVG = elvitegravir; FTC = emtricitabine; LPV = lopinavir; NC = not calculated; RAL = raltegravir; RPV = rilpivirine; /r = boosted with ritonavir;

RTV = ritonavir; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate; TFV = tenofovir.

Ninety percent CIs of the GLSM ratio were within (↔), extended above (↑), or extended below (↓) the predetermined lack of PK alteration boundaries of 70% to 143% (except for RAL: 50% to 200 %) for Studies GS US 342 1167 and GS US 342-1326.

2.4.3. Discussion on clinical pharmacology

Discussion on clinical pharmacokinetics

Basic pharmacokinetic characteristics for velpatasvir such as absorption, distribution and elimination have been sufficiently investigated, however the Applicant committed to perform and provide a few studies post-authorisation.

Pharmacokinetics

The velpatasvir PK is considered straightforward. The mean faecal and urinary recovery of [^{14}C]-radioactivity was 94% and 0.4%, respectively. Velpatasvir was the major compound found in faeces accounting for on average 77% of the administered dose and it is unknown if this is unabsorbed velpatasvir or excreted via bile. The elimination of VEL is not fully understood, partly due to that the bioavailability is unknown. Two scenarios are considered: The first scenario being if the fraction absorbed is >25%, only unchanged parent compound via bile will be considered a major elimination pathway. This is the case since the largest metabolic pathway (M18) under these circumstances will only be responsible for maximum 23.6% (5.9%/25%) of the VEL elimination. A second scenario is if the fraction absorbed is less than 25%, then both unchanged parent compound eliminated via bile and metabolism to M18 are considered as important elimination pathways (i.e. contributes to ≥25% of drug elimination). The Applicant has not provided unambiguous data to show that the fraction absorbed (fa) is >25%. However there is evidence indicating that fa is >25% as radioactivity excreted after 48 h can be considered systematically available. Based on mean data approximately at least 35% was excreted >48 h after administration. Also, velpatasvir has been shown to be a substrate of P-gp and BCRP and in vivo inhibition with e.g. cyclosporine or ATR/r resulted in a 2 to 2.4-fold increase in exposure. Even so, it cannot be concluded that biliary excretion of parent is the only major elimination pathway. Therefore it the suggested investigations to identify the enzyme responsible for formation of M18 is supported.

Special populations

Renal Impairment

The exposure (AUC) of VEL was ca. 50% higher, with similar Cmax, in the subjects with severe renal impairment as compared to subjects with normal renal function. This was somewhat unexpected since renal elimination of VEL is negligible. However, the change in exposure due to renal impairment may be a result of renal impairment affecting VEL intestinal and hepatic metabolism and/or transport. The VEL half-life was not altered by renal impairment which indicates that the increase in exposure is due to an alteration in the absorption/first-pass process. Independent of the reason for increased exposure of VEL in severe renal impaired subjects, the change is not considered clinically relevant as the safety margin for VEL is wide and therefore no dose adjustments for patients with any grade of renal impairment are needed. This is adequately reflected in the SmPC. Of note, the efficacy and safety for sofosbuvir have not been established in patients with severe renal impairment (eGFR <30 ml/min/1.73 m^2) and end stage renal disease (ESRD).

Hepatic impairment

For the total plasma concentration, AUC is marginally affected by grade of hepatic impairment (HI) and C_{max} is lowered. The half-life of VEL increased in subjects with HI (from 18 h in HV to 31 h in severe HI) which indicates that the elimination of VEL is prolonged in subjects with HI. Unbound plasma concentration in subjects with hepatic impairment (HI) was determined in vitro and was found to be similar (0.2-0.3%) in plasma from in subjects with mild, moderate or severe HI compared to subjects with

normal liver function. No dose adjustment for patients with any grade of HI is needed of exposure reasons. The study data is adequately reflected in the SmPC.

Age, sex, race, body weight

Female subjects had a lower CL/F of VEL and SOF compared to male subjects, resulting in approximately 50% and 20%, respectively, higher exposure compared to male subjects. No apparent relation has been observed between adverse events and exposure. The increased exposure in women is therefore not considered clinically relevant. The Population PK did not suggest a significant effect of age, race and weight. The safety and efficacy of SOF/VEL in children and adolescents aged <18 years have not yet been established. The SOF/VEL FDC is not indicated in patients less than 18 years. These intrinsic factors have been reflected in the SmPC.

Drug- drug interactions

Effects of VEL and SOF/VEL on PK of other medical products

In vitro, velpatasvir showed no direct inhibition on the CYPs, however it has not been investigated whether velpatasvir is as a mechanism based inhibitor. The Applicant should perform an in vitro study to investigate if there is any inhibitory effect of pre-incubation with NADPH for all individual CYPs (i.e. the same CYPs as for competitive inhibition should be investigated) according to Guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/rev 1, 2012). The data from the in vitro study will be provided post approval and depending on outcome further DDI studies could be requested.

There was a clear in vitro induction signal for CYP3A4 (PXR) with gene expression increases of 2.7 to 30-fold in the hepatocyte lots investigated. No dedicated in vivo DDI was performed, however a DDI study with FTC/RPV/TDF is considered to be relevant, as RPV is a relatively sensitive CYP3A4 substrate and it is unlikely that FTC or TDF has an effect on RPV exposure. The duration of VEL dosing was 8 days and is short, but sufficient to detect any clinically relevant induction potential. No overt treatment sequence effect was observed. Although, the DDI study was not optimally designed to investigate CYP induction, the data indicate that the risk of in vivo induction of PXR appears to be low.

In vitro, VEL showed a concentration-dependent inhibition of OATP1B1, OATP1B3, P-gp and BCRP and clinically relevant interactions between VEL and substrates of these transporters cannot be excluded. Clinical DDI studies with statins (pravastatin OATP1B1, MRP2, possibly CYP3A substrate; rosuvastatin OATP1B1/3 and BCRP substrate) and digoxin (P-gp substrate) were performed.

In vivo, the exposure was 1.4-fold higher and 2.7-fold for pravastatin and rosuvastatin, respectively, following co-administration with VEL, relative to statin administration alone. The similar median half-life for both pravastatin and rosuvastatin with and without VEL, indicates the lack of effect of VEL on the systemic clearance of the probe drugs. The in vivo increase of rosuvastatin exposure is likely mostly dependent on BCRP and/or OATP1B3 inhibition, since both pravastatin and rosuvastatin are suitable OATP1B1 probes and the effect on pravastatin was minor. In the SmPC no dose adjustment is recommended for pravastatin, while the highest recommended dose of rosuvastatin is 10 mg. These recommendations are acceptable. A general text is added in the SmPC regarding risk of increase in exposure of substrates of P-gp, BCRP and OATP1B1/3.

The exposure of digoxin, AUC and Cmax was 34% and 88% higher, respectively, following co-administration with VEL, relative to digoxin administration alone. In the SmPC caution and concentration monitoring is recommended as SOF/VEL may increase the digoxin concentration. This is acceptable. Dabigatran etexilate, which is the recommended in vivo probe to study intestinal P-gp inhibition, was not studied. Dabigatran etexilate has a narrow therapeutic interval and the SmPC recommendation is clinical monitoring and signs of bleeding and anaemia.

It can be concluded that VEL is an in vivo P-gp, BCRP and OATP1B inhibitor.

The effect of VEL on the PK of a representative hormonal oral contraceptive (OC) medication was studied. Similar systemic exposures of ethinyl estradiol, norelgestromin and norgestrel were achieved following OC co-administration with and without VEL. No loss in contraceptive efficacy containing norgestimate/ethinyl estradiol is expected based on PK and PD results. In the study of OC in combination with SOF the duration was only 7 days. The study is considered to be too short to fully exclude a minor induction, however no sign of reduced exposure was observed and a clinically relevant effect is not expected based on the data. This is adequately reflected in the SmPC.

SOF/VEL has been studied in several combinations of antiretroviral treatments. Summaries of the effect of SOF/VEL 400/100 mg on the PK of HIV ARV regimens in healthy subjects are given in Table 30. It is considered to be no clinically relevant change in exposure of EFV, RPV, DTG, EVG, FTC, COBI, or TAF when co-administered with SOF/VEL in the antiretroviral combinations. The exposure of tenofovir (TFV) increased in all cohorts with the highest increase with the EFV/FTC/TDF combination. A caution involving renal monitoring is included in the SmPC when TFV is part of the regimen.

Effects of other medical products on the PK of VEL and SOF/VEL

DDI studies with ketoconazole (P-gp and CYP3A4 inhibitor), single dose rifampicin (OATP1B1/3 inhibitor at single dose) and cyclosporine (multiple transporter inhibitor) have been performed, resulting in an increase of VEL AUC and C_{max} of 71% and 29%, 46% and 28%, 103% and 56%, respectively. The VEL half-life was similar when co-administered with rifampicin and CsA and was slightly prolonged with ketoconazole, i.e. both pre-systemic and systemic inhibition is likely involved in the latter case. The P-gp and CYP3A4 contributions cannot be separated as the inhibitor used is not selective and both are expressed in the liver and intestine. The in vivo results confirm that VEL is an OATP1B, P-gp/CYP3A4 substrate and that BCRP could be involved in the transport of VEL. The safety margin of velpatasvir is very wide and no safety issue is expected with this magnitude of increase in exposure. The data and recommendation are adequately reflected in the SmPC.

The solubility of VEL is pH dependent and therefore medical products that increase gastric pH are expected to decrease plasma concentration of VEL. When SOF/VEL was combined with famotidine simultaneously or staggered (12 hours after famotidine) the decrease in exposure was approximately 10-20% for both SOF and VEL. The SmPC includes the recommendation is that no dose adjustment is needed for famotidine up to 40 mg BID.

Sofosbuvir/Velpatasvir was co-administered with omeprazole 20 mg simultaneously in the fasting state, the decrease of SOF and VEL exposure (AUC) was 29% and 46%, respectively, and staggered (12 hours after omeprazole), 44% and 55% respectively. The decrease in SOF/VEL exposure in fasting state was considered to be large, which led to a new study; SOF/VEL was co-administered with omeprazole in the fed state. However, there is a caveat in the study design, namely that the reference treatment was given in the fasted state, while the test with PPI was given in the fed state. As both compounds VEL and SOF have a higher exposure in the fed state, the fasted state exposure is not a straightforward comparison. Administering SOF/VEL with food increases VEL exposure and in essence, PPI with food will diminish the decrease in absolute exposure terms. The Applicant presented data indicating that subjects with highest exposure in reference treatment showed the largest decrease in VEL exposure with PPI. As VEL shows a pH dependent solubility a mechanistic plausible explanation is that the patients with low exposure already had relatively high gastric pH and thus the pH was not changed to same extent as in a subject with lower gastric pH.

Since the Applicant has not clearly described the efficacious plasma levels, it is difficult to judge an acceptable magnitude of decrease in exposure. Consequently a cautious approach is needed regarding co-medication with PPIs to not compromise the efficacy and therefore co- medication with proton pump inhibitors is not recommended. If it is considered necessary to co-administer, then SOF/VEL should be

administered with food and taken 4 h before proton pump inhibitor (as studied) at max doses comparable to omeprazole 20 mg.

When velpatasvir was administered with repeated dosing (7 days) of rifampicin the resulting decrease in exposure was large (70-80% decrease). The duration of the rifampicin dosing was a bit short as the recommended duration to obtain full induction effect is 10-14 days. The effect of rifampicin was extensive also on the SOF exposure (decrease of 70-80% when studied as the mono-component Sovaldi®, EMEA/H/C/002798/II/0018). The large decrease in exposure for both SOF and VEL in combination with the short duration of dosing of rifampicin, i.e. the decrease of exposure of VEL could be even larger, should lead to a contraindication with strong P-pg inducers (e.g. rifampicin, rifabutin, St. John's wort, carbamazepine, phenobarbital and phenytoin). Also, medicinal products that are moderate P-gp inducers (e.g. oxcarbazepine and modafinil) are not recommended to be co-administered with SOF/VEL. This is reflected in the SmPC.

SOF/VEL (400/100 mg) has been studied in several combinations of antiretroviral treatments. Summaries of the effect of HIV ARV regimens on the SOF/VEL PK in healthy subjects are given in Table 30. The safety margin for VEL is wide and no dose adjustment of SOF/VEL is required for these six ARV combinations. This is adequately reflected in the SmPC. Ribavirin is commonly co-medicated with SOF/VEL. There is no clinically relevant effect on the exposure of SOF and VEL.

When SOF/VEL was administered with EFV/FTC/TDF the exposure of VEL decreased 50-60%. An effect on P-pg and possibly enzymes by EFV (inducer) is likely the mechanism for the change in exposure of VEL. As reflected in the SmPC co-administration of SOF/VEL with efavirenz containing regimens is not recommended.

Population PK

The popPK model for velpatasvir was overall able to describe the data and it can be concluded that the model could be used for predicting individual exposure and further used in PK/PD modelling. There were some issues with the PopPK model for sofosbuvir e.g. some model misspecification was observed in the VPCs, a high η shrinkage and high residual error. Although the popPK model is not optimal it is considered acceptable for describing the data. The SOF PopPK model can currently not be used for predictive purpose. The issue will not be further pursued, however if the Applicant wishes to use the popPK model for simulations and further product claims, an updated model is requested.

The pharmacodynamics of SOF has been well characterized and has been presented during the approval of Sovaldi (SOF as single agent). In brief summary, SOF has pangenotypic activity and carries a high resistance barrier. There seems to be only one key mutation (S282T) which has relevant impact on susceptibility. However, S282T has a profound effect on viral fitness, and mutant virus was shown to rapidly revert to WT virus when drug pressure is stopped SOF can therefore be used in re-treatment of patients who failed SOF-containing treatment.

VEL is highly potent in vitro against HCV genotype 1 to 6, with mean EC50 values ranging from 0.002 to 0.13 nM. In addition, VEL retains high potency against a broad range of NS5A polymorphisms observed across HCV genotypes 2a, 2b, 3a, and 4a, including the M31 polymorphism in NS5A, in genotype 2 virus. The majority of variants across genotypes 1-6 conferred a greater fold increase in EC50 to LDV and DCV than to VEL.

Y93H remains a challenge in specific genotypes; this variant showed 46-fold reduced susceptibility in genotype 2a (seemingly without relevance), very high fold changes in genotypes 1a (609), 2b (4582), and 3a (724), but <3.3-fold resistance in genotype 1b and 4a. Considering the prevalence of Y93H in different genotypes, the clinical relevance in patients without prior NS5A therapy seems restricted to genotype-3 infection.

Three-day VEL monotherapy resulted in 3-4 log reductions in viral load. Treatment-emergent NS5A RAVs were seen in the majority of viral isolates post baseline. Whether this is an effect of de novo resistance development, or rather an effect selection effect where pre-existing variants are seen when wild type virus is cleared is hard to tell.

In longer-term follow-up, Y93H persisted in patients with genotype 1a, 1b, and 3-virus, after only three days of monotherapy with therapeutic doses.

The Y93H mutation in genotype-3 infection is the only naturally occurring NS5A RAV that had a relevant impact on treatment outcome with SOF/VEL. This RAV is seen at baseline in slightly less than 10% of genotype-3 isolates in previously untreated patients, and has apparently no relevant impact on viral fitness. Y93H is universally found in genotype-3 infected patients who failed therapy with SOF + an NS5A-inhibitor, VEL included. This is of great clinical importance when discussing re-treatment options, in particular for patients with cirrhosis, where a delayed effective re-treatment may have severe consequences. At present, the issue in practice mostly concern genotype-3 infected patients with cirrhosis. How to re-treat such patients after a failure with SOF/VEL is not so clear.

It was shown that NS5A RAVs (single and multiple) typically seen in genotype-1 infected patients failing available NS5A-containing with few exceptions have no relevant impact on velpatasvir susceptibility in vitro (genotype-1 virus). Furthermore, the cure rate with SOF/VEL therapy was in practice not affected by the presence of such NS5A RAVs in baseline virus in genotype-1 infected patients in the ASTRAL-1 study (discussed next section). In some cases effective re-treat options may be very limited, and SOF/VEL + RBV for a prolonged treatment duration of 24 weeks may therefore be a regimen that could be considered in such special cases in need of therapy. A proposal for wordings was given by the company, which is endorsed by the Rapporteurs. Of note, that regimen will be evaluated in an ongoing study (GS-US-342-1553).

2.4.4. Conclusions on clinical pharmacology

The CHMP considers the following measures necessary to address the issues related to pharmacology:

The clinical pharmacology data for sofosbuvir/velpatasvir FDC is considered acceptable. The CHMP recommended to investigate the mechanism based CYP inhibition caused by velpatasvir. The applicant has committed to undertake such investigation. In addition, the results from the already initiated study of investigating the responsible enzymes for the formation of the major metabolite M18 will be provided post-authorisation.

2.5. Clinical efficacy

The dose of SOF was 400 mg in all studies. Supportive studies for SOF as single agent is not discussed in this report.

SOF + VEL 25/100 mg +/- RBV was used in the phase 2 programme, while the fixed dose SOF/VEL (400/100 mg) was used in the four phase 3 studies. Dedicated studies were done for genotype 2 (presently lacking a RBV-free regimen), genotype 3 (most problematic genotype with IFN-free regimens) and for the special population with decompensated cirrhosis.

The total number of enrolled patients was 2603; 1302 in phase 3, 802 in phase 2 and 499 in phase 1.

Table 31: Clinical Studies that Support Efficacy for the SOF/VEL Clinical Program

Study Number	Study Design	Treatment Regimen	Subject Population		
			HCV Genotype (N)	Prior HCV Treatment	Cirrhosis Status
SOF+VEL Phase 2 Dose/Duration Evaluation and Efficacy Studies					
GS-US-342-0102	randomized, open-label	SOF + VEL 25/50 mg without RBV for 12 weeks, or +/- RBV for 8 weeks	1, 2, 3, 4, 5, or 6 GT 1: 175; GT 2: 124; GT 3: 54; GT 4: 14; GT 5: 1; GT 6: 9	TN	None had cirrhosis
GS-US-342-0109	randomized, open-label	SOF + VEL 25/50 mg +/- RBV for 12 weeks.	1 or 3 Genotype 1: 112; Genotype 3: 211	TE	~50% had cirrhosis
GS-US-337-0122 (ELECTRON-2; Cohort 4)	open-label	SOF + VEL 25/50 mg +/- RBV for 8 weeks.	3 (n=104)	TE	None had cirrhosis
SOF/VEL Phase 3 Efficacy Studies					
GS-US-342-1138 (ASTRAL-1)	randomized, double-blind, placebo-controlled	SOF/VEL or placebo for 12 weeks	1, 2, 4, 5, or 6 (GT 1: 393; GT 2: 125; GT 4: 138; GT 5: 35; GT 6: 49)	TN/TE	Up to 20% may have had cirrhosis
GS-US-342-1139 (ASTRAL-2)		SOF/VEL or SOF+RBV for 12 weeks	2 (n=266)		
GS-US-342-1140 (ASTRAL-3)		SOF/VEL 12 weeks or SOF+RBV 24 weeks	3 (n=552)		
GS-US-342-1137 (ASTRAL-4)	randomized, open-label	SOF/VEL 12 wks SOF/VEL+ RBV 12 wks SOF/VEL 24 weeks	1, 2, 3, 4, or 6 (Genotype 1: 207; Genotype 2: 12; Genotype 3: 39; Genotype 4: 8; Genotype 6: 1)	TN/TE	All had decompensated cirrhosis, Child Pugh B

2.5.1. Dose response studies

As a single-agent, the VEL PK/PD relationship for efficacy was examined in a Phase 1 study following administration of VEL monotherapy for 3 days to HCV infected subjects. Using PK and antiviral response data following VEL monotherapy, Emax modeling predicted VEL exposures at a 100-mg dose would achieve near maximal (99.5%) antiviral effect, and doses above 100 mg were considered unlikely to cause further meaningful reductions in HCV RNA.

Phase 2 studies evaluated SOF 400 mg with VEL 25 mg or 100 mg + /-RBV for 8 or 12 weeks. High SVR12 rates were achieved across all HCV genotypes in subjects receiving SOF 400 mg + VEL 100 mg for 12 weeks.

Study 342-0102 included TN patients without cirrhosis, infected with genotype 1-6. The study showed clearly that 8 weeks of therapy is not sufficient (genotype 1 and 2 tested), regardless addition of ribavirin.

With 12 weeks of SOF + VEL therapy there was no obvious difference in cure rates with VEL dosed 25 or 100 mg in this study.

Table 32: SVR12 by regimen and genotype, non-cirrhotic patients in study GS-US-342-0102

	GT1	GT2	GT3	GT4	GT5	GT6
SOF + VEL (dosed 25 or 100 mg) for 12 weeks						
25 mg	26/27	10/11	25/27	7/7	1/1	4/4
100 mg	28/28	10/10	25/27	6/7	-	5/5
SOF + VEL (dosed 25 or 100 mg) + RBV for 8 weeks						
25 mg	26/30	20/26				
100 mg	26/29	23/26				
SOF + VEL (dosed 25 or 100 mg) without RBV for 8 weeks						
25 mg	25/30	22/25				
100 mg	25/31	23/26				

In ELECTRON-2 cohort 4, the 8 week regimens with or without ribavirin was studied in non-cirrhotic genotype-3 infected patients, with a prior treatment failure.

Table 33: SVR12 rates in genotype-3 infected patients in GS-US-337-0122 (ELECTRON-2, cohort 4)

	8 weeks of SOF 400 mg +			
	VEL 25mg	VEL 25mg + RBV	VEL 100 mg	VEL 100 mg + RBV
Non-cirrhotic patients	27/27 (100.0%)	21/24 (87.5%)	26/27 (96.3%)	26/26 (100.0%)

GS-US-342-0109 included hard to cure genotype-3 infected patients. All patients were treatment experienced (i.e. selected for negative predictive factors for cure) and half of them had cirrhosis. In this study the need for (at least) a 100 mg dose is clearer.

Table 34: SVR12 rates in genotype-3 infected patients with or without cirrhosis in GS-US-342-0109

	12 weeks of SOF 400 mg +			
	VEL 25mg	VEL 25mg + RBV	VEL 100 mg	VEL 100 mg + RBV
Noncirrhotic patients	22/26 (84.6%)	27/28 (96.4%)	27/27 (100.0%)	26/26 (100.0%)
Cirrhotic patients	15/26 (57.7%)	21/25 (84.0%)	23/26 (88.5%)	25/26 (96.2%)

In summary, the results obtained in the phase 2 studies supported the evaluation of SOF/VEL 400/100 mg for 12 weeks in the phase 3 studies.

2.5.2. Main studies

ASTRAL-1 was placebo-controlled and blinded, while ASTRAL-2, -3 and -4 used active comparators and were of open-label design. In ASTRAL-1, -2 and -3 approximately 20% of patients could be treatment experienced and approximately 20% of patients could have compensated cirrhosis. ASTRAL-4 was conducted in patients with decompensated cirrhosis (CPT Class B).

Main exclusion criteria

- Prior exposure to SOF or other nucleotide analogue HCV NS5B inhibitor or any HCV NS5A Inhibitor
- Infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV)

Efficacy endpoints

The following efficacy endpoints were common to all phase 3 studies:

The primary efficacy endpoint was SVR12, defined as HCV RNA <LLOQ 12 weeks after discontinuation of the study drug, in all randomized and treated subjects (FAS = the full analysis set).

Secondary efficacy endpoints include:

- The proportion of subjects with: HCV RNA < LLOQ at 4 and 24 weeks after cessation of therapy (SVR4 and SVR24)
- The proportion of subjects with HCV RNA < LLOQ on treatment
- HCV RNA change from Baseline/Day 1
- The proportion of subjects with virologic failure
- Kinetics of circulating HCV RNA during treatment and after cessation of treatment
- Emergence of viral resistance to SOF and VEL during treatment and after cessation of treatment

ASTRAL-1, -2 and -3 share the following additional features:

Criteria used for cirrhosis determination:

A. Cirrhosis was defined as any 1 of the following:

- Liver biopsy showing cirrhosis (eg, Metavir score = 4 or Ishak score ≥ 5)
- FibroTest® score > 0.75 and an AST : platelet ratio index (APRI) > 2 during screening
- Fibroscan® with a result of > 12.5 kPa

B. Absence of cirrhosis was defined as any 1 of the following:

- Liver biopsy within 2 years of screening showing absence of cirrhosis
- FibroTest score ≤ 0.48 and APRI ≤ 1 performed during screening
- Fibroscan with a result of ≤ 12.5 kPa within ≤ 6 months of baseline/Day 1

In the absence of a definitive diagnosis of presence or absence of cirrhosis by FibroTest/APRI using the above criteria, a liver biopsy or Fibroscan was required. Liver biopsy results superseded FibroTest/APRI or Fibroscan results and were considered definitive.

Clinical hepatic decompensation (ie, ascites, encephalopathy or variceal hemorrhage) constituted an exclusion criterion.

The following laboratory parameters had to be fulfilled at screening:

- ALT and AST $\leq$ 10 x the upper limit of normal (ULN)
- Direct bilirubin $\leq$ 1.5 x ULN
- Platelets $\geq$ 50,000/microL
- Hemoglobin A1c (HbA1c) $\leq$ 8.5%
- Creatinine clearance (CLcr) $\geq$ 60 mL /min as calculated by the Cockcroft-Gault equation
- Hemoglobin $\geq$ 11 g/dL for female subjects; $\geq$ 12 g/dL for male subjects
- Albumin $\geq$ 3 g/dL
- International normalized ratio (INR) $\leq$ 1.5 × ULN unless subject had known hemophilia or was stable on an anticoagulant regimen affecting INR

GS-US-342-1138 (ASTRAL-1) - Study title: A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Investigate the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed Dose Combination for 12 Weeks in Subjects with Chronic HCV

Study participants

Patients were to be enrolled across 81 study sites in the United States (US), Canada, Europe, and Asia and were to have chronic genotype 1, 2, 4, 5, or 6 HCV infection.

Treatments

Patients were randomized in a 5:1 ratio in a double-blind manner to 1 of the following 2 treatment groups:

1) SOF/VEL 12 Week group (Group 1): SOF/VEL FDC (400/100 mg) tablet once daily for 12 weeks

2) Placebo 12 Week group (Group 2): SOF/VEL placebo tablet once daily for 12 weeks

Patients were stratified by HCV genotype (1, 2, 4, 6, and indeterminate) infection and the presence or absence of cirrhosis at screening. Patients with genotype 5 HCV infection were not randomized but were enrolled into the SOF/VEL 12 Week group.

Results

In ASTRAL-1, 625 patients were randomized to the different treatment groups of which 624 were treated. Of these 622/624 patients completed study treatment. In practice all patients came to the follow-up visits (including 12 weeks post therapy). Randomization to the placebo group comprised 116 patients, all of whom received placebo.

Demographics and baseline characteristics were generally balanced between the SOF/VEL and Placebo 12 Week groups. The majority of subjects were male (59.7%), white (78.8%), and non-Hispanic/Latino (94.6%), with a mean age of 54 years (range: 18 to 82). The mean (SD) baseline body mass index (BMI) was 26.6 (4.93) kg/m2 and 21.4% of patients had a BMI $\geq$ 30 kg/m2. Genotype 1, of which 2/3 of the patients had genotype 1a, dominated. The proportion of the cirrhotic subgroup varied between 10 and 23% in different genotypes. Between 55 and 93% of patients were treatment naïve, depending on genotype.

SVR12 rates were high and uniform across genotypes, irrespective of treatment experience, cirrhosis status or other baseline characteristics. All except 2 patients in the active groups who did not achieve SVR12 experienced virologic relapse.

Table 35: SVR12 Overall and by Genotype and Cirrhosis Status (Full Analysis Set; SOF/VEL 12 Week Group)

	Total (All Genotypes) (N = 624)	**GT1a (N = 210)**	**GT1b (N = 118)**	**GT1 Total (N = 328)**
Overall	618/624 (99.0%)	206/210 (98.1%)	117/118 (99.2%)	323/328 (98.5%)
Cirrhosis Yes	120/121 (99.2%)	49/49 (100.0%)	23/24 (95.8%)	72/73 (98.6%)
No	496/501 (99.0%)	157/161 (97.5%)	94/94 (100.0%)	251/255 (98.4%)
Missing	2/2 (100.0%)	0/0	0/0	0/0
	GT2 (N = 104)	**GT4 (N = 116)**	**GT5 (N = 35)**	**GT6 (N = 41)**
	104/104 (100.0%)	116/116 (100.0%)	34/35 (97.1%)	41/41 (100.0%)
Cirrhosis Yes	10/10 (100.0%)	27/27 (100.0%)	5/5 (100.0%)	6/6 (100.0%)
No	93/93 (100.0%)	89/89 (100.0%)	28/29 (96.6%)	35/35 (100.0%)
Missing	1/1 (100.0%)	0/0	1/1 (100.0%)	0/0

Prevalence of NS5A/NS5B RAVs and Impact on Treatment Outcome

Having results in mind, NS5A RAVs at baseline did not have a relevant impact on cure rates. Overall, a total of 257 of 616 patients (42%) in the Resistance Analysis Population had detectable (≥ 1%) NS5A RAVs at baseline and 255 of 257 patients (99.2%) achieved SVR12.

GS-US-342-1139 (ASTRAL-2) - Study title: A Phase 3, Multicenter, Randomized, Open-Label Study to Compare the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed Dose Combination for 12 Weeks with Sofosbuvir and Ribavirin for 12 Weeks in Subjects with Chronic Genotype 2 HCV Infection

Study participants

Patients with chronic genotype 2 HCV infection were to be enrolled across 51 sites in the United States.

Treatments

Approximately 240 patients were randomized (1:1) to 1 of the following 2 treatment groups:

- SOF/VEL 12 Week (Group 1): SOF/VEL FDC (400/100 mg) tablet once daily for 12 weeks
- SOF+RBV 12 Week (Group 2): SOF (400 mg) tablet once daily + RBV (1000 or 1200 mg/day divided twice daily) tablets for 12 weeks

Randomization was stratified by the presence or absence of cirrhosis at screening and prior treatment experience (treatment naive versus [vs] treatment experienced).

Results

In ASTRAL-2, 269 patients were randomized and 266 were treated. All but two patients completed study treatment. In practice all patients came to the follow-up visits (including 12 weeks post therapy).

Demographics and baseline characteristics were generally balanced across both treatment groups. Overall, the majority of patients were male (59.4%), white (88.3%), and non-Hispanic/Latino (79.3%),

with a mean age of 57 years (range: 23-81). The mean (SD) baseline body mass index (BMI) value for patients was 28.6 (6.08) kg/m2, and 32.7% of patients had a BMI $\geq$ 30 kg/m2.

Genotype 2b was predominant (78% of the patients). The cirrhotic subgroup constituted 14% of the patient population and approximately 15% of the patients were treatment naïve.

Table 36: SVR12 (Full Analysis Set)

	SOF/VEL 12 Weeks (N = 134)	**SOF+RBV 12 Weeks (N = 132)**	**SOF/VEL 12 Weeks vs SOF+RBV 12 Weeks Prop Diff (95% CI)**
Overall	133/134 (99.3%)	124/132 (93.9%)	5.2% (0.2% to 10.3%)
95% CI	95.9% to 100%	88.4% to 97.3%	
P-value	0.018		

SOF/VEL is concluded to be superior to SOF + RBV for the primary endpoint SVR12.

GS-US-342-1140 (ASTRAL-3) - Study title: A Phase 3, Multicenter, Randomized, Open-Label Study to Compare the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed Dose Combination for 12 Weeks with Sofosbuvir and Ribavirin for 24 Weeks in Subjects with Chronic Genotype 3 HCV Infection

Study participants

Patients with chronic genotype 3 infection were to be enrolled across 76 sites: 8 in Australia, 7 in Canada, 11 in France, 9 in Germany, 2 in Italy, 2 in New Zealand, 11 in the United Kingdom, and 26 in the United States.

Treatments

Patients were randomized (1:1) to 1 of the following 2 treatment groups:

- SOF/VEL 12 Week group (Group 1): SOF/VEL fixed-dose combination (FDC) (400/100 mg) tablet once daily for 12 weeks
- SOF+RBV 24 Week group (Group 2): SOF (400 mg) tablet once daily + RBV (1000 or 1200 mg/day divided twice daily) tablets for 24 weeks

Randomization was stratified by the presence or absence of cirrhosis at screening and prior treatment experience (treatment-naive versus [vs] treatment-experienced).

Results

A total of 552 patients were randomized and treated. In the SOF/VEL group 275/277 completed study treatment, as compared to 254/275 in the SOF + RBV group. Two patients in the SOF/VEL group discontinued. All but one of the patients in the SOF/VEL group came to the follow-up visits (including 12 weeks post therapy).

Demographics and baseline characteristics were generally balanced across both treatment groups. Overall, the majority of subjects were male (62.3%), white (88.6%), and non-Hispanic/Latino (95.8%), with a mean age of 50 years (range: 19-76). The majority of patients were from countries outside the US (78.3%). The mean (SD) baseline BMI value for patients was 26.5 (5.21) kg/m2, and 20.3% of patients had a BMI $\geq$ 30 kg/m2. Subtype 3a dominated, comprising 96% of patients in the SOF/VEL group and 91% in the SOF +RBV group. Thirty per cent of patients had cirrhosis and 26% were treatment-experienced.

In the subgroup with compensated cirrhosis, the mean (SD, Q1, Q3) baseline platelets were 145 (63.4, 100, 179). The same values for albumin were 3.9 (0.39, 3.7, 4.2) and for Fibroscan 22.5 (11.81, 14.3, 26.6).

SOF/VEL 12 W is concluded to be superior to SOF + RBV 24W for the primary endpoint (Table 37).

Table 37: SVR12 Overall and by Baseline Disease Characteristics Subgroups (Full Analysis Set)

	SOF/VEL 12 Weeks (N=277)	**SOF+RBV 24 Weeks (N=275)**
Overall	264/277 (95.3%)	221/275 (80.4%)
P-value	< 0.001	
95% CI	92.1% to 97.5%	75.2% to 84.9%
Cirrhosis		
Yes	73/80 (91.3%)	55/83 (66.3%)
95% CI	82.8% to 96.4%	55.1% to 76.3%
No	191/197 (97.0%)	163/187 (87.2%)
Prior HCV Treatment Experience		
Treatment-Naive	200/206 (97.1%)	176/204 (86.3%)
Treatment-Experienced	64/71 (90.1%)	45/71 (63.4%)

Although numbers are limited, there seemed to be a trend towards an increased risk of relapse in patients with more severe (yet compensated cirrhosis); 4/25 in those with a scanning values >20 kPa versus 0/32 in those with a value <20 kPa. This is to be expected, having results in ASTRAL-4 in mind.

Table 38: SVR12 by Liver Disease Severity (Cirrhotic Patients Only) Full Analysis Set

	SOF/VEL 12 Weeks (N=80)	**SOF+RBV 24 Weeks (N=83)**
Overall	73/80 (91.3%)	55/83 (66.3%)
95% CI	82.8% to 96.4%	55.1% to 76.3%
Baseline Albumin (g/dL)		
<= 3.5	9/10 (90.0%)	11/16 (68.8%)
95% CI	55.5% to 99.7%	41.3% to 89.0%
> 3.5	64/70 (91.4%)	44/67 (65.7%)
95% CI	82.3% to 96.8%	53.1% to 76.8%
Baseline Platelets ($x10^3$/uL)		
<= 100	20/24 (83.3%)	11/20 (55.0%)
95% CI	62.6% to 95.3%	31.5% to 76.9%
> 100	53/56 (94.6%)	44/63 (69.8%)
95% CI	85.1% to 98.9%	57.0% to 80.8%
Fibroscan (kPa)		
>= 20 kPa	21/25 (84.0%)	22/32 (68.8%)
95% CI	63.9% to 95.5%	50.0% to 83.9%
< 20 kPa	32/32 (100.0%)	18/27 (66.7%)
95% CI	89.1% to 100.0%	46.0% to 83.5%

This latter issue was raised during the procedure, where the company was asked to discuss the adequacy of adding ribavirin when treating genotype-3 infected patients with severe, yet compensated, cirrhosis, having uncertain re-treatment options in mind in case of treatment failure with sof/vel. The company presented SVR12-data for those ASTRAL-3 patients who had a baseline Fibroscan value of >20 kPa (n=32). In summary, using Fibroscan values does not seem to be a valid decision tool for deciding what genotype-3 infected patients that may need the addition of ribavirin to minimize the risk for relapse.

Table 39: GS-US-342-1140: FibroScan Results and SVR12 for Subjects with FibroScan ≥ 20 kPa Treated with SOF/VEL in ASTRAL-3 (Full Analysis Set)

Subjects with SVR12		Subjects with SVR12	
Subject ID	FibroScan (kPA)	Subject ID	FibroScan (kPA)
02080-62099	75.0	01065-62504	24.5
02080-62121	46.4	04139-62031	24.5
05170-62320	45.0	06819-62061	23.9
05730-62252	45.0	05873-62131	22.5
00451-62230	42.8	01069-62349	21.8
00972-62146	35.3	00595-62473	21.1
05295-62183	35.3	00472-62434	20.6
01126-62321	33.8		

06819-62060	33.8	Subjects without SVR12	
01815-62172	27.7	Subject ID	FibroScan (kPA)
05294-62450	26.6	02080-62118	35.3
05868-62160	26.1	05873-62186	29.5
04421-62014	25.7	00529-62069	29.1
00519-62032	24.8	00472-62512	21.1

It was also explored whether traditional parameters, s-albumin and thrombocytes, were useful predictors for the risk of relapse in cirrhotic patients who were treated with SOF/VEL in ASTRAL-3. That was not the case. Since ribavirin added considerably to the efficacy in genotype-3 infection in decompensated patients in ASTRAL-4 (discussed below), the Rapporteurs concluded that ribavirin should be considered also in the setting of compensated cirrhosis, in genotype-3 infection.

Prevalence of NS5A RAVs and Impact on Treatment Outcome

Overall, a total of 43 of 274 patients (16%) in the SOF/VEL 12 Week group with NS5A deep sequencing data had detectable (≥ 1%) NS5A RAVs at baseline.

Baseline presence of the Y93H RAV had a clear impact on treatment outcome, in particular in cirrhotic patients (albeit based on small numbers).

Table 40: SVR12 in Patients with and without baseline Y93H, 1% Cut-off (Full Analysis Set)

	SOF/VEL 12 Weeks		
	All Subjects (N=277)	**Cirrhotic (N=80)**	**Non-Cirrhotic (N=197)**
Overall	264/277 (95.3%)	73/80 (91.3%)	191/197 (97.0%)
95% CI	92.1% to 97.5%	82.8% to 96.4%	93.5% to 98.9%
SVR with Y93H	21/25 (84.0%)	**2/4 (50.0%)**	**19/21 (90.5%)**
95% CI	63.9% to 95.5%	6.8% to 93.2%	69.6% to 98.8%
SVR without Y93H	242/251 (96.4%)	**71/76 (93.4%)**	**171/175 (97.7%)**
95% CI	93.3% to 98.3%	85.3% to 97.8%	94.3% to 99.4%
SVR with 'not determined' Y93H	1/1 (100.0%)	0/0	1/1 (100.0%)
95% CI	2.5% to 100.0%		2.5% to 100.0%

Baseline NS5B RAVs did not impact treatment outcome in the SOF/VEL group, while they appear to impact treatment with SOF/RBV (50% SVR12 in 8 patients with NS5B RAVS as compared to 86% in patients without).

Virologic failures in the SOF/VEL group

As previously discussed, the Y93H mutation was a universal finding at time of failure.

Table 41: GT3 patients with Virologic Failure Following Treatment with SOF 400mg/VEL 100 mg for 12 Weeks

Subject	Cirrhosis	Baseline albumin (g/dL)	Baseline platelets (x10^3/uL)	Prior treatment experience	Baseline NS5A RAV (%)	Virologic Failure NS5A RAV (%)
04472-62202	Yes	4.1	159	PEG+RBV	A30K (> 99%)	A30K (> 99%), Y93H (97.2%)
03314-62107	No	4.6	204	PEG+RBV	Y93H (2.8%)	Y93H (> 99%)
00529-62147	Yes*	4.2	100	Naive	Y93H (> 99%)	Y93H (> 99%)
01589-62011	No	4.4	271	Naive	Y93H (> 99%)	Y93H (> 99%)
00472-62512	Yes	3.8	158	Naive	Y93H (15.2%)	Y93H (> 99%)
00529-62069	Yes	4.0	57	PEG+RBV	None	Y93H (> 99%)
01065-62502	No	3.7	125	PEG+RBV	None	Y93H (> 99%)
02080-62118	Yes	4.1	90	PEG+RBV	None	Y93H (> 99%)
05873-62186	Yes	3.4	98	PEG+RBV	None	Y93H (> 99%)
05730-62185	Yes	3.8	109	Naive	None	Y93H (> 99%)
01069-62225	No	3.9	172	PEG+RBV	None (GT3a)	None (GT1a reinfection)

GS-US-342-1137 (ASTRAL-4) - Study Title: A Phase 3, Multicenter, Open-Label Study to Investigate the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed-Dose Combination in Patients with Chronic HCV Infection and Child-Pugh Class B Cirrhosis

Study participants

Patients with chronic HCV infection (genotypes 1-6) were to be enrolled across 47 sites in the United States (US).

Important inclusion criteria

- Confirmation of cirrhosis by any 1 of the following methods:
    - Liver biopsy showing cirrhosis (eg, Metavir score = 4 or Ishak score ≥5)
    - Fibroscan (in countries where locally approved) showing cirrhosis or results > 12.5 kPa
    - FibroTest® score of > 0.75
- Confirmed CPT class B (7–9) at screening
- Additional criteria shared across all phase 3 studies are described in the beginning of Main study section in this document.

Important exclusion criteria

- Haematological and biochemical parameters, including the following:
    - Haemoglobin < 10 g/dL
    - Platelets ≤ 30,000/mm^3
    - ALT, AST, or ALP ≥ 10 x ULN
    - Sodium < 125 mEq/L
    - Total bilirubin > 5 mg/dL
    - Creatinine clearance (CLcr) < 50 mL/min as calculated by the Cockcroft-Gault equation

- Additional criteria shared across all phase 3 studies are described in the beginning of Section 3.4 in this document.

Treatments

Participants were randomized (1:1:1) to 1 of the following 3 treatment groups:

- SOF/VEL 12 Week group (Group 1): SOF/VEL FDC (400/100 mg) tablet once daily for 12 weeks
- SOF/VEL+RBV 12 Week group (Group 2): SOF/VEL FDC tablet once daily + RBV (1000 or 1200 mg/day divided twice daily) tablets for 12 weeks
- SOF/VEL 24 Week group (Group 3): SOF/VEL FDC tablet once daily for 24 weeks

Randomization was stratified by HCV genotype (1, 2, 3, 4, 5, 6, and indeterminate).

Endpoints

In addition to the endpoints described for the ASTRAL 1-3 studies, changes in CPT and MELD Scores constituted a secondary endpoint.

Results

Demographics and baseline disease characteristics were generally balanced across all treatment groups. The majority of patients were male (69.7%), white (89.5%), and non-Hispanic/Latino (85.4%), with a mean age of 58 years (range: 40-73). The mean (SD) baseline BMI value for patients was 30.4 (6.74) kg/m2, and 42.3% of patients had a BMI $\geq$ 30 kg/m2.

Table 42: Baseline Disease Characteristics (Safety Analysis Set)

Disease Characteristics	**SOF/VEL 12 Weeks (N = 90)**	**SOF/VEL +RBV 12 Weeks (N = 87)**	**SOF/VEL 24 Weeks (N = 90)**	**Total (N = 267)**
HCV Genotype, n (%)				
Genotype 1	68 (75.6%)	68 (78.2%)	71 (78.9%)	207 (77.5%)
Genotype 1a	50 (55.6%)	54 (62.1%)	55 (61.1%)	159 (59.6%)
Genotype 1b	18 (20.0%)	14 (16.1%)	16 (17.8%)	48 (18.0%)
Genotype 2	4 (4.4%)	4 (4.6%)	4 (4.4%)	12 (4.5%)
Genotype 3	14 (15.6%)	13 (14.9%)	12 (13.3%)	39 (14.6%)
Genotype 4	4 (4.4%)	2 (2.3%)	2 (2.2%)	8 (3.0%)
Genotype 6	0	0	1 (1.1%)	1 (0.4%)
Baseline HCV RNA Category, n (%)				
≥ 800,000 IU/mL	59 (65.6%)	45 (51.7%)	45 (50.0%)	149 (55.8%)
Prior HCV Treatment Response by Prior HCV Treatment, n (%)				
Treatment-Experienced	58/90 (64.4%)	47/87 (54.0%)	42/90 (46.7%)	147/267 (55.1%)
Baseline CPT Score Category, n (%)				
CPT A [5-6]	3 (3.3%)	6 (6.9%)	7 (7.8%)	16 (6.0%)
CPT B [7-9]	86 (95.6%)	77 (88.5%)	77 (85.6%)	240 (89.9%)
CPT C [10-12]	1 (1.1%)	4 (4.6%)	6 (6.7%)	11 (4.1%)
Baseline MELD Score Category, n (%)				
< 10	36 (40.0%)	29 (33.3%)	26 (28.9%)	91 (34.1%)
10-15	50 (55.6%)	54 (62.1%)	59 (65.6%)	163 (61.0%)
16-20	3 (3.3%)	4 (4.6%)	5 (5.6%)	12 (4.5%)
21-25	1 (1.1%)	0	0	1 (0.4%)
Baseline Ascites, n (%)				

None	16 (17.8%)	22 (25.3%)	15 (16.7%)	53 (19.9%)
Mild/Moderate	72 (80.0%)	61 (70.1%)	74 (82.2%)	207 (77.5%)
Severe	2 (2.2%)	4 (4.6%)	1 (1.1%)	7 (2.6%)
Baseline Encephalopathy, n (%)				
None	38 (42.2%)	33 (37.9%)	31 (34.4%)	102 (38.2%)
Grade 1−2	52 (57.8%)	54 (62.1%)	59 (65.6%)	165 (61.8%)
Grade 3−4	0	0	0	0

Despite their decompensated liver disease the vast majority of patients completed study treatment. Very few discontinuations were considered to be due to the study drugs.

Table 43: Subject Disposition (Screened Patients)

n (%)	SOF/VEL	SOF/VEL +RBV	SOF/VEL	Total
Subjects Screened				438
Subjects Not Randomized				170
Subjects in Safety Analysis Set	90	87	90	267
Subjects in PK Analysis Set	90	87	90	267
Subjects in PK Substudy Analysis Set	14	9	14	37
Study Treatment Status				
Completed Study Treatment	89 (98.9%)	82 (94.3%)	84 (93.3%)	255 (95.5%)
No FU-4 HCV RNA Assessment	1	1	0	2
With FU-4 but No FU-12 HCV RNA Assessment	3	0	2	5
Discontinued Study Treatment	1 (1.1%)	5 (5.7%)	6 (6.7%)	12 (4.5%)
No FU-4 HCV RNA Assessment	0	2	3	5
With FU-4 but No FU-12 HCV RNA Assessment	0	0	0	0
Reason for Premature Discontinuation of Study Treatment				
Adverse Event	1 (1.1%)	4 (4.6%)	4 (4.4%)	9 (3.4%)
Lack of Efficacy	0	1 (1.1%)	1 (1.1%)	2 (0.7%)
Noncompliance with Study Drug	0	0	1 (1.1%)	1 (0.4%)

Overall 12 weeks of therapy that included RBV yielded the highest cure rates. This was the case for genotype-1 infection, and in particular for genotype-3 infection. Hence, the addition of RBV had a clearly higher impact on efficacy (lowering the risk for relapse) than prolonging therapy of SOF/VEL to 24 weeks. Relapse in practice constituted the all virologic failures.

Patients with genotypes 2 and 4 did well with all three regimens, however numbers are low and the data is only descriptive.

Table 44: SVR12 and virologic failures by Genotype (Full Analysis Set)

	Total	GT-1a	GT-1b	GT-1 Total	GT-2	GT-3	GT-4	GT-6
SOF/VEL 12 Week								
SVR12	75/90 (83.3%)	44/50 (88.0%)	16/18 (88.9%)	60/68 (88.2%)	4/4 (100.0%)	7/14 (50.0%)	4/4 (100.0%)	0
95% CI	74.0% to 90.4%	75.7% to 95.5%	65.3% to 98.6%	78.1% to 94.8%	39.8% to 100.0%	23.0% to 77.0%	39.8% to 100.0%	–
Relapse	11/90 (12.2%)	3/50 (6.0%)	2/18 (11.1%)	5/68 (7.4%)	0/4	6/14 (42.9%)	0/4	0
Non-virologic failure	4/90 (4.4%)	3/50 (6.0%)	0/18	3/68 (4.4%)	0/4	1/14 (7.1%)	0/4	0
SOF/VEL+RBV 12 Week								
SVR12	82/87	51/54	14/14	65/68	4/4	11/13	2/2	0
95% CI	87.1% to	84.6% to	76.8% to	87.6% to	39.8% to	54.6% to	15.8% to	–
Relapse	2/85 (2.4%)	1/53 (1.9%)	0/14	1/67 (1.5%)	0/4	1/12 (8.3%)	0/2	0
Non-virologic failure	2/87 (2.3%)	2/54 (3.7%)	0/14	2/68 (2.9%)	0/4	0/13	0/2	0
SOF/VEL 24 Week								
SVR12	77/90	51/55	14/16	65/71	3/4	6/12	2/2	1/1
95% CI	76.6% to	82.4% to	61.7% to	82.5% to	19.4% to	21.1% to	15.8% to	2.5% to
Relapse	7/88 (8.0%)	2/55 (3.6%)	1/16 (6.3%)	3/71 (4.2%)	0/4	4/10 (40.0%)	0/2	0/1
Non-virologic failure	5/90 (5.6%)	2/55 (3.6%)	1/16 (6.3%)	3/71 (4.2%)	1/4 (25.0%)	1/12 (8.3%)	0/2	0/1

Changes in CPT stage and MELD score

Over the treatment course CPT stage improved in around half of patients overall. This was mainly driven by decreased bilirubin and increased albumin values. Patients in the small subgroup with MELD scores of 15 or more at baseline were most likely to experience MELD score improvements during the study period. Long-term impact on liver function will be followed in a registry study where all patients who achieved SVR12 are eligible.

Although deterioration in liver function was rare, around 10% of patients who were cured in fact had a worsened CPT score or a MELD score increasing by 2 points or more, and a few patients experienced a considerable increase in MELD score, despite the cure. The Applicant was asked to provide further analyses on the latter patients.

Table 45: MELD Change from Baseline at Posttreatment Week 12 (BL MELD < 15), ASTRAL 4

	SOF/VEL 12w		SOF/VEL+RBV 12w		SOF/VEL 24w		TOTAL	
-11	0/67		0/71		0/65		0/203	
-8	0/67		0/71		0/65		0/203	
-7	2/67	(3.0%)	0/71		1/65	(1.5%)	3/203	(1.5%)
-6	0/67		2/71	(2.8%)	0/65		2/203	(1.0%)
-5	3/67	(4.5%)	2/71	(2.8%)	4/65	(6.2%)	9/203	(4.4%)
-4	3/67	(4.5%)	1/71	(1.4%)	0/65		4/203	(2.0%)
-3	4/67	(6.0%)	4/71	(5.6%)	6/65	(9.2%)	14/203	(6.9%)
-2	13/67	(19.4%)	10/71	(14.1%)	9/65	(13.8%)	32/203	(15.8%)
-1	15/67	(22.4%)	15/71	(21.1%)	11/65	(16.9%)	41/203	(20.2%)
0	17/67	(25.4%)	10/71	(14.1%)	16/65	(24.6%)	43/203	(21.2%)
1	4/67	(6.0%)	12/71	(16.9%)	12/65	(18.5%)	28/203	(13.8%)
2	3/67	(4.5%)	11/71	(15.5%)	5/65	(7.7%)	19/203	(9.4%)
3	2/67	(3.0%)	0/71		0/65		2/203	(1.0%)
4	0/67		3/71	(4.2%)	1/65	(1.5%)	4/203	(2.0%)
7	1/67	(1.5%)	0/71		0/65		1/203	(0.5%)
11	0/67		1/71	(1.4%)	0/65		1/203	(0.5%)

Table 46: MELD Change from Baseline at Posttreatment Week 12 (BL MELD > 15), ASTRAL 4

	SOF/VEL 12w		SOF/VEL+RBV 12w		SOF/VEL 24w		TOTAL	
-11	1/6	(16.7%)	0/10		0/10		1/26	(3.8%)
-8	0/6		1/10	(10.0%)	0/10		1/26	(3.8%)
-7	0/6		0/10		0/10		0/26	
-6	0/6		0/10		1/10	(10.0%)	1/26	(3.8%)
-5	0/6		0/10		2/10	(20.0%)	2/26	(7.7%)
-4	1/6	(16.7%)	1/10	(10.0%)	2/10	(20.0%)	4/26	(15.4%)
-3	1/6	(16.7%)	2/10	(20.0%)	2/10	(20.0%)	5/26	(19.2%)
-2	1/6	(16.7%)	0/10		0/10		1/26	(3.8%)
-1	2/6	(33.3%)	3/10	(30.0%)	2/10	(20.0%)	7/26	(26.9%)
0	0/6		2/10	(20.0%)	0/10		2/26	(7.7%)
1	0/6		0/10		1/10	(10.0%)	1/26	(3.8%)
2	0/6		0/10		0/10		0/26	
3	0/6		1/10	(10.0%)	0/10		1/26	(3.8%)
4	0/6		0/10		0/10		0/26	
7	0/6		0/10		0/10		0/26	
11	0/6		0/10		0/10		0/26	

Changes in MELD (based on creatinine, bilirubin, and INR) and CPT scores (albumin, bilirubin, INR, ascites and encephalopathy), scores that are predicative of survival in the untreated, were further discussed during the procedure. Overall, scores generally improved or showed no change with the 24 weeks of follow-up that was available. Improvement was mainly driven by a decrease in bilirubin (both scoring systems). Median albumin levels were also improved (CPT). During 24 weeks of follow-up improvement of INR and the clinical parameters (ascites and encephalopathy) was less marked (Table 47).

Table 47: Change in CPT Score Parameters; All Subjects Who Achieved SVR in ASTRAL-4 (Full Analysis Set)

	Albumin	Bilirubin	INR	Ascites	Encephalopathy
Posttreatment Week 12 (N=236), n/N (%)					
Decreased score (Improvement)	79/229 (34.5%)	41/229 (17.9%)	5/229 (2.2%)	18/229 (7.9%)	12/229 (5.2%)
No change	138/229 (60.3%)	175/229 (76.4%)	221/229 (96.5%)	204/229 (89.1%)	209/229 (91.3%)
Increased score (Worsening)	12/229 (5.2%)	13/229 (5.7%)	3/229 (1.3%)	7/229 (3.1%)	8/229 (3.5%)
No assessment	7	7	7	7	7
Posttreatment Week 24 (N=236), n/N (%)					
Decreased score (Improvement)	84/213 (39.4%)	35/213 (16.4%)	5/213 (2.3%)	32/213 (15.0%)	20/213 (9.4%)
No change	115/213	172/213	202/213	173/213	188/213
Increased score (Worsening)	14/213 (6.6%)	6/213 (2.8%)	6/213 (2.8%)	8/213 (3.8%)	5/213 (2.3%)
No assessment	23	23	23	23	23

Note: Baseline frequency of ascites was: 20% none, 77% mild/moderate, 3% severe
Baseline frequency of encephalopathy was: 38% none, 62 % grade 1-2.

Prevalence of NS5A RAVs and Impact on Treatment Outcome

In genotype 1 HCV-infected patients, the SVR12 rates in patients with or without pre-treatment RAVs were similar in the SOF/VEL+RBV 12 Week group, in contrast to the SOF/VEL 12 and 24 Week groups, where patients with baseline RAVs had lower SVR12 rates (80% and 90%) compared to patients without RAVS (96% and 98%), respectively.

Interpretation of the results in patients with genotype 3 HCV infection is limited by the small number of patients with NS5A RAVs in each treatment group. In GT3 patients without baseline NS5A RAVs, SVR12 rates were superior in the SOF/VEL/RBV group (91%) compared to the two SOF/VEL groups (60 and 50%, in the 12 and 24 week groups, respectively).

All patients with genotype 2, 4, or 6 HCV infection achieved SVR12 irrespective of the presence of pre-treatment NS5A RAVs.

Patients with Virologic Failure

The majority of patients with virologic failure had genotype-3 infection and with the Y93H RAV detected posttreatment. None of the patients with virologic failure had pre-treatment NS5B RAVs.

Table 48: Patients with Virologic Failure by Treatment Group

Treatment Group	HCV GT	Prior treatment experience	Pre-treatment NS5A RAV (%)	Posttreatment NS5A RAV (%)	Posttreatment NS5B RAV (%)
SOF/VEL 12 Weeks	1a	Naive	M28V (6.1%)	None	None
	1a	PEG+RBV	None	Y93N (> 99%)	None
	1a	PEG+RBV	None	None	None
	1b	Naive	L31I (8.3%), L31M (2.6%),Y93H (60.0%)	L31M (89.8%), L31V (10.1%), Y93H (> 99%)	L159F (13.9%) S282T (3.6%)
	1b	PEG+RBV	Y93H (80.2%)	L31M (49.9%) L31V (49.8%) Y93H (>99%)	None
	3a	PEG+RBV	None	Y93H (> 99%)	None
	3a	PEG+RBV	None	Y93H (> 99%)	None
	3a	PEG+RBV	Y93H (>99%)	Y93H (> 99%)	L320I (1.1%)
	3a	Naive	None	Y93H (> 99%)	None
	3a	Naive	None	Y93H (> 99%)	None
	3a	PEG+RBV	Y93H (4.9%)	Y93H (> 99%)	None
SOF/VEL +RBV 12 Weeks	1a	PEG+RBV	None	None	None
	3a	Naive	None	Y93H (> 99%)	None
	3a	Naive	Y93H (2.9%)	Y93H (> 99%)	N142T (3.1%) E237G (2.3%)
SOF/VEL 24 Weeks	1a	Naive	None	Q30R (94.5%), H58D (94.5%), Y93N (4.2%)	None
	1a	Naive	Q30H (64.6%), Y93H (57.4%),Y93N (1.2%)	Q30H (> 99%), Y93H (> 99%)	L159F (96.3%) S282T (3.0%)
	1b	DAA+PEG+ RBV	L31M (> 99%)	L31M (97.9%), L31V (1.7%), Y93H (> 99%)	None
	3a	Naive	None	Y93H (97.8%)	E237G (1.5%)
	3a	PEG+RBV	None	Y93H (98.8%)	None
	3a	PEG+RBV	None	Y93H (98.9%)	None
	3a	PEG+RBV	None	M28T (2.2%), Y93H (> 99%)	None
	3a	PEG+RBV	None	Y93H (> 99%)	None

2.5.3. Discussion on clinical efficacy

The clinical development program follows the established principles in terms of efficacy endpoints as well as inclusion and exclusion criteria. The phase 3 program comprised four clinical studies: ASTRAL-1, -2 and -3 mainly included patients without cirrhosis, while approximately 20% of the participants had compensated cirrhosis. ASTRAL-4 was conducted in patients with decompensated cirrhosis.

The predominant HCV genotypes are well represented in the overall patient population. For example, ASTRAL-3 is by far the largest study to date in patients with genotype 3. Still, some subgroup analyses are hampered by low numbers of patients, in particular those with several baseline predictors of virologic failure (commented below).

Although the entry criteria used in the phase 3 studies are typical and accepted, the cirrhotic patients included in ASTRAL-1 – 3 may be considered to have fairly mild cirrhosis with modest baseline Fibroscan values in the majority of these patients. Consequently, patients with more severe (yet compensated) cirrhosis are in a way underrepresented in the study program.

The study in decompensated patients with CPT class B cirrhosis (ASTRAL-4) therefore serves as an important point of reference. In this study, SOF/VEL (without ribavirin) for 12 weeks yielded high efficacy outcomes in these very sick patients – with genotypes other than 3. Hence, ASTRAL-4 can be considered support for efficacy in severe compensated cirrhosis.

The choice of comparators for the individual studies is overall considered relevant.

Overall, premature discontinuation of study treatment was rare, illustrating the excellent tolerability and safety profile of the study regimes. Patients with decompensated cirrhosis had slightly higher rates, but this should be considered a reflection of their severe disease state rather than related to the study drugs (see the section on clinical safety).

Efficacy data in different genotypes

Table 49 shows the proportion of patients who achieved SVR12 following treatment with SOF/VEL for 12 weeks across all four phase 3 studies. For ASTRAL-4, both comparator arms are included. The recommended treatments for compensated and decompensated liver disease are highlighted.

Table 49: Pooled analysis – overall SVR12 rates [95% CI] and per genotype in phase 3 program

	Regime	GT 1	GT 2	GT3	GT4	GT5	GT6	Total
ASTRAL-1, -2 and -3 (compensated liver disease)								
Overall	SOF/VEL 12wk	323/328 (98.5%) [96.5-99.5%]	237/238 (99.6%) [97.7%-100.0%]	264/277 (95.3%) [92.1-97.5%]	116/116 (100.0%) [96.9-100.0%]	34/35 (97.1%) [85.1-99.9%]	41/41 (100.0%) [91.4-100.0%]	1015/1035 (98.1%) [97.0-98.8%]
No cirrhosis		251/255 (98.4%) [96.0-99.6%]	207/208 (99.5%) [97.4-100.0%]	191/197 (97.0%) [93.5-98.9%]	89/89 (100.0%) [95.9-100.0%]	28/29 (96.6%) [82.2-99.9%]	35/35 (100.0%) [90.0-100.0%]	801/813 (98.5%) [97.4-99.2%]
Compensated cirrhosis		72/73 (98.6%) [92.6-100.0%]	29/29 (100.0%) [88.1-100.0%]	73/80 (91.3%) [82.8-96.4%]	27/27 (100.0%) [87.2-100.0%]	5/5 (100.0%) [47.8%-100.0%]	6/6 (100.0%) [54.1-100.0%]	212/220 (96.4%) [93.0%-98.4%]
ASTRAL-4 (decompensated liver disease, Child Pugh B))								
Decompensated cirrhosis	SOF/VEL 12wk	60/68 (88.2%) [78.1-94.8%]	4/4 (100.0%) [39.8-100.0%]	7/14 (50.0%) [23.0-77.0%]	4/4 (100.0%) [39.8-100.0%]	-	0	75/90 (83.3%) [74.0-90.4%]
	SOF/VEL + RBV 12wk	65/68 (95.6%) [87.6-99.1%]	4/4 (100.0%) [39.8-100.0%]	11/13 (84.6%) [54.6-98.1%]	2/2 (100.0%) [15.8-100.0%]	-	0	82/87 (94.3%) [87.1-98.1%]
	SOF/VEL 24wk	65/71 (91.5%) [82.5-96.8%]	3/4 (75.0%) [19.4-99.4%]	6/12 (50.0%) [21.1-78.9%]	2/2 (100.0%) [15.8-100.0%]	-	1/1 (100%) [2.5%-100.0%]	77/90 (85.6%) [76.6-92.1%]

GT1

GT1 is well represented in the clinical development program. SVR12 rates in patients without as well as with compensated cirrhosis are high, including in patients with multiple baseline predictors of virologic failure. Patients with decompensated cirrhosis receiving 12 weeks SOF/VEL had an increased risk of virologic failure (7.4%) compared to patients with less severe liver disease. The addition of RBV, in contrast to prolonged SOF/VEL therapy, minimized the risk for relapse.

GT3

Patients with GT3 represent the biggest remaining challenge for SOF/VEL treatment. Virologic failures in patients without cirrhosis were infrequent (2.0%) but increased to 8.8% in the group with compensated cirrhosis. The same pattern was seen for baseline NS5A RAVs (2.6 vs 11.6%), treatment experience (1.9 vs 9.9%) and HCV ≥ 800,000 IU/ml (1.2 vs 5.2%).

In patients with combinations of these predictors SVR12 rates were reduced proportionally to the number of predictors present. In patients with cirrhosis and the NS5A resistance-associated variant Y93H only 2/4 achieved SVR12, in contrast to 19/21 of the non-cirrhotic subgroup carrying Y93H at baseline.

A treatment failure would be considered particularly problematic in patients with cirrhosis (compensated cirrhosis included), since re-treatment options for genotrype-3 infected patients who failed therapy with SOF/VEL are not so clear at present. The Applicant was not able to find cut off values for Fibroscan values, or baseline albumin/thrombocytes that were predictive for relapse in patients with compensated cirrhosis (ASTRAL-3). The results in ASTRAL-4 therefore favour the addition of ribavirin also in patients with compensated cirrhosis (GT-3), as an approach to minimize the risk for relapse, in the present lack of randomized data. A study is planned where SOF/VEL +/- ribavirin will be given for 12 weeks to genotype 3-infected patients with compensated cirrhosis (GS-US-342-2097, with preliminary results are expected by the end of 2017).

The relative vulnerability of genotype 3 is further pronounced in patients with CPT B decompensated cirrhosis (ASTRAL-4), where 6/14 (42.9%) GT3 patients in the SOF/VEL 12 week group experienced

relapse. GT3 patients with baseline NS5A RAVs were few in all treatment groups (totally 6) but outcomes in these patients indicate as expected a further increased risk of failure. The addition of RBV improves results considerably, reducing virologic failures in patients with GT3 to 15.4% (2/13 patients). The latter finding justifies a discussion regarding the adequacy of adding ribavirin to SOF/VEL also in patients with compensated cirrhosis of the higher magnitude.

The starting dose of RBV used in ASTRAL-4 was higher than used in previous studies with IFN-free regimens in decompensated patients. This is discussed in the section on clinical safety.

When summarizing efficacy yielded in genotype-3 infected patients, it is reasonable to believe that the addition of RBV to patients with compensated cirrhosis would optimize results. This issue should be further discussed (LoQ, Efficacy).

GT2, GT4, GT5, GT6

Results in patients without as well as with compensated cirrhosis are excellent: This includes subgroups of patients with multiple baseline factors that historically have been associated with high risk of virologic failure. In decompensated cirrhosis, the small number of patients complicates assessment. However, the complete lack of virologic failures among patients with GT2 (n=12), GT-4 (n=8) and GT-6 (n=1), across all treatment groups, adds further support to the 100% cure rates in patients with compensated cirrhosis, (including patients with multiple baseline predictors of virologic failure).

2.5.4. Conclusions on the clinical efficacy

SOF/VEL treatment for 12 weeks is a highly effective therapy for chronic HCV patients with or without compensated cirrhosis across all genotypes. In patients with liver decompensation, SOF/VEL + RBV results in unprecedented high cure rates (overall 94.3%), and that is the recommended regimen for all such patients (i.e. for all genotypes).

There is no clinical data for patients with Child Pugh C cirrhosis, or for patients with a prior liver transplant; such studies are planned, with an expected start in second half of 2016.

The remaining Achilles heel of 12 week SOF/VEL therapy concerns genotype-3 infected patients with negative predictors of cure (prior treatment experience and cirrhosis). Of note, there are limited re-treatment options for these patients, and a treatment failure in a cirrhotic patient should be considered a severe event. On the basis of available data, the CHMP recommended the addition of ribavirin in patients with compensated cirrhosis in genotype-3 infection.

2.6. Clinical safety

Patient exposure

SOF/VEL

A total of 2603 patients have received at least 1 dose of SOF/VEL or SOF+VEL, including 1302 patients in the phase 3 studies (ASTRAL 1 to 4), 802 patients in phase 2 studies, and 499 as part of phase 1.

SOF 400 mg + VEL 100 mg (separate or as fixed dose) administered for at least 12 weeks was given to 1539 patients, including 90 who received SOF/VEL for 24 weeks (ASTRAL 4), and 167 patients who received SOF/VEL+RBV for 12 weeks (ASTRAL-4 plus the phase 2 study GS US 342 0109), see Table 50.

The phase 2/3 program included 325 patients with compensated cirrhosis (phase 2 + ASTRAL 1-3) and another 267 patients with decompensated cirrhosis (ASTRAL-4).

The number of patients aged 65 years and above treated with SOF/VEL +/- RBV in the phase 3 studies (ASTRAL 1-4) was 156/1302 (12%).

Of note, a substantial number of patients have been exposed to SOF, as part of Sovaldi (SOF, first approval December 2013) and Harvoni (SOF/ledipasvir, first approval October 2014). In addition to large scale phase 3 studies for both products, the estimated accumulated post marketing exposure to Sovaldi was 160.000-320.000 patients by June 2015 (on the basis of 24 or 12 weeks of therapy). The predicated post marketing exposure of Harvoni was around 24.000 patient-years by April 2015, equivalent to well over 50,000 patients treated, and likely more close to 100.000 patients.

Table 50: SOF/VEL exposure in the clinical development program

Study	**Regimen**	**Total (N = 2603)**	**Number of cirrhotics**
Phase 3 Studies	SOF/VEL FDC		
ASTRAL-1	SOF/VEL for 12 weeks (single arm)	624	220 (compensated)
ASTRAL-2	SOF/VEL for 12 weeks (vs placebo)	134	
ASTRAL-3	SOF/VEL for 12 weeks (vs SOF + RBV)	277	
ASTRAL-4	SOF/VEL for ≥ 12 weeks	267	267 (decompensated, Child Pugh B)
	SOF/VEL for 12 weeks	90	
	SOF/VEL FDC + RBV for 12 weeks	87	
	SOF/VEL FDC for 24 weeks	90	
	Total	**1302**	
Phase 2 Studies	SOF + VEL		
GS-US-342-0102, GS-US-342-0109, GS-US-337-0122	SOF + VEL 100 mg ± RBV for 12 weeks	237	105 (compensated)
	SOF + VEL 100 mg for 12 weeks	157	
	SOF + VEL 100 mg + RBV for 12 weeks	80	
	SOF + VEL 25 mg ± RBV for 8 weeks	162	
	SOF + VEL 100 mg ± RBV for 8 weeks	165	
	SOF + VEL 25 mg ± RBV for 12 weeks	238	
	Total	**802**	**105**
Phase 1 Studies	SOF/VEL FDC		
GS-US-342-0104, GS-US-342-1167, GS-US-342-1326, GS-US-342-1346, GS-US-342-1709	SOF/VEL FDC (dosed to evaluate bioavailability, food effects, and DDIs with ARVs, PPIs, and H2RAs)	499	
	Total	**499**	
Total Exposure to SOF/VEL and SOF+VEL in Phase 1, 2, and 3 Clinical Studies		**2603**	**Compensated 325 Decompensated 267**

The ASTRAL 1-3 studies compared SOF/VEL 400/100 mg (without RBV) to control regimens (placebo and SOF + RBV) in patients without cirrhosis and in patients with compensated cirrhosis.

ASTRAL-4 concerns patients with decompensated cirrhosis (Child Pugh B). Child Pugh C patients and patients with a prior liver transplant were not included. On the basis of ASTRAL-4, the Applicant proposed that SOF/VEL + RBV 1000/1200 mg (arm 2 of the study) should be given to all patients with decompensated disease. Of note, this RBV starting dose is higher than that used in previous studies on patients with decompensated liver disease, where the RBV starting dose was 600 mg (Gilead´s SOLAR-1/2 studies and the ALLY-1 study of BMS).

Adverse events

There was no relevant difference in the frequency of reported AEs (all reported, or possibly related) in patients treated with SOF/VEL as compared to those treated with placebo in the ASTRAL 1-3 studies (Table 51).

For patients treated with SOF + RBV, control regimen in ASTRAL 2 (12 weeks, n=132) and ASTRAL-3 (24 weeks, n=275) a higher frequency of fatigue and anemia were the main differences as compared to the other regimens (SOF/VEL and placebo).

Table 51: AEs reported for ≥5% of patient for any treatment regimen*, ASTRAL 1-3.

	All reported		Possibly related	
Preferred Term	**SOF/VEL 12 Week (N = 1035)**	**Placebo 12 Week (N = 116)**	**SOF/VEL 12 Week (N = 1035)**	**Placebo 12 Week (N = 116)**
Numbers, (%) with Any AE	822 (79.4%)	89 (76.7%)	520 (50.2%)	52 (44.8%)
Headache	296 (28.6%)	33 (28.4%)	218 (21.1%)	25 (21.6%)
Fatigue	217 (21.0%)	23 (19.8%)	163 (15.7%)	18 (15.5%)
Nausea	135 (13.0%)	13 (11.2%)	98 (9.5%)	10 (8.6%)
Insomnia	87 (8.4%)	11 (9.5%)	56 (5.4%)	7 (6.0%)
Nasopharyngitis	121 (11.7%)	12 (10.3%)		
Diarrhoea	73 (7.1%)	8 (6.9%)		
Cough	57 (5.5%)	4 (3.4%)	4 (0.4%)	0
Irritability	49 (4.7%)	4 (3.4%)	36 (3.5%)	3 (2.6%)
Arthralgia	56 (5.4%)	9 (7.8%)		
Back pain	56 (5.4%)	11 (9.5%)		
Asthenia	58 (5.6%)	9 (7.8%)	41 (4.0%)	4 (3.4%)
Pruritus	33 (3.2%)	5 (4.3%)	23 (2.2%)	3 (2.6%)
Dizziness	44 (4.3%)	5 (4.3%)	31 (3.0%)	2 (1.7%)
Constipation	47 (4.5%)	3 (2.6%)		
Dyspepsia	33 (3.2%)	4 (3.4%)		
Abdominal pain	41 (4.0%)	2 (1.7%)		
Myalgia	38 (3.7%)	6 (5.2%)		
Vomiting	34 (3.3%)	1 (0.9%)		
Rash	33 (3.2%)	1 (0.9%)		
Anxiety	23 (2.2%)	1 (0.9%)		
Muscle spasms	29 (2.8%)	4 (3.4%)		
Decreased appetite	28 (2.7%)	5 (4.3%)		
Dyspnoea	20 (1.9%)	2 (1.7%)	12 (1.2%)	1 (0.9%)
Pyrexia	28 (2.7%)	2 (1.7%)		
Sleep disorder	16 (1.5%)	5 (4.3%)		
Dry skin	12 (1.2%)	0	5 (0.5%)	0
Disturbance in attention	19 (1.8%)	2 (1.7%)		
Anaemia	1 (<0.1%)	0	1 (< 0.1%)	0
Dyspnoea exertional	6 (0.6%)	2 (1.7%)		

* SOF + RBV control regimen not shown in this table.

In ASTRAL 1-3 AEs of grade 3 were reported for a total of 3% of patients treated with SOF/VEL (n=1035). It concerned headache (0.5%), anxiety 0.3%, acute myocardial infarction 0.2%, and common AEs at a frequency of <0.1% each.

Two patients (0.2%) in the SOF/VEL group had Grade 4 AEs (1 lung cancer, 1 who died in his sleep on posttreatment Day 8); both events were assessed as unrelated to study drug.

In ASTRAL-4, a similar pattern of common AEs were seen in the decompensated patients treated with SOF/VEL. AEs of grade 3 and 4 were more common in this treatment population, and clearly linked to the severe liver disease status per se (Table 52). Grade 3/4 events were not more frequent with the RBV-containing regimen, than with SOF/VEL alone.

Table 52: Grade 3 or 4 Adverse Events Reported for > 1 Patient, ASTRAL-4

Number (%) of Patients Experiencing	SOF/VEL 12 Weeks (N = 90)	SOF/VEL+RBV 12 Weeks (N = 87)	SOF/VEL 24 Weeks (N = 90)
Any Grade 3 or 4 AE	16 (17.8%)	11 (12.6%)	17 (18.9%)
Hepatic encephalopathy	2 (2.2%)	2 (2.3%)	1 (1.1%)
Sepsis	1 (1.1%)	3 (3.4%)	1 (1.1%)
Gastrointestinal haemorrhage	3 (3.3%)	0	0
Hepatocellular carcinoma	0	0	3 (3.3%)
Hyponatraemia	1 (1.1%)	2 (2.3%)	0
Nausea	1 (1.1%)	1 (1.1%)	1 (1.1%)
Acute kidney injury	0	1 (1.1%)	1 (1.1%)
Asthenia	0	2 (2.3%)	0
Gastric varices haemorrhage	1 (1.1%)	0	1 (1.1%)
Peritonitis bacterial	0	2 (2.3%)	0
Upper gastrointestinal haemorrhage	1 (1.1%)	0	1 (1.1%)
Vomiting	1 (1.1%)	1 (1.1%)	0

Serious adverse event/deaths/other significant events

SAEs

In ASTRAL 1-3 SAEs were reported in 2.2% (23 patients) in the SOF/VEL 12 Week group, none of which were assessed as related to study drug. The only SAEs reported for >1 patient treated with SOF/VEL concerned acute myocardial infarction in two patients, at days 10 and 24 days post treatment. Both patients had typical cardiovascular risk factors.

Also in ASTRAL-4, in practice all serious adverse events were not considered related to study drug (Table 53). One patient (03055-64017, SOF/VEL 24 weeks), experienced SAEs assessed as related to SOF/VEL by the investigator: this male patient had a prior history of varices, ascites, encephalopathy, and portal vein thrombosis. He experienced hepatorenal syndrome (Grade 4), peritonitis (Grade 3), sepsis (Grade 4), and hypotension (Grade 4) on Day 35 leading to study drug discontinuation. At the time of event the MELD score was 38, and underwent a liver transplantation on posttreatment Day 8. He subsequently achieved SVR12.

No other patient in ASTRAL-4 was transplanted during study or follow-up.

Table 53: Serious AEs reported in >1 patient in ASTRAL-4

Number (%) of Patients Experiencing	SOF/VEL 12 Weeks (N = 90)	SOF/VEL+RBV 12 Weeks (N = 87)	SOF/VEL 24 Weeks (N = 90)
Any SAE	17 (18.9%)	14 (16.1%)	16 (17.8%)
Hepatic encephalopathy	2 (2.2%)	2 (2.3%)	1 (1.1%)
Sepsis	1 (1.1%)	3 (3.4%)	1 (1.1%)
Gastrointestinal haemorrhage	3 (3.3%)	0	0
Hepatocellular carcinoma	0	0	3 (3.3%)
Hyponatraemia	1 (1.1%)	2 (2.3%)	0
Anaemia	1 (1.1%)	1 (1.1%)	0

Cellulitis	1 (1.1%)	1 (1.1%)	0
Escherichia infection	0	1 (1.1%)	1 (1.1%)
Gastric varices haemorrhage	1 (1.1%)	0	1 (1.1%)
Hip fracture	0	1 (1.1%)	1 (1.1%)
Nausea	2 (2.2%)	0	0
Seizure	1 (1.1%)	1 (1.1%)	0
Upper gastrointestinal haemorrhage	1 (1.1%)	0	1 (1.1%)
Urinary tract infection	0	2 (2.3%)	0

As mentioned in the efficacy section, around 10% of patients had a worsening of MELD/CPT score, despite curative therapy. A specific analysis, requested during the procedure, revealed no particular AEs suggestive of causality to therapy in these patients.

Deaths

In ASTRAL 1-3, three treatment-emergent and 3 non-treatment emergent deaths were reported, none consider related to therapy by the investigator. Two cases of treatment emergent deaths had no clear causative events; autopsies were not performed. Having the overall safety profile in mind, it is not likely that these deaths were related to therapy.

Treatment emergent	Therapy Patient ID	History
Yes	**SOF/VEL 12 wks** 01386-63561	55-year old male with dyslipidemia, treated with ezetimibe/simvastatin. Died at sleep, 8 days after the completion of the 12 weeks of therapy (no events or incidences during therapy).
	SOF+RBV 24 wks 04262-62067	Died from multiple gunshot wounds on day 74.
	SOF+RBV 24 wks 01154-62556	58-year-old female with ongoing depression. Found dead on day 141. Autopsy not performed. Death considered due to natural causes
No	**SOF/VEL 12 wks** 03054-65012	58-year-old male diagnosed with metastatic lung cancer (including brain) after end of therapy, died posttreatment day 112.
	SOF/VEL 12 wks 02111-65015	56-year-old female with a history of depression and drug abuse. Unwitnessed cardiac arrest at home on posttreatment day 130, died next day. Toxicology reports positive for opiates, benzodiazepines, and ethanol. Autopsy not performed.
	SOF+RBV 24 wks 3902-62126	66-year-old male with a history of myocardial ischemia, chronic obstructive pulmonary disease, and venous thromboembolism. Found dead at home on posttreatment Day 118. Autopsy determined the cause of death as epilepsy with coronary artery disease.

In ASTRAL-4 there were 9 deaths; 2 subjects died within 30 days of discontinuing study drug (ie, treatment-emergent death) and did not complete posttreatment visits, 4 subjects died prior to posttreatment Week 12 and 3 subjects died subsequent to posttreatment Week 12.

Treatment-emergent:

- **(1) Sepsis** (after 3 weeks of SOF/VEL + RBV)

69-yer old male patient, complicated hospitalization course with bacterial and fungal peritonitis, ischemic colitis, pneumoperitoneum, atrial fibrillation, and pneumonia.

- **(2) Acute myocardial infarction (**after 9 days of SOF/VEL).

52-year old male, long term smoker, no amiodarone therapy.

Non-treatment emergent

- **(3) Liver failure** (3 months after fulfilling 12 weeks SOF/VEL+RBV).

55-year-old female patient underwent surgery for right hip fracture (due to alcohol-related fall) and subsequently deteriorated with hematomas, disseminated intravascular coagulation, and atrial fibrillation. Patient placed in hospice and died of liver failure.

- **(4) Liver failure** (39 days after 4 weeks of (halted) SOF/VEL therapy)

67-year-old male patient admitted with incarcerated umbilical hernia leading to study drug discontinuation; prolonged hospitalization with eventual death from liver failure.

- **(5) Liver failure** (5 months after completing 12 weeks of SOF/VEL+RBV).

51-year-old male patient admitted with alcoholic liver disease with ascites, hypercoaguable state, acute kidney disease, and hyponatremia; subsequently developed cardiopulmonary arrest, and was placed in hospice care.

- **(6) Pneumonia** (1 month after fulfilling 12 weeks of SOF/VEL + RBV).

65-year old male patient admitted to hospital for hyponatremia and aspiration pneumonia.

- **(7) Sepsis** (1 month after fulfilling 12 weeks of SOF/VEL)

58-year-old female patient, died from sepsis with multi-organ failure during hospitalization for spontaneous bacterial peritonitis and pneumonia.

- **(8) Sepsis** (2 months after fulfilling 12 week of SOF/VEL)

59-year-old male patient declined medical treatment of osteomyelitis and subsequently developed septic shock resulting in death.

- **(9) Sepsis** (3 months after completing 24 weeks of SOF/VEL

53-year old female patient admitted with spontaneous bacterial peritonitis, with E coli sepsis, and multi-organ failure.

The causes of death (9 out of 267 patients treated) in ASTRAL-4 were not indicative of causality to therapy.

Adverse events of special interest

Adverse events of interest were defined as AEs that have previously been associated with administration of nucleoside/nucleotide inhibitors or other DAAs.

Cardiac Safety

Cardiac safety assessments included analysis of cardiac failure events, cardiac arrhythmia/bradycardia events, the effect of beta blockers and calcium-channel blockers on heart rate, and any safety events in patients with amiodarone use during treatment.

Cardiac Failure
No on-treatment events were reported in ASTRAL 1-3.

Cardiac arrhythmias/bradycardia
Four patients had cardiac arrhythmias/bradycardia events in the ASTRAL 1-3 studies: 3/1035 in the SOF/VEL 12 Week group and 1/132 in the SOF+RBV 12 Week group. The four adverse events observed were considered as non-related to SOF/VEL therapy and are summarised as follows:

1) minimal QT increase,

2) palpitations without ECG changes,

3) asymptomatic atrial fibrillation in patient with prior history of arrhythmia,

4) supraventricular tachycardia and sinus arrhythmia, in patient with severe ribavirin-induced anemia (Hb 53 g/L).

Adverse Events by use of beta- and calcium channel blockers
For all patients who received (A) a beta blocker, (B) a calcium-channel blocker with chronotropic effects (diltiazem or verapamil), or (C) neither at any time during the first 2 weeks of study treatment, an analysis was performed to identify patients with (1) any AE in the SOC of cardiac disorders and/or (2) the preferred terms of syncope and dizziness during that time.

Of note, in ASTRAL-4 more than half (58%) of patients were on stable beta-blocker therapy when entering the study (evenly distributed between the three treatment arms). Only 3 patients were receiving a calcium channel blocker.
No notable changes in vital signs (systolic blood pressure, diastolic blood pressure, and pulse) were reported during the studies. No trends in electrocardiogram findings suggestive of cardiotoxicity were observed.
No relevant findings were seen in those treated with betablockers/calcium-blockers; the frequency of AEs was similar in these patients as compared to the frequency in those without such drugs. The few cases reported concerned dizziness without relevant changes in the ECGs.

The maximum on-treatment pulse decrease was fully similar for patients with betablockers and patients without any such drugs. Numbers treated with calcium channel blockers were too few for a meaningful assessment. Hence, for all groups (A-C), there were no meaningful changes in pulse rate or maximal decrease at any time.

Amiodarone Use
No patients in ASTRAL 1-4 received amiodarone during the study treatment period.

One patient in the phase 2 received amiodarone prior to study treatment; SOF/VEL + RBV was started 3 weeks later, without events and no meaningful changes in heart rate.

Other Adverse Events of Interest

No patients in the SOF/VEL 12 Week group experienced any of the "Other Events of Interest": dermatologic events, pancytopenia (including aplastic anemia) events, psychiatric events relevant to suicide ideation or attempt, pancreatitis events, rhabdomyolysis/myopathy events, and renal failure events.

Discontinuation due to adverse events

SOF/VEL

AEs that lead to discontinuation of SOF/VEL were very infrequent and not indicative for causality to the treatment regimens.
In ASTRAL 1-3 only 2/1035 treated prematurely discontinued SOF/VEL for reasons of AEs:

- *Anxiety grade 3 on day 4.* The AE resolved the same day; assessed as unrelated to study drug.
- *Difficulty concentrating, headache, and anxiety of grade 3, on day 1*, resolved the following day. The patient had a medical history of depression, insomnia, and posttraumatic stress disorder. Assessed as related by the investigator.

In ASTRAL-4, nine patients (3.3%) had AEs leading to discontinuation of all study drugs (Table 54). In one case this was consider as related to study drugs by the investigator (patient 03055-64017).

Table 54: AEs leading to premature discontinuation of all study drugs, ASTRAL-4.

Subject Number	Preferred Term	SAE	Severity	Relationship to Study Treatment
SOF/VEL 12 Weeks				
07275-64023	Diffuse large B-cell lymphoma	Yes	Grade 3	Not related
SOF/VEL+RBV 12 Weeks				
02760-64074	Urinary tract infection	Yes	Grade 3	Not related
03060-64241[a]	Duodenal ulcer perforation	Yes	Grade 4	Not related
04421-64166	Nausea	No	Grade 2	Not related
	Vomiting	No	Grade 2	Not related
07585-64119	Ileus	Yes	Grade 2	Not related
SOF/VEL 24 Weeks				
02760-64102[b]	Incarcerated umbilical hernia	Yes	Grade 3	Not related
03055-64017	Hepatorenal syndrome	Yes	Grade 4	Related
	Peritonitis	Yes	Grade 3	Related
	Sepsis	Yes	Grade 4	Related
	Hypotension	Yes	Grade 4	Related
	Escherichia infection	Yes	Grade 1	Not related
03060-64200[c]	Acute myocardial infarction	Yes	Grade 4	Not related
	Acute kidney injury	No	Grade 3	Not related
	Acute respiratory failure	No	Grade 3	Not related
05275-64229	Hyperbilirubinaemia	Yes	Grade 3	Not related

RBV dose reductions or discontinuations (ASTRAL-4)
In ASTRAL-4, dose reductions of ribavirin in the SOF/VEL+RBV group were common; 32/87 (37%) lowered the dose (>3 days), mainly for reasons of anaemia (n=20) and 15/87 (17%) stopped ribavirin completely.

As mentioned in the exposure section, the Applicant selected a standard weight based starting dose of RBV in ASTRAL-4 (1000/1200 mg/d), in contrast to the 600 mg starting dose used in previous studies in decompensated patients (SOLAR 1+2 studies by Gilead; ALLY-1 study by BMS). On the basis of ASTRAL-4 (which excluded patients with Child Pugh C, and those with a prior transplant), the Applicant proposed that SOF/VEL + RBV 1000/1200 mg should be recommended to all patients with decompensated cirrhosis (including those populations not studied in ASTRAL-4).

SOLAR-1 (SOF/ledipasvir + RBV 600 mg starting dose) included all aspects of decompensated liver disease (Child Pugh B/C, and transplant patients with decompensated disease). It should be noted that these patients are more vulnerable for RBV-associated anaemia and might have difficulties to keep even the 600 mg dose.

Therefore the adequacy of a 1000/1200 mg starting dose of ribavirin for patients with CPT C cirrhosis, and for post-transplant patients with Child Pugh B or C cirrhosis, was further discussed during the procedure. The Applicant concluded that a 600 mg starting dose was a more reasonable option for these mentioned patients although not studied within the SOF/VEL program), which was endorsed by the CHMP.

Laboratory findings

ASTRAL 1-3

There were no meaningful changes from baseline in haematology parameters. A few cases of lowered white blood cells were seen in the active arms, none considered clinically important.

The most common Grade 3-4 abnormalities for other blood chemistry for patients in the SOF/VEL 12 Week group were elevated lipase, elevated serum glucose, and elevated creatine kinase.

- All cases of lipase increases were asymptomatic and generally isolated or transient and intermittent; no cases of pancreatitis were reported. With regards the new drug, velpatasvir, there was no tendency for a dose dependent effect on lipase in the dose finding study in phase 2, comparing 25 and 100 mg dosing.
- Out of 20 patients who had hyperglycaemia in the SOF/VEL group, 18 had diabetes mellitus and for the other 2 cases the increase was transient.
- The 8 cases of grade 3 or 4 CK increases in the SOF/VEL group were transient and related to exercise or physical exertion, according to the investigator, with no cases of rhabdomyolysis.

There were no cases of unexplained increases of transaminases during therapy with SOF/VEL.

ASTRAL-4

As expected, abnormal lab chemistry values were more common in ASTRAL-4 reflecting the severe liver disease status (particularly low haemoglobin, lymphopenia, thrombocytopenia and hyperbilirubinemia).

Amylase increases of grade 3-4 were seen in total 7 patients (2.7%), all asymptomatic (no cases of pancreatitis). One case of grade 4 CK increase was seen (<1%), following a surgical procedure where other drugs were given that were deemed the inciting factor.

Safety in special populations

Gender

The proportion of female patients was around 40% in the ASTRAL 1-3 studies (405 females treated with SOF/VEL), and 30% of the patients in the ASTRAL-4 study.

The incidence of overall AEs was slightly higher for female patients compared with male patients (primarily due to a higher incidence of nausea, headache, and asthenia) – for all regimens (SOF/VEL, placebo, sof + rbv).

The incidence of Grade 3 or 4 AEs and SAEs was similar for males and females receiving SOF/VEL.

Age

The proportion of patients with an age >65 was 11% in ASTRAL 1-3 (n=123 treated with SOF/VEL), and 12% in ASTRAL-4 (33 treated with SOF/VEL +/- RBV).

Age >65 did not have any apparent relevant effect on the incidence of AEs, Grade 3 or 4 AEs or SAEs.

Race

The majority of patients in the SOF/VEL program were white. In ASTRAL 1-3 around 16% of patients were non-white, with 8.3% Asian (86 treated with SOF/VEL) and 5.9% black (61 treated with SOF/VEL) patients.

Graded laboratory abnormalities for low neutrophils and elevated creatine kinase were more frequently seen in black patients than in white and "other" patients – for all regimens (SOF/VEL, placebo, SOF + RBV).

Renal impairment

The safety of SOF/VEL has not been studied in patients with renal impairment. A creatinine clearance <60 ml/min was an exclusion criteria in ASTRAL 1-3 and <50 ml/min in ASTRAL-4 studies. The proportion of patients with a clearance <90 was around 25%, and a total of around 30 patients with a clearance <60 has been treated. No apparent difference in overall AEs were not by degree of renal function in these patients

The exposure of SOF and VEL has been studied in patients with renal impairment(as single agents).

The exposure of VEL is not much affected by renal function, and this agent could be given also in the setting of severe renal impairment.

However, the main metabolite of sofosbuvir increases markedly (several hundred-fold) in patients with severe renal impairment. The safety and efficacy of SOF administered at 200 or 400 mg are presently being evaluated in subjects with severe renal impairment or ESRD requiring dialysis in Study GS-US-334-0154. The company is asked for any updates on this study in order to understand whether SOF/VEL (400/100 mg fixed dose) in fact may be used in patients with severe renal impairment. That is a population presently lacking optimal treatment alternatives (LoQ, Clinical Safety, other concern)

At present, sofosbuvir/velpatasvir should not be given to patients with a creatinine clearance < 30 ml/min.

Severe hepatic impairment and liver transplant patients

SOF/VEL has been studied in a large number of patients with decompensated Child Pugh B cirrhosis (ASTRAL-4), without any particular safety signals (previous pages).

Data on safety in patients with Child Pugh C cirrhosis is lacking. However, SOF has been studied in patients with Child Pugh C cirrhosis, without safety issues, and VEL exposure was similar in patients without cirrhosis as compared to exposures in patients with Child Pugh B cirrhosis (ASTRAL 1-4 studies). Likewise similar levels were seen after single dose in health subjects with normal liver function as compared to levels in HCV-negative patients with Child pugh B and C cirrhosis.

Hence, SOF/VEL 400/100 mg qd can be given to patients without regards to liver function.

Liver transplant patients have not been studied. However, DDI studies with agents typically used were done; there is no need for dose adjustments during co-treatment with tacromilus.

HIV/HCV co-infection

Specific safety data in patients co-infected with HIV is presently pending. A Phase-3 study (GS-US-342-1202[ASTRAL-5]) is currently ongoing.

The safety would not be expected to be different from that in HIV-negative patient, provided that drug interactions (antiretroviral therapy) are taken into account.

Safety related to drug-drug interactions and other interactions

Within the study program, no new safety issues related to drug-drug interactions emerged.

Other safety issues

Recently there were some literature case reports on events of hepatitis B-reactivation during IFN-free hep C therapy (Colline et al, Ende et al, Takayama et al; all in 2015). Two cases concerned patients with occult hepatitis B (serology: HBsAg negative, anti-HBc positive), normally regarded as having a "prior hep B

infection" without a risk for reactivation with the exception of advanced immunosuppressive therapy where this is a well-known risk. Another two cases concerned pronounced re-activation of a previously silent hepatitis B co-infection (serology: HBsAg positive); a phenomenon that is well known from the past, subsequent to successful therapy with IFN-based therapy of such patients. These cases have triggered a PRAC investigation on hep B reactivation during therapy with the new hep C agents, since this issue has not been studied within the developmental program of any agents, SOF/VEL included.

Hep B co-infection (HBsAg positive) was an exclusion criteria in the SOF/VEL program. However, patients with prior "occult" hepatitis B infection have likely been treated in high numbers, since around 30 % (or higher) of hepatitis C patients show markers for a prior hep B-infection (frequency varying by region and setting).

Although there is no clear signal for HBV reactivation in the SOF/VEL studies, the company was asked to further discuss the issue, including providing any data available on HBV viral dynamics during HCV therapy in patients with HBV co-infection:

The Applicant has recently presented a cumulative review in the issue within the latest Sovaldi PSUR (submitted Feb 2016). No SAEs suggestive of HBV reactivation have been observed in Gilead-sponsored clinical studies in which 12,157 subjects have been treated with a SOF-containing regimen. In the post-marketing setting there were 7 reported cases of HBV reactivation in the context of 191,533 patient years of exposure to SOF-containing regimens. Two of these cases involved HBV flares with associated elevations in liver laboratory tests; 1 of these cases involved fulminant hepatic flare in a patient with other risk factors for HBV reactivation.

The Applicant had no data on the number of patients with "prior HBV infection" that were part of the SOF/VEL program (serology not analyzed during the studies). A small number of patients had a documented prior HBV-infection by medical history and safety data in these patients were not suggestive of any HBV-reactivation during study.

Furthermore, results from a prior study with Harvoni (sofosbuvir/ledipasvir) undertaken in South East Asia were provided. None of 103 HCV-infected patients with prior HBV infection (documented HBsAg negative, HBcAb positive) showed any signs of HBV reactivation during therapy with Harvoni given for 12 weeks, on the basis of transaminases (normalized during therapy, where in practice all patients were cured for the hep C infection).

With regards HCV/HBV co-infection (HbsAg positive), the Applicant provided data from a small cohort of such treated with Harvoni in the ELECTRON-2 study (cohort 6), including HBV dynamics during therapy and follow-up. Patients were HBeAg-negative and without therapy for their HBV infection. All 8 patients were cured from the hepatitis C-infection. A modest increase in HBV-DNA levels were seen overall, but without any other signs of clinical HBV flare. Transaminases were improved, and there were no cases of grade 3/4 increases of liver enzymes.

Although HBV-DNA increases were modest, in some cases these increases resulted in levels that would justify concomitant HBV therapy, as recommended in treatment guidelines.

Gilead has also initiated Study GS-US-337-1655, an open-label study of LDV/SOF for 12 weeks in 100 subjects with chronic genotype 1 or 2 HCV infection and HBV coinfection in Taiwan. The impact of effective HCV treatment on concurrent HBV infection and HBV disease progression will be evaluated throughout treatment and for a follow-up period of 2 years after treatment completion.

2.6.1. Discussion on clinical safety

In the clinical program for the fixed dose combination of sofosbuvir/velpatasvir (SOF/VEL) around 1300 patients were exposed to at least 12 weeks of SOF/VEL 400/100 mg as part of the phase 3, including 220 patients with compensated cirrhosis (as part of ASTRAL 1-3 studies) and 267 patients with decompensated cirrhosis, Child Pugh B (ASTRAL-4). In the phase 2 studies another 800 patients were given SOF/VEL (400/25 mg and 400/100 mg) for 8-12 weeks.

A favourable safety profile of SOF 400 mg is already well established; a substantial number of patients have been exposed as part of Sovaldi (sofosbuvir, first approval December 2013) and Harvoni (sofosbuvir/ledispasvir, first approval October 2014).

The active substance VEL is not previously approved. Although VEL has not been studied as a single agent for the treatment of hepatitis C outside short term monotherapy studies, it is noted that VEL showed no particular signs of toxicity in pre-clinical studies (no target organ of toxicity found) apart from a possible risk of teratogenicity. That is in line with other agents of this class.

The safety profile for SOF/VEL was unremarkable, with similar frequencies of adverse events (overall as well as possibly treatment-related) as compared to that seen with placebo (control in ASTRAL-1). The 3 most commonly reported AEs being headache (20%), fatigue (15%), and nausea (10%).

In ASTRAL 1-3 (patients with compensated liver disease) there was no patient reported to have had any serious adverse events related to therapy with SOF/VEL, out of 1035 treated. In these studies there were 6 deaths (0.4%), none of which was considered related to therapy. No relevant adverse effects were seen on laboratory parameters.

In ASTRAL-4, the same common AEs were seen in patients with decompensated Child Pugh B cirrhosis. Also in this population serious adverse events were not indicative for causal relationship to therapy. One patient out of the 267 treated worsened in liver function after a month of therapy, with a subsequent need for liver transplantation (and subsequently achieved SVR12). The event occurred during an episode of E-coli sepsis (with subsequent hepatorenal failure), which seems to be the triggering event.

On the basis of efficacy and safety in ASTRAL-4 the company proposes that SOF/VEL should be given in combination with RBV 1000/1200 mg (for 12 weeks) to all patients with decompensated liver disease. That would include patients with Child Pugh C cirrhosis and decompensated patients with a prior liver transplant; populations not studied in ASTRAL-4.

That stands in contrast to what is recommended in the Harvoni SmPC, Gilead´s similar preceding product, which recommends SOF/ledipasvir + RBV 600 mg starting dose, on the basis of efficacy and safety from the SOLAR-studies, which included the full spectrum of decompensated patients, transplanted included.

For patients with decompensated cirrhosis, SOF/VEL should be combined with ribavirin in order to maximize efficacy. The company proposes that the ribavirin starting dose should be 1000/1200 mg per day, in line with what was used in the ASTRAL-4 study. According to the present proposal, that dose would apply to all patients with decompensated cirrhosis, Child Pugh C-patients and also for those with a prior liver transplant. Of note, such patients were not included in ASTRAL-4, and it is known from previous studies with similar regimens that the problems with ribavirin-associated anemia increases by disease severity, in particular in the setting of transplanted patients. In ASTRAL-4 (Child Pugh B, no transplanted patients) dose reductions and discontinuations of ribavirin were common, but manageable (37% and 17%, respectively). In previous studies with similar regimens that included the full spectrum of decompensated cirrhotic patieints, such as the SOLAR-1 (Harvoni + Ribavirin), a 600 mg starting dose was used. It should be noted that these patients are more vulnerable for RBV-associated anaemia and might have difficulties to keep even the 600 mg dose. The adequacy of a 1000/1200 mg starting dose of ribavirin for patients with CPT C cirrhosis, and for post-transplant patients with Child Pugh B or C cirrhosis,

was further discussed during the procedure. The Applicant concluded that a 600 mg starting dose was a more reasonable option for these mentioned patients although not studied within the SOF/VEL program), which was endorsed by the CHMP.

A number of AEs of special interest were followed, in particular cardiac safety having in mind a recently reported signal for arrhythmic events during therapy with sofosbuvir-based regimens in combination with amiodarone (mechanism unknown). No patient in the ASTRAL-4 studies used amiodarone, and of those patients on stable beta-blocker (58% in ASTRAL-4) or calcium blocker therapy (very few patients) no notable changes in vital signs or electrocardiogram findings suggestive of cardiotoxicity were observed. There was no arrythmic events or considered related to therapy, and no events of cardiac failure.

HBV-reactivation during hep C therapy in patients with prior HBV-infection, or a silent non-treated HBV co-infection, is presently reviewed in an article 20 procedure. Within the SOF/VEL program there were no cases suggestive of such events in patients that, on the basis of epidemiological data, to a substantial part would have had a prior HBV infection. The Applicant provided limited data on HBV dynamics in HBV co-infected patients during and following therapy with sofosbuvir/ledipasvir. Although HBV-DNA increases were modest, in some cases these increases resulted in levels that would justify HBV therapy, according to acknowledged treatment guidelines. The CHMP considered that the results, despite the limited numbers, justify a recommendation in the SmPC for the monitoring of HBV DNA levels during and following therapy with SOF/VEL.

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

SOF/VEL has been studied in a sufficient number of patients, including substantial numbers compensated and decompensated cirrhosis. The safety profile is indeed favourable, seemingly similar to that of placebo.

For patients with decompensated cirrhosis SOF/VEL should be combined with ribavirin in order to maximize efficacy. The optimal RBV dose for that population with regards to anaemic complications, in particular for patients with Child Pugh C cirrhosis and liver transplant patients, was discussed during the procedure and where the final proposal is endorsed (starting dose 1000/1200 mg in Child Pugh B without prior liver transplant; 600 mg in Child Pugh C without prior transplant and for patients with Child Pugh B or C post transplant).

HBV-reactivation during hep C therapy in patients with prior hep B infection, or in those with a silent non-treated HBV co-infection, is presently reviewed as part of an article 20 procedure for all authorised DAAS. The data provided by the Applicant within this application does not warrant any specific warning for the former subset of patients. However, for patients with HBV co-infection a recommendation for HBV-DNA monitoring is considered justified as part of section 4.4 of the SmPC.

2.7. Risk Management Plan

Safety concerns

Important Identified Risks	Severe bradycardia and heart block when used with concomitant amiodarone
Important	Drug-drug interaction with potent Pgp inducers

Potential Risks	with moderate and potent inducers of CYP2B6, CYP2C8, or CYP3A4
	with PPIs
	with TDF
	with rosuvastatin
	with digoxin
Missing Information	Safety in children
	in pregnant or breastfeeding women
	in patients with HCV/HIV coinfection
	in patients with HCV/HBV coinfection
	in post-transplant patients
	in HCV patients with severe renal impairment or end-stage renal disease
	Development of resistance

Severe bradycardia and heart block when used with concomitant amiodarone was moved from "Important Potential Risk to "Important Identified Risk" to align with other sofosbuvir-containing products.

Having considered the data in the safety specification, the CHMP agrees that the updated safety concerns listed by the applicant are appropriate.

Pharmacovigilance plan

Study/Title	**Objectives**	**Safety Concerns Addressed**	**Status (Planned, Started)**	**Date for Submission of Interim or Final Reports (Planned or Actual)**
Category 3 (Interventional Studies)				
GS-US-342-1143 Open label single arm trial to evaluate pharmacokinetics, safety, antiviral activity and acceptability/palatability of SOF/VEL in children from 3 to less than 18 years of age with chronic hepatitis C genotype 1-6 infection	To evaluate the pharmacokinetics (PK), efficacy, and safety of sofosbuvir (SOF)/velpatasvir (VEL) in adolescents and children	Safety of SOF/VEL in children	Planned	Final study report October 2020

Study/Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
GS-US-342-1202 (ASTRAL-5) A Phase 3, Open-label Study to Investigate the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed Dose Combination for 12 weeks in in Subjects with Chronic Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV)-1 Co-infection	To evaluate the safety and efficacy of SOF/VEL in subjects with chronic HCV who are coinfected with HIV-1	Safety in patients with HCV/HIV coinfection Drug-drug interaction (DDI) between SOF/VEL and TDF	Started	Final study report June 2017
GS-US-334-0154 A Phase 2b, Open-Label Study of 200 mg or 400 mg Sofosbuvir+RBV for 24 Weeks in Genotype 1 or 3 HCV-Infected Subjects with Renal Insufficiency	To evaluate the safety, efficacy and pharmacokinetics of treatment with SOF+ ribavirin (RBV) for 24 weeks in subjects with chronic genotype 1 or 3 HCV infection and severe renal impairment	Safety in patients with severe renal impairment or end-stage renal disease	Started	Final study report July 2017
Category 3 (Non-interventional Studies)				
GS-US-248-0123 A Long Term Follow-up Registry Study of Subjects Who Did Not Achieve Sustained Virologic Response in Gilead-Sponsored Trials in Subjects with Chronic Hepatitis C Infection	To evaluate HCV viral sequences and the persistence or evolution of treatment-emergent viral mutations in subjects who fail to achieve an SVR after treatment with a Gilead oral antiviral containing regimen in a previous Gilead-sponsored hepatitis C study	Development of resistance	Started	Final study report July 2020
GS-US-334-1113 A Long Term Follow-up	To evaluate long-term efficacy in	Evaluation of viral relapse.	Planned	To be determined

Study/Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
Registry for Pediatric Subjects Who Received Treatment in Gilead-Sponsored Chronic Hepatitis C Infection Trials	adolescents and children who received SOF/VEL in study GS-US-342-1143			
Category 3 (Nonclinical Studies)				
Studies to assess the potential for transporter and enzyme based interactions and pharmacodynamic effects	To assess the potential for a pharmacokinetic interaction via transporter or enzyme based inhibition	Severe bradycardia and heartblock when SOF and other direct-acting antiviral (DAAs) are used concomitantly with amiodarone	Planned	To be determined

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
Important identified risk(s)		
Severe bradycardia and heart block when used with concomitant amiodarone	The Summary of Product Characteristics (SmPC [Sections 4.4, 4.5, and 4.8]) includes information that cases of severe bradycardia and heart block have been observed when sofosbuvir in combination with another direct acting antiviral (DAA), is used with concomitant amiodarone with or without other drugs that lower heart rate, that amiodarone should only be used in patients on SOF/VEL when other alternative anti-arrhythmic treatments are not tolerated or are contraindicated, and that patients who must take amiodarone with SOF/VEL should be closely monitored.	None
Important potential risk(s)		

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Drug-drug interaction with moderate and potent Pgp inducers	The SmPC (Sections 4.3 and 4.5) includes information that potent Pgp inducers (eg, rifampicin, rifabutin, St. John's wort, carbamazepine, phenobarbital and phenytoin) should not be used with SOF/VEL due to the potential for significant decreases in SOF or VEL plasma concentrations, which may lead to loss of efficacy of SOF/VEL, and that the use of such medicinal products with SOF/VEL is not recommended. The SmPC (Section 4.4) includes information that moderate Pgp inducers (e.g. oxcarbazepine, modafinil) are not recommended with SOF/VEL as they can reduce the plasma concentrations of SOF or VEL leading to reduced therapeutic effect of SOF/VEL.	None
Drug-drug interaction with moderate and potent inducers of CYP2B6, CYP2C8, or CYP3A4	The SmPC (Section 4.3, 4.4 and 4.5) includes information that moderate (e.g.efavirenz, oxcarbazepine, modafinil) inducers of CYP are not recommended for coadministration with SOF/VEL, and potent (e.g.rifampicin) inducers of CYP are contraindicated with SOF/VEL due to to the potential for significant decreases in SOF orVEL plasma concentrations, which may lead to loss of effect or reduced therapeutic effect of SOF/VEL.	None
Drug-drug interaction with PPIs	The SmPC (Section 4.5) includes information about the maximum allowed dose for PPIs, and recommendation for coadministration of PPIs with SOF/VEL under fed conditions, as coadministration of PPIs above the recommended dose with SOF/VEL under fasted conditions has the potential to decrease VEL plasma concentrations, which may lead to reduced therapeutic effect of SOF/VEL	None
Drug-drug interaction with TDF	The SmPC (Section 4.5) includes information that when coadministered with TDF, SOF/VEL increases the concentration of tenofovir and that patients receiving TDF and SOF/VEL concomitantly should be monitored for adverse reactions associated with TDF.	None
Drug-drug interaction with rosuvastatin	The SmPC (Section 4.5) includes information that coadministration of SOF/VEL with rosuvastatin may increase the concentration of rosuvastatin, which is associated with increased risk of myopathy, including rhabdomyolysis, and that rosuvastatin may be administered with SOF/VEL at a dose that does not exceed 10 mg.	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Drug-drug interaction with digoxin	The SmPC (Section 4.5) includes information that coadministration of SOF/VEL with digoxin may increase the concentration of digoxin, and that caution is warranted and therapeutic concentration monitoring of digoxin is recommended when co-administered with SOF/VEL.	None
Missing information		
Safety in children	The SmPC states that the safety and efficacy of SOF/VEL in pediatric subjects have not been established and that SOF/VEL is not recommended for use in children and adolescents < 18 years of age (Sections 4.2, 4.4, 4.8) and that the PK of SOF, GS-331007 and VEL have not been established in children (Section 5.2).	None
Safety in pregnant or breastfeeding women	The SmPC (Section 4.6) states that there are no or limited amount of data from the use use of SOF/VEL in pregnant women, that animal studies do not indicate direct or indirect harmful effects for reproductive toxicity or fetal development, and that, as a preventive measure, use of SOF/VEL should be avoided during pregnancy. The SmPC also states that it is unknown whether sofosbuvir, its metabolites or velpatasvir are excreted in human milk, that available PK data in animals have shown excretion of velpatasvir and metabolites of sofosbuvir in milk, and that, a risk the newborns/infants cannot be excluded. Therefore, SOF/VEL should not be used during breast-feeding.	None
Safety in patients with HCV/HIV coinfection	The SmPC section 4.4 provides warning on coadministration of SOF/VEL with efavirenz. The SmPC (Section 4.5) provides warnings and information on coadministration of SOF/VEL with many common HIV medicines.	None
Safety in patients with HCV/HBV coinfection	The SmPC(Section 4.4) states that there are no data in this population.	None
Safety in post-transplant patients	The SmPC(Section 4.4) states that there are no data in this population.	None
Safety in HCV patients with severe renal impairment or end-stage renal disease	The SmPC states that no dose adjustment of SOF/VEL is required for patients with mild, moderate or severe renal impairment (Section 4.2) and that the use of SOF/VEL is not recommended in patients with ESRD requiring hemodialysis (Section 4.4).	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Development of resistance	No risk minimization measures are considered necessary.	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 0.2 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

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to Harvoni. The bridging report submitted by the applicant has been found acceptable.

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Epclusa (sofosbuvir / velpatasvir) 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

In patients with chronic HCV infection with or without compensated cirrhosis (ASTRAL 1-3 studies), 12 weeks of SOF/VEL therapy yielded SVR (sustained virological response = viral clearance) in practically all patients, regardless of HCV genotype. The only exception concerned patients with genotype 3-infection

and cirrhosis, where the SVR rate was slightly lower (around 90%). The company proposes that all patients with compensated cirrhosis, regardless of HCV genotype, should be given SOF/VEL for 12 weeks.

For patients with decompensated cirrhosis (Child Pugh B), the addition of ribavirin (i.e. SOF/VEL + RBV for 12 weeks) optimized SVR rates, in particular for patients with genotype-3 infection. Although numbers are low, it is clear that ribavirin adds to the efficacy in patients with this genotype.

The results in decompensated patients with the 12 week triple regimen are impressive across genotypes. As a consequence the recommendation for all patients with decompensated cirrhosis is SOF/VEL + RBV for 12 weeks.

	Regime	GT 1	GT 2	GT3	GT4	GT5	GT6	Total
ASTRAL-1, -2 and -3 (compensated liver disease)								
Overall	SOF/VEL 12wk	323/328 (98.5%) [96.5-99.5%]	237/238 (99.6%) [97.7%-100.0%]	264/277 (95.3%) [92.1-97.5%]	116/116 (100.0%) [96.9-100.0%]	34/35 (97.1%) [85.1-99.9%]	41/41 (100.0%) [91.4-100.0%]	1015/1035 (98.1%) [97.0-98.8%]
No cirrhosis		251/255 (98.4%) [96.0-99.6%]	207/208 (99.5%) [97.4-100.0%]	191/197 (97.0%) [93.5-98.9%]	89/89 (100.0%) [95.9-100.0%]	28/29 (96.6%) [82.2-99.9%]	35/35 (100.0%) [90.0-100.0%]	801/813 (98.5%) [97.4-99.2%]
Compensated cirrhosis		72/73 (98.6%) [92.6-100.0%]	29/29 (100.0%) [88.1-100.0%]	73/80 (91.3%) [82.8-96.4%]	27/27 (100.0%) [87.2-100.0%]	5/5 (100.0%) [47.8%-100.0%]	6/6 (100.0%) [54.1-100.0%]	212/220 (96.4%) [93.0%-98.4%]
ASTRAL-4 (decompensated liver disease, Child Pugh B))								
Decompensated cirrhosis	SOF/VEL 12wk	60/68 (88.2%) [78.1-94.8%]	4/4 (100.0%) [39.8-100.0%]	7/14 (50.0%) [23.0-77.0%]	4/4 (100.0%) [39.8-100.0%]	-	0	75/90 (83.3%) [74.0-90.4%]
	SOF/VEL + RBV 12wk	65/68 (95.6%) [87.6-99.1%]	4/4 (100.0%) [39.8-100.0%]	11/13 (84.6%) [54.6-98.1%]	2/2 (100.0%) [15.8-100.0%]	-	0	82/87 (94.3%) [87.1-98.1%]
	SOF/VEL 24wk	65/71 (91.5%) [82.5-96.8%]	3/4 (75.0%) [19.4-99.4%]	6/12 (50.0%) [21.1-78.9%]	2/2 (100.0%) [15.8-100.0%]	-	1/1 (100%) [2.5%-100.0%]	77/90 (85.6%) [76.6-92.1%]

For the vast majority of patients, the proposed treatment recommendations will lead to SVR. In genotype-3 infected patients with compensated cirrhosis it seems likely that the addition of RBV would further optimize results, in particular for those with more severe, yet compensated, cirrhosis.

Uncertainty in the knowledge about the beneficial effects

Taking into consideration that the study population with compensated cirrhosis in the clinical development program represents fairly mild cirrhosis, it could be anticipated that outcomes with SOF/VEL for 12 weeks in patients with more pronounced (but still compensated) liver disease might be slightly lower. One might assume that the addition of RBV to SOF/VEL treatment would maximize SVR for these patients, in particular in the case of genotype-3 infection.

Risks

Unfavourable effects

Exposure data, both in terms of total numbers of patients (n=2603) as well as coverage of genotypes and patients with compensated and decompensated cirrhosis, is sufficient for the regulatory safety evaluation in the context of establishing a positive benefit/risk balance. A favourable safety profile of sofosbuvir (400 mg) is already well established; a substantial number of patients have been exposed as part of Sovaldi (sofosbuvir, first approval December 2013) and Harvoni (sofosbuvir/ledispasvir, first approval October 2014).

SOF/VEL is well tolerated and the emerging safety profile is similar to placebo, with comparable frequencies of adverse events (overall as well as possibly treatment-related).

The most important unfavorable effect was relapse after therapy with SOF/VEL in certain subgroups of hard-to-treat patients, i.e. patients with genotype 3 and cirrhosis, since NS5A resistance (Y93H mutation) is a universal finding in these cases. That resistance, with a substantial impact on the activity of all agents of this class, has been shown to persist long term and is presently considered an obstacle with regard to highly effective re-treatment options.

Uncertainty in the knowledge about the unfavourable effects

Virologic failure in patients with genotype 3 and decompensated or severe compensated cirrhosis constitutes an important adverse event which presently leaves these seriously ill patients with no clear effective options for retreatment. The full impact of this is unknown.

For patients with decompensated cirrhosis, SOF/VEL should be combined with ribavirin in order to maximize efficacy. The Applicant initially proposed that the ribavirin starting dose should be 1000/1200 mg per day, in line with what was used in the ASTRAL-4 study, for all patients with decompensated cirrhosis (CPC and post transplants included). This was further discussed during the procedure and where the final proposal is endorsed (starting dose 1000/1200 mg in Child Pugh B without prior liver transplant; 600 mg in Child Pugh C without prior transplant and for patients with Child Pugh B or C post transplant).

HBV-reactivation during HCV therapy in patients with prior hep B infection, or in those with a silent non-treated HBV co-infection, is presently reviewed as part of an article 20 procedure. The data presented by the company within this application does not warrant any specific warning for the former subset of patients. However, for patients with HBV co-infection a recommendation for HBV-DNA monitoring is considered justified as part of section 4.4 of the SmPC.

Effects table

	Effect	Short Description	Outcome	Control	Uncertainties/ Strength of evidence
Favourable	SVR12 (clinical cure based on virological endpoint) in patients with compensated liver disease	Proportion of patients with SVR12 when treated with applicant's recommended regimen	Overall >95% across all genotypes (SOF/VEL 12W))	Placebo (no spontaneous cure) SOF/VEL superior to SOF+RBV 12/24 w in GT2/GT3	Cure rates in compensated cirrhotic patients with GT3 may be suboptimal (see Unfavourable effects)
	SVR12 in patients with decompen-sated cirrhosis		>95% in GT1, 2 and 4; 84.6% in GT3 (SOF/VEL + RBV 12 W)	SOF/VEL + RBV was better than comparator treatments (SOF/VEL 12/24W)	
	Treatment-emerg ent adverse events		Frequency is the same as for placebo (ASTRAL-1)		
Unfavourable	Relapse after SOF/VEL therapy in cirrhotic patients with GT3	Suboptimal cure rates in cirrhotic patients with GT3	8.8% in compensated cirrhotic patients with GT3		Data from patients with decompensated cirrhosis indicate that patients with GT3 and severe yet compensated cirrhosis might be sub-optimally treated.
	Dose-dependent RBV-associated anemia	RBV dose of 1000/1200 mg per day for	Resulted in need for RBV dose reduction (37%)		The risk of pronounced anaemia increases by liver disease severity, and is

	Effect	Short Description	Outcome	Control	Uncertainties/ Strength of evidence
		patients with decompensated cirrhosis presently proposed, on the basis of the ASTRAL-4 study	and RBV discontinuation (17%) in ASTRAL-4.		further increased in patients after liver transplantation. ASTRAL-4 did not include patients with Child Pugh C, or those with a prior liver transplant. The suggested RBV dose is likely too high for certain groups of decompensated patients.

Benefit-risk balance

Importance of favourable and unfavourable effects

SVR marks the clearance of HCV infection, and has been associated with a decrease risk of cirrhosis, decompensation, cancer, and liver-related deaths. The adverse effect profile of SOF/VEL does not stand out as different from placebo.

Benefit-risk balance

Discussion on the benefit-risk balance

Treatment outcomes with SOF/VEL in patients without cirrhosis and with compensated cirrhosis as well as with SOF/VEL/RBV in patients with decompensated cirrhosis represent unprecedented SVR rates in chronic HCV infection.

The emerging safety profile is very favourable and the recommended treatment combinations will be suitable for the majority of patients with chronic HCV infection.

Virologic failures mainly occurred in already hard to treat patients (genotype-3 infected patients with cirrhosis) leaving unclear re-treatment options. However, the risk of treatment failure is most likely lowered by the addition of ribavirin to SOF/VEL for genotype-3 infected patients with compensated cirrhosis as well as those with decompensated cirrhosis. The recommendations of triple therapy in these patient populations are reflected in section 4.2 of the SmPC.

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 Epclusa 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 medical prescription.

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 velpatasvir is qualified as a new active substance.