Report on the Deliberation Results

June 26, 2014

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name]	Daklinza Tablets 60 mg
[Non-proprietary name]	Daclatasvir Hydrochloride (JAN*)
[Name of applicant]	Bristol-Myers K.K.
[Date of application]	October 29, 2013

[Results of deliberation]
In the meeting held on June 26, 2014, the Second Committee on New Drugs concluded that the product may be approved and that this result should be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period is 8 years, the drug substance and the drug product are both classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

**Japanese Accepted Name (modified INN)*

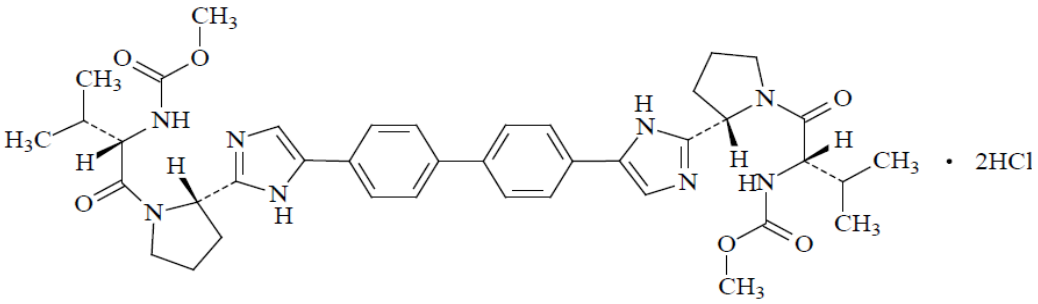
Review Report

June 6, 2014
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical products submitted for registration are as follows.

[Brand name]	(a) Daklinza Tablets 60 mg
	(b) Sunvepra Capsules 100 mg
[Non-proprietary name]	(a) Daclatasvir Hydrochloride
	(b) Asunaprevir
[Applicant]	Bristol-Myers K.K.
[Date of application]	October 29, 2013
[Dosage form/Strength]	(a) Each tablet contains 66 mg of Daclatasvir Hydrochloride (60 mg as Daclatasvir)
	(b) Each capsule contains 100 mg of Asunaprevir
[Application classification]	(1) Drug with a new active ingredient
[Chemical structure]	

(a) Daclatasvir Hydrochloride



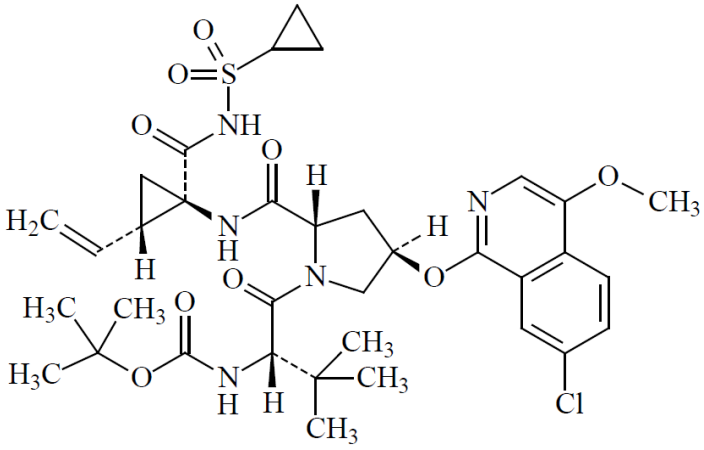
Molecular formula: $C_{40}H_{50}N_8O_6 \cdot 2HCl$

Molecular weight: 811.80

Chemical name:

Dimethyl*N,N'*-([1,1'-biphenyl]-4,4'-diylbis{1*H*-imidazole-5,2-diyl-[(2*S*)-pyrrolidine-2,1-diyl][(1*S*)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate dihydrochloride

(b) Asunaprevir



Molecular formula: $C_{35}H_{46}ClN_5O_9S$

Molecular weight: 748.29

Chemical name:

1,1-Dimethylethyl{(2*S*)-1-[(2*S*,4*R*)-4-({7-chloro-4-methoxyisoquinolin-1-yl}oxy)-2-({(1*R*,2*S*)-1-[(cyclopropanesulfonyl)carbamoyl]-2-ethenylcyclopropyl}carbamoyl) pyrrolidin-1-yl]-3,3-dimethyl-1-oxobutan-2-yl}carbamate

[Items warranting special mention]
Priority Review (Notification No. 1225-1 of Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated December 25, 2013)

[Reviewing office] Office of New Drug IV

Review Results

June 6, 2014

[Brand name]	(a) Daklinza Tablets 60 mg
	(b) Sunvepra Capsules 100 mg
[Non-proprietary name]	(a) Daclatasvir Hydrochloride
	(b) Asunaprevir
[Applicant]	Bristol-Myers K.K.
[Date of application]	October 29, 2013

[Results of review]

Based on the submitted data, the efficacy of the products in patients with chronic hepatitis C, or chronic hepatitis C with compensated cirrhosis has been demonstrated and their safety is acceptable in view of their observed benefits.

As a result of its review, the Pharmaceuticals and Medical Devices Agency concluded that the products may be approved for the following indications and dosage and administration.

[Indications]	Improvement of viraemia in either of the following patients with chronic hepatitis C serogroup 1 (genotype 1), or chronic hepatitis C serogroup 1 with compensated cirrhosis: (1) patients who are treatment-naïve and ineligible for or who are intolerant of interferon-based therapy, or (2) patients who have failed to respond to interferon-based therapy.
[Dosage and administration]	[Daklinza Tablets 60 mg] The usual adult dosage is 60 mg of Daclatasvir orally administered once daily. Daklinza should be used in combination with Asunaprevir for a duration of 24 weeks. [Sunvepra Capsules 100 mg] The usual adult dosage is 100 mg of Asunaprevir orally administered twice daily. Sunvepra should be used in combination with Daclatasvir Hydrochloride for a duration of 24 weeks.

Review Report (1)

April 23, 2014

I. Products Submitted for Registration

[Brand name]	(a) Daklinza Tablets 60 mg
	(b) Sunvepra Capsules 100 mg
[Non-proprietary name]	(a) Daclatasvir Hydrochloride
	(b) Asunaprevir
[Name of applicant]	Bristol-Myers K.K.
[Date of application]	October 29, 2013
[Dosage form/Strength]	(a) Each tablet contains 66 mg of Daclatasvir Hydrochloride (60 mg as Daclatasvir) per tablet
	(b) Each capsule contains 100 mg of Asunaprevir per capsule

[Proposed indications]

Improvement of viraemia in either of the following patients with chronic hepatitis C serogroup 1 (genotype 1), or chronic hepatitis C serogroup 1 with compensated cirrhosis:

- patients who are treatment-naïve and ineligible for, or who are intolerant of interferon therapy, or
- patients who have failed to respond to interferon therapy.

[Proposed dosage and administration]

(a) The usual adult dosage is 60 mg of Daclatasvir orally administered once daily.

Daklinza should be used in combination with Asunaprevir for a duration of 24 weeks.

(b) The usual adult dosage is 100 mg of Asunaprevir orally administered twice daily.

Sunvepra should be used in combination with Daclatasvir Hydrochloride for a duration of 24 weeks.

II. Summary of the Submitted Data and Outline of Review

A summary of the submitted data and the outline of a review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or background of discovery and usage conditions in foreign countries etc.

Daclatasvir Hydrochloride (DCV) and Asunaprevir (ASV) are a selective hepatitis C virus (HCV) NS5A replication complex inhibitor and a selective HCV NS3/4A serine protease inhibitor, respectively, developed as treatments for HCV infection by Bristol-Myers Squibb Company and Bristol-Myers K.K.

There are estimated to be approximately 150 million people infected with HCV globally[1)] and 1.5 to 2 million in Japan.[2),3)] After the initial infection, 70% to 80% will become chronically infected and if the HCV infection is left untreated, liver fibrosis will progress slowly and develop into liver cirrhosis. Liver cirrhosis causes liver failure and furthermore, patients with liver cirrhosis are at high risk for hepatocellular carcinoma and it has been reported that the hepatocellular carcinogenesis rate in patients with chronic hepatitis C was 12.4% at the end of the 10th year[4)] and that the hepatocellular carcinogenesis rate in patients with cirrhosis infected with HCV was 53.9% after a mean observation period of 9.2 years.[5)] In Japan, approximately 70% of about 30,000 people who die of hepatocellular carcinoma each year are those infected with HCV[6),7)] and many of patients with chronic hepatitis C are elderly patients, who are at an increased risk of developing hepatocellular carcinoma.[8)]

Currently in Japan, interferon (IFN) preparations, pegylated IFN (PegIFN) preparations, ribavirin (RBV) preparations, and HCV NS3/4A serine protease inhibitors (telaprevir and simeprevir sodium) have been approved as drugs for chronic hepatitis C to achieve viral clearance and the Japanese guidelines recommend a triple therapy of an HCV NS3/4A serine protease inhibitor and PegIFN/RBV.[2)] PegIFN/RBV combination therapy has been approved for the treatment of patients with HCV with compensated cirrhosis.

However, even these therapies are not adequately effective in patients with chronic hepatitis C with previous treatment failure or patients with HCV with compensated cirrhosis. In addition, there are problems with the triple therapy of an HCV NS3/4A serine protease inhibitor and PegIFN/RBV: patients have to visit their physician weekly to receive PegIFN; dose reduction or discontinuation of PegIFN or RBV may be needed depending on the patient's condition; and there are many patients who have difficulty tolerating side effects associated with the therapy. Especially, elderly patients and patients with comorbidities (hypertension, heart disease, psychiatric disorder, cytopenia, etc.) are difficult to treat with PegIFN/RBV combination therapy.

Based on the above, there is a high medical need for new treatments in patients with chronic hepatitis C who are either IFN-ineligible or -intolerant, patients with chronic hepatitis C with previous treatment failure, and patients with HCV with compensated cirrhosis.

Anti-HCV activity of a combination of DCV and ASV was determined in a non-clinical study, which demonstrated their combined effects. Foreign clinical studies showed that resistance mutations may emerge in patients with chronic hepatitis C treated with DCV or ASV alone, but each drug produced reductions in HCV RNA and there were no particular safety issues. Therefore, it was considered that the combination of DCV and ASV, which have different mechanisms of action, has an enhanced anti-viral effect, can offer a new treatment option to HCV-infected patients with no or poor response to current therapies, and reduces patients' burden

1) http://www.who.int/mediacentre/factsheets/fs164/en/ (April 2014)
2) Drafting Committee for Hepatitis Management Guidelines, the Japan Society of Hepatology. *Guidelines for the Management of Hepatitis C Virus Infection (Second edition)*, 2013
3) http://www.vhfj.or.jp/06.qanda/about_ctype.html#syou44 (April 2014)
4) Kobayashi M, et al. *J Med Virol*. 2006;78:459-465.
5) Ikeda K, et al. *Hepatology*. 1999;29(4):1124-1130.
6) Vital Statistics 2011 http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei11/dl/11_h7.pdf (April 2014)
7) Follow-up Committee, Liver Cancer Study Group of Japan. *Acta Hepatologica Japonica*. 2010;51(8):460-484.
8) Hamada H, et al. *Cancer*. 2002;95(2):331-339.

(adverse drug reactions, frequent visit) and healthcare professionals' burden (monitoring and management of adverse events), and the development of the new IFN-free, dual therapy was planned by Bristol-Myers Squibb Company overseas and Bristol-Myers K.K. in Japan.

In a Japanese clinical study, the Daklinza Tablets (DCV tablets) + Sunvepra Capsules (ASV softgel capsules) dual therapy achieved high efficacy in IFN-ineligible or -intolerant patients and nonresponders to IFN-based therapies and there were no major safety concerns. Thus, a marketing application has been filed.

Although these proposed products have not been approved overseas as of April 2014, an EU marketing authorization application for DCV and US new drug applications for DCV and ASV have been submitted and are currently undergoing regulatory review.

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance (DCV)

2.A.(1).1) Characterization

The drug substance is a white to yellow powder and has been characterized by melting point, thermal analysis, hygroscopicity, specific rotation, solubility, pH, dissociation constant (imidazole ring), partition coefficient, and crystalline polymorphism. [redacted].

The chemical structure of the drug substance has been confirmed by ultraviolet-visible spectroscopy, infrared spectrophotometry (IR), Raman spectroscopy, nuclear magnetic resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR), mass spectrometry, and single-crystal x-ray crystallography.

2.A.(1).2) Manufacturing process

[redacted].

Quality by Design (QbD) approaches were utilized and the following studies were mainly performed.

- [redacted]
- Identification of process parameters that may affect the critical quality attributes (CQAs) of the drug substance through a quality risk assessment of each step of the manufacturing process

[redacted]

[redacted].

2.A.(1).3) Control of drug substance

[redacted].

2.A.(1).4) Stability of drug substance

Stability studies on the drug substance are as shown in Table 1. Based on the results of a photostability study, the drug substance is photostable.

Table 1. Stability studies on drug substance

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	3 pilot-scale batches	25°C	60%RH	double polyethylene bags/fiber drum	12 months
Accelerated	3 pilot-scale batches	40°C	75%RH		6 months

Based on "Guideline for Evaluation of Stability Data" (PMSB/ELD Notification No. 0603004 dated June 3, 2003, "ICH Q1E Guideline"), a re-test period of [redacted] months has been proposed for the drug substance when stored at room temperature in double polyethylene bags in a fiber drum. The long-term stability study will be continued up to [redacted] months.

2.A.(2) Drug product (DCV)

2.A.(2).1) Description and composition of the drug product and formulation development

DCV is formulated as a film-coated tablet. Each tablet contains 66 mg of Daclatasvir Hydrochloride (60 mg as Daclatasvir).

[redacted].

2.A.(2).2) Manufacturing process

[redacted].

QbD approaches were utilized and the following studies also were mainly performed.

- [redacted]
- Formulation selection and establishment of the range of the process parameters through quality risk assessment, design of experiments, etc.

2.A.(2).3) Control of drug product

The proposed drug product specifications include strength, description, identity (IR, LC), purity (related substances [LC]), uniformity of dosage units (content uniformity [LC]), dissolution, and assay (LC).

2.A.(2).4) Stability of drug product

Stability studies on the drug product are as shown in Table 2. A photostability study showed the drug product is photostable.

Table 2. Stability studies on drug product

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	3 pilot-scale batches	25°C	60%RH	PTP	12 months
Accelerated	3 pilot-scale batches	40°C	75%RH		6 months

Based on the ICH Q1E Guideline, a shelf-life of 24 months has been proposed for the drug product when stored at room temperature in PTP (polyvinyl chloride film laminated to polychlorotrifluoroethylene film/aluminum foil). The long-term stability study will be continued up to [redacted] months.

2.A.(3) Drug substance (ASV)

2.A.(3).1) Characterization

The drug substance is a white to pale yellowish white powder and has been characterized by melting point, thermal analysis, hygroscopicity, particle size, solubility, pH, dissociation constant, partition coefficient, and crystalline polymorphism. [redacted].

The chemical structure of the drug substance has been elucidated by ultraviolet-visible spectroscopy, infrared spectrophotometry (IR), nuclear magnetic resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR), mass spectrometry, and single-crystal x-ray crystallography. Theoretically, 32 stereoisomers exist for the drug substance and 2 diastereomers have been detected in production batches.[10)]

2.A.(3).2) Manufacturing process

[redacted].

QbD approaches were utilized and the following studies were mainly performed.

- [redacted]
- Identification of process parameters that may affect the CQAs of the drug substance through a quality risk assessment of each step of the manufacturing process

9) [redacted]

10) These diastereomers are controlled by the specification (related substances).

[redacted]

2.A.(3).3) Control of drug substance

The proposed drug substance specifications include content, description, identity (IR, LC), heavy metals, related substances (LC), residual solvents (gas chromatography), and assay (LC).

2.A.(3).4) Stability of drug substance

Stability studies on the drug substance are as shown in Table 3. In long-term and accelerated stability studies on pilot-scale drug substance batches, water content increased over time. A photostability study showed the drug substance is photolabile.

Table 3. Stability studies on drug substance

Study	Primary batches (Manufacturing process [a)])	Temperature	Humidity	Storage package	Storage period
Long-term	2 pilot-scale batches (drug substance produced by Process X)	25°C	60%RH	double polyethylene bags/cylindrical high-density polyethylene drum (without desiccant)	18 months
	3 production batches (drug substance produced by Process X)			double polyethylene bags/cylindrical high-density polyethylene drum (with desiccant)	9 months
	3 pilot-scale batches (drug substance produced by Process Y)			double polyethylene bags/cylindrical high-density polyethylene drum (without desiccant)	18 months
Accelerated	2 pilot-scale batches (drug substance produced by Process X)	40°C	75%RH	double polyethylene bags	6 months
	3 production batches (drug substance produced by Process X)				6 months
	3 pilot-scale batches (drug substance produced by Process Y)				6 months

a) As in Process Y, a reagent, which remained as a genotoxic impurity, was used, Process X, free of this reagent, was employed as a commercial-scale manufacturing process. Batch analyses have ascertained that the drug substances produced in Process Y and in Process X are comparable in terms of quality.

Based on the ICH Q1E Guideline, a re-test period of [redacted] months has been proposed for the drug substance when stored at room temperature in double polyethylene bags (with desiccant) in a high-density polyethylene drum, protected from light. The long-term stability study will be continued up to [redacted] months.

2.A.(4) Drug product (ASV)

2.A.(4).1) Description and composition of the drug product and formulation development

ASV is formulated as a softgel capsule. Each softgel capsule contains 100 mg of Asunaprevir. The excipients used are medium-chain triglycerides, monoglycerides of caprylic and capric acids, polysorbate 80, dibutylhydroxytoluene, gelatin, sugar alcohol solution derived from corn starch, concentrated glycerin, and titanium oxide.

2.A.(4).2) Manufacturing process

The drug product manufacturing process consists of the preparation of a capsule content solution, the preparation

of gel for capsules, the preparation of a capsule shell solution, capsule filling, drying, washing, testing and storage, and packaging, labeling, storage, and testing. [redacted].

QbD approaches were utilized and the following studies were mainly performed.

- [redacted]
- Formulation selection and establishment of the range of the process parameters through risk assessment of the manufacturing process and design of experiments

2.A.(4).3) Control of drug product

The proposed drug product specifications include strength, description, identity (IR, LC), purity (related substances [LC]), uniformity of dosage units (content uniformity [LC]), dissolution, microbial limits, and assay (LC).

2.A.(4).4) Stability of drug product

Stability studies on the drug product are as shown in Table 4. At the accelerated storage condition, increases in related substances over time (3 pilot-scale batches only) and a decrease in dissolution rate were observed. A photostability study showed the drug product is photolabile.

Table 4. Stability studies on drug product

Study	Primary batches [a)]	Temperature	Humidity	Storage package	Storage period
Long-term	3 production batches (drug product manufactured using drug substance produced by Process X)	25°C	60%RH	PTP	3 months
	3 pilot-scale batches (drug product manufactured using drug substance produced by Process Y)				15 months
Accelerated	3 production batches (drug product manufactured using drug substance produced by Process X)	40°C	75%RH		3 months
	3 pilot-scale batches (drug product manufactured using drug substance produced by Process Y)				6 months

a) Drug product is manufactured at a commercial-scale using drug substance produced in Process X. Batch analyses have ascertained that the drug product manufactured using drug substance produced in Process Y is comparable to the drug product manufactured using drug substance produced in Process X in terms of quality.

Based on the above, a shelf-life of 15 months has been proposed for the drug product when stored at room temperature in PTP (polyvinyl chloride film laminated to polychlorotrifluoroethylene film/aluminum foil), protected from light. The long-term stability study will be continued up to [redacted] months.

2.B Outline of the review

Based on the submitted data, PMDA concluded that the quality of the drug substance and drug product of DCV is adequately controlled. Based on the submitted data and the following reviews, PMDA also concluded that the quality of the drug substance and drug product of ASV is adequately controlled.

2.B.(1) Dissolution acceptance criteria for the drug product (ASV)

The applicant explained the basis for selecting dissolution acceptance criteria for the drug product as follows:

Although cross-linking of the gelatin in the capsule shell resulted in a decreased dissolution rate at 6 months in an accelerated stability study on the drug product,[11)] the results from clinical pharmacology studies[12)] indicate that even if there are some differences in dissolution rate, therapeutic activity will not be altered. Thus, the acceptance criterion for drug product dissolution was set as "a Q-value of [redacted]% in [redacted] minutes".

PMDA considered as follows:
Given that the pharmacokinetics of the gelatin capsules with and without cross-linking has not been compared in non-clinical or clinical studies, the effects of a decreased dissolution rate due to cross-linking of the gelatin in the capsule shell on the pharmacokinetics, efficacy, and safety of ASV capsules have not been determined. Thus, the acceptance criterion for drug product dissolution that can detect a decreased dissolution rate due to cross-linking of the gelatin in the capsule shell, should be established.

PMDA asked the applicant to explain the appropriateness of the established acceptance criterion.

The applicant explained as follows:
According to batch analysis of the drug product, the dissolution rates of [redacted] batches in [redacted], [redacted], and [redacted] minutes were [redacted]% to [redacted]%, [redacted]% to [redacted]%, and [redacted]% to [redacted]%, respectively. As it is preferable from the standpoint of quality control (i.e. detect the drug product with poor quality) to measure dissolution at a time point when a plateau of the mean dissolution rate is reached, either [redacted] minutes or [redacted] minutes is considered acceptable. In order to more reliably detect delayed dissolution due to cross-linking of the gelatin in the capsule shell,[11)] dissolution will be measured at the [redacted]-minute time point and the acceptance criterion for dissolution testing will be changed as follows: "a Q-value of [redacted]% in [redacted] minutes".

Based on the above explanation by the applicant, PMDA concluded that "a Q-value of [redacted]% in [redacted] minutes" is acceptable as the acceptance criterion that can detect a decreased dissolution rate due to cross-linking of the gelatin in the capsule shell.

2.B.(2) Novel excipient (ASV)

[redacted]. PMDA also concluded based on the submitted data that there is no particular problem with stability or safety.

3. Non-clinical data

3.(i) Summary of pharmacology studies

11) Dissolution [redacted]% to [redacted]% in [redacted] minutes, Dissolution [redacted]% to [redacted]% in [redacted] minutes
12) [redacted].

3.(i).A Summary of the submitted data

For this application, primary pharmacodynamic studies of DCV were performed to evaluate its anti-HCV activity, cytotoxic effect, activity against viruses other than HCV, effects when combined with other anti-HCV agents, and drug resistance. Safety pharmacology studies were performed to assess the effects of DCV on the cardiovascular and respiratory systems, and central nervous system (CNS).

Primary pharmacodynamic studies of ASV were performed to determine structure by crystallography and evaluate its enzyme inhibitory activity, anti-HCV activity, cytotoxic effect, activity against viruses other than HCV, effects when combined with other anti-HCV agents, and drug resistance. A secondary pharmacodynamic study was performed to evaluate the effect of ASV on IFN production. Safety pharmacology studies were performed to assess the effects of ASV on the cardiovascular and respiratory systems, and CNS. Doses of DCV are expressed in terms of Daclatasvir.

3.(i).A.(1) Primary pharmacodynamics (DCV)

3.(i).A.(1).1) *In vitro* studies

3.(i).A.(1).1).(a) Anti-viral activity and specificity (4.2.1.1.1)

The mechanism of action of DCV has been explained as follows: since NS5A exists as dimers[13] and DCV is a biphenyl-based C-2 symmetric homodimeric molecule, DCV is modeled to bind in a cleft at the dimer interface of NS5A. Using cell-based HCV replicon assays (detection method: Luciferase, FRET, or Taqman),[14] the anti-viral activity (50% effective concentration [EC_{50}]) of DCV against different HCV genotypes and clinical isolates[15] was determined. The results were as shown in Table 5. DCV exhibited lower activity against genotype 2a (HC-J6), which has been explained as follows: the amino acid residue at position 31 of NS5A from the HC-J6 strain is methionine; and genotype 1a and genotype 1b viruses with the L31M/V substitution are known to be resistant to DCV.

13) Tellinghuisen TL, et al. *Nature*. 2005;435: 374-379.
14) Lohmann V, et al. *Science*. 1999;285:110-113.
15) Hybrid replicons constructed using the genetic background of a genotype 1b (Con1) or genotype 2a (JFH-1) replicon were utilized in the study.

Table 5. Anti-viral activity of DCV in replicon cell lines

HCV genotype (Viral strain)	Detection method	EC_{50} (nM)		
		DCV	BMS-805215	BMS-795853
1a (H77c)	Luciferase	0.020 ± 0.009	ND	ND
1a (H77c)	FRET	0.050 ± 0.013	19 ± 5	14 ± 2
1a (H77c)	Taqman	0.003 ± 0.0006	17 ± 5	14 ± 2
1b (Con1)	Luciferase	0.004 ± 0.002	0.4 ± 0.20	ND
1b (Con1)	FRET	0.009 ± 0.004	0.82 ± 0.23	0.07 ± 0.03
1b (Con1)	Taqman	0.0012 ± 0.0007	0.63 ± 0.10	ND
2a (JFH-1)	Luciferase	0.034 ± 0.019	ND	ND
2a (HC-J6) [a)]	Luciferase	18 ± 5.1	ND	ND
2a (clinical isolates) [a)]	Luciferase	8.8 - 19 [c)]	ND	ND
3a [a)]	FRET	0.146 ± 0.034	3.8 ± 1	50 ± 30
3a (1-100) [a)]	Luciferase	0.19 ± 0.07	ND	ND
3a (clinical isolates) [a)]	Luciferase	0.14 - 1.25 [d)]	ND	ND
4a [a)]	FRET	0.012 ± 0.004	0.66 ± 0.13	1.6 ± 1.4
4a (full length) [a)]	Luciferase	0.007 ± 0.003	ND	ND
4a (clinical isolates) [a)]	Luciferase	0.007 - 0.013 [d)]	ND	ND
5a [a)]	FRET	0.033 ± 0.010	0.0019 ± 0.0004	0.416 ± 0.065
5a (1-100) [a)]	Luciferase	0.019 ± 0.007	ND	ND
5a (clinical isolates) [a)]	Luciferase	0.003 - 0.004 [c)]	ND	ND
6a (1-429) [a)]	Luciferase	0.054 ± 0.008	ND	ND
CC_{50} in 1b (Con1) cell line	Fluorescence	17 ± 1 μM	17 ± 1 μM	> 30 μM
Therapeutic index [b)]		1,900,000	21,000	> 330,000

Mean ± Standard Deviation (SD)
BMS-805215, the major metabolite in monkeys (a compound produced by pyrrolidine ring hydroxylation and rearrangement of DCV)
BMS-795853, the major metabolite in dogs (decarboxymethylated form of DCV); ND, not determined
a) Hybrid replicons were utilized in the study (The numbers in parentheses refer to the sequence positions of the amino acids of NS5A from each genotype in replicon cell line [Con1 strain or JFH-1 strain]).
b) 50% cytotoxic concentration (CC_{50})/EC_{50} of DCV in genotype 1b (Con1) replicon cell line (FRET)
c) EC_{50} range for 2 clinical isolates
d) EC_{50} range for 3 clinical isolates

The effect of 40% human serum protein on the anti-viral activity of DCV was studied. The results were as shown in Table 6.

Table 6. Effect of 40% human serum protein on DCV activity

HCV genotype	EC_{50} (nM)		Effect of human serum [a)]
	In the absence of human serum	In the presence of human serum	
1a	0.041 ± 0.013	0.068 ± 0.016	1.7
1b	0.007 ± 0.002	0.012 ± 0.003	1.7

Mean ± SD
a) EC_{50} in the presence of human serum/EC_{50} in the absence of human serum

With respect to the anti-viral selectivity of DCV, its anti-viral activity against various RNA and DNA viruses, including bovine viral diarrhea virus (BVDV) classified in the genus Pestivirus, which is closely related to HCV, was determined. The results were as shown in Table 7. The cytotoxicity of DCV was investigated in multiple cell lines derived from various tissues.[16)] As a result, the CC_{50} of DCV was ≥17 μM in all cell lines tested.

16) Liver (Huh-7), kidney (Vero and MDBK), lung fibroblast (MRC5), and T-lymphocyte (MT2) cells were tested.

Table 7. Anti-viral activity of DCV against various RNA and DNA viruses

Replicon/Virus	Cell line	EC_{50} (nM)	Selectivity index [a)]
HCV replicon (1b)	Huh-7	0.009	-
HCV genotype 2a (JFH-1)	Huh-7	0.020	-
BVDV replicon	Huh-7	9000	1,000,000
BVDV	MDBK	12,000	1,300,000
HIV	MT2	> 20,000	> 2,200,000
HSV-1/HSV-2	Vero	> 11,000	> 1,200,000
Influenza	MDBK	> 100,000	> 11,000,000
CPIV	Vero	26,000	2,900,000
Human Rhinovirus	MRC5	> 29,000	> 3,200,000
Coxsackie virus	MRC5	> 29,000	> 3,200,000
Poliovirus	MRC5	> 29,000	> 3,200,000
Human Coronavirus	MRC5	> 29,000	> 3,200,000

HSV, herpes simplex virus; CPIV, canine parainfluenza virus
a) EC_{50} against other replicons or viruses/EC_{50} against HCV replicon (genotype 1b)

3.(i).A.(1).1).(b) Resistance (4.2.1.1.2, 5.3.3.2.2)

Replicon cells (genotypes 1a and 1b) were incubated with DCV, DCV-resistant variants were identified and relative replication capacity and anti-viral activity were determined. The results were as shown in Table 8 and Table 9.

Table 8. Relative replication capacity of DCV-resistant variants and anti-viral activity (Genotype 1a)

Genotype 1a [a)]	Wild type	M28T	Q30H	Q30R	L31V	Y93C	M28T/Y93C
Relative replication capacity (%)	100	6	80	34	113	23	5
EC_{50} (pM)	7	305	533	1017	3385	658	7914

a) Genotype 1b hybrid replicons carrying the amino acids 1-100 of NS5A from genotype 1a were utilized.

Table 9. Relative replication capacity of DCV-resistant variants and anti-viral activity (Genotype 1b)

Genotype 1b	Wild type	L31F	L31V	Y93H	Y93N	L31V/Y93H
Relative replication capacity (%)	100	132	127	16	25	0.7
EC_{50} (pM)	3	13	49	41	74	13,843

A foreign clinical study (Study AI444004)[17)] evaluated the efficacy and safety of DCV alone and the major mutations observed in subjects with viral breakthrough or relapse were also introduced into replicons and relative replication capacity and anti-viral activity were determined. The results were as shown in Table 10 and Table 11.

[17)] A foreign early phase II study in which foreign patients with chronic hepatitis C received orally DCV 1, 10, 30, 60, or 100 mg once daily or DCV 30 mg twice daily for 14 days.

Table 10. Resistance profiles of DCV in an *in vitro* replicon system (Genotype 1a)

Amino acid substitution	Relative replication capacity (%)	EC_{50} (ng/mL)
Wild type	100	0.0044 ± 0.0028
T21I	169 ± 9	0.0034 ± 0.0003
M28A	27 ± 25	20.2 ± 13.3
M28I	200 ± 38	0.0055 ± 0.0003
M28T	31 ± 23	3.0 ± 0.3
M28V	16 ± 11	0.0055 ± 0.0019
Q30D	171 ± 134	> 1481
Q30E	130 ± 56	110.9 ± 66.0
Q30G	54 ± 22	37.8 ± 11
Q30H	75 ± 31	6.5 ± 1.4
Q30K	19 ± 9	108 ± 52
Q30L	37 ± 11	0.016 ± 0.003
Q30P	114 ± 19	0.0067 ± 0.0007
Q30R	41 ± 16	5.4 ± 0.8
Q30 deletion	ND	ND
L31M	55 ± 15	1.5 ± 0.5
L31**P**/Q/**R**	ND	ND
L31V	117 ± 29	14.9 ± 4.4
P32L	18 ± 5	1.0 ± 0.15
V37A	13 ± 2	0.0037 ± 0.0006
V37M	117 ± 5	0.004 ± 0.0009
H58D	92 ± 9	2.2 ± 0.3
H58P	266 ± 261	0.0053 ± 0.0006
E62D	103 ± 32	0.017 ± 0.005
Y93C	11 ± 7	8.2 ± 3.0
Y93H	18 ± 11	23.9 ± 7.0
Y93N	13 ± 8	208.9 ± 47.9
T21I-L31M	82 ± 5	2.39 ± 0.018
M28A-Q30R	45 ± 11	1262 ± 273
M28T-Q30H	31 ± 22	461 ± 233
M28T-Q30K	15 ± 2	> 1481
M28T-Q30R	76 ± 23	264 ± 9
M28T-Y93H	—	ND
M28V-Q30R	147 ± 55	1.4 ± 0.01
Q30E-Y93H	6 ± 1	299 ± 45.6
Q30H-H58D	28 ± 2	> 1481
Q30H-Y93H	20 ± 6	409.8 ± 153.6
Q30R-L31M	54 ± 15	868 ± 679
Q30R-H58D	60 ± 12	1867 ± 46
Q30R-E62D	30 ± 14	111.9 ± 6.7
Q30R-Y93H	6 ± 1	252.5 ± 30.2
L31M-H58D	41 ± 4	294.5 ± 10.5
L31V-V37A	10 ± 0.1	47.1 ± 6.6
L31V-H58P	100 ± 0	54 ± 6
L31V-Y93H	20 ± 2	> 741
H58P-Y93H	22 ± 0.7	5.3 ± 0.06

Mean ± SD

ND, not determined; —,a marked decrease in replication capacity

Standard letters, resistance mutations observed in replicon cells

Bold letters, resistance mutations observed in replicon cells and clinical study

Italic letters, resistance mutations observed in clinical study

Table 11. Resistance profiles of DCV in an *in vitro* replicon system (Genotype 1b)

Amino acid substitution	Relative replication capacity (%)	EC_{50} (ng/mL)
Wild type	100	0.0019 ± 0.0002
R30Q	91 ± 1	0.0011 ± 0.0002
L31F	146 ± 44	0.009 ± 0.0009
L31I	54 ± 12	0.0027 ± 0.0011
L31M	99 ± 23	0.0062 ± 0.0014
L31V	158 ± 54	0.053 ± 0.015
L31W	191 ± 9	0.175 ± 0.047
P32L	18 ± 6	0.031 ± 0.016
P32 deletion	29.1 ± 3.2	> 741
F37L	151 ± 32	0.0013 ± 0.0001
Q54H	83 ± 18	0.0024 ± 0.0003
Q54N	83 ± 29	0.0027 ± 0.0006
P58S	121 ± 17	0.0017 ± 0.0003
Y93C	61.9 ± 4.4	0.0063 ± 0.0014
Y93H	27 ± 16	0.046 ± 0.020
Y93N	19 ± 5	0.096 ± 0.07
L23F-L31F	65 ± 5	0.025 ± 0.004
R30Q-L31F	224 ± 39	0.17 ± 0.073
L31I-Y93H	43 ± 11	4.8 ± 2.5
L31M-Y93H	70 ± 68	13.5 ± 12.2
L31V-Y93H	49.9 ± 38	28.1 ± 24.7
P32del-Y93H	—	ND
F37L-Y93H	34 ± 4	0.036 ± 0.005
Q54H-Y93H	22 ± 7	0.018 ± 0.005
L31V-Q54H-Y93H	189 ± 25	36.1 ± 7.7

Mean ± SD
ND, not determined; —, not replicated
Standard letters, resistance mutations observed in replicon cells
Bold letters, resistance mutations observed in replicon cells and clinical study
Italic letters, resistance mutations observed in clinical study

Cross-resistance between DCV and other anti-HCV agents was studied. The results were as shown in Table 12.

Table 12. Anti-viral activities of anti-HCV agents against wild-type and DCV-resistant mutant viruses

Mutant cell line	EC_{50} (nM)				
	DCV	ASV	[redacted]	HCV-796	pegIFNα
1a wild type	0.021	2.9	[redacted]	43.2	1.7
Q30E/K/D	239/82/ > 2000	2.1/3.4/2.4	[redacted]	27.8/ND/ND	2.3/ND/ND
Y93N/H	465/52	1.4/2.3	[redacted]	17.4/ND	1.7/ND
M28A-Q30R	> 2000	2.4	[redacted]	ND	ND
M28T-Q30H	768	3	[redacted]	25.2	2.9
Q30R-L31M	442	1.4	[redacted]	ND	ND
Q30H-Y93H	561	1.6	[redacted]	15.5	3.0
Q30R-H58D	> 2000	2.9	[redacted]	ND	ND

Mean
[redacted]; HCV-796, NS5B polymerase site II inhibitor; ND, not determined
The unit of pegIFNα concentrations is ng/mL.

3.(i).A.(1).1).(c) Combination studies (4.2.1.1.1, 4.2.1.1.3)

[redacted].[18] The results were as shown in Table 13.

[18] The EC_{50}, EC_{75}, and EC_{90} values of DCV as a single agent and of DCV in combination with other agents were calculated. Combination indices and 95% confidence intervals were calculated with the isobologram equation and the lower limit of the confidence interval >1, the upper limit of the confidence interval ≤1, and the confidence interval containing 1 indicated antagonism, synergism, and additive effect, respectively (Chou TC. *Pharmacological Reviews*. 2006;58(3):621-681).

Table 13. DCV combination studies in HCV replicon cells

	IFNα	IFNλ	ASV	[redacted]	[redacted]	[redacted]
Combination index (Min.) [95% CI]	0.47 [0.40, 0.54]	0.82 [0.75, 0.89]	0.57 [0.51, 0.63]	[redacted]	[redacted]	[redacted]
Combination index (Max.) [95% CI]	1.11 [0.99, 1.23]	1.20 [0.85, 1.55]	1.07 [0.79, 1.36]	[redacted]	[redacted]	[redacted]
Assessment	Additive or synergistic activity	Additive or synergistic activity	Additive or synergistic activity	[redacted]	[redacted]	[redacted]

3.(i).A.(2) Secondary pharmacodynamics (DCV)

No secondary pharmacodynamic data have been submitted in the application.

3.(i).A.(3) Safety pharmacology (DCV) (Reference data 4.2.1.3.1-4.2.1.3.5, 4.2.3.1.1, 4.2.3.1.2, 4.2.3.2.2-4.2.3.2.9, 4.2.3.7.3.2)

Safety pharmacology studies assessed the effects of DCV on the CNS and cardiovascular and respiratory systems. The results were as shown in Table 14.

Table 14. Summary of safety pharmacology studies

Organ systems evaluated	Animal species/ Cell species	Method of administration	Doses or concentrations	Gender and No. per Group	Noteworthy findings
Receptors, enzymes, and ion channels	Human, rat, and guinea pig cells	*In vitro*	10 μM	—	65% inhibition of sodium ion channel
			BMS-805215: 10 μM	—	None
Cardiovascular	HEK-293 cells	*In vitro*	10, 30 μM	—	hERG potassium channel: 26.2% inhibition at 10 μM and 50.6% inhibition at 30 μM (IC_{50}, 29.2 μM).
			10 μM	—	Sodium channel (SCN5A): 50.5% inhibition (1-Hz stimulation) and 59.4% inhibition (4-Hz stimulation).
			10, 30 μM	—	L-type calcium channel ($Ca_v1.2$): 31.9% inhibition at 10 μM and 42.6% inhibition at 30 μM.
			BMS-795853: 1, 3, 10 μM	—	hERG potassium channel: 40.0% inhibition at 10 μM.
			BMS-795853: 10 μM	—	Sodium channel (SCN5A): 22.6% inhibition (1-Hz stimulation) and 22.9% inhibition (4-Hz stimulation).
	Rabbit/ Purkinje fibers	*In vitro*	3, 10, 30 μM	—	None
	Rabbits (NZW)	Intravenous	0, 1, 3, 10, 30 mg/kg	3M	At 30 minutes post-dose, QRS interval prolongation (29%), PR interval prolongation (19%), AH interval prolongation (16%), HV interval prolongation (10%), increases in MAP (7%), and no effects on QTcF or QTcv interval at 30 mg/kg
	Beagle dogs (Telemetry)	Oral	0, 15, 100 mg/kg	3M, 3F	Vomiting (4 animals), reversible increases in systemic blood pressures (4 animals), and reversible decreases in +dp/dt (an index of cardiac contractility) (4 animals) at 100 mg/kg
Respiratory, CNS	Mice (ICR)	Oral	0, 100, 300, 1000 mg/kg	5M, 5F	None
	Rats (SD)	Oral	0, 100, 300, 1000 mg/kg	5M, 5F	None
	Rats (SD)	Oral	0, 10, 30, 100 mg/kg	15M, 15F	None
			DCV: 0, 10, 60 mg/kg ASV: 0, 30, 60 mg/kg	10M, 10F	None
	Rats (SD)	Oral	0, 12.5, 25, 50 mg/kg	25M, 25F	None

Organ systems evaluated	Animal species/ Cell species	Method of administration	Doses or concentrations	Gender and No. per Group	Noteworthy findings
Cardiovascular, CNS, Respiratory	Beagle dogs	Oral	0, 3, 15, 100/50 mg/kg	5M, 5F	Increased body temperature and heart rate in dead animals in the 100/50 mg/kg/day group (it has been discussed that these findings were secondary to other DCV-related effects).
	Cynomolgus monkeys	Oral	0, 10, 30, 100, 300 mg/kg	2M, 2F	None
			DCV: 0, 15, 50 mg/kg ASV: 0, 72, 129.5 mg/kg	4M, 4F	None
			DCV: 0, 15, 50 mg/kg ASV: 0, 45, 80 mg/kg	4M, 4F	None
			0, 15, 50, 300 mg/kg	4M, 4F	None
			0, 15, 30, 150 mg/kg	6M, 6F	None

BMS-805215, M2 metabolite of DCV; BMS-795853, M4 metabolite of DCV
Hz, Hertz; AH, interval between atrial wave to His wave; HV, interval between the His wave to ventricular wave; MAP, mean arterial blood pressure; QTcF, QT interval corrected for heart rate using Fridericia's formula; QTcv, QT interval corrected for heart rate using Van de Water's formula

The ratios of exposure levels at which DCV-related cardiovascular effects were observed in animals to the human exposure (C_{max}),[19] were 6.3 for dogs and 91 for rabbits. The ratio of *in vitro* to human exposure was ≥214. In the studies that assessed the effects of DCV on the CNS and respiratory system, DCV exposures in the rat, dog, and monkey were 6.9-, 4.3-, and 2.3-fold higher, respectively, than the human exposure (C_{max}), and no DCV-related effects on the CNS or respiratory system were observed.

3.(i).A.(4) Primary pharmacodynamics (ASV)

3.(i).A.(4).1) *In vitro* studies

3.(i).A.(4).1).(a) Enzyme activity and specificity (4.2.1.1.1)

The inhibitory activities of ASV and telaprevir (50% inhibitory concentration [IC_{50}]) against HCV NS3/4A proteases representing different genotypes were determined. The results were as shown in Table 15.

Table 15. *In vitro* inhibitory activity against HCV NS3/4A protease

Genotype (Strain)	IC_{50} (nM)	
	ASV	Telaprevir
1a (BMS)	1.8 ± 0.2	12 ± 2
1a (H77)	0.7 ± 0.06	33 ± 7
1b (J4L6S)	0.3 ± 0.02	22 ± 6
2a (HC-J6)	15 ± 1.2	95 ± 17
2b (HC-J8)	78 ± 2.0	12 ± 1
3a (S52)	320 ± 13	537 ± 4
4a (ED43)	1.6 ± 0.1	12 ± 1
5a (SA13)	1.7 ± 0.2	38 ± 3
6a (HK-6A)	0.9 ± 0.09	86 ± 2

Mean ± SD

The inhibitory activity of ASV against various proteases was determined. The IC_{50} values of ASV against HCV genotype 1a NS3/4A protease and the closely related GB virus-B NS3/4A protease were 0.001 and 21.2 μM, respectively, and the IC_{50} values against human elastase, porcine pancreatic elastase, human chymotrypsin, and human cathepsin B were all >50 μM.

3.(i).A.(4).1).(b) Anti-viral activity (4.2.1.1.1, 4.2.1.1.6)

Using cell-based HCV replicon assays (detection method, Luciferase or FRET),[20] the anti-viral activity of ASV

19) Animal to human exposure ratios were calculated using the steady-state C_{max} of DCV at the recommended clinical dose (60 mg once daily (QD)), 1.73 μg/mL [Study AI444004, see "4.(ii).A.(3).1).(b) Early phase II study in foreign patients with chronic hepatitis C"] and the ratio of in vitro to human exposure was calculated using the C_{max} of free DCV in humans (0.0346 μg/mL), taking into account protein binding of DCV (98.0%).

20) Lohmann V, et al. *Science*. 1999;285:110-113.

against different genotypes and clinical isolates[21] was determined. The results were as shown in Table 16.

Table 16. Genotypic coverage of Asunaprevir in HCV replicon assays

HCV genotype (Viral strain)	Detection method	EC_{50} (nM)	
		ASV	Telaprevir
1a (H77)	Luciferase	4.0 ± 0.3	995 ± 11
1b (Con 1)	Luciferase	1.2 ± 0.3	427 ± 3
1b (Con 1)	FRET	2.9 ± 0.5	573 ± 21
2a (JFH-1)	FRET	230 ± 74	229 ± 32
3a (S52)	Luciferase	1162 ± 274	2731 ± 188
2a (JFH-1)	Luciferase	67 ± 23	108 ± 12
2b (HC-J8)	Luciferase	480 ± 104	1236 ± 96
1b (Con 1)	Luciferase	1.3 ± 0.1	266 ± 77
4a (ED43)	Luciferase	1.8 ± 0.2	1137 ± 223
CC_{50} in 1b (Con 1) cell line	Fluorescence	27,000 ± 2000	> 100,000
Therapeutic index [a]	-	9300	> 175

Mean ± SD

a) CC_{50}/EC_{50} in genotype 1b (Con 1) replicon cell line (FRET)

Anti-viral activity against infectious HCV genotype 2a (JFH-1) virus was determined. The EC_{50} values of ASV and telaprevir were 39 nM and 116 nM, respectively.

The EC_{50} values of ASV against BVDV, HIV-1, human rhinovirus, and human coronavirus were 12 μM, 14 μM, >100 μM, and >100 μM, respectively.

The effect of serum protein on the anti-viral activity of ASV was studied in an HCV genotype 1b replicon assay supplemented with 40% human serum. The EC_{50} of ASV against a genotype 1b (Con 1) replicon supplemented with 40% human serum was 6.5-fold the EC_{50} without 40% human serum.

The cytotoxicity of ASV was investigated in multiple cell lines derived from various tissues.[22] As a result, the CC_{50} of ASV was ≥11 μM in all cell lines tested.

3.(i).A.(4).1).(c) Mechanism of action (4.2.1.1.2)

Using recombinant enzyme assays, the inhibition constant (K_i) values for HCV genotype 1 NS3/4A protease complexes were calculated. ASV competitively binds to the NS3/4A protease complex, with K_i values of 0.24 to 1.03 nM for the viral strains tested (3 strains). X-ray crystallography studies (2.2Å resolution) confirmed that ASV binds to the active site of the NS3 protease domain.

3.(i).A.(4).1).(d) Resistance (4.2.1.1.2, 4.2.1.1.3, 4.2.1.1.6)

Replicon cells (genotypes 1a and 1b) were incubated with ASV; ASV-resistant variants were identified; and anti-viral activity was determined. The results were as shown in Table 17.

[21] Hybrid replicons constructed using the genetic background of a genotype 1b (Con1) or genotype 2a (JFH-1) replicon were utilized in the study.

[22] Liver (Huh-7 and HepG2), T-lymphocyte (MT2), lung fibroblast (MRC5), cervical (HeLa), and embryonic kidney (HEK-293) cells were tested.

Table 17. Anti-viral activity against ASV-resistant replicons and amino acid substitutions identified

HCV replicon strain	EC_{50} (nM) [a] (Fold Change compared with wild type)	Major amino acid substitutions in the NS3 region	Frequency [b]
Wild type 1a (H77)	6.5 ± 2.2 (1)	—	—
Genotype 1a resistance selection in the presence of ASV at 10 times the EC_{50} value (Colony 1)	91 ± 39 (13)	V51A	1/9
		D79E	1/9
		T95A	1/9
		R155K	8/9
		I170T	1/9
Genotype 1a resistance selection in the presence of ASV at 10 times the EC_{50} value (Colony 2)	77 ± 26 (11)	Q41E	1/9
		R62K	1/9
		I114V	1/9
		D168G	3/9
		I170T	4/9
		N174Y	1/9
Genotype 1a resistance selection in the presence of ASV at 30 times the EC_{50} value	315 ± 72 (49)	T40A	1/10
		R123G	1/10
		R155K	4/10
		I170T	5/10
		L175P	1/10
		G176E	1/10
Wild type 1b (Con 1)	3 ± 0.5 (1)	Q41R	—
		Q86R	—
Genotype 1b resistance selection in the presence of ASV at 10 times the EC_{50} value [c]	592 ± 75, 1100 ± 141 (197, 367)	Q41R	6/10
		Q86R	10/10
		D168G/A/H	5/10;1/10;1/10
		E173G	1/10
		E176G	1/10
Genotype 1b resistance selection in the presence of ASV at 30 times the EC_{50} value [c]	509 ± 47, 767 ± 33, 1200 ± 141 (170, 400)	Q41R	7/12
		Q80R	1/12
		Q86R	10/12
		P89L	1/12
		Y105C	1/12
		D168V/G/A/Y	7/12; 3/12; 1/12; 1/12

Fold Change: EC_{50} against the mutant/EC_{50} against the wild type
a) Mean ± SD
b) Number of clones with amino acid substitutions/Total number of clones
c) Selected cells were assayed for susceptibility multiple times. Range for Fold Change.

As reduced responses to similar drugs, telaprevir and boceprevir,[23] have been associated with the emergence of mutations at amino acids V36 and A156, the amino acid substitutions reported in clinical studies[24] were introduced into replicons and the anti-viral activity of ASV against these replicons was determined. The results were as shown in Table 18.

Table 18. Anti-viral activity of ASV or telaprevir against replicons carrying mutations

NS3 variant replicon	Relative replication capacity (%)	ASV EC_{50} (nM)	Fold Change compared with wild type	Telaprevir EC_{50} (nM)
Wild type 1a (H77) [a]	100	0.76 ± 0.3	—	181 ± 87
V36A	89	2.3 ± 0.7	3	2689 ± 241
V36L	137	1.8 ± 0.6	2	1248 ± 427
V36M	171	1.5 ± 0.5	2	2989 ± 1813
T54A	39	0.3 ± 0.1	0.4	847 ± 209
T54S	36	0.7 ± 0.2	1	664 ± 150
Q80K	119	2.5 ± 1	3	152 ± 75
R155K	7	16 ± 11	21	860 ± 256
V36M + R155K	5	42 ± 19	55	> 6900
D168G	2	11 ± 4	14	133 ± 76
D168V	33	283 ± 4	373	162 ± 10
Q80K + D168V	46	542 ± 111	713	130 ± 37
I170T	12	3.6 ± 2	5	647 ± 202

23) Unapproved in Japan
24) Incivek (telaprevir) Prescribing label approved by US Food and Drug Administration, May 2011. http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/201917lbl.pdf (April 2014)

NS3 variant replicon	Relative replication capacity (%)	ASV EC_{50} (nM)	Fold Change compared with wild type	Telaprevir EC_{50} (nM)
Wild type 1b (Con 1)	100	0.86 ± 0.3	1	176 ± 41
V36A	124	1.6 ± 0.6	2	933 ± 281
V36L	77	0.7 ± 0.1	1	395 ± 19
T54A	101	0.4 ± 0.1	0.4	718 ± 31
T54S	15	1.6 ± 0.3	2	> 3353
Q80K	139	5.6 ± 1	6.5	ND
Q80L	98	0.9 ± 0.1	1	167 ± 14
Q80R	12	4.0 ± 1.7	5	121 ± 19
A156S	120	5.9 ± 0.9	7	2515 ± 624
A156T	17	5.4 ± 1	6	> 10,000
A156V	3	17 ± 2	20	> 10,000
D168A	37	109 ± 19	127	21 ± 4
D168G	29	13 ± 4	16	56 ± 12
D168V	29	241 ± 17	280	42 ± 7
D168Y	2	205 ± 29	238	71 ± 43
V170A	96	1.6 ± 0.4	2	699 ± 237

Mean ± SD for EC_{50}

3.(i).A.(4).1).(e) Combination studies (4.2.1.1.1, 4.2.1.1.4-4.2.1.1.6)

[redacted]. エラー! ブックマークが定義されていません。) As a result, as in "3.(i).A.(1).1).(c) Combination studies", additive or synergistic activity was observed.

A wild-type genotype 1a replicon and a genotype 1a replicon carrying the R155K mutation were treated with a triple therapy of ASV, DCV, and IFNα for 25 days. As a result, replication of both replicons was inhibited. On the other hand, when these replicons were treated with a dual combination therapy of ASV and DCV for 25 days, replicon replication was not inhibited.[25)]

3.(i).A.(5) Secondary pharmacodynamics (ASV) (4.2.1.1.2)

Persistent HCV infections reflect the ability of HCV to circumvent host immunity and antagonize IFNα/β-mediated anti-viral defense. HCV NS3 protease has been reported to suppress IFN-stimulated gene transcription by blocking IRF-3 (IFN regulatory factor-3) activation and nuclear translocation.[26)] The effect of ASV on IFN-stimulated gene transcription was investigated. As a result, inhibition of HCV NS3 protease by ASV relieved the blockage of IRF-3 activation and restored IFN-stimulated gene transcription.

25) Friborg J, et al. *Antimicrob Agents Chemother*. 2013;57(3):1312-1322.
26) Foy E, et al. *Science*. 2003;200:1145-1148.

3.(i).A.(6) Safety pharmacology (ASV) (Reference data 4.2.1.3.1-4.2.1.3.5, 4.2.3.1.1, 4.2.3.1.2, 4.2.3.2.2-4.2.3.2.6, 4.2.3.2.8, 4.2.3.2.9)

Safety pharmacology studies assessed the effects of ASV on the CNS and cardiovascular and respiratory systems. The results were as shown in Table 19.

Table 19. Summary of safety pharmacology studies

Organ systems evaluated	Animal species/Cell species	Method of administration	Doses or concentrations	Gender and No. per Group	Noteworthy findings
Receptors, enzymes, and ion channels	Human, rat, and guinea pig cells	*In vitro*	10 μM	—	None
Cardiovascular	HEK-293 cells	*In vitro*	10, 30 μM	—	hERG potassium channel: 8.2%-20.6% inhibition at ≥10 μM (IC_{50} >30 μM).
			10, 30 μM	—	Sodium channel (SCN5A): 26.6%-65.9% inhibition (1-Hz stimulation) and 29.8%-71.6% inhibition (4-Hz stimulation) at ≥10 μM.
			30 μM	—	L-type calcium channel ($Ca_v1.2$): 18.3% inhibition at 30 μM.
	Rabbit/ Purkinje fibers	*In vitro*	3, 10, 30 μM	—	None
	Langendorff isolated rabbit heart	*In vitro*	0, 10 μM	2-3F	None
	Beagle dogs (Telemetry)	Oral	0, 100 mg/kg	3M, 3F	stool changes (unformed stool, liquid stool, hemorrhagic stool, mucous stool) at 100 mg/kg
	Rabbits (NZW)	Intravenous	0, 3, 10, 30 mg/kg	3M	Dose-dependent increases in blood pressure (10%-24%).
Respiratory, CNS	Mice (ICR)	Oral	0, 200, 600, 2000 mg/kg	5M, 5F	None
	Rats (SD)	Oral	0, 200, 600, 2000 mg/kg	5M, 5F	None
			0, 30, 100, 600 mg/kg	15M, 15F	None
			0, 40, 80, 200 mg/kg	25M, 25F	None
			ASV: 0, 30, 60 mg/kg DCV: 0, 10, 60 mg/kg	10M, 10F	None
Cardiovascular, CNS, Respiratory	Beagle dogs	Oral	0, 20, 60, 300 mg/kg	3M, 3F	None
			0, 15, 50, 100 mg/kg	6M, 6F	None
	Rats (SD)	Oral	ASV: 0, 30, 60 mg/kg DCV: 0, 10, 60 mg/kg	10M, 10F	None
	Cynomolgus monkeys	Oral	ASV: 0, 72, 129.5 mg/kg DCV: 0, 15, 50 mg/kg	4M, 4F	None
			ASV: 0, 45, 80 mg/kg DCV: 0, 15, 50 mg/kg	4M, 4F	None

The ratios of exposure levels at which ASV-related cardiovascular effects were observed in animals to the human exposure (C_{max}),[27] were 120 for dogs and 36 for rabbits. The ratio of *in vitro* (ASV 10 μM [7.5 μg/mL]) to human exposure was 1500. In the studies that assessed the effects of ASV on the CNS and respiratory systems, ASV exposures in the rat, dog, and monkey were 11-, 82-, and 18-fold higher, respectively, than the human exposure (AUC) and no ASV-related effects on the CNS or respiratory system were observed.

27) Animal to human exposure ratios were calculated using the steady-state human exposure to ASV at the recommended clinical dose (100 mg twice daily (BID)), C_{max} 0.419 μg/mL or AUC 3.69 μg·h/mL (Study AI447016). The ratio of *in vitro* to human exposure was calculated using the C_{max} of free ASV in humans (0.005 μg/mL).

3.(i).B Outline of the review

3.(i).B.(1) Anti-viral activities of DCV and ASV

PMDA's view on the anti-HCV activities of DCV and ASV is as follows:

DCV and ASV were shown to exert anti-HCV activity by inhibiting the HCV NS5A replication complex and the HCV NS3/4A serine protease, respectively, and a combination of DCV and ASV also exhibited additive or synergistic anti-viral activity. Thus, there should be no particular problems with the anti-viral activity of DCV or ASV. The efficacy of the DCV + ASV dual therapy in patients with chronic hepatitis C including those with compensated cirrhosis will be discussed in "4.(iii).B.(1) Efficacy".

3.(i).B.(2) Resistance to DCV or ASV

The applicant explained the resistance profiles of DCV as follows:

For genotype 1b, the frequently observed resistance substitutions identified were at amino acid residues L31 and Y93. Variants with single amino acid substitutions and variants with double amino acid substitutions such as the Q54H-Y93H substitution displayed minimal resistance, while variants with double amino acid substitutions such as the L31V-Y93H substitution conferred moderate or high levels of resistance. For genotype 1a, the major resistance substitutions identified were at residues M28, Q30, L31, and Y93 and even variants with single amino acid substitutions conferred high levels of resistance.

PMDA asked the applicant to explain whether or not variants resistant to other HCV NS3/4A serine protease inhibitors approved in Japan show cross-resistance to ASV.

The applicant explained as follows:

The major amino acid substitutions associated with resistance to other HCV NS3/4A serine protease inhibitors have been identified at residues 43, 80, 122, 155, 156, and 168 of NS3, and the anti-viral activities of ASV, simeprevir, and telaprevir against replicon cell lines containing these mutations are as shown in Table 20. The EC_{50} values of ASV against R155K, A156V, and D168V were lower than the EC_{50} values of simeprevir. Taking also account of anti-viral activity against replicons containing the amino acid substitutions reported in clinical studies[24] [see "3.(i).A.(4).1).(d) Resistance"], ASV is considered to show better anti-viral activity than telaprevir.

Table 20. Anti-viral activities of NS3 protease inhibitors against variant replicons

NS3 variant replicon	EC_{50} (nM)		
	ASV	Simeprevir	Telaprevir
Genotype 1a wild type	7.3 ± 3	5.8 ± 1	623 ± 51
Genotype 1a-R155K	150 ± 29	335 ± 23	5719 ± 1237
Genotype 1a-Q80K	2.5 ± 1.0	— [a]	152 ± 75
Genotype 1b wild type	1.2 ± 0.2	0.83 ± 0.1	263 ± 53
Genotype 1b-F43S	3.2 ± 0.3	83 [b]	213 ± 36
Genotype 1b-Q80K	5.6 ± 1.2	62 [b]	ND
Genotype 1b-A156V	20 ± 5	372 ± 28	4973 ± 472
Genotype 1b-D168V	187 ± 26	2753 ± 362	79 ± 29

Mean ± SD
ND, not determined
a) An approximately 10-fold reduction in susceptibility (FDA background package for simeprevir [NDA 205123])
b) Cited from Lenz et al.'s report[28]

[28] Lenz O, et al. *Antimicrob Agents Chemother*. 2010;54(5):1878-1887.

PMDA considers as follows:

Variants resistant to each drug emerged *in vitro*; single or multiple mutations conferred high levels of resistance; and as in the case of similar drugs, the major resistance mutations such as R155K, A156V, and D168V resulted in reduced susceptibility to ASV. The association between the emergence of these mutations and the efficacy of the DCV + ASV dual therapy will be discussed in "4.(iii).B.(1) Efficacy". Since resistance mutations are considered to significantly influence efficacy and the information on the DCV + ASV dual therapy in HCV-infected patients is limited, it is important to continue to collect post-marketing information on drug resistance and provide the information to healthcare providers in clinical settings when a new finding becomes available.

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

For this application, DCV and ASV pharmacokinetic studies were conducted to evaluate the pharmacokinetics following intravenous or oral administration of ^{3}H-DCV, ^{14}C-DCV, and unlabeled DCV and ^{14}C-ASV and unlabeled ASV to mice, rats, rabbits, dogs, and monkeys under fasting or non-fasting conditions. Doses and pharmacokinetic parameters of DCV are expressed in terms of Daclatasvir. Concentrations of DCV, ASV, and their metabolites in biological samples were determined using liquid chromatography/tandem mass spectrometry (LC/MS/MS), radioactivity levels in biological samples were determined by liquid scintillation counter, tissue radioactivity levels were determined by quantitative whole-body autoradiography, and metabolites were analyzed by LC, LC/MS, LC/MS/MS, and nuclear magnetic resonance spectroscopy.

Unless otherwise specified, pharmacokinetic parameters are expressed in mean values.

3.(ii).A.(1) Absorption (DCV)

3.(ii).A.(1).1) Single-dose administration (4.2.2.2.1)

Following single intravenous doses of DCV 3 mg/kg in FVB mice (3 males/time point), DCV 2 or 5 mg/kg in SD rats (3 males/group), DCV 1 mg/kg in beagle dogs (5 males), and DCV 1.13 mg/kg in cynomolgus monkeys (3 males), the total plasma clearance (CL) was 9.3, 9.1 to 14.8, 20.3, and 12.4 mL/min/kg, respectively, and the steady-state volume of distribution (V_{ss}) was 1.2 to 3.6 L/kg in rats, 5.4 L/kg in dogs, and 2.2 L/kg in monkeys. The area under the plasma concentration-time curve (AUC)[29)] was 5.4 μg·h/mL in mice, 3.7 and 5.66 μg·h/mL in rats, 1.31 μg·h/mL in dogs, and 1.63 μg·h/mL in monkeys.

Following intraportal administration of DCV 2 mg/kg to SD rats (3 males), the AUC from time zero to infinity (AUC_{inf}) was 2.8 μg·h/mL.

Following single oral doses of DCV 3 mg/kg in FVB mice (3 males/time point), DCV 5 mg/kg in SD rats (3 males), DCV 3 mg/kg in beagle dogs (5 males), and DCV 2.83 mg/kg in cynomolgus monkeys (3 males), the AUC[29)] was 6.6, 3.6, 5.66, and 1.45 μg·h/mL, respectively, and the bioavailability (BA) was 123%, 50.4%,

29) The AUC from time zero to 8 hours (AUC_{0-8}) for mice and the AUC_{inf} for rats, dogs, and monkeys were calculated.

144%, and 38%, respectively.

Following oral coadministration of DCV 3 mg/kg with pentagastrin or famotidine to beagle dogs (3 males/group), the AUC_{inf} was 9.1 or 5.4 μg·h/mL, respectively, and BA was 89% or 48%, respectively.

3.(ii).A.(1).2) Repeat-dose administration (Toxicokinetics) (4.2.3.2.2, 4.2.3.2.4, 4.2.3.2.5, 4.2.3.2.8, 4.2.3.2.9, 4.2.3.7.3.2)

Following repeat oral doses of DCV in SD rats, beagle dogs, and cynomolgus monkeys, the plasma AUC from time zero to time T ($AUC_{0\text{-}T}$) was as shown in Table 21 and there was no marked accumulation after multiple doses and no gender differences were also observed.

Table 21. $AUC_{0\text{-}T}$ after multiple oral doses

Species	Duration of dosing	Dose (mg/kg/day)	Number of animals	$AUC_{0\text{-}T}$ (μg·h/mL) after the last dose	
				Males	Females
Rats	1 month	10	15M, 15F	5.39	4.75
		30	15M, 15F	26.9	23.9
		100	15M, 15F	109	105
	6 months	12.5	25M, 25F	7.97	10.3
		25	25M, 25F	15.6	17.2
		50	25M, 25F	43.8	74.9
Dogs	1 month	3	5M, 5F	1.23	2.36
		15	5M, 5F	28.6	24.0
		100/50 [a)]	5M, 5F	186	105
Monkeys	1 month	10	2M, 2F	2.31	1.65
		30	2M, 2F	14.0	13.6
		100	2M, 2F	38.3	21.5
		300	2M, 2F	54.5	88.5
	4 months	15	4M, 4F	2.55	2.07
		50	4M, 4F	19.3	25.7
		300	4M, 4F	48.0	34.4
	9 months	15	6M, 6F	2.91	3.61
		30	6M, 6F	11.3	11.8
		150	6M, 6F	39.6	37.9

a) DCV at a dose of 100 mg/kg/day was administered from Day 1 to Day 8 (females) or Day 9 (males); DCV was interrupted from Day 9 to Day 13 (females) or from Day 10 to Day 14 (males); and DCV at a dose of 50 mg/kg/day was administered from Day 14 (females) or Day 15 (males) to Day 28.

3.(ii).A.(2) Distribution (DCV)

3.(ii).A.(2).1) Protein binding and distribution in blood cells (4.2.2.2.1, 4.2.2.3.1, 4.2.2.4.8)

When DCV (7.39 μg/mL) was added to mouse, rat, dog, rabbit, monkey, and human serum, the protein binding was 98.2%, 98.3%, 96.5%, 99.5%, 95.1%, and 95.6%, respectively. The human plasma protein binding of DCV at concentrations of 0.074, 0.739, and 7.39 μg/mL was 97.9%, 98.0%, and 97.7%, respectively. The protein binding of DCV (0.001-2.030 μg/mL) in plasma from patients with chronic hepatitis C[30)] was 98.9% to 99.3%.

When DCV (7.39 μg/mL) was added to mouse, rat, dog, monkey, and human blood, the blood to plasma concentration ratio was 0.56 to 1.08 in animal blood and 0.77 to 0.82 in human blood.

30) A foreign phase II study in foreign patients with chronic hepatitis C (Study AI444004) [see "4.(ii).A.(3) Studies in patients with chronic hepatitis C"].

3.(ii).A.(2).2) Tissue distribution (4.2.2.3.4, 4.2.2.3.5)

Following single or repeat oral doses of ^{14}C-DCV 30 mg/kg in albino SD rats (a single dose, 1 male and 1 female/time point; repeat doses, 1 male/time point), tissue radioactivity levels were determined. After a single dose, there were no gender differences in the tissue distribution of radioactivity; C_{max} was reached at 2 to 8 hours post-dose in most tissues; the bile, liver, adrenal gland, and Harderian gland exhibited high levels of radioactivity in both males and females (74.2, 21.7, 19.3, and 19.1 μg eq./g, respectively, in males; 57.6, 22.3, 15.8, and 16.5 μg eq./g, respectively, in females); and radioactivity was eliminated from most tissues by 168 hours post-dose.[31)] After multiple doses, there was no accumulation of radioactivity in any tissue; the tissue distribution and elimination of radioactivity were similar to those after a single dose; and radioactivity levels were high in the bile, Harderian gland, and liver (104, 22.1, and 13.9 μg eq./g, respectively). Radioactivity levels peaked at 8 hours post-dose in most tissues, and radioactivity was eliminated from most tissues by 2016 hours (84 days) post-dose except for the exorbital lacrimal gland, intraorbital lacrimal gland, thymus, and thyroid gland.

The tissue distribution of radioactivity following a single oral dose of ^{14}C-DCV 10.5 mg/kg was similar between pigmented Long-Evans rats (1 male/time point) and albino SD rats (1 male/time point), except for the pigmented skin and ocular tissue.[32)]

3.(ii).A.(2).3) Distribution in liver (4.2.2.2.1, 4.2.2.3.6)

Following oral or intravenous administration of DCV to mice, rats, dogs, and monkeys,[33)] the liver to serum or plasma ratio of the DCV AUC was 2.35 (intravenous administration) and 1.9 (oral administration) in mice, 5.9 (intravenous administration) and 6.8 (oral administration) in rats, 10.6 (oral administration) in dogs, and 17 (oral administration) in monkeys.

3.(ii).A.(2).4) Placental transfer to fetus (4.2.2.3.5)

The tissue distribution of a single oral dose of ^{14}C-DCV 30 mg/kg was similar between SD rats (gestation day 18, 1 female/time point) and non-pregnant female rats and C_{max} of radioactivity was reached at approximately 2 hours post-dose in maternal tissues including the placenta. Although radioactivity was detectable in the amniotic sac at 0.5 to 48 hours post-dose (1.23-7.68 μg eq./g), radioactivity levels in the amniotic fluid were below the lower limit of quantification (LLOQ) (0.248 μg eq./g). In fetal tissues, radioactivity was detectable only in the liver at 4 hours post-dose (0.473 μg eq./g) and radioactivity was below the LLOQ or undetectable in other tissues including blood at all sampling points.

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31) At 168 hours post-dose, radioactivity was detectable in the adrenal gland, exorbital lacrimal gland, Harderian gland, intraorbital lacrimal gland, kidney, renal cortex, pituitary gland, spleen, thymus, and thyroid gland in males and in the kidney, renal cortex, preputial gland, spleen, thymus, and thyroid gland in females (<1 μg eq./g).

32) In the ocular tissue, radioactivity was still detectable at 840 hours (35 days) post-dose in pigmented Long-Evans rats, while radioactivity levels were below the LLOQ (0.069 μg eq./g) by 24 hours post-dose in albino SD rats.

33) FVB mice (3 males/time point) orally or intravenously received DCV 3 mg/kg, SD rats (2 males/time point) orally received DCV 5 mg/kg or intravenously received DCV 2 mg/kg, beagle dogs (1 male/time point) orally received DCV 3 mg/kg, and rhesus monkeys (4 males) orally received DCV 2.8 mg/kg.

3.(ii).A.(3) Metabolism (DCV)

3.(ii).A.(3).1) *In vivo* metabolism (4.2.2.4.2, 4.2.2.4.3)

Following a single oral dose of ^{14}C-DCV in mice, rats, rabbits, dogs, and monkeys,[34] *in vivo* metabolism was investigated and the possible metabolic pathways of DCV are as shown in Figure 1.

The unchanged drug represented 74% to 94% of the plasma radioactivity and the main component in plasma was DCV in all animal species tested. In excreta, 17% to 36% of the administered dose was recovered as metabolites and among the detected metabolites,[35] the major metabolites in feces were BMS-952328 in mice and rats (7.3% and 9.4%, respectively, of the administered dose) and BMS-805215 in rabbits and monkeys (3.6% and 16.8%, respectively, of the administered dose) and the major metabolite in bile was BMS-805215 in rats, dogs, and monkeys (10.1%, 2.9%, and 7.2%, respectively, of the administered dose). In urine, 1.2% to 1.6% of the radioactive dose was detected and trace amounts of metabolites were found.

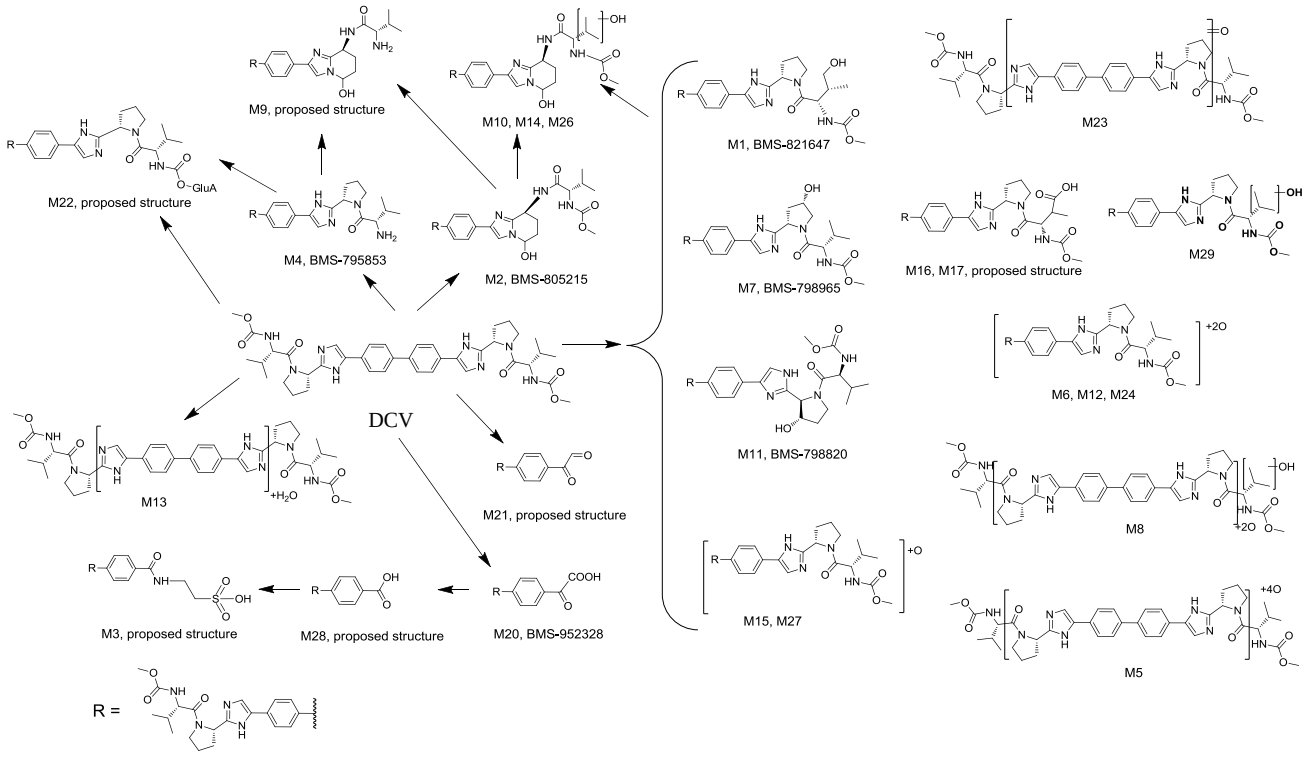

Figure 1. Possible metabolic pathways of DCV

3.(ii).A.(3).2) *In vitro* metabolism (4.2.2.2.1, 4.2.2.4.1, 4.2.2.4.4)

When ^{14}C-DCV (10 µM) was added to mouse, rat, rabbit, dog, monkey, and human liver microsomes (in the presence of NADPH and glutathione), mouse, rat, dog, monkey, and human liver S9 fractions (in the presence of NADPH), and mouse, rat, dog, monkey, and human hepatocytes, 11 metabolites[36] were detected. The major metabolites were BMS-805215, BMS-795853, BMS-821647, BMS-798820, and M27, and there were no unique

34) ^{14}C-DCV was given as single oral doses of 50 mg/kg to rasH2 mice (9 males/time point), 30 mg/kg to SD rats (3 males/time point), 30 mg/kg to bile duct cannulated SD rats (3 males), 40 mg/kg to NZW rabbits (3 females), 50 mg/kg to bile duct cannulated beagle dogs (3 males), 30 mg/kg to cynomolgus monkeys (3 males), and 100 mg/kg and 1 mg/kg to bile duct cannulated cynomolgus monkeys (3 males).

35) BMS-821647, BMS-805215, M3, BMS-795853, M5, M6, BMS-798965, M8, M9, BMS-798820, M11, M12, M13, M14, M15, M16, M17, BMS-952328, M21, M22, M23, M24, M26, M27, M28, M29

36) BMS-821647, BMS-805215, BMS-795853, M6, M9, BMS-798820, M12, M13, BMS-952328, M27, M28

human DCV metabolites detected.

A metabolic inhibition study in human liver microsomes with or without specific inhibitors and a metabolism study using human cytochrome P450 (CYP) expression system in the presence of NADPH were conducted to identify CYP isoforms involved in the metabolism of DCV, which indicated a major contribution of CYP3A4 and a minor contribution of CYP3A5 to the metabolism of DCV into BMS-805215 and a major contribution of CYP2C8 to the metabolism of DCV into BMS-821647.

3.(ii).A.(4) Excretion (DCV)

3.(ii).A.(4).1) Urinary and fecal excretion and biliary excretion (4.2.2.2.1, 4.2.2.4.2, 4.2.2.5.1-4.2.2.5.6)

Following a single oral dose of ^{14}C-DCV (mice, 50 mg/kg; rats and monkeys, 30 mg/kg; rabbits, 40 mg/kg) in rasH2 mice (9 males), SD rats (3 males), NZW rabbits (3 females), and cynomolgus monkeys (3 males), the urinary and fecal excretion of radioactivity over 168 hours post-dose were 1.40% and 87.4%, respectively, of the dose in mice, 1.55% and 91.1%, respectively, of the dose in rats, 0.73% and 91.6%, respectively, of the dose in rabbits, and 1.35% and 69.4%, respectively, of the dose in monkeys. Following a single oral dose of ^{14}C-DCV (rats, 30 mg/kg; dogs, 50 mg/kg; monkeys, 100 mg/kg) in bile duct cannulated SD rats, beagle dogs, and monkeys (3 males each), the biliary excretion of radioactivity[37)] was 38.5%, 24.5%, and 14.7%, respectively, of the dose.

3.(ii).A.(4).2) Excretion in milk (4.2.2.3.5)

Following a single oral dose of ^{14}C-DCV 30 mg/kg in SD rats (day 4 postpartum, 3 females/time point), the milk to plasma ratios of radioactivity were 1.27 and 1.55 based on C_{max} and $AUC_{inf,}$[38)] respectively.

3.(ii).A.(5) Pharmacokinetic drug interactions (DCV)

3.(ii).A.(5).1) Enzyme inhibition and induction (4.2.2.2.1, 4.2.2.4.7, 4.2.2.7.2)

Inhibition of the activities of CYP isoforms (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) and uridine diphosphate-glucuronyltransferase (UGT) 1A1 by DCV (0.002-40 μM) in human liver microsomes was assessed. The IC_{50} values of DCV for CYP3A4 were 31.8 and 11.0 μM[39)] and the IC_{50} value of DCV for UGT1A1 was 12.7 μM while the IC_{50} values for other CYP isoforms were >40 μM. The IC_{50} values for CYP3A4 decreased from 31.8 μM to 13.5 μM and from 11.0 μM to 8.9 μM with 30 minutes of preincubation, suggesting that DCV is a time-dependent CYP3A inhibitor.

The CYP (CYP1A2, 2B6, 3A4) induction potential of DCV in human hepatocytes was evaluated. DCV did not induce CYP1A2 and dose-dependently increased CYP3A4 and 2B6 mRNA levels and activities.

37) Biliary excretion over 48 hours post-dose in rats, and biliary excretion over 72 hours post-dose in dogs and monkeys
38) As a terminal elimination phase was not observed and $T_{1/2}$ or AUC_{inf} could not be calculated, AUC_{inf} was calculated based on AUC_{0-72}.
39) As CYP3A4 substrates, midazolam and testosterone were used.

3.(ii).A.(5).2) Characterization of DCV as a potential substrate for drug transporters (4.2.2.2.1, 4.2.2.7.1-4.2.2.7.3)

In Caco-2 cell monolayers, the efflux ratio (the ratio of the apparent permeability coefficient in the basolateral to apical direction to the apparent permeability coefficient in the apical to basolateral direction) of DCV (0.3 μM) was >24, which was reduced to 1.6 and 0.8 in the presence of ketoconazole and cyclosporine (both P-glycoprotein [P-gp] inhibitors), respectively. When DCV 3 mg/kg was orally administered to wild-type mice and P-gp knockout (*mdr 1a/1b*) mice (3 males/time point/group), the AUC_{0-8} in P-gp knockout mice was 1.7-fold higher than that in wild-type mice. In wild-type MDCK cells and MDCK cells expressing human breast cancer resistance protein (BCRP), the efflux ratios of ^{3}H-DCV (1 μM) were 12.4 and 10.8, respectively. Furthermore, the uptake of ^{3}H-DCV (5 μM) into membrane vesicles from Sf9 cells expressing human multidrug resistance protein (MRP2) was similar in the presence of adenosine monophosphate (AMP, energy-independent) or adenosine triphosphate (ATP, energy-dependent) and was not affected by an MRP2 inhibitor, MK-571 (P-gp and MRP2 inhibitor). The above results indicated that DCV is a substrate for P-gp and is not a substrate for BCRP or MRP2.

It was suggested that the uptake of DCV into rat hepatocytes is rapid and saturable (K_m >50 μM). A study using Xenopus laevis oocytes expressing rat organic anion transport polypeptide (rOatp) 1, rOatp2, or rOatp4 indicated that the uptake of ^{3}H-DCV (1 μM) is partially mediated by rOatp1 and rOatp2, in addition to passive diffusion.

The uptake of ^{3}H-DCV (0.01-25 μM) into human hepatocytes was rapid and was not inhibited by rotenone or BSP (both cause ATP depletion) or bromosulfophthalein (a transporter inhibitor), suggesting that the uptake is not energy- or transporter-dependent. Moreover, the uptake of ^{3}H-DCV (10 nM) into HEK-293 cells expressing OATP1B1, OATP2B1, or OATP1B3 in the presence or absence of BSP, and the uptake of ^{3}H-DCV (1 μM) into Xenopus laevis oocytes expressing OATP1B3 were studied, which indicated that DCV is not a substrate for OATP1B1, OATP2B1, or OATP1B3.

3.(ii).A.(5).3) Inhibition of drug transporters (4.2.2.2.1, 4.2.2.7.4-4.2.2.7.9)

Inhibition of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OATP2B1, sodium taurocholate cotransporting polypeptide (NTCP), organic anion transporter (OAT) 1, OAT3, organic cation transporter (OCT) 1, OCT2, and a bile acid transporter (BSEP) by DCV was assessed and the results were as shown in Table 22. When foreign patients with chronic hepatitis C receivedDCV capsules 60 mg once daily (QD) for 14 days, the C_{max} was 2.34 μM (1.73 μg/mL).[40),41),42)] Therefore, it was considered that DCV can interact with P-gp, BCRP, OATP1B1, OATP1B3, and BSEP substrates and is unlikely to interact with other transporters.

40) A foreign phase II study in foreign patients with chronic hepatitis C (Study AI444004) [see "4.(ii).A.(3) Studies in patients with chronic hepatitis C"].
41) FDA Guidance for Industry. Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. DRAFT GUIDANCE, 2012
42) Giacomini, et al. *Nat Rev Drug Discovery*. 2010;9:215-236.

Table 22. *In vitro* inhibition of transporters by DCV

Cells	Transporter	Substrate	IC_{50} value (μM)
Caco-2		Digoxin	4.4
MDCK	P-gp	Digoxin	>7
	BCRP	Genistein	10.9 ± 8.6
HEK-293	OATP1B1	BMS-791553	2.3
	OATP1B3	Cholecystokinin-8	5.7 ± 1.3
	OATP2B1	Estrone sulfate	41.8 ± 4.0
	OAT1	p-aminohippuric acid	>8
	OAT3	Estrone sulfate	>8
	OCT1	Metformin	1.4
	OCT2	Metformin	7.3
Membrane vesicles	MRP2	Estradiol-17β-D-glucuronide	32.0 ± 7.7
	BSEP	Taurocholic acid	6.39
CHO	NTCP	Taurocholic acid	-

Mean or Mean ± SD

3.(ii).A.(6) Absorption (ASV)

3.(ii).A.(6).1) Single-dose administration (4.2.2.2.1, 4.2.2.2.2, Reference data 4.2.3.1.3)

Following single intravenous doses of ASV 2 mg/kg in FVB mice (3 males/time point), ASV 1, 3, or 5 mg/kg in SD rats (3 males/group), ASV 1 or 2 mg/kg in beagle dogs (2 males and 3 males, respectively), and ASV 1 mg/kg in cynomolgus monkeys (3 males), the total serum or plasma CL was 57.3, 38.4 to 38.9, 15.4 to 18.7, and 18.3 mL/min/kg, respectively, and the V_{ss} was 12.6, 7.4 to 7.9, 0.2 to 0.6, and 0.5 L/kg, respectively. The serum or plasma AUC_{inf} was 0.58 μg·h/mL in mice, 0.53, 1.62, and 2.27 μg·h/mL, respectively, in rats, 0.90 and 2.62 μg·h/mL, respectively, in dogs, and 0.95 μg·h/mL in monkeys.

Following intraportal administration of ASV 5 mg/kg to SD rats (2 males), the AUC_{inf} was 2.44 μg·h/mL.

Following single oral doses of ASV or the potassium salt of ASV in the form of solution, suspension, or capsules in FVB mice, SD rats, beagle dogs, and cynomolgus monkeys (all males), serum or plasma pharmacokinetic parameters were as shown in Table 23 and BA tended to increase with increasing dose in rats and dogs.

Table 23. Pharmacokinetic parameters after a single oral dose

Species	Formulation	Dose (mg/kg)	N	C_{max} (μg/mL)	AUC_{inf} (μg·h/mL)	$t_{1/2}$ (h)	Absolute BA (%)
Mice	ASV solution	5	3 (per time point)	0.096 ± 0.059	0.405	NA	28
Rats	ASV solution	5	3	0.032 ± 0.020	0.227 ± 0.173	4.5 [a)]	10
		10	3	0.031 ± 0.009	0.317 ± 0.203	6.1 [a)]	7
		15	3	0.137 ± 0.063	0.945 ± 0.220	8.3 ± 2.2	14
	ASV suspension	10	3	0.122 ± 0.112	0.300 ± 0.053	2.8 [a)]	7
		30	3	1.44 ± 0.820	4.03 ± 2.63	3.7 ± 1.4	30
		90	3	4.59 ± 2.59	16.8 ± 4.90	2.5 ± 0.1	41
Dogs	ASV solution	3	2	0.45	1.65	2	42
		6	3	5.60 ± 2.60	16.4 ± 4.83	2.4 ± 0.2	>100
	Potassium salt of ASV capsules	4	4	0.35 ± 0.16	0.80 ± 0.37	0.7 ± 0.1	15
		10	3	4.32 ± 2.26	12.2 ± 7.19	2.8 ± 0.8	93
Monkeys	ASV solution	3	3	0.14 ± 0.13	0.31 ± 0.21	1.1 ± 0.3	10

Mean or Mean ± SD

C_{max}, maximum concentration; $t_{1/2}$, elimination half-life; NA, not available

a) N = 2

The effect of food was studied following oral administration of ASV solution, ASV tablets, and the potassium salt of ASV capsules to beagle dogs (4 males/time point/group). No food effect was observed with the solution or capsule formulation, and compared to fasted administration, administration of the tablet formulation with food increased the plasma C_{max} and AUC (2.1-fold and 3.6-fold, respectively).

3.(ii).A.(6).2) Repeat-dose administration (Toxicokinetics) (4.2.3.2.2, 4.2.3.2.4-4.2.3.2.6, Reference data 4.2.3.2.7)

Following repeated oral doses of ASV in SD rats, beagle dogs, and cynomolgus monkeys, the plasma $AUC_{0\text{-}T}$ was as shown in Table 24 and there was no marked accumulation after multiple doses. In rats and dogs, the plasma $AUC_{0\text{-}T}$ tended to be higher in females than in males.

Table 24. $AUC_{0\text{-}T}$ after multiple oral doses

Species	Duration of dosing	Dose (mg/kg/day)	Number of animals	$AUC_{0\text{-}T}$ (μg·h/mL) after the last dose	
				Males	Females
Rats	1 month	30	15M, 15F	1.88	1.34
		100	15M, 15F	83.2	98.2
		600	15M, 15F	227	371
	6 months	40	25M, 25F	3.99	11.6
		80	25M, 25F	39.3	144
		200	25M, 25F	321	684
Dogs	1 month	20	3M, 3F	4.58	16.3
		60	3M, 3F	102	98.5
		300	3M, 3F	1410	1360
	9 months	15	6M, 6F	5.46	15.6
		50	6M, 6F	47.2	80.9
		100	6M, 6F	223	380
Monkeys	1 week	30	2M, 2F	0.483	0.24
		150	2M, 2F	5.44	6.16
		300	2M, 2F	982	411

3.(ii).A.(7) Distribution (ASV)

3.(ii).A.(7).1) Protein binding and distribution in blood cells (4.2.2.2.1, 4.2.2.3.1, 4.2.2.3.2, 5.3.3.2.2)

When ASV (7.48 μg/mL) was added to mouse, rat, dog, monkey, and human serum, the protein binding was 99.2%, 98.8%, 98.5%, 97.2%, and 98.8%, respectively. The human plasma protein binding of ASV at concentrations of 0.075, 0.75, and 7.48 μg/mL was 99.8%, 99.7%, and 99.8%, respectively. The protein binding of ASV (0.050-1.588 μg/mL) in plasma from patients with chronic hepatitis C[43)] was 99.8%.

When ASV (0.75 μg/mL) was added to rat, dog, monkey, and human blood, the blood to plasma concentration ratio was 0.34 to 0.82 in animal blood and 0.55 in human blood.

3.(ii).A.(7).2) Tissue distribution (4.2.2.3.3, 4.2.2.3.4)

Following single or repeated oral doses of ^{14}C-ASV 80 mg/kg in albino SD rats (a single dose, 1 male and 1 female/time point; repeated doses, 1 male/time point), tissue radioactivity levels were determined. There were no gender differences in the tissue distribution of radioactivity and C_{max} was reached in most tissues at 4 to 8 hours post-dose and radioactivity was eliminated from all the tissues analyzed by 168 hours post-dose. After a single dose, the distribution of radioactivity was highest in the liver in both males and females and high levels of radioactivity were present in the cecum, renal cortex, renal medulla, kidney, and adrenal gland in males and in the small intestine, brown fat, renal cortex, and cecum in females and the liver (184 μg eq./g) in males and

43) A foreign phase II study in foreign patients with chronic hepatitis C (Study AI447004) [see "4.(ii).A.(3) Studies in patients with chronic hepatitis C"].

the liver and small intestine (206 and 29.0 µg eq./g, respectively) in females had radioactivity concentrations higher than plasma at the C_{max} (males: 23.9 µg eq./g; females: 20.2 µg eq./g). After repeated doses, the liver (114 µg eq./g), cecum (22.0 µg eq./g), small intestine (13.0 µg eq./g), large intestine (11.2 µg eq./g), gastric mucosa (8.36 µg eq./g), renal cortex (6.90 µg eq./g), and kidney (5.91 µg eq./g) had radioactivity concentrations higher than plasma at the C_{max} (5.25 µg eq./g). Radioactivity was not detected in the CNS tissues.

There were no marked differences in the tissue distribution of a single oral dose of ^{14}C-ASV 10 mg/kg between pigmented Long Evans rats (1 male/time point) and albino SD rats (1 male/time point).

3.(ii).A.(7).3) Distribution in liver (4.2.2.2.1)

Following oral or intravenous administration of ASV to rats, dogs, and monkeys,[44] the liver to serum or plasma ratios of the ASV AUC were 772 and 257 (intravenous administration) and 1240 and 360 (oral administration) in rats, 40 (oral administration) in dogs, and 216 (oral administration) in monkeys.

3.(ii).A.(7).4) Placental transfer to fetus (4.2.2.3.4)

Following a single oral dose of ^{14}C-ASV 80 mg/kg in SD rats (gestation day 18, 1 female/time point), radioactivity was detectable in the amniotic sac at 1 to 72 hours post-dose (0.851-24.9 µg eq./g) while radioactivity was below the LLOQ (0.766 µg eq./g) in the amniotic fluid. In fetal tissues, radioactivity was detectable in the liver at 4 to 12 hours post-dose (1.46-1.58 µg eq./g) and in the alimentary tract at 24 and 48 hours post-dose (1.99 and 0.951 µg eq./g, respectively). Fetal tissue radioactivity levels, except for the liver radioactivity level at 12 hours post-dose (1.46 µg eq./g), were lower than maternal blood levels (0.822-13.8 µg eq./g).

3.(ii).A.(8) Metabolism (ASV)

3.(ii).A.(8).1) *In vivo* metabolism (4.2.2.4.3-4.2.2.4.5)

Following a single oral dose of ^{14}C-ASV in mice, rats, rabbits, dogs, and monkeys,[45] *in vivo* metabolism was investigated and the possible metabolic pathways of ASV are as shown in Figure 2.

The main component in plasma was ASV in all animal species tested and all metabolites[46] represented <6% each of the plasma radioactivity. In excreta, 35% to 76% of the administered dose was recovered as metabolites, and among the detected metabolites,[47] the major metabolites in feces were BMS-558364 in mice and rats (8.3% and 7.0%, respectively, of the administered dose), M7 in rabbits (3.1% of the administered dose), and BMS-558364 and M3 in dogs (both 2.3% of the administered dose) and the major metabolites in bile were M7 in rats (0.8% of the administered dose) and BMS-558364 in monkeys (2.3% of the administered dose). In urine,[48]

[44] SD rats (2 males/time point) orally received ASV 1 and 5 mg/kg or intravenously received ASV 5 and 15 mg/kg, beagle dogs (6 males) orally received ASV 6 mg/kg, and rhesus monkeys (3 males) orally received ASV 10 mg/kg.

[45] ^{14}C-ASV was given as single oral doses of 100 mg/kg to rasH2 mice (5-9 males/time point), 80 mg/kg to SD rats (3 males/time point), 80 mg/kg to bile duct cannulated SD rats (3 males), 100 mg/kg to NZW rabbits (3 females), 50 mg/kg to beagle dogs (3 males), and 100 mg/kg to bile duct cannulated cynomolgus monkeys (3 males).

[46] M3, M4, M5, M7, M8, BMS-558364, M12, M15, M19, M20, M21, M22, M24, and M25

[47] M1, M3, M4, M5, M7, M8, BMS-558364, M11, M12, M15, M19, M20, M21, M22, M24, M25, and M26

[48] Metabolites in urine were analyzed for mice only.

1.42% of the radioactive dose was detected and the main component was ASV (0.5% of the administered dose) and trace amounts of metabolites were found.

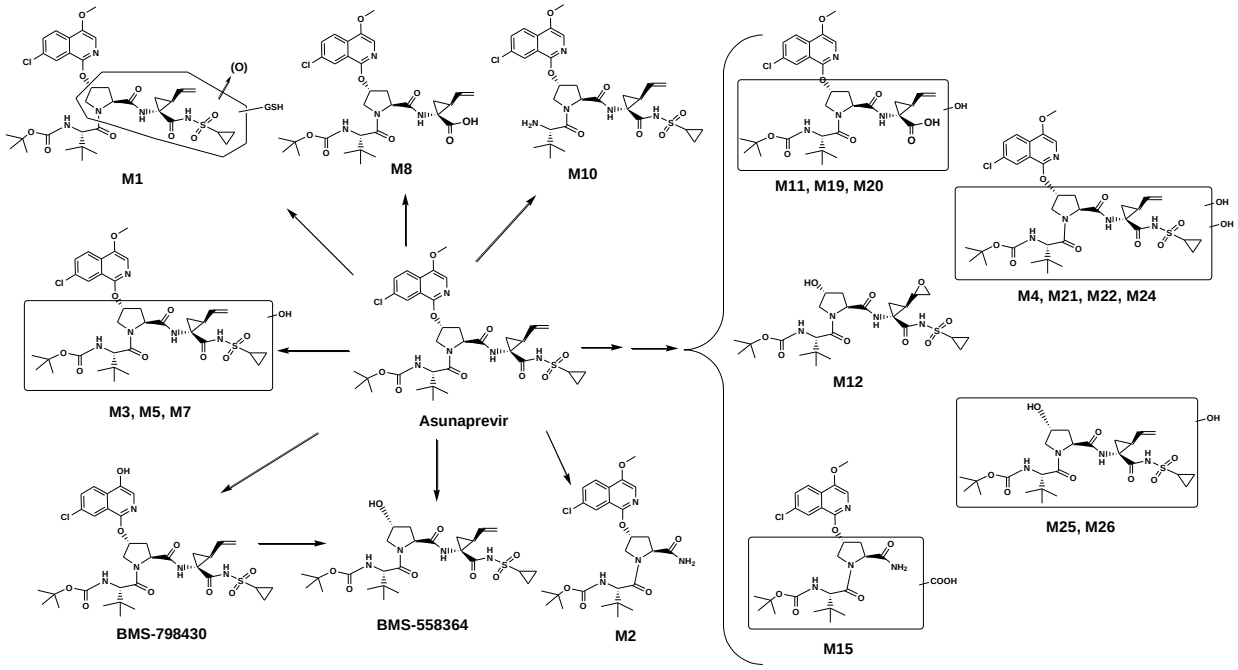

Figure 2. Possible metabolic pathways of ASV

3.(ii).A.(8).2) *In vitro* metabolism (4.2.2.2.1, 4.2.2.4.1, 4.2.2.4.2)

When ^{14}C-ASV (10 μM) was added to mouse, rat, dog, monkey, and human liver microsomes (in the presence of NADPH and glutathione), rat liver S9 fraction (in the presence of NADPH), and mouse, rat, dog, monkey, and human hepatocytes, 13 metabolites[49)] were detected and the major metabolites were BMS-798430, M7, and BMS-558364 and there were no unique human ASV metabolites detected.

A metabolic inhibition study in human liver microsomes with or without specific inhibitors and a metabolism study using human CYP expression system in the presence of NADPH were conducted to identify CYP isoforms involved in the metabolism of ASV, which indicated that ASV is metabolized primarily by CYP3A4 and CYP3A5 and that CYP2A6, CYP2B6, CYP2C9, CYP2C19, and CYP2D6 are also involved in the metabolism of ASV.

49) M1, M2, M3, M4, M5, BMS-798430, M7, M8, BMS-558364, M10, M11, M12, and M15

3.(ii).A.(9) Excretion (ASV)

3.(ii).A.(9).1) Urinary and fecal excretion and biliary excretion (4.2.2.2.1, 4.2.2.4.3, 4.2.2.4.4, 4.2.2.5.1-6)

Following a single oral dose of ^{14}C-ASV (mice and rabbits, 100 mg/kg; rats, 80 mg/kg; dogs, 50 mg/kg) in rasH2 mice, SD rats, NZW rabbits, and beagle dogs (3 males each), the urinary and fecal excretion of radioactivity over 168 hours were 1.42% and 87.9%, respectively, of the dose in mice, 0.29% and 86.9%, respectively, of the dose in rats, 0.92% and 84.5%, respectively, of the dose in rabbits, and 0.42% and 77.1%, respectively, of the dose in dogs. Following a single oral dose of ^{14}C-ASV (rats, 80 mg/kg; monkeys, 6 and 100 mg/kg) in bile duct cannulated SD rats (3 males) and cynomolgus monkeys (1 male and 3 males, respectively), the biliary excretion of radioactivity[50)] was 25.5% of the dose in rats and 32.9% and 26.3%, respectively, of the dose in monkeys.

3.(ii).A.(9).2) Excretion in milk (4.2.2.3.4)

Following a single oral dose of ^{14}C-ASV 80 mg/kg in SD rats (day 9 or 10 postpartum, 3 females/time point), the milk-to-plasma ratios of radioactivity were 0.174 and 0.454 based on C_{max} and AUC_{inf}, respectively.

3.(ii).A.(10) Pharmacokinetic drug interactions (ASV)

3.(ii).A.(10).1) Enzyme inhibition and induction (4.2.2.2.1, 4.2.2.7.4-4.2.2.7.6, 4.2.2.7.8, 4.2.2.7.9, 4.2.2.7.16, 4.2.2.7.17)

Inhibition of the activities of CYP isoforms (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A) and UGT1A1 by ASV (0.002-40.0 μM) in human liver microsomes was assessed. The IC_{50} values of ASV for CYP2B6, 2C8, 3A, and UGT1A1 were 31.8, 30.9, 27.3,[51)] and 14.9 μM, respectively, and the results of an analysis using a model[52)] indicated that ASV is not an inhibitor of CYP2B6 or 2C8. The IC_{50} values for other CYP isoforms were >40 μM. The IC_{50} value for CYP2D6 decreased from >40 μM to 5.7 μM and the IC_{50} value for CYP3A decreased from 27.3 μM to 5.4 μM with 30 minutes of preincubation, suggesting that ASV is a time-dependent inhibitor of CYP2D6 and CYP3A. The maximal inactivation rate constant (k_{inact}) and inhibition constant (K_i, inhibitor concentration necessary to achieve 50% the maximum inactivation rate) of ASV against CYP2D6 were 0.0182 min^{-1} and 6.62 μM, respectively, and the k_{inact} and K_i values of ASV against CYP3A were 0.0318 min^{-1} and 40.4 μM, respectively.

The CYP (CYP1A2, 2B6, 3A4) induction potential of ASV in human hepatocytes was evaluated. ASV did not induce CYP1A2 or 2B6 and increased CYP3A4 mRNA level and activity by up to 41% and 16%, respectively, showing that ASV is a weak inducer of CYP3A4.

3.(ii).A.(10).2) Characterization of ASV as a potential substrate for drug transporters (4.2.2.2.1, 4.2.2.7.1-4.2.2.7.3)

In Caco-2 cell monolayers, the efflux ratio of ASV (5 μM) was 31, which was reduced to 1.9, 1.4, and 20 in the

50) Biliary excretion over 48 hours post-dose in rats; and biliary excretion over 72 hours post-dose in monkeys
51) The IC_{50} value obtained using midazolam as a substrate. The IC_{50} value obtained using testosterone as a substrate was >45 μM.
52) Basic models and mechanistic static models in an FDA Guidance (draft). FDA Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, DRAFT GUIDANCE, 2012

presence of GF120918 (P-gp and BCRP inhibitor), MK-571 (P-gp and MRP2 inhibitor), and FTC (BCRP inhibitor), respectively. When ASV 5 mg/kg was orally administered to wild-type mice and P-gp knockout (*mdr 1a/1b*) mice (3 males/time point/group), the AUC_{inf} in P-gp knockout mice was 5.9-fold higher than that in wild-type mice. In wild-type MDCK cells and MDCK cells expressing human BCRP, the efflux ratios of ^{3}H-ASV (1 μM) were 12.1 and 5.0, respectively. The above results indicated that ASV is a substrate for P-gp and MRP2 and is not a substrate for BCRP.

The uptake of ASV into rat hepatocytes was rapid and increased linearly within the concentration range of 0.5 to 100 μM. The uptake of ASV (1 μM) into rOatp1- or rOatp4-expressing Xenopus laevis oocytes was studied. As a result, increased uptake of ASV into the cells expressing Oatp1 or Oatp4 was observed. Based on the above, the uptake of ASV into rat hepatocytes is partially mediated by Oatp1 and Oatp4 and an involvement of an uptake process not mediated by transporters, such as passive diffusion, was also suggested.

The uptake of ^{3}H-ASV into human hepatocytes was rapid and saturable (K_m = 0.69 μM). The uptake of ^{3}H-ASV (10 nM) into OATP1B1-, OATP2B1-, or OATP1B3-expressing HEK-293 cells and the uptake of ^{14}C-ASV (1 μM) into OATP1B3-expressing Xenopus laevis oocytes were studied. As a result, increased uptake of ASV into the cells expressing OATP1B1 or OATP2B1 was observed and the uptake of ASV into the cells expressing OATP1B1 or OATP2B1 was more decreased in the presence of rifampicin (OATP inhibitor) than in its absence, suggesting that ASV is a substrate for OATP1B1 and OATP2B1 and is not a substrate for OATP1B3.

3.(ii).A.(10).3) Inhibition of drug transporters (4.2.2.2.1, 4.2.2.7.10-4.2.2.7.15, 4.2.2.7.18)

Inhibition of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OATP2B1, NTCP, OAT1, OAT3, OCT1, OCT2, and BSEP by ASV was assessed and the results were as shown in Table 25. When Japanese patients with chronic hepatitis C received ASV softgel capsules 100 mg twice daily (BID) for 14 days, the C_{max} was 0.87 μM (0.65 μg/mL).[53)] Therefore, it was considered that ASV can interact with OATP1B1 and OATP2B1 substrates and is unlikely to interact with other transporters.

Table 25. *In vitro* inhibition of transporters by ASV

Cells	Transporter	Substrate	IC_{50} value (μM)
Caco-2		Digoxin	11
MDCK	P-gp	Digoxin	50.6 ± 17
	BCRP	Genistein	>50
HEK-293	OATP1B1	BMS-791553	0.3
	OATP1B3	Cholecystokinin-8	3.0 ± 0.7
	OATP2B1	Estrone sulfate	1.2 ± 0.3
	OAT1	p-aminohippuric acid	11.8
	OAT3	Estrone sulfate	69.6
	OCT1	Metformin	77.6
	OCT2	Metformin	>80
Membrane vesicles	MRP2	Estradiol-17β-d-glucuronide	94.8 ± 23.6
	BSEP	Taurocholic acid	8.81
CHO	NTCP	Taurocholic acid	3.56

Mean or Mean ± SD

53) The Japanese phase III study in Japanese patients with chronic hepatitis C (Study AI447026) [see "4.(ii).A.(3) Studies in patients with chronic hepatitis C"].

3.(ii).B Outline of the review

PMDA concluded that there are no particular problems with the submitted non-clinical pharmacokinetic data on DCV or ASV.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The results from the following toxicity studies of DCV were submitted in the application: single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and other toxicity studies (phototoxicity studies, a mechanistic study, studies on impurities). The results from the following toxicity studies of ASV were submitted: single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and other toxicity studies (phototoxicity studies, safety evaluation of excipients). The results from oral combination toxicity studies of DCV with ASV (repeat-dose toxicity studies) were also submitted. Doses of DCV are expressed in terms of Daclatasvir.

Unless otherwise specified, 75% 0.1M phosphate buffer (pH 3.0), 5% polyvinylpyrrolidone (PVP-K-30), 5% d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), and 15% polyethylene glycol 400 (PEG-400) for DCV, and 60% PEG-400 and 40% TPGS for ASV were used as vehicles.

3.(iii).A.(1) Single-dose toxicity (DCV) (4.2.3.1.1, 4.2.3.1.2, Reference data 4.2.3.1.3, Reference data 4.2.3.1.4)

Single oral dose toxicity studies in CD-1 mice (n = 5/sex/group; DCV 0 [vehicle], 100, 300, 1000 mg/kg), SD rats (n = 5/sex/group; DCV 0 [vehicle], 100, 300, 1000 mg/kg), dogs[54] (n = 2/sex/group; DCV 0 [vehicle], 15, 50, 150 mg/kg), and cynomolgus monkeys[54] (n = 1/sex/group; DCV 0 [vehicle], 15, 50, 150 mg/kg) were conducted. No deaths occurred in any animal species. In rats, reduced food consumption at ≥100 mg/kg, reduced body weight at ≥300 mg/kg, and stained fur at 1000 mg/kg were observed transiently. In dogs, vomiting and reduced food consumption were observed at ≥50 mg/kg.

Based on the above, the approximate lethal doses were determined to be >1000 mg/kg for mice and rats and >150 mg/kg for dogs and monkeys.

3.(iii).A.(2) Repeat-dose toxicity (DCV)

The results from oral repeat-dose toxicity studies of DCV in rats (1 month and 6 months), dogs (1 month), and monkeys (1 month, 4 months, 9 months) were submitted. Although the oral BA was higher in dogs than in monkeys (>100% in dogs; 38% in monkeys) and the plasma drug concentrations were also higher in dogs than in monkeys, the concentrations of a metabolite that was not detected in humans (BMS-795853) were higher[55] in dogs than in monkeys. Thus, monkeys, in which the metabolic profile of DCV is more similar to that of humans, were selected for toxicologic evaluation. The primary target organs for the toxicity of DCV were the liver (rats, dogs, monkeys), adrenal gland (rats, monkeys), bone marrow (dogs, monkeys), and prostate gland

54) Conducted as a single oral dose toxicokinetic and tolerability study.
55) The metabolite to unchanged drug AUC ratio was 10% to 32%.

and testes (rats, dogs). The main findings in the target organs for toxicity included perivascular inflammation, hepatocellular degeneration, etc., (the liver), cortical hypertrophy/hyperplasia in the zona fasciculata or reticularis, changes in cytoplasmic vacuolation, etc., (the adrenal gland), decreases in red blood cells and granulocytic cells or lymphoid cell hyperplasia, etc., in the rib and sternal bone marrow (bone marrow), decreases in prostate gland weight and atrophic glandular epithelium (the prostate gland), and seminiferous tubule degeneration (the testis). A dog 9-day oral administration study was conducted to determine changes in the liver and bone marrow over time and the mechanism of development. The effects of DCV on the immune system were assessed by adding immunotoxicity endpoints to dog and monkey repeat-dose toxicity studies. When the DCV exposure levels (AUC) at the doses of no observed adverse effect level (NOAEL) in repeat-dose toxicity studies (rat 6-month study, 25 mg/kg/day; monkey 9-month study, 15 mg/kg/day) were compared to the steady-state human exposure at the recommended clinical dose (60 mg QD) (AUC, 15.1 μg·h/mL),[56)] the rat to human exposure ratio was 1.1 and the monkey to human exposure ratio was <1.

The results from oral repeat-dose combination studies of DCV with ASV in rats (1 month) and monkeys (1 month and 3 months) were submitted, which showed no apparent changes suggestive of toxicologic interaction.

3.(iii).A.(2).1) Rat 1-month oral toxicity study (4.2.3.2.2)

SD rats (n = 15/sex/group) orally received DCV 0 (vehicle), 10, 30, or 100 mg/kg/day for 1 month and the reversibility of toxicity following a 1-month recovery period was evaluated in 5 males and 5 females/group. Reduced food consumption, increases in triglycerides, cholesterol, and total bilirubin, decreases in aspartate aminotransferase (AST), increases in absolute adrenal gland weight, and adrenal cortical hypertrophy/hyperplasia at ≥30 mg/kg/day were observed. Staining of the head/the nose and mouth, staining around the genital organ, stained limbs, reduced body weight gain, increased water consumption, decreases in reticulocyte count and platelet count, increases in glucose and alanine aminotransferase (ALT), increased urine volume and its secondary changes (decreases in urea nitrogen, urine specific gravity, and osmolality, increases in the fractional excretion of sodium and chloride, decreases in urinary creatinine, protein, and electrolyte concentrations, decreases in the fractional excretion of potassium), increases in urinary corticosterone/creatinine ratio, increases in urinary total corticosterone, increases in absolute liver weight, decreases in absolute prostate gland weight, multifocal discoloration of the gastric mucosa, and erosion in the glandular stomach at 100 mg/kg/day were observed. All findings were reversible or tended to be reversible. The changes in the gastric mucosa were considered related to stress associated with dosing, and the decreases in reticulocyte count and platelet count and the increases in glucose on Day 7 were considered related to the transient changes in body weight and food consumption observed during the early phase of dosing. The effects of DCV on urine were assessed in animals used for toxicokinetic assessment under water deprivation. As a result, there were no effects on urine specific gravity, osmolality, or urine volume at doses up to 100 mg/kg/day. Thus, it has been discussed that although the cause for the findings in rats only is not clear, increased urine volume was associated with increased water consumption.

56) Study AI444004 [see "4.(ii).A.(3).1).(b) Early phase II study in foreign patients with chronic hepatitis C"].

Based on the above, as effects on the adrenal gland, the target organ of DCV, (hypertrophy/hyperplasia) were observed at ≥30 mg/kg/day, the NOAEL in this study was determined to be 10 mg/kg/day.

3.(iii).A.(2).2) Rat 6-month oral toxicity study (4.2.3.2.4)

SD rats (n = 25/sex/group) orally received DCV 0 (vehicle), 12.5, 25, or 50 mg/kg/day for 6 months and the reversibility of toxicity following a 2-month recovery period was evaluated in 5 males and 5 females/group. Increases in water consumption and urine volume and their secondary changes (decreases in urine specific gravity, urinary creatinine concentration, and urine osmolality, increases in the total corticosterone excretion, increases in corticosterone/creatinine ratio), increases in cytoplasmic microvacuolation in the adrenal cortical zona fasciculata, and an increased incidence and severity of adrenal vasodilation (extension/dilation of blood vessels) at ≥12.5 mg/kg/day were observed. Salivation, prolongation of activated partial thromboplastin time, increases in cholesterol, and increases in absolute adrenal gland weight at ≥25 mg/kg/day, and increases in triglycerides and glucose and minimal adrenal cortical hypertrophy/hyperplasia at 50 mg/kg/day were observed. Except for the increases in water consumption and urine volume at 50 mg/kg/day, all findings were reversible or tended to be reversible.

Based on the above, as effects on the adrenal gland (hypertrophy/hyperplasia) were observed at 50 mg/kg/day, the NOAEL was determined to be 25 mg/kg/day.

3.(iii).A.(2).3) Dog 1-month oral toxicity study (4.2.3.2.5)

Beagle dogs (n = 5/sex/group) orally received DCV 0 (vehicle), 3, 15, or 100/50 mg/kg/day[57)] for 1 month and the reversibility of toxicity following a 1-month recovery period was evaluated in 2 males and 2 females from all groups excluding the 100/50 mg/kg/day group. Stool changes (liquid stool, white stool, black stool, or mucous stool) and vomitus (clear, food eaten, white, brown, or yellow), extramedullary hematopoiesis in the spleen, and perivascular inflammation in the liver [58)] and its secondary effects (Kupffer cell hypertrophy/hyperplasia and pigmentation) at ≥15 mg/kg/day were observed. In surviving animals in the 100/50 mg/kg/day group, vomiting (decreased after dose reduction), salivation, stained fur, and decreases in bone marrow cells were noted. Mainly in decedents in the 100/50 mg/kg/day group, decreases in red blood cell parameters,[59)] reticulocyte count, platelet count, neutrophil count, lymphocyte count, eosinophil count, and basophil count, increases in fibrinogen, prolongation of activated partial thromboplastin time, increases in total bilirubin, alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), ALT, AST, globulin, triglycerides, and cholesterol, decreases in albumin, etc., increases in urine protein and granular casts, rough surface of the liver, mottled liver, yellowish-brown liver, darkening, etc., of splenic lymph nodes (pancreatic lymph nodes), hepatocellular degeneration[60)] and its secondary changes (sinusoidal congestion and neutrophil infiltration in

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57) As 2 males and 2 females treated with 100 mg/kg/day were sacrificed moribund due to serious deterioration of general condition, DCV was interrupted from Day 9 (females) or Day 10 (males) for 5 days and resumed at a reduced dose of 50 mg/kg/day on Day 14 (females) or Day 15 (males). It has been discussed that the main cause for deterioration of general condition in the decedents was the hepatic (perivascular inflammation, hepatocellular degeneration, etc., in the liver) and bone marrow toxicity of DCV.

58) Inflammation primarily mediated by mononuclear cells and a small number of neutrophils around the central vein or portal vein.

59) Red blood cell count, hemoglobin, hematocrit

60) Cytoplasmic eosinophilic change or vacuolation and nuclear condensation and constriction in the cells in the vicinity of perivascular inflammation and in the hepatic parenchyma.

splenic lymph nodes [pancreatic lymph nodes][61]), decreases in erythroid and granulocytic cells, increases in the number of MAC387[62]-positive myeloid cells, extramedullary hematopoiesis in the spleen, decreases in the number of lymphoid cells in the spleen and thymus, seminiferous tubule degeneration in the testis and its secondary changes (abnormal epididymal content [sperm-forming cells]), atrophy of the prostate gland, and vacuolation of acinar cells of the pancreas were observed. All of the findings observed at 15 mg/kg/day were reversible.

Based on the above, the NOAEL in this study was determined to be 15 mg/kg/day.

3.(iii).A.(2).4) Monkey 1-month oral toxicity study (Reference data 4.2.3.7.3.2)

Cynomolgus monkeys (n = 2/sex/group) orally received DCV 0 (vehicle), 10, 30, 100, or 300 mg/kg/day for 1 month.[63] Inflammation around the hepatic central vein[64] and increases in serum amyloid A (SAA) were noted at ≥30 mg/kg/day. Although these findings were considered related to treatment, the incidence and severity of inflammation around the central vein were not dose-dependent and SAA values were not directly related to inflammation around the central vein. An analysis of hepatic transcription factors could not elucidate the mechanism of development of inflammation around the central vein. The hepatic findings observed in monkeys were morphologically similar to, but less severe than those in dogs.

Based on the above, the NOAEL in this study was determined to be 10 mg/kg/day.

3.(iii).A.(2).5) Monkey 4-month oral toxicity study (4.2.3.2.8)

Cynomolgus monkeys (n = 4/sex/group) orally received DCV 0 (vehicle), 15, 50, or 300 mg/kg/day for 4 months. Loose stool or liquid stool, which was considered related to vehicle, light-colored areas or light-colored foci in the liver, bile duct hyperplasia, Kupffer cell hypertrophy/hyperplasia, cytoplasmic rarefaction of centrilobular hepatocytes, decreases in cytoplasmic vacuolation in the adrenal cortical zona fasciculata, and lymphoid cell hyperplasia of the bone marrow at ≥50 mg/kg/day were observed. Changes in red blood cell parameters,[59] increases in ALT and AST, decreases in albumin and its secondary changes (changes in total protein and globulin), mononuclear cell infiltration in the centrilobular zone of the liver, and increases in absolute adrenal gland weight, adrenal hypertrophy, and cortical hyperplasia in the zona reticularis at 300 mg/kg/day were observed. The findings in the adrenal gland (decreases in vacuolation in the cortex and cortical hyperplasia) were morphologically similar to stress-induced changes. Electron microscopy of the livers of some animals treated with 300 mg/kg/day revealed accumulation of non-crystalline granular materials in the dilated endoplasmic reticulum of hepatocytes.

In order to evaluate the immunotoxic potential of long-term treatment with DCV, SAA and inflammatory

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61) Since these lymph nodes drain lymph fluid from the liver, it has been discussed that the observed changes in these lymph nodes were secondary to hepatic inflammation.

62) An antibody that recognizes monocytes/macrophages.

63) The study was conducted to assess 1-month repeat-dose toxicity of DCV in monkeys and to determine whether hepatic and bone marrow toxicity as observed in a dog repeat-dose toxicity study occurs also in monkeys.

64) Mild focal or multifocal inflammation primarily mediated by mononuclear cells.

mediators[65] were measured. cTnI[66] was also measured to assess the effect of DCV on cardiac muscle. As a result, IL-8 was reduced at ≥50 mg/kg/day without associated findings, which was considered of little biological significance. There were no DCV-related changes in SAA or cTnI. No histopathological changes in the hearts of surviving animals were observed in monkey 4-month and 9-month toxicity studies.

Based on the above, the NOAEL in this study was determined to be 15 mg/kg/day.

3.(iii).A.(2).6) Monkey 9-month oral toxicity study (4.2.3.2.9)

Cynomolgus monkeys (n = 6/sex/group) orally received DCV 0 (vehicle), 15, 30, or 150 mg/kg/day for 9 months and the reversibility of toxicity following a 2-month recovery period was evaluated in 2 males and 2 females/group. One male in the 150 mg/kg/day group was euthanized on Day 28 due to deteriorated condition associated with inflammatory changes in lymphoid tissue, liver, and skin. In the decedent, atrophy, necrosis, or inflammation of lymphoid tissue, hepatocellular necrosis and portal and periportal inflammation with thrombi, skin inflammation and epithelial necrosis and shedding with crust formation (ulceration), and infiltration of inflammatory cells including a small number of multinucleated giant cells (mostly Langhans giant cells) in multiple organs were observed and although the cause of death was not identified, a relationship to DCV was suggested. Loose stool/liquid stool, which was considered related to vehicle, adrenal hypertrophy, increases in absolute adrenal gland weight, decreases in cytoplastic vacuolation in the cortical zona fasciculata, and light-colored foci and Kupffer cell hypertrophy/hyperplasia[67] in the liver at ≥ 30 mg/kg/day and increases in ALT and AST, increases in C-reactive protein, bile duct hyperplasia, and mononuclear cell infiltration and hepatocellular vacuolation in the liver at 150 mg/kg/day were observed. The findings other than loose stool/liquid stool and Kupffer cell hypertrophy/hyperplasia in the liver were reversible.

Based on the above, the NOAEL in this study was determined to be 15 mg/kg/day.

Immunotoxicological evaluation of DCV included T-cell-dependent antibody response to keyhole-limpet hemocyanin (KLH), measurement of serum IL-8 as an inflammatory mediator (at Week 40), and bone marrow immunophenotyping.[68] As a result, KLH-specific antibody response (total of IgA, IgM, and IgG antibodies) was observed in all animals treated with DCV. T-cell-dependent antibody response to KLH was not affected in males. In females, KLH-specific antibody response decreased over time in the 150 mg/kg/day group, which was considered of little toxicological significance. There were no effects on serum IL-8 or the phenotype of bone marrow cells from the femur.

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65) IL-2, MCP-1, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-12/3 p40, TNFα, G-CSF, IFN-γ, GM-CSF, MIP-1α, MIP-1β

66) cTnI (a biomarker of cardiac injury) was measured to obtain additional information because 1 monkey in the 150 mg/kg/day group had chronic inflammation (minimal to moderate) in multiple organs/tissues (liver, skin, lymph nodes, spleen, thymus, kidney, heart, pancreas) and was euthanized due to deteriorated condition in an ongoing monkey 9-month oral toxicity study.

67) Hypertrophic Kupffer cells were found in centrilobular areas, which had abundant cytoplasm and often contained eosinophilic, light-colored materials. Hypertrophic Kupffer cells with syncytial or multinuclear giant cells were also sporadically found.

68) The percentage of cells expressing CD117 (a marker for precursor cells, c-kit) and the percentage of leukocytes (CD45+) expressing CD3 (a T-cell marker), CD20 (a B-cell marker), or CD14 (a monocyte/macrophage marker) were determined and monocytes/macrophages were further phenotyped by intracellular staining using the antibody MAC387.

3.(iii).A.(2).7) Combination toxicity studies

For combination toxicity studies of DCV and ASV, doses of DCV and ASV associated with AUCs that were relevant to the human exposure range in clinical settings (DCV, 1.9-2.4 times the human AUC; ASV, 6.2-18 times the human AUC) were selected.

3.(iii).A.(2).7).(a) Rat 1-month oral combination toxicity study (4.2.3.2.3)

SD rats (n = 10/sex/group) orally received DCV/ASV at doses of 0/0 (vehicle[69)]), 10/30, or 60/60 mg/kg/day for 1 month. Decreases in lymphocyte count, basophil count, and white blood cell count and adrenal cortical vacuolation at ≥10/30 mg/kg/day were observed. Iincreased urine volume and associated decreases in urine specific gravity, decreases in hemoglobin and mean corpuscular volume (MCV), and whitish discolored adrenal gland at 60/60 mg/kg/day were observed. The findings observed in this study were all mild in severity and these changes were also observed in toxicity studies with DCV or ASV alone. Based on the above, there was no evidence of toxicologic interaction in the combination study of DCV and ASV at doses up to 60/60 mg/kg/day.

3.(iii).A.(2).7).(b) Monkey 1-month oral combination toxicity study (DCV, 4.2.3.2.6)

Cynomolgus monkeys (n = 4/sex/group) orally received DCV/ASV at doses of 0/0 (vehicle), 15/72, or 50/129.5 mg/kg/day for 1 month. One animal in the 15/72 mg/kg/day group had subacute inflammation in the intestinal tract. Liquid stool, stained fur, and decreased lymphocytes in the thymus at ≥15/72 mg/kg/day were observed. Vomiting, necrosis and enlargement of the crypt with mixed cell infiltration[70)] or eosinophil infiltration in the large intestine, darkening of the adrenal gland, and decreases in vacuolation in the cortical zona fasciculata with cortical hyperplasia in the zona reticularis in the adrenal gland at 50/129.5 mg/kg/day were observed. The findings observed in this study were all mild in severity and these changes were also observed in toxicity studies with DCV or ASV alone. Based on the above, there was no evidence of toxicologic interaction in the combination study of DCV and ASV at doses up to 50/129.5 mg/kg/day.

3.(iii).A.(2).7).(c) Monkey 3-month oral combination toxicity study (DCV, 4.2.3.2.7)

Cynomolgus monkeys (n = 4/sex/group) orally received DCV/ASV at doses of 0/0 (vehicle), 15/45, or 50/80 mg/kg/day for 3 months. Darkening of the adrenal gland and decreases in vacuolation in zona fasciculata cells of the adrenal cortex at 50/80 mg/kg/day were observed. The findings observed in this study were all mild in severity and these changes were also observed in toxicity studies with DCV or ASV alone. Based on the above, there was no evidence of toxicologic interaction in the combination study of DCV and ASV at doses up to 50/80 mg/kg/day.

3.(iii).A.(3) Genotoxicity (DCV) (4.2.3.3.1.1, 4.2.3.3.1.2, 4.2.3.3.2.1)

As genotoxicity studies of DCV, bacterial reverse mutation tests, chromosomal aberration assays in Chinese hamster ovary cells, and a rat bone marrow micronucleus test were performed, all of which were negative.

3.(iii).A.(4) Carcinogenicity (DCV)

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69) 50% 0.1M phosphate buffer (pH 3), 30%PEG-400, and 20%TPGS were used as vehicles.
70) Cell infiltration consisting of lymphocytes, histiocytes, and eosinophils.

3.(iii).A.(4).1) CByB6F1/Tg rasH2 transgenic mouse 26-week oral carcinogenicity study (4.2.3.4.2.2)

CbyB6F1/Tg rasH2 mice (n = 25/sex/group) orally received DCV 0 (water), 0 (vehicle), 30, 100, or 300 mg/kg/day for 26 weeks. As a result, no DCV-related tumors were observed. In the positive control[71)] group, the incidences of skin papilloma in the right auricle[72)] and lymphoma were increased. As non-neoplastic changes, an increased incidence of extramedullary hematopoiesis in the spleen[73)] was observed in females at ≥100 mg/kg/day. It has been reported that in the strain of mice used in this study, extramedullary hematopoiesis in the spleen occurs more frequently in females than in males.[74)] In addition, there were no specific increases in hematopoietic lineage cells (myeloid, erythroid, megakaryocytic) in the region of extramedullary hematopoiesis, the degree of the change was not prominent, and such change was observed also in the control groups. Thus, it has been discussed that this finding was unrelated to tumor development.

Based on the above, it has been concluded that DCV is not carcinogenic in Tg-rasH2 mice.

3.(iii).A.(4).2) Rat 2-year carcinogenicity study (4.2.3.4.1.1)

SD rats (n = 65/sex/group) orally received DCV 0 (water), 0 (vehicle), 5, 15, or 50 mg/kg/day for 2 years. As the number of surviving animals was reduced to 20 for the male water control group and the male and female vehicle control groups, surviving animals in all groups were necropsied at Week 94 for males and at Week 92 for females. The survival rates at necropsy in the water control, vehicle control, 5, 15, and 50 mg/kg/day groups were 31%, 31%, 35%, 23%, and 29%, respectively, for males and 34%, 31%, 46%, 46%, and 37%, respectively, for females.[75)] No DCV-related tumors were observed. As non-neoplastic changes, an increased incidence of salivation, increased incidences of light-colored adrenal gland and enlarged adrenal gland, and an increased incidence of microvacuolation/cytoplasmic rarefaction of adrenal cortical cells were observed at 50 mg/kg/day. It has been discussed that the findings in the adrenal gland were non-adverse because there were no deaths, proliferative changes, or neoplastic changes associated with these findings.

Based on the above, it has been concluded that DCV is not carcinogenic in SD rats.

3.(iii).A.(5) Reproductive and developmental toxicity (DCV)

The results from the following reproductive and developmental toxicity studies of DCV were submitted: a rat study of fertility and early embryonic development to implantation; rat and rabbit embryo-fetal development studies; and a rat study for effects on pre- and postnatal development, including maternal function. In the rat study of fertility and early embryonic development to implantation, although decreases in prostate gland and seminal vesicle weights and dysmorphic sperm in males and effects on clinical observations, body weight, food

71) N-nitrosomethyl urea 75 mg/kg was intraperitoneally administered on Day 1 only.
72) Sites of metallic ear tags.
73) The numbers of animals with the finding in the water control, vehicle control, 30, 100, 300 mg/kg/day, and positive control groups were 3/25 (12%), 1/25 (4%), 3/25 (12%), 7/25 (28%), 5/25 (20%), and 3/15 (20%), respectively, for males and 6/25 (24%), 2/25 (8%), 4/25 (16%), 14/25 (56%), 19/25 (76%), and 1/14 (7%), respectively, for females.
74) Mean incidences (710 males and 710 females) were 10% (range, 4%-28%) in males and 21% (range, 8%-68%) in females (*Toxicol Pathol*. 2013;41: 1137-1145). The laboratory background incidences (78 males and 81 females, 2011 to 2012) were 1.3% in males and 14.8% in females.
75) It has been discussed that the number of animals evaluated and the duration of study met the requirements in the FDA Guidance (Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals) and this did not affect carcinogenicity assessments.

consumption, etc., in females were observed at the high dose level, there were no effects on the reproductive function of adult male and female animals or early embryonic development (males, fertility). In the embryo-fetal development studies, maternal toxicity (mortality, moribund sacrifice, abortion, etc.) and developmental toxicity (fetal lethality, and teratogenic effects, skeletal abnormalities, skeletal variations, etc., notably affecting the developing brain, skull, and limbs) were observed in both rats and rabbits. In the rat study for effects on pre- and postnatal development, including maternal function, salivation, decreases in body weight and food consumption, increases in adrenal gland weight, and adrenal hypertrophy, etc., were seen in maternal animals, and an increased number of stillborn pups, decreased survival rate, and decreases in pup body weight were noted in the F1 generation. The ratio of the exposure (AUC) associated with the NOAEL dose for embryo-fetal developmental toxicity (rats, 50 mg/kg/day; rabbits, 40/20 mg/kg/day) to the steady-state human exposure (AUC, 15.1 μg·h/mL) at the recommended clinical dose (60 mg QD) was 4.6 for rats and 16 for rabbits. DCV has been shown to cross the placenta and be excreted in milk in rats [see "3.(ii).A.(2).4) Placental transfer to fetus and 3.(ii).A.(4).2) Excretion into milk"].

3.(iii).A.(5).1) Rat study of fertility and early embryonic development to implantation (4.2.3.5.1.1)

SD rats (25 males and 35 females/group) orally received DCV 0 (vehicle), 15, 50, or 200 mg/kg/day. Males were dosed from 28 days prior to mating until the day of necropsy and females were dosed from 14 days prior to mating until gestation day 7. Salivation (wet fur), red/brown staining of fur (mainly, the nose and mouth, lower jaw, and forelimb) in males and females at ≥15 mg/kg/day, increases in absolute and relative adrenal gland weights, light-colored adrenal gland, and adrenal hypertrophy in males and females at ≥50 mg/kg/day, and decreases in food consumption in males and females and reduced body weight gain, light-colored stomach and hard protrusion in the stomach, decreases in absolute and relative weights of the prostate gland and seminal vesicle, and dysmorphic sperm (abnormal shape of the head) in males at 200 mg/kg/day were observed. In males, changes in reproductive organ weights and increased dysmorphic sperm were observed,[76)] but there were no effects on copulation index. In females, there were no effects on the reproductive function or early embryonic development.

Based on the above, the NOAELs in this study were determined to be 50 mg/kg/day for paternal general and reproductive toxicity, 200 mg/kg/day for maternal general and reproductive toxicity, and 200 mg/kg/day for early embryonic development (males, fertility).

3.(iii).A.(5).2) Embryo-fetal development studies

3.(iii).A.(5).2).(a) Rat study (4.2.3.5.2.3)

Pregnant SD rats (n = 21-22/group) orally received DCV 0 (vehicle), 50, 200, or 1000 mg/kg/day from gestation day 6 to gestation day 15. One of 22 dams in the 200 mg/kg/day group and 1 of 21 dams in the 1000 mg/kg/day group were sacrificed moribund on gestation days 12 and 14, respectively. In the decedents, decreased body weight, decreased food consumption or loss of appetite, decreased stool output or unformed stool, stained fur (red), and red material around the nose and mouth were observed and the cause of death was not identified, but

[76)] The applicant has discussed that the mechanism of effects on the male reproductive system is unknown.

was considered related to DCV. Decreased stool output/no feces, stained fur, material around the nose and mouth and urine stained fur, decreases in body weight and food consumption, and adrenal hypertrophy at ≥200 mg/kg/day and red material around the vagina and splenic hypertrophy at 1000 mg/kg/day were observed. In the embryos/fetuses, malformations and variations, notably of the brain, skull, and limbs (smaller and deformed cerebrum, enlarged cerebral ventricle, shortened lower jaw, incomplete ossification of the calvaria and frontal bone, enlarged fontanelle, deformed/fused sternum, an extra phalanx in the hind-limb, etc.) at ≥200 mg/kg/day and embryonic lethality and associated reductions in litter size, reductions in the number of live fetuses, decreases in fetal body weight, malformations and abnormalities (In addition to the findings at 200 mg/kg/day, anophthalmos/microphthalmia and malposition of the eyeball with visceral or skeletal abnormalities, enlarged olfactory bulb, nasal atresia or no nostrils with visceral abnormalities, exencephaly, cleft lip and cleft palate, fore- and hind-limb polydactyly with skeletal abnormalities, shortened upper jaw [shortened premaxilla and shortened nasal bone], deformed tympanic ring, the premaxilla fused to the nasal bone), and skeletal abnormalities (fore limb girdle, sternum, vertebrae, ribs) and skeletal variations at the same sites at 1000 mg/kg/day were observed.

Based on the above, the NOAEL in this study was determined to be 50 mg/kg/day for maternal general toxicity and embryo-fetal developmental toxicity.

3.(iii).A.(5).2).(b) Rabbit study (4.2.3.5.2.4)

Pregnant NZW rabbits (n = 22/group) orally received DCV 0 (vehicle), 40/20, 200/99, or 750/370[77)] mg/kg/day from gestation day 7 to gestation day 19. One of 22 dams in the 200/99 mg/kg/day group was sacrificed moribund due to poor general condition on gestation day 17, 7 of 22 dams in the 200/99 mg/kg/day group had abortion. Dams had decreases in body weight and food consumption at ≥200/99 mg/kg/day, and 2 of 22 dams in the 750/370 mg/kg/day group died by gestation days 11 to 15 and the remaining dams were all sacrificed moribund due to poor general condition or euthanized due to marked decreases in body weight and food consumption. In the premature decedents, loss of righting reflex, ataxia, dyspnoea, decreased activity, and hypothermia were noted and necropsy revealed blood coagulation in the aorta and thinning or discoloration and focal coloration of the gastric inner wall. In the 750/370 mg/kg/day group, 6 of 22 dams had live fetuses at necropsy. In the fetuses, decreased body weight and increased incidences of malformations of the ribs and skeletal variations (vertebrae and skull) at 200/99 mg/kg/day were observed. At 40/20 mg/kg/day, the total numbers of fetuses and litters with skeletal variations were both increased, which were considered non-adverse, because fetal skeletal variations are commonly observed and resolve postnatally and are not chronic in nature.

Based on the above, the NOAEL in this study was determined to be 40/20 mg/kg/day for maternal toxicity and embryo-fetal developmental toxicity.

77) Since changes in stools (decreased stool output or unformed stool etc.), which were considered related to vehicle, were observed after 3 to 6 doses, the administration volume was to be reduced by 51%.

3.(iii).A.(5).3) Rat study for effects on pre- and postnatal development, including maternal function (4.2.3.5.3.1)

Pregnant SD rats (n = 25/group) orally received DCV 0 (vehicle), 25, 50, or 100 mg/kg/day from gestation day 6 to lactation day 20. In the dams, red staining of fur/wet fur and increases in adrenal gland weight/adrenal hypertrophy at ≥25 mg/kg/day were observed and 1 of 25 dams in the 100 mg/kg/day group died due to dystocia and this dam delivered 9 pups and there were 4 full-term fetuses in the uterus. In the surviving dams in the 100 mg/kg/day group, sporadic salivation and dehydration-like symptoms, decreases in body weight and food consumption, and light-colored adrenal gland were observed. In the F1 offspring, an increased number of stillborn pups, decreases in survival between postnatal day 1 and postnatal day 4, and decreased body weight were observed at 100 mg/kg/day and moribund pups exhibited decreased activity, decreased respiratory rate, dyspnoea, recumbency, and loss of gastric content.

Based on the above, the NOAEL in this study was determined to be 50 mg/kg/day for maternal general toxicity and for F1 developmental and reproductive toxicity.

3.(iii).A.(6) Other toxicity studies (DCV)

3.(iii).A.(6).1) Phototoxicity

As DCV absorbs light in the wavelength range of 290 to 700 nm and a tissue distribution study [see "3.(ii).A.(2).2) Tissue distribution"] demonstrated its binding to the pigmented skin and pigmented ocular tissue of rats, phototoxicity studies were performed.

3.(iii).A.(6).1).(a) Neutral Red Uptake phototoxicity assay with the Balb/c 3T3 mouse fibroblast cell line (4.2.3.7.7.1)

Balb/c 3T3 mouse fibroblast cells were treated with DCV (Assays 1 and 2, 0.112-20.0[78)] mg/L) and cell viability was determined by Neutral Red Uptake in the presence or absence of ultraviolet A (UVA) irradiation (5 J/cm^2 for 2.5 hours). As a result, DCV concentration-dependently reduced cell viability in the presence of UVA irradiation in Assays 1 and 2 and the IC_{50} values of DCV were 13.13 and 13.89 mg/L, respectively. DCV was not cytotoxic when tested in the absence of UVA irradiation. Based on the above, it was concluded that DCV has phototoxic potential.

3.(iii).A.(6).1).(b) Single oral dose phototoxicity study on eyes and skin of Long Evans pigmented rats (4.2.3.7.7.2)

Long-Evans pigmented rats (5 males/group) were treated with single oral doses of DCV 0, 10, 30, or 100 mg/kg and exposed 1 hour later to UVA (6.5 kw, 30 minutes; wavelengths, 290-790 nm), and then the eyes and back skin of each rat were observed at 1, 4, 24, 48, and 72 hours after exposure. As a result, in rats treated with DCV, there were no skin reactions or no effects on ophthalmologic parameters or histopathology of the eyes after UVA exposure. Based on the above, it was concluded that DCV is not phototoxic in pigmented rats.[79)] The ratio of

78) Maximum solubility of DCV in vehicle (1% DMSO phosphate-buffered saline)

79) It has been explained that no phototoxicity-related adverse events such as photosensitivity were reported in Japanese clinical studies of DCV in combination with ASV (Study AI447026 and Study AI447017).

the exposure associated with the highest dose of 100 mg/kg in this study (C_{max}, 14.8 μg/mL) to the steady-state human exposure at the recommended clinical dose (60 mg QD) (C_{max}, 1.73 μg/mL)[56)] was 8.6.

3.(iii).A.(6).2) Mechanistic studies

In order to elucidate the mechanism of hepatic and bone marrow toxicity of DCV observed in dogs, early change over time and nature of these toxicities were studied based on clinicopathological, histopathological, and immunotoxicological examinations[80)] and changes in hepatic transcription (gene expression).

3.(iii).A.(6).2).(a) Dog 9-day oral dose study (4.2.3.7.3.1)

Beagle dogs (8 males/group) orally received DCV 0 (vehicle), 50, or 100 mg/kg/day for 3 or 9 days and 4 dogs/group were necropsied on Day 4 or 10. As a result, many of the hepatic and bone marrow findings observed in a dog 1-month oral toxicity study [see "3.(iii).A.(2).3) Dog 1-month oral toxicity study"] were reproduced. Liquid stool and intermittent vomiting, decreases in peripheral blood cell counts (neutrophil count, reticulocyte count, etc.), increases in serum liver enzymes [AST, ALT, glutamate dehydrogenase (GDH)], increases in blood biomarkers of acute inflammation (C-reactive protein and fibrinogen), decreases in serum albumin, discoloration (red or darkening) of the stomach, intestine, and lymph nodes, and colonic hemorrhage at ≥50 mg/kg/day. Decreased body weight, increases in leukoblast count, total lymphocyte count, promyelocyte count, and monocyte count in the bone marrow and associated increases in CD5+ T cells, CD117+ precursor cells, and CD34+ precursor cells, decreases in red blood cell parameters,[59)] and increases in GGT at 100 mg/kg/day were observed. The decreases in peripheral blood cell counts, increases in serum liver enzymes, and increases in blood biomarkers of acute inflammation at 100 mg/kg/day occurred on Day 2 or 4 and it was found that both bone marrow and hepatic effects occur during the early phase of dosing. As the decreases in peripheral blood cell counts (on Day 2 or 4) preceded the changes in liver enzymes (on Days 6-10) at 50 mg/kg/day, it has been discussed that bone marrow and hepatic effects may independently occur during the early phase of dosing. In addition, keratinocyte chemoattractant[81)] in the liver was measured and analyses of 14 inflammatory mediators in serum and liver homogenates and of hepatic transcription factors (gene expression) and metabonomic analysis of the liver were performed, which gave no clear results about the biochemical background of hepatic inflammation and the mechanism of hepatic toxicity.

3.(iii).A.(6).3) Studies on impurities (4.2.3.7.6.2, 4.2.3.7.6.1)

Among impurities contained within the DCV drug substance, safety assessment studies to qualify 3 impurities with specific acceptance criteria included in the specifications for the drug substance (Related Substance A, Related Substance B, Related Substance C) were conducted because the criteria exceeded the qualification threshold (0.15%)[82)].

A rat 3-month oral dose study was conducted to compare the toxicological profile of DCV (50 mg/kg/day)

80) Analysis of cytokines in serum and liver, measurement of serum complement C3, bone marrow apoptosis, bone marrow immunophenotyping by flow cytometry, cell type classification

81) An inflammatory mediator involved in chemotaxis of neutrophils

82) "Revision of the Guideline on Impurities in New Drug Substances" (PMSB/ELD Notification No.1216001 dated December 16, 2002, supplemented by PMSB/ELD Notification No.0624001 dated June 24, 2003)

containing up to [redacted]% and [redacted]% of Related Substance A and Related Substance C, respectively, with that of previously qualified material. As a result, there were no differences in toxicological or pathological findings between DCV containing the impurities and previously qualified material and all of the findings were similar to those observed in other rat toxicity studies. A rat 6-month oral dose study with a batch containing Related Substance B at a level exceeding the specification limit [see "3.(iii).A.(2).2) Rat 6-month oral toxicity study"] was conducted and all of the findings were similar to those observed in other rat toxicity studies.

For assessment of the genotoxic potential of the impurities, bacterial reverse mutation tests were performed with DCV (up to 5000 μg/plate) containing approximately 5% of each impurity (up to 250 μg/plate, a total of 2000 μg/plate). As a result, these impurities did not induce reverse mutations. The clastogenic and carcinogenic potential of the impurities were evaluated in a rat micronucleus test of DCV [see "3.(iii).A.(3) Genotoxicity (DCV)"] and Tg-rasH2 mouse and rat carcinogenicity studies of DCV [see "3.(iii).A.(4).1) CByB6F1-Tg rasH2 transgenic mouse 26-week oral carcinogenicity study and 3.(iii).A.(4).2) Rat 2-year carcinogenicity study"]. As a result, it was concluded that these impurities have no genotoxic potential.

Based on the above, it has been concluded that no safety concerns regarding the 3 impurities have been identified for humans.

3.(iii).A.(7) Single-dose toxicity (ASV) (4.2.3.1.1, 4.2.3.1.2, Reference data 4.2.3.1.3)

Single oral dose toxicity studies in CD-1 mice (n = 5/sex/group; ASV 0 [vehicle], 200, 600, 2000 mg/kg), SD rats (n = 5/sex/group; ASV 0 [vehicle], 200, 600, 2000 mg/kg), and dogs[54)] (n = 1/sex/group; ASV 0 [vehicle], 30, 100, 300 mg/kg) were conducted. In mice, mortalities due to poor general condition occurred at 2000 mg/kg and effects on the gastrointestinal tract (stomach, small intestine, and large intestine distended with fluid and gases, flat gastric mucosa and swelling/vacuolation of the surface mucosal epithelial cells of the glandular stomach, decreased exfoliation of enterocytes at the tip of villi and swelling/vacuolation of enterocytes in the small intestine, single cell necrosis of enterocytes in the cecum, etc.) were observed in the decedents. Unformed stool, mucous stool, reduced body weight, etc., at ≥200 mg/kg and decreased activity, incomplete eyelid opening, etc., at 2000 mg/kg were observed. In rats, trunk staining at ≥600 mg/kg and decreased stool output, decreased activity, incomplete eyelid opening, etc., at 2000 mg/kg were observed. In dogs, vomiting was noted at 300 mg/kg.

Based on the above, the approximate lethal doses were determined to be 2000 mg/kg for mice, >2000 mg/kg for rats, and >300 mg/kg for dogs.

3.(iii).A.(8) Repeat-dose toxicity (ASV)

The results from oral repeat-dose toxicity studies of ASV in rats (1 month and 6 months) and dogs (1 month and 9 months) were submitted. The primary target organs for the toxicity of ASV in both rats and dogs were the liver and gastrointestinal tract. Increases in ALT, GGT, and total bilirubin and hepatocellular necrosis were noted in the liver. In the gastrointestinal tract, small and large intestines distended with fluid and gases, enterocyte hypertrophy in the small intestine and cecum, and decreases in goblet cells in the cecum and colon

in rats and vomiting in dogs were observed. No enhanced toxicities were observed and no new target organs were identified with long-term administration. It has been discussed that the gastrointestinal findings were due to the local effects of the presence of a large amount of ASV in the gastrointestinal tract. The ratio of the ASV exposure (AUC) associated with the NOAEL dose in a repeat-dose toxicity study (rat 6-month study, 200 mg/kg/day; dog 9-month study, 100 mg/kg/day) to the steady-state human exposure at the recommended clinical dose (100 mg BID) (AUC, 3.69 μg·h/mL)[83] was 136 for rats and 82 for dogs.

The formation of a glutathione adduct (M1) and covalent binding to liver microsomal proteins were detected *in vitro*,[84] indicating the possible formation of a reactive intermediate metabolite. However, as rat 6-month and dog 9-month toxicity studies (82-136 times the human AUC) showed no changes suggestive of hepatic toxicity, it has been discussed that the formation of a reactive intermediate metabolite is of little toxicological significance.

The results from oral combination studies of DCV with ASV in rats (1 month) and monkeys (1 month and 3 months) were submitted, which showed no apparent changes suggestive of toxicologic interaction.

3.(iii).A.(8).1) Rat 1-month oral toxicity study (4.2.3.2.2)

SD rats (n = 15/sex/group) orally received ASV 0 (vehicle), 30, 100, or 600 mg/kg/day for 1 month and the reversibility of toxicity following a 2-week recovery period was evaluated in 5 males and 5 females/group. Decreases in serum creatinine and increases in liver weight at ≥100 mg/kg/day and decreases in body weight and food consumption, changes in red blood cell parameters (decreases in hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin [MCH], and mean cell hemoglobin concentration and increases in red cell distribution width), decreases in fibrinogen, increases in ALT, ALP, total bilirubin, and chloride, increases in albumin/globulin ratio, decreases in total protein, triglycerides, albumin, globulin, glucose, urea nitrogen, calcium, creatinine, and total cholesterol, increases in urine volume and associated findings (decreases in urine specific gravity and increases in urine pH), increases in thyroid gland, adrenal gland, and liver weights, small and large intestines distended with fluid and gases, thickened small intestinal wall, enterocyte hypertrophy in the small and large intestines, decreases in goblet cells in the cecum and colon, and multifocal cell degeneration[85] in the adrenal cortical zona fasciculata at 600 mg/kg/day were observed. These findings have been discussed as follows: (a) it is considered that the changes in red blood cell parameters were associated with smaller red blood cells[86] though the mechanism is unknown, (b) it is considered that the increases in urine volume were due to increased water consumption and not associated with renal disorders because dehydration was not observed and there is little urinary excretion of ASV and its metabolites, (c) it is considered that the increases in liver weight were adaptive changes associated with the induction of liver drug metabolizing enzymes due to high levels of ASV distributed in the liver, and (d) it is considered that the changes in the adrenal gland resulted from the enhancement of spontaneous changes in rats[87] by ASV. All the findings were reversible.

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83) ASV exposure at 100 mg BID (Study AI447016).
84) Covalent binding of ^{14}C-ASV to microsomal proteins was detected and this binding decreased in the presence of glutathione (see CTD 2.6.4.5.1).
85) A single cell or small cluster of cells with an aggregate of eosinophilic material, which were mostly found along the border with the zona reticularis.
86) It has been discussed that this is a finding commonly observed in anemia and considered to indicate changes in iron metabolism.
87) Similar findings were observed in a small number of rats in the control group as well.

Based on the above, the NOAEL in this study was determined to be 100 mg/kg/day for both males and females.

3.(iii).A.(8).2) Rat 6-month oral toxicity study (4.2.3.2.4)

SD rats (n = 25/sex/group) orally received ASV 0 (vehicle), 40, 80, or 200[88)] mg/kg/day for 6 months and the reversibility of toxicity following a 1-month recovery period was evaluated in 5 males and 5 females/group. Increases in liver weight at ≥40 mg/kg/day and loose stool, stained fur, increases in body weight and food consumption, and decreases in total cholesterol at 200 mg/kg/day were observed without associated histopathological findings, and all the findings were reversible.

Based on the above, the NOAEL in this study was determined to be 200 mg/kg/day for both males and females.

3.(iii).A.(8).3) Dog 1-month oral toxicity study (4.2.3.2.5)

Beagle dogs (n = 3/sex/group) orally received ASV 0 (vehicle), 20, 60, or 300 mg/kg/day for 1 month. Vomiting, unformed/watery stool, decreases in body weight and food consumption, changes in red blood cell parameters (decreases in MCV, MCH, and hemoglobin and increases in red cell distribution width), the appearance of nucleated erythrocytes and erythrocytes with minute basophilic cellular inclusions that were iron-positive (Pappenheimer bodies[89)]) in blood smear specimens, increases in ALT, GGT, total bilirubin, and chloride, decreases in total protein, albumin, globulin, calcium, and total cholesterol, increases in adrenal gland and spleen weights, smaller thymus, hepatocellular coagulation necrosis in the liver, decreases in secretory granules in the pancreatic exocrine portion, and increased incidence and severity of thymus involution at 300 mg/kg/day were observed. It has been discussed that the pancreatic finding was of little toxicological significance because the degree of change was minimal and most of secretory granules in the pancreas were retained and that the thymic findings were due to the enhancement of spontaneous changes and stress.

Based on the above, the NOAEL in this study was determined to be 60 mg/kg/day for both males and females.

3.(iii).A.(8).4) Dog 9-month oral toxicity study (4.2.3.2.6)

Beagle dogs (n = 6/sex/group) orally received ASV 0 (vehicle), 15, 50, or 100[90)] mg/kg/day for 9 months and the reversibility of toxicity following a 2-month recovery period was evaluated in 2 males and 2 females/group. Increases in ALP at ≥50 mg/kg/day and salivation and decreases in MCV and MCH at 100 mg/kg/day were observed without associated histopathological findings. It has been discussed that these findings were of little toxicological significance. All the findings were reversible.

Based on the above, the NOAEL in this study was determined to be 100 mg/kg/day for both males and females.

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88) Based on the findings from a rat 1-month oral toxicity study, a dose level that provides adequate exposure was selected, taking account of possible enhanced toxicity with prolongedadministration [AUC, 503 μg·h/mL; 136 times the AUC at the recommended clinical dose (3.69 μg·h/mL)].

89) A relationship to impaired hemoglobin synthesis has been reported (Stiene-Martin EA, et al. In, Clinical Hematology: principles, procedures, correlations, 2nd ed. 1998; 87-105). Although it is considered that this finding was related to ASV, as this was observed at high exposure (375 times the AUC at the recommended clinical dose) and not observed in a dog 9-month oral toxicity study (82 times the AUC at the recommended clinical dose), it has been discussed that this is of little safety concern to humans.

90) Based on the findings from a dog 1-month oral toxicity study, a dose level that provides adequate exposure was selected, taking account of possible enhanced toxicity with prolonged administration [AUC, 302 μg·h/mL (82 times the AUC at the recommended clinical dose)].

3.(iii).A.(8).5) Monkey 1-week oral toxicity study (Reference data 4.2.3.2.7)

Cynomolgus monkeys (n = 2/sex/group) orally received ASV 0 (vehicle), 30, 150, or 300 mg/kg/day for 1 week. Decreases in total cholesterol at ≥150 mg/kg/day and increases in total bilirubin, decreases in total protein and albumin, and increases in the number of bone marrow cells in the sternum and ribs at 300 mg/kg/day were observed. As no changes in white blood cell parameters or other hematopoietic tissues were observed in the animals with affected bone marrow, it has been discussed that this finding was of little toxicological significance.

Based on the above, the NOAEL in this study was determined to be 300 mg/kg/day for both males and females.

3.(iii).A.(8).6) Combination toxicity studies (4.2.3.2.3, 4.2.3.2.8, 4.2.3.2.9)

The results from these studies are summarized in "3.(iii).A.(2).7) Combination toxicity studies."

3.(iii).A.(9) Genotoxicity (ASV) (4.2.3.3.1.1, 4.2.3.3.1.2, 4.2.3.3.2.1)

As genotoxicity studies of ASV, bacterial reverse mutation tests, chromosomal aberration assays in Chinese hamster ovary cells, and a rat bone marrow micronucleus test were performed, all of which were negative.

3.(iii).A.(10) Carcinogenicity (ASV)

3.(iii).A.(10).1) CByB6F1-Tg rasH2 transgenic mouse 26-week oral carcinogenicity study (4.2.3.4.2.2)

CbyB6F1/Tg rasH2 mice (n = 25/sex/group) orally received ASV 0 (water), 0 (vehicle), 25, 100, or 200 mg/kg/day for 26 weeks. As a result, no ASV-related tumors were observed. In the positive control[71)] group, skin papilloma in the auricle[72)] and skin cancer were sporadically found and the incidence of lymphoma was increased. As non-neoplastic changes, centrilobular hepatocellular hypertrophy and chronic thrombus in mesenteric white adipose tissue[91)] were observed in males at 200 mg/kg/day and the increase in the incidence of hepatocellular vacuolation tended to be greater in males. Although it is considered that centrilobular hepatocellular hypertrophy was an adaptive change reflecting the induction of microsomal enzymes by ASV, that chronic thrombus in mesenteric white adipose tissue[92)] resulted from the enhancement of spontaneous lesions that are commonly seen in male Tg-rasH2 mice by ASV, and that hepatocellular vacuolation resulted from the enhancement of hepatocellular vacuolation, which occurred also in the control group, by ASV, it has been discussed that all of these changes occurred at the high dose level (AUC [males], 983 μg·h/mL, was 266 times the AUC [3.69 μg·h/mL] at the recommended clinical dose [100 mg BID]) and are unlikely to raise safety concerns for humans.

Based on the above, it has been concluded that ASV is not carcinogenic in Tg-rasH2 mice.

3.(iii).A.(10).2) Rat 2-year carcinogenicity study (4.2.3.4.1.1)

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91) The numbers of animals with the finding in the water control, vehicle control, ASV 25, 100, 200 mg/kg/day, and positive control groups were 5/25 (20%), 7/25 (28%), 9/25 (36%), 5/25 (20%), 14/25 (56%), and 0/15 (0%), respectively, for males and 1/25 (4%), 0/25 (0%), 0/25 (4%), 1/25 (4%), 1/25 (4%), and 0/15 (0%), respectively, for females.

92) Mean incidences (710 males and 710 females) were 15% (range, 8%-36%) in males and 1.8% (range, 0%-4%) in females (*Toxicol Pathol*. 2013;41: 1137-1145). It has been discussed that no findings related to vascular injury or coagulation disorder were reported in toxicity studies in other animal species.

Male and female SD rats (n = 65/sex/group) orally received ASV at doses of 0 (water), 0 (vehicle), 50, 75, or 125 mg/kg/day for males and at doses of 0 (water), 0 (vehicle), 40, 60, or 80 mg/kg/day for females for 2 years. As the number of surviving animals was reduced to 20 for the male and female vehicle control groups, surviving animals in all groups were necropsied at Week 84 or 85 for males and at Week 92 or 93 for females. The survival rates at necropsy were 51% in the water control group, 31% in the vehicle control group, 46% in the 50 mg/kg/day group, 31% in the 75 mg/kg/day group, and 52% in the 125 mg/kg/day group for males and 37% in the water control group, 31% in the vehicle control group, 37% in the 40 mg/kg/day group, 34% in the 60 mg/kg/day group, and 48% in the 80 mg/kg/day group for females.[75)] No ASV-related tumors were observed. As non-neoplastic changes, an increased incidence of bile duct hyperplasia was observed in males at 125 mg/kg/day and in females at 80 mg/kg/day. As there were no deaths or neoplastic lesions associated with this finding and no proliferative or neoplastic lesions in the bile duct were observed also in repeat-dose toxicity studies, it has been discussed that this finding was non-adverse.

Based on the above, it has been concluded that ASV is not carcinogenic in SD rats.

3.(iii).A.(11) Reproductive and developmental toxicity (ASV)

The results from the following reproductive and developmental toxicity studies of ASV were submitted: a rat study of fertility and early embryonic development to implantation; mouse[93)] and rabbit[94)] embryo-fetal development studies; and a rat study for effects on pre- and postnatal development, including maternal function. In the rat study of fertility and early embryonic development to implantation, effects on clinical observations and liver weight were noted, but there were no effects on the parental reproductive competence or early embryonic development. In the mouse embryo-fetal development study, 1 dam was euthanized due to poor general condition, but no teratogenic effects were observed. In the rat study for effects on pre- and postnatal development, including maternal function, increases in adrenal gland weight, abdominal distension, and enlarged gastrointestinal tract were seen in maternal animals and decreased survival rate and decreases in body weight and food consumption were noted in the F1 generation. ASV has been shown to cross the placenta and be excreted in milk in rats [see "3.(ii).A.(7).4) Placental transfer to fetus and 3.(ii).A.(9).2) Excretion into milk"].

3.(iii).A.(11).1) Rat study of fertility and early embryonic development to implantation (4.2.3.5.1.1)

SD rats (n = 25/sex/group) received ASV by oral gavage at doses of 0 (vehicle), 50, 200, or 600 mg/kg/day. Males were dosed from 28 days prior to mating until the day of necropsy and females were dosed from 14 days prior to mating until gestation day 7. Salivation and coloration of fur in males and females and loose stool in males at ≥50 mg/kg/day were observed and these changes were dose-dependent.[95)] Decreased food consumption up to Week 2 in males and females and increases in food consumption and liver weight after administration on

93) As mice were more sensitive to the embryo-fetal toxicity of an HCV protease inhibitor with a similar structure to ASV than rats in an earlier study, mice were selected as a rodent species for an embryo-fetal development study.

94) Although high ASV exposure could not be achieved in rabbits, as the exposure in rabbits (200 mg/kg/day; AUC, 4.4 μg·h/mL) was at least equivalent to (1.2-fold) the human exposure at the recommended clinical dose (AUC, 3.69 μg·h/mL) and high exposure was achieved in mice, it has been discussed that effects on embryo-fetal development were appropriately evaluated.

95) Although no increases in the plasma drug concentration (AUC) were observed at ≥200 mg/kg/day, effects on clinical observations, body weight, and food consumption were dose-dependent and it has been discussed that this was due to a much greater amount of ASV unabsorbed in the gastrointestinal tract in the 600 mg/kg/day group than in the 200 mg/kg/day group.

gestation day 7 in females at ≥200 mg/kg/day were observed. Decreased body weight on Days 1 to 8 and decreased body weight gain during the dosing period in males and loose stool and decreased body weight gain during the premating period (Study Days 1-11) and on Study Days 8 to 15 in females at 600 mg/kg/day were observed. There were no ASV-related effects on the reproductive function or early embryonic development.

Based on the above, the NOAELs in this study were determined to be 50 mg/kg/day for parental general toxicity and 600 mg/kg/day for parental reproductive function and early embryonic development.

3.(iii).A.(11).2) Embryo-fetal development studies

3.(iii).A.(11).2).(a) Mouse study (4.2.3.5.2.3)

Pregnant CD-1 mice (n = 22/group) orally received ASV 0 (vehicle), 10, 50, 250, or 500 mg/kg/day from gestation day 6 to gestation day 15. One of 22 dams in the 500 mg/kg/day group was euthanized on gestation day 7 due to poor general condition (decreased activity, incomplete eyelid opening, piloerection, tremor, decreased respiratory rate, etc.). Although necropsy could not identify the cause for poor general condition, it has been discussed that the symptoms were related to ASV. There were no effects on maternal animals or embryo-fetal development at any dose level.

Based on the above, the NOAELs in this study were determined to be 250 mg/kg/day for maternal general toxicity and 500 mg/kg/day for embryo-fetal developmental toxicity.

3.(iii).A.(11).2).(b) Rabbit study (4.2.3.5.2.4)

Pregnant NZW rabbits (n = 22-32/group) orally received ASV 0 (vehicle), 50, 100, or 200[96)] mg/kg/day from gestation day 7 to gestation day 19. There were no effects on maternal animals or embryo-fetal development at any dose level.

Based on the above, the NOAEL in this study was determined to be 200 mg/kg/day for maternal toxicity and embryo-fetal developmental toxicity.

3.(iii).A.(11).3) Rat study for effects on pre- and postnatal development, including maternal function (4.2.3.5.3.1)

Pregnant SD rats (n = 25-30/group) orally received ASV 0 (vehicle), 40, 125, or 400 mg/kg/day from gestation day 6 to lactation day 20. In maternal animals, abdominal distension, wet fur of the lower jaw and coloration of fur, increases in adrenal gland weight, and hypertrophy of the small and large intestines at ≥125 mg/kg/day were observed. Reduced body weight gain on gestation days 6 to 21 and reduced body weight on gestation day 21, decreased food consumption during gestation and on lactation days 0 to 14, and distended small and large intestines at 400 mg/kg/day were observed. In the F1 offspring, decreases in survival to postnatal day 14 or to postnatal day 21, decreased skin temperature before weaning, emaciation and loss of gastric content, reduced body weight gain from birth to postnatal day 87 or 125, and reduced food consumption on postnatal days 21 to

96) The high dose was selected as the maximum feasible dose based on the solubility of ASV in vehicle and as the maximum daily dose of PEG-400 for rabbits, as recommended by the laboratory.

77 at 400 mg/kg/day were observed. There were no ASV-related effects on postnatal survival rate, litter size, behavior, female age of sexual maturity, estrous cycle, and reproductive competence at any dose level.

Based on the above, the NOAELs in this study were determined to be 40 mg/kg/day for maternal general toxicity, 125 mg/kg/day for F1 developmental toxicity, and 400 mg/kg/day for F1 reproductive toxicity.

3.(iii).A.(12) Other toxicity studies (ASV)

3.(iii).A.(12).1) Phototoxicity

As ASV absorbs light in the wavelength range of 290 to 700 nm, phototoxicity studies were performed.

3.(iii).A.(12).1).(a) Neutral Red Uptake phototoxicity assay with the Balb/c 3T3 mouse fibroblast cell line (4.2.3.7.7.1)

Balb/c 3T3 mouse fibroblast cells were treated with ASV (Assay 1, 0.2-65.0 mg/L; Assay 2, 0.11-65.0[97] mg/L) and cell viability was determined by Neutral Red Uptake in the presence or absence of UVA irradiation (5 J/cm^2 for 2.5 hours). As a result, ASV reduced cell viability, either in the presence or absence of UVA irradiation in Assays 1 and 2. The IC_{50} values of ASV in the presence of UVA irradiation were 0.46 and 0.40 mg/L, respectively, and the IC_{50} values of ASV in the absence of UVA irradiation were 21.3 and 24.2 mg/L, respectively. Based on the above, as ASV treatment with UVA irradiation caused greater reductions in the viability of Balb/c 3T3 mouse fibroblast cells than did ASV treatment alone, it was concluded that ASV has phototoxic potential.

3.(iii).A.(12).1).(b) Single oral dose phototoxicity study on eyes and skin of Long Evans pigmented rats (4.2.3.7.7.2)

Long-Evans pigmented rats (5 males/group) were treated with single oral doses of ASV 0, 60, 325, or 600 mg/kg and exposed 4 hours later to UVA (6.5 kw, 30 minutes; wavelengths, 290-790 nm) and then the back skin of each rat was observed at 1, 4, 24, 48, and 72 hours and the eyes of each rat were examined ophthalmologically and histopathologically 3 days after administration. As a result, in rats treated with ASV, there were no skin reactions or no effects on the eyes after UVA exposure. Based on the above, it was concluded that ASV is not phototoxic in pigmented rats.[79] The ratio of the exposure associated with the highest dose used in this study which produced no phototoxic effects (600 mg/kg) (C_{max}, 115 μg/mL) to the steady-state human exposure at the recommended clinical dose (100 mg BID) (C_{max}, 0.419 μg/mL) was 274.

3.(iii).B Outline of the review

3.(iii).B.(1) Embryo-fetal effects (DCV)

Embryo-fetal developmental toxicity studies of DCV showed increased incidences of fetal malformations, skeletal abnormalities, and skeletal variations throughout the entire body. PMDA asked the applicant to explain the mechanism of development and the association with maternal toxicity, human relevance, precaution regarding the use of DCV in pregnant women or in women who may possibly be pregnant, and risk management

[97] Maximum solubility of ASV in vehicle (1% DMSO phosphate-buffered saline).

for women of childbearing potential.

The applicant explained as follows:

● The mechanism of development of malformations, skeletal abnormalities, skeletal variations, etc., and the association with maternal toxicity

Although the cause for malformations, skeletal variations, etc., observed in rat and rabbit embryo-fetal development studies [see "3.(iii).A.(5).2).(a) Rat study and 3.(iii).A.(5).2).(b) Rabbit study"] is not clear, as malformations, skeletal abnormalities, and skeletal variations occurred only in association with maternal toxicity, it is considered that these findings were all due to nonspecific effects and that no selective developmental toxicity was observed. The increase in the total number of fetuses/litters with skeletal variations noted at 40/20 mg/kg/day in the rabbit embryo-fetal development study is considered related to DCV while no maternal toxicity was found at the same dose.The fetal skeletal variations are commonly observed, resolve postnatally and hence are not chronic in nature.Thus, these findings are considered non-adverse.

● Human relevance

As malformations observed in rats and rabbits and skeletal abnormalities observed in rats all occurred only in association with maternal toxicity and no clinically significant toxicity occurred in humans treated with the recommended clinical dose of DCV, the malformations and skeletal abnormalities are unlikely to have relevance for humans.

● Precaution regarding the use of DCV in pregnant women or in women who may possibly be pregnant

The exposure (AUC, 70.1 μg·hr/mL) associated with the NOAEL dose for both maternal toxicity and fetal developmental toxicity (50 mg/kg/day) in a rat embryo-fetal development study was 4.6 times the human AUC at the recommended clinical dose and maternal and fetal effects were observed at >4.6 times the human AUC. Therefore, for safety considerations, it is recommended that DCV not be used in pregnant women or in women who may possibly be pregnant. On the other hand, DCV is not a selective developmental toxic substance and the exposure (AUC, 375 μg·hr/mL) at 200 mg/kg/day, at which effects on fetal development were observed, was 25 times the human AUC at the recommended clinical dose and toxic doses are estimated to be >4.6 to 25 times the clinical dose based on exposure. Thus, the risk is not serious enough to contraindicate DCV in pregnant women. As HCV treatment in pregnant women can often be postponed until after delivery and lactation, the use of DCV during pregnancy is not assumed.

● Risk management for women of childbearing potential

Women of childbearing potential will be advised to use contraception during treatment with DCV and for 5 weeks[98)] after treatment. It is not necessary for the package insert to mandate pregnancy testing prior to the use of DCV because DCV will not be contraindicated in pregnant women or in women who may possibly be pregnant.

PMDA considers as follows:

In both animal species tested in embryo-fetal developmental toxicity studies, fetal effects such as malformations with unknown mechanism were observed even at dose levels producing no severe maternal toxicity; the affected

98) 5 days (5 times the elimination half-life of DCV [the estimated time required for the elimination of ≥95% of DCV]) plus 30 days (ovulation cycle).

sites were located throughout the body (including the brain, skull, and limbs) in rats; and the ratio of the exposure at which no malformations were observed to the human exposure at the proposed dosage regimen (4.6 in terms of AUC) does not represent an adequate margin of safety. Taking account of these findings, the risk of DCV to induce developmental toxicity including teratogenic effects in pregnant patients and patients who may possibly be pregnant cannot be denied. As to skeletal variations, the total number of fetuses/litters with variations in the skull, vertebrae, etc., was significantly increased in rabbits at a low dose level producing no overt maternal toxicity and their relationship to DCV cannot be denied, and their reversibility and effects on fetal growth have not been evaluated. Taking account of these points, the possible relevance for human safety cannot be ruled out. Furthermore, treatment of chronic hepatitis C and chronic hepatitis C with compensated cirrhosis in pregnant patients or in patients who may possibly be pregnant can be postponed until after delivery and lactation, the situation where the benefits of DCV with teratogenic potential outweigh its risks is unlikely, and it is difficult to infer the clinical safety of DCV in theses patients. Based on the above, DCV should be contraindicated in pregnant women and in women who may possibly be pregnant. For women of childbearing potential, a negative pregnancy test result should be confirmed before starting treatment.

Advising women of childbearing potential to use contraception for a specified period of time, as proposed by the applicant, is appropriate.

The above conclusions by PMDA will be finalized, taking account of comments from the Expert Discussion.

3.(iii).B.(2) Effects on bone marrow (DCV)

There were effects on bone marrow in dog and monkey repeat-dose toxicity studies. PMDA asked the applicant to explain human relevance and the possibility that the patient's immune function, resistance to infections, etc., are affected.

The applicant explained as follows:

In dogs, marked decreases in the number of bone marrow cells with decreases in circulating erythroid and granulocytic cells were observed. Bone marrow toxicity occurred in dogs at ≥9.7 times the human AUC at the recommended clinical dose and no clinically significant changes in hematological parameters or no signs of altered immune response (increased infections etc.) have been observed in clinical studies to date.

In a monkey 4-month toxicity study, bone marrow effects (lymphoid cell hyperplasia) occurred at ≥1.5 times the human AUC at the recommended clinical dose. This change occurred in males only and not in females at similar exposure levels and no associated laboratory findings were observed. There were no overt effects on bone marrow in a monkey 1-month or 9-month toxicity study. It has been reported that lymphoid hyperplasia can occur as a spontaneous lesion in the bone marrow of monkeys[99),100)] and the finding was possibly a physiological change etc. Therefore, the bone marrow lesions observed in the monkey 4-month toxicity study are considered of little toxicological significance.

99) Sato J, Doi T, et al. *J Toxicol Pathol*. 2012; 25: 63-101.
100) Chamanza R, et al. *Toxicol Pathol*. 2010; 38: 642-657.

In Japanese clinical studies of DCV in combination with ASV (pooled analysis of a Japanese phase II study [Study AI447017] and the Japanese phase III study [Study AI447026]), hematological parameters were largely normal during treatment and most of abnormal values were of Grade 1 or 2.[101)] In Japanese clinical studies of DCV or placebo in combination with PegIFNα/RBV (Study AI444021 and Study AI444022), there were no differences in the incidence of hematological abnormalities or infections between subjects treated with DCV + PegIFNα/RBV and those treated with placebo + PegIFNα/RBV[102)] and the safety profile of DCV + PegIFNα/RBV was similar to the known safety profile of PegIFNα/RBV. Based on the above, clinical studies did not suggest a causal relationship between DCV and bone marrow toxicity (hematological abnormalities) and non-clinical findings were not confirmed in clinical studies.

PMDA considers as follows:
As the applicant's discussion on non-clinical data is understood and clinical studies did not suggest the effects of DCV on bone marrow, there is no particular concern about bone marrow effects at present. However, as a dog repeat-dose toxicity study demonstrated DCV-related bone marrow toxicity and there is limited clinical experience in Japan and overseas, if bone marrow effects are reported in future, the information should be provided to healthcare providers in clinical settings.

3.(iii).B.(3) Effects on adrenal gland (DCV)
There were effects on the adrenal gland in rat and monkey repeat-dose toxicity studies. PMDA asked the applicant to explain the mechanism of development, possible effects on human hormone balance via the hypothalamic-pituitary-adrenal axis, and human relevance.

101) As to hematological parameters (hemoglobin, platelet count, leukocyte count, absolute neutrophil count, absolute lymphocyte count, INR), Grade 3 or 4 hematological abnormalities with an incidence of >1% were hemoglobin decreased (2.7%), absolute lymphocyte count decreased (2.7%), and platelet count decreased (1.6%).

102) Grade 3 or 4 hematological abnormalities were absolute lymphocyte count decreased (33.3% in the DCV+pegIFNα/RBV group and 43.8% in the placebo+pegIFNα/RBV group), absolute neutrophil count decreased (19.4% and 37.5%, respectively), hemoglobin decreased (11.1% and 6.3%, respectively), and leukocyte count decreased (11.1% and 25.0%, respectively). The incidences of infections and infestations (SOC) were 50.0% in the DCV+pegIFNα/RBV group and 50.0% in the placebo+pegIFNα/RBV group.

The applicant explained as follows:

Although the detailed mechanism of development of changes in the adrenal glands of rats and monkeys is undefined, based on the following points, the effects on the adrenal gland are unlikely to represent a concern for the clinical use of DCV.

● No adrenal insufficiency or findings of direct toxicity to the adrenal gland have been observed in any animal species.

● Increases in the urinary corticosterone concentration in rats were consistent with adrenal hypertrophy, which is a functional response that can be monitored.

● Changes in the adrenal gland did not progress during up to 6 months of treatment in rats or during up to 9 months of treatment in monkeys and resolved following a recovery period.

● In mouse 6-month and rat 2-year carcinogenicity studies, no findings suggestive of tumor development in the adrenal gland and other tissues[103)] were observed.

● The observed findings were morphologically similar to adaptive stress responses[104)] as observed in laboratory animals.

● Based on 24-hour urine collection from humans treated with the clinical dose of DCV for 14 days, the urine cortisol level was within the reference range in most subjects (a foreign phase I study AI444003 and a Japanese phase I study AI444007). The levels of 24-hour urine cortisol were within the reference range also in a study of DCV in combination with ASV (Study AI447028).

PMDA's view on effects on the adrenal gland is as follows:

As clinical studies did not suggest effects on the adrenal gland, there should be no particular concern about effects on the adrenal gland at present. However, in rats and monkeys, effects on the adrenal gland occurred in a dose-dependent manner, starting from the low dose used, even in the absence of overt effects on parameters or organs/tissues that are responsive to stress (clinical observations, body weight and food consumption, thymus, spleen, accessory reproductive organs, etc.,[105)]) and the possibility that DCV directly affects the adrenal gland also cannot be ruled out, and there is limited clinical experience in Japan and overseas. Thus, if effects on the adrenal gland are reported in future, the information should be provided to healthcare providers in clinical settings.

3.(iii).B.(4) Potential toxicologic interactions of a combination of DCV and ASV

Decreases in leukocytic cells or inflammatory and necrotic changes in the intestinal tract were observed in rat and monkey combination toxicity studies. PMDA asked the applicant to explain the potential toxicologic interactions of a combination of DCV and ASV and its safety in patients with underlying disease in the gastrointestinal tract, such as enteritis.

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103) It has been explained that the absence of findings suggestive of CNS effects is supported by limited distribution of DCV into the brain in a rat distribution study [see "3.(ii).A.(2).1) Tissue distribution after a single oral dose in rats"].

104) It has been explained that increases in adrenal gland weight, enlarged adrenal gland, cortical hypertrophy in the zona fasciculata or reticularis, changes in cytoplasmic vacuolation, and increases in urinary corticosterone concentration observed in rats were similar to adaptive stress responses. It has also been explained that decreases in vacuolation in adrenal cortical cells observed in monkeys were due to depletion of cholesterol or cholesteryl esters stored in cells and such change is known to occur after stimulation with adrenocorticotropic hormone (*J Lipd Res*. 1978;19:570-577, *Braz J Med Biol Res*. 2004; 37:193-199) and is stress-related.

105) *Toxicol Pathol*. 2013;41(4):560-614.

The applicant explained as follows:
A relationship between decreases in lymphocyte count and basophil count observed in a rat 1-month combination toxicity study [see "3.(iii).A.(2).7).(a) Rat 1-month oral combination toxicity study"] and a combination of DCV and ASV (2.3 times and 11 times the human AUC, respectively) cannot be denied. However, as no associated changes in clinical observations or organic changes were noted in this study and similar changes did not occur in a monkey 1-month or 3-month oral combination toxicity study of DCV with ASV (2.4 times and 18 times the human AUC, respectively) [see "3.(iii).A.(2).7).(b) Monkey 1-month oral combination toxicity study and 3.(iii).A.(2).7).(c) Monkey 3-month oral combination toxicity study"], these findings are considered of little toxicological significance. In clinical studies, no clinically significant hematological findings have been reported to date in patients treated with DCV or ASV in combination with PegIFN/RBV or patients treated with DCV + ASV dual therapy.

Although inflammatory and necrotic changes in the intestinal tract as observed in the monkey 1-month combination toxicity study did not occur in toxicity studies with DCV or ASV alone and the mechanism of development is not clear, as there were effects on the gastrointestinal tract in rats and dogs in toxicity studies with ASV alone, it is considered that these changes were related to ASV. However, these findings were observed at a dose level approximately 18 times the recommended clinical dose in humans based on AUC and similar to inflammation in the gastrointestinal tract reported as a spontaneous finding in cynomolgus monkeys[106), 107)] and changes in the gastrointestinal tract did not occur at a dose level 6 times the recommended clinical dose in humans based on AUC in the monkey 3-month combination toxicity study. Thus, these findings are considered of little toxicological significance. Since cortical hypertrophy in the zona reticularis in the adrenal gland, decreases in vacuolation in the adrenal cortical zona fasciculata, and decreased lymphocytes in the thymus, which are known to be related to stress, were observed in this study, it is considered that increased incidences of minimal inflammatory and necrotic lesions in the intestinal tract (which were observed also in 1 female in the control group) in the high dose group were possibly related to stress.

In Japanese clinical studies of DCV in combination with ASV (pooled analysis of a Japanese phase II study [Study AI447017] and the Japanese phase III study [Study AI447026]), the incidence of diarrhoea was 9.8% (25 of 255 subjects), the incidence of nausea was 5.9% (15 of 255 subjects), the incidence of vomiting was 2.4% (6 of 255 subjects), the incidence of abdominal pain upper was 2.7% (7 of 255 subjects), and the incidence of gastrointestinal disorders[108)] was 7.8% (20 of 255 subjects) during treatment. All events were of Grade 1 or 2 and none of the gastrointestinal disorders were serious or led to discontinuation. Furthermore, as clinically significant worsening of gastrointestinal disorders were not observed in subjects with prior or concurrent conditions related to gastroenteritis in the Japanese phase III study (Study AI447026),[109)] the safety and efficacy of DCV + ASV dual therapy in patients with acute or chronic gastroenteritis have not been established, but the risk of gastrointestinal toxicity in humans should be low.

106) Reindel RJF, et al. *Vet Pathol*. 1999;36:1-13.
107) Chamanza R, et al. *Toxicol Pathol*. 2010;38: 624-657.
108) Nausea, vomiting, and anorexia
109) In 111 subjects with prior or concurrent conditions related to gastroenteritis, the incidence of diarrhoea was 13.5% (15 of 111 subjects), the incidence of nausea was 5.4% (6 of 111 subjects), the incidence of vomiting was 2.7% (3 of 111 subjects), and the incidence of abdominal pain was 5.4% (6 of 111 subjects) during study treatment, and all events were of Grade 1.

PMDA considers as follows:

As the applicant's discussion on non-clinical data is understood and clinical studies did not suggest bone marrow or gastrointestinal effects, there is no particular concern about the DCV + ASV dual therapy at present. However, as bone marrow effects were observed in DCV toxicity studies, gastrointestinal effects were observed in ASV toxicity studies, and there is limited clinical experience in Japan and overseas, if bone marrow and gastrointestinal effects are reported in future, the information should be provided to healthcare providers in clinical settings.

4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

The results from the following biopharmaceutical studies were submitted in the application: 3 clinical studies of DCV in foreign healthy adult subjects[110)] and 4 clinical studies of ASV in foreign healthy adult subjects. In this section, biopharmaceutical studies with the DCV phase III tablet formulation and those with the ASV softgel capsule formulation are mainly described. Doses and pharmacokinetic parameters of DCV are expressed in terms of Daclatasvir.

DCV and ASV concentrations in human plasma and urine and samples for protein binding studies were determined using LC/MS/MS (LLOQ, 0.05-2.0 ng/mL in plasma, 1.0 ng/mL for DCV in urine, 5.0 ng/mL for ASV in urine, 0.05 ng/mL in samples for protein binding studies).

DCV formulations used were an oral solution formulation and an immediate-release capsule formulation for earlier phase I studies and the phase II tablet formulation manufactured by Process A for other phase I studies and phase II studies and the phase III tablet formulation containing [redacted] of DCV compared with the phase II tablet formulation for phase III studies and the phase III tablet formulation has been proposed for marketing. ASV formulations used in phase I and II studies in early clinical development were an immediate-release film-coated tablet formulation (ASV tablets), a suspension formulation, an oral solution formulation, and a hard gelatin capsule formulation (ASV hard capsules). As marked food effects were observed with these formulations, the ASV softgel capsule formulation with reduced food effect and better BA was used in phase III studies and has been proposed for marketing.

Unless otherwise specified, pharmacokinetic parameters are expressed in geometric mean value.

110) A coadministration study of DCV and a proton pump inhibitor submitted as a biopharmaceutic study of DCV (Study AI444024) is described in "4.(ii).A.(5) Drug interaction studies".

4.(i).A.(1) Administration of DCV alone

4.(i).A.(1).1) Relative BA and food effect study (5.3.1.1.2, Study AI444039 [20 to 20])

A 4-treatment, 4-period, crossover study was conducted in foreign healthy adult male and female subjects (23 subjects included in pharmacokinetic assessment) to assess the relative BA of the DCV phase III tablet formulation to the DCV phase II tablet formulation and food effects.[111] The results were as shown in Table 26 and the BA was comparable between the phase II and III tablet formulations. With regard to food effects, a high-fat meal decreased exposure while the BA after a low-fat meal was similar to the BA under fasting conditions.

Table 26. Comparison of pharmacokinetic parameters between phase II and III tablet formulations (DCV 60 mg)

Treatment	N	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)	t_{max} a) (h)	$t_{1/2}$ b) (h)	Adjusted geometric mean ratio [90% CI]	
						C_{max}	AUC_{inf}
Phase II tablet formulation under fasting conditions	22	1474 (33)	15,493 (39)	1.0 [1.0, 4.0]	14.5 ± 4.03	-	-
Phase III tablet formulation under fasting conditions	22	1477 (38)	15,412 (39)	1.0 [1.0, 4.0]	14.1 ± 2.79	(Phase III tablet formulation under fasting conditions/Phase II tablet formulation under fasting conditions)	
						1.00 [0.92, 1.09]	1.00 [0.95, 1.04]
Phase III tablet formulation after a high-fat meal	23	1066 (30)	11,931 (38)	1.5 [1.0, 4.0]	15.0 ± 3.96	(Phase III tablet formulation after a high-fat meal/Phase III tablet formulation under fasting conditions)	
						0.72 [0.66, 0.79]	0.77 [0.73, 0.80]
Phase III tablet formulation after a low-fat meal	23	1438 (25)	15,502 (35)	2.0 [1.0, 4.0]	14.5 ± 3.50	(Phase III tablet formulation after a low-fat meal/Phase III tablet formulation under fasting conditions)	
						0.97 [0.89, 1.06]	1.00 [0.95, 1.05]

Geometric mean (CV%)
C_{max}, maximum plasma concentration; AUC_{inf}, area under the plasma concentration-time curve from time zero extrapolated to infinity; t_{max}, time of maximum plasma concentration; $t_{1/2}$, elimination half-life
a) Median [range], b) Mean ± SD

4.(i).A.(1).2) Absolute oral BA study (5.3.1.1.3, Study AI444044 [20 to 20])

Foreign healthy adult male and female subjects (8 subjects included in pharmacokinetic assessment) received a single oral dose of DCV 60 mg and single intravenous 100 μg doses of ^{13}C-DCV and ^{15}N-DCV and the absolute BA of oral DCV was determined. The absolute BA of oral DCV [90% CI] was 67.0 [56.2, 79.8]%. Following single intravenous doses of 100 μg of ^{13}C-DCV and ^{15}N- DCV, the V_{ss} (mean) was 47.1 L and the CL was 4.24 L/h.

4.(i).A.(2) Administration of ASV alone

4.(i).A.(2).1) Relative BA and food effect studies (5.3.1.1.2, Study AI447024 [20 to 20]; 5.3.1.1.4, Study AI447043 [20 to 20])

An 8-treatment, 5-period, crossover study was conducted in foreign healthy adult male and female subjects (35

[111] Each subject received the following treatments and at least a 4-day washout period was included between the treatment periods.
- Treatment A: a single dose of 60 mg of DCV as the phase II tablet formulation under fasting conditions
- Treatment B: a single dose of 60 mg of DCV as the phase III tablet formulation under fasting conditions
- Treatment C: a single dose of 60 mg of DCV as the phase III tablet formulation after a high-fat meal (52% fat/951 kcal)
- Treatment D: a single dose of 60 mg of DCV as the phase III tablet formulation after a low-fat meal (15% fat/277 kcal)

subjects included in pharmacokinetic assessment).[112)] The primary objective of the study was to assess the relative BA following a single oral dose of ASV tablets 200 mg administered after a standard meal (36% fat/423 kcal) and following a single oral dose of ASV softgel capsules 200 mg administered under fasting conditions or after a standard meal. The pharmacokinetic parameters of the ASV phase II tablet formulation under fed conditions and those of the ASV softgel capsule formulation under fasting or fed conditions were as shown in Table 27 and the BA of the ASV softgel capsule formulation under either fasting or fed conditions was higher than the BA of the ASV tablet formulation under fed conditions. With regard to food effects, administration of ASV softgel capsules under fed conditions resulted in shorter t_{max} and higher exposure, but ASV softgel capsules were shown to have a smaller food effect than ASV tablets.[113)] The food effect for ASV softgel capsules was similar, regardless of meal type (a high-fat meal or a standard meal).[114)]

Table 27. Comparison of pharmacokinetic parameters between ASV tablet and softgel capsule formulations (ASV 200 mg)

Treatment	N	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)	t_{max}[a)] (h)	$t_{1/2}$[b)] (h)	Adjusted geometric mean ratio [90% CI]	
						C_{max}	AUC_{inf}
ASV tablet formulation after a standard meal	33	62.3 (123)	476 (74)	4.0 [1.5, 8.0]	11.6 ± 6.18	-	-
ASV softgel capsule formulation under fasting conditions	19	252 (98)	1059 (71)	3.0 [1.0, 4.0]	12.0 ± 6.26	(Softgel capsule formulation under fasting conditions/Tablet formulation after a meal)	
						4.09 [3.08, 5.43]	2.23 [1.86, 2.67]
ASV softgel capsule formulation after a standard meal	19	287 (51)	1060 (36)	1.5 [1.0, 4.0]	10.4 ± 4.86	(Softgel capsule formulation after a meal/Tablet formulation after a meal)	
						5.36 [4.03, 7.12]	2.60 [2.17, 3.12]
						(Softgel capsule formulation after a meal/Softgel capsule formulation under fasting conditions)	
						1.31 [0.95, 1.82]	1.17 [0.95, 1.44]

Geometric mean (CV%)
a) Median [range] , b) Mean ± SD

4.(i).A.(2).2) Absolute oral BA study (5.3.1.1.3, Study AI447027 [[redacted] 20[redacted] to [redacted] 20[redacted]])

Foreign healthy adult male subjects (10 subjects included in pharmacokinetic assessment) received a single oral dose of ASV softgel capsules 100 mg or a single intravenous dose of 100 μg of ^{14}C-ASV. The absolute oral BA of ASV softgel capsules [90% CI] was 9.3 [7.0, 12.5]%. Following a single intravenous dose of ^{14}C-ASV, the V_{ss} (mean) was 194 L and the CL was 49.5 L/h.

112) The following treatments were administered and a 5-day washout period was included between the treatment periods.
- Treatment A: ASV tablet formulation manufactured by Process A administered after a standard meal
- Treatment B: ASV tablet formulation manufactured by Process B using Drug Substance 1 administered under fasting conditions
- Treatment C: ASV tablet formulation manufactured by Process B using Drug Substance 2 administered under fasting conditions
- Treatment D: ASV tablet formulation manufactured by Process B using Drug Substance 2 administered after a standard meal
- Treatment E: ASV [redacted] tablet formulation administered under fasting conditions
- Treatment F: ASV [redacted] tablet formulation administered after a standard meal
- Treatment G: ASV softgel capsule formulation administered under fasting conditions
- Treatment H: ASV softgel capsule formulation administered after a standard meal

113) Following administration of a single oral dose of ASV tablets after a high-fat meal (51% fat/1038 kcal) compared to under fasting conditions, the C_{max} and AUC_{inf} were increased 29.6-fold and 11.5-fold, respectively (CTD5.3.1.1.1, Study AI447008).

114) A 2-period crossover study was conducted in 28 foreign healthy adult male and female subjects to assess the effect of a high-fat meal (52% fat/951 kcal) on the pharmacokinetic parameters of a single oral dose of ASV softgel capsules 100 mg. The adjusted geometric mean ratios of the C_{max} and AUC_{inf} of ASV administered after a high-fat meal vs. under fasting conditions [90% CIs] were 1.34 [1.08, 1.66] and 1.20 [1.07, 1.34], respectively. Administration after a high-fat meal resulted in shorter t_{max}.

4.(i).B Outline of the review

4.(i).B.(1) Pharmacokinetic parameters of ASV tablets and ASV softgel capsules

The applicant explained the reason for selecting the 100 mg BID regimen of ASV softgel capsules as being equivalent to 200 mg BID of ASV tablets as follows:

In Study AI447024, the AUC after a single 200 mg dose of ASV softgel capsules was approximately 2-fold that after a single 200 mg dose of ASV tablets [see "4.(i).A.(2).1) Relative BA and food effect studies"]. Assuming that the ASV exposure after administration of softgel capsules is linear, half the exposure after administration of ASV softgel capsules 200 mg was compared with the exposure after administration of ASV tablets 200 mg. As a result, the adjusted geometric mean ratios of the C_{max} and AUC_{inf} [90% CIs] were 2.68 [2.02, 3.56] and 1.30 [1.09, 1.56], respectively, and it was predicted that the C_{max} would be higher, but the AUC_{inf} would be almost comparable after administration of ASV softgel capsules 100 mg compared to ASV tablets 200 mg and the 100 mg BID regimen of ASV softgel capsules was chosen for a phase III study.

The exposure after administration of ASV softgel capsules increased more than dose-proportionally [Study AI447030, see "4.(ii).A.(2).2) ASV alone"]. PMDA asked the applicant to compare the actual pharmacokinetic parameters after administration of ASV tablets 200 mg BID with those after administration of ASV softgel capsules 100 mg BID and then explain the differences in exposure.

The applicant explained as follows:

Following oral administration of ASV tablets 200 mg BID and ASV softgel capsules 100 mg BID to Japanese patients with chronic hepatitis C, the pharmacokinetic parameters[115)] were as follows: the C_{max} values were 711 and 647 ng/mL, respectively; the plasma concentrations at 12 hours post-dose (C_{min}) were 65.7 and 31.3 ng/mL, respectively; the t_{max} values (median) were 4.0 and 2.0 hours, respectively; and the values of AUC during the dosing interval (AUC_{tau}) were 2947 and 2155 ng·h/mL, respectively. The C_{max} after administration of ASV softgel capsules 100 mg BID was lower than that expected from linearity assumption and the exposure after administration of ASV softgel capsules 100 mg BID was similar to the exposure after administration of ASV tablets 200 mg BID. Non-linear pharmacokinetics of ASV softgel capsules were considered associated with saturation of the process of excretion in the intestinal tract or hepatic uptake with increasing dose.

PMDA considers as follows:

With respect to the exposure to ASV after administration of softgel capsules, linear pharmacokinetics were assumed at the time of dose selection, but this assumption was not true. On the other hand, with respect to the pharmacokinetic parameters after administration of ASV tablets 200 mg BID or ASV softgel capsules 100 mg BID to Japanese patients with chronic hepatitis C, as the exposure after administration of ASV tablets 200 mg BID was almost comparable to that after administration of ASV softgel capsules 100 mg BID, the applicant's explanation that the 100 mg BID regimen of ASV softgel capsules was selected for the Japanese phase III study (Study AI447026) is acceptable. The dosage regimen for ASV will be determined based on clinical data [see "4.(iii).B.(4) Dosage and administration"].

115) Pharmacokinetic parameters in Study AI447017 (ASV tablets administered) and Study AI447026 (ASV softgel capsules administered) [see "4.(ii).A.(3).3) Combination of DCV and ASV"].

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

The results from the following pharmacokinetic studies with DCV alone were submitted in the application: 2 Japanese phase I studies (including 1 drug interaction study), 20 foreign phase I studies (including 15 drug interaction studies), 2 Japanese phase II studies, and 5 foreign phase II studies. The results from the following pharmacokinetic studies with ASV alone were submitted: 1 Japanese phase I study, 16 foreign phase I studies (including 10 drug interaction studies), and 2 foreign phase II studies. The results from the following pharmacokinetic studies of DCV in combination with ASV were submitted: 3 foreign phase I studies (including 2 drug interaction studies), 1 Japanese phase II study, 1 foreign phase II study, and 1 Japanese phase III study. The results of population pharmacokinetic (PPK) and exposure-response (E-R) analyses of Japanese phase II and III studies were also submitted.

Unless otherwise specified, pharmacokinetic parameters are expressed in the geometric mean and doses and pharmacokinetic parameters of DCV are expressed in terms of Daclatasvir.

4.(ii).A.(1) *In vitro* studies using human biomaterials

In vitro studies of DCV and ASV using human biomaterials conducted include human serum or plasma protein binding studies, *in vitro* metabolism studies using human liver microsomes, primary hepatocytes, and human CYP expression system, and drug metabolizing enzyme inhibition and induction studies using human liver microsomes or primary hepatocytes [see "3.(ii).A.(2) Distribution (DCV), 3.(ii).A.(3) Metabolism (DCV), 3.(ii).A.(5) Pharmacokinetic drug interactions (DCV), 3.(ii).A.(7) Distribution (ASV), 3.(ii).A.(8) Metabolism (ASV), and 3.(ii).A.(10) Pharmacokinetic drug interactions (ASV)" for summaries of study data].

4.(ii).A.(2) Studies in healthy adult subjects

4.(ii).A.(2).1) Administration of DCV alone

4.(ii).A.(2).1).(a) Phase I single-dose/multiple-dose study in Japanese healthy adult subjects (5.3.3.1.4, Study AI444007 [[redacted] 20[redacted] to [redacted] 20[redacted]])

The pharmacokinetics of DCV were investigated following single oral doses of 1, 10, 50, 100, or 200 mg of DCV solution and 14-day oral administration of DCV capsules at doses of 1, 10, or 100 mg QD in Japanese healthy adult male subjects [48 subjects included in pharmacokinetic assessment (6 subjects/group)] under fasting conditions. The results were as shown in Table 28 and the single-dose C_{max} and AUC of DCV increased dose-proportionally and the increases in the multiple-dose C_{max} and AUC were slightly more than dose-proportional. Elimination from plasma was multiphasic and the accumulation ratios for C_{max} and AUC_{tau} were 1.22 to 1.36 and 1.36 to 1.55, respectively. Trough values indicated that steady-state is nearly achieved by Day 4 or 5.

Table 28. Pharmacokinetic parameters of DCV after single or multiple oral doses (Japanese healthy adult subjects)

	Dose	N	C_{max} (ng/mL)	AUC[a] (ng·h/mL)	CL/F (mL/min)	$t_{1/2}$[b] (h)
Solution Single dosing	1 mg	6	18.7 (19)	171 (18)	97.7 (19)	10.2 ± 1.1
	10 mg	6	207 (15)	1811 (20)	92.0 (22)	9.1 ± 0.9
	50 mg	6	1090 (21)	10509 (19)	79.3 (21)	9.3 ± 0.8
	100 mg	6	1864 (19)	20912 (21)	79.7 (20)	8.8 ± 0.5
	200 mg	6	2929 (24)	34030 (25)	98.0 (29)	10.1 ± 3.7
Capsules	1 mg QD	6	13.2 (49)	111 (34)	150.3 (36)	16.1 ± 8.9[c]
	10 mg QD	6	226 (24)	1760 (29)	94.7 (27)	16.8 ± 9.4[c]
Multiple dosing Day 14	100 mg QD	6	1853 (23)	17115 (30)	97.4 (25)	12.8 ± 4.0[c]

Geometric mean (CV%); CL/F, oral clearance
a) AUC_{inf} for single dosing, AUC_{tau} for multiple dosing, b) Mean ± SD, c) Calculated from the accumulation ratio

4.(ii).A.(2).1).(b) Phase I single-dose and multiple-dose studies in foreign healthy adult subjects (5.3.3.1.1, Study AI444001 [20 to 20]; 5.3.3.1.2, Study AI444003 [20 to 20])

The pharmacokinetics of DCV were investigated following single oral doses of 1, 10, 25, 50, 100, or 200 mg of DCV solution in foreign healthy adult male and female subjects (36 subjects included in pharmacokinetic assessment [6 subjects/group]) under fasting conditions (Study AI444001) and following 14-day oral administration of DCV capsules at 1, 10, 30, or 60 mg QD[116] in foreign healthy adult male and female subjects (24 subjects included in pharmacokinetic assessment [6 subjects/group]) under fasting conditions (Study AI444003). Multiple-dose pharmacokinetic parameters on Day 14 were as shown in Table 29 and the C_{max} and AUC_{tau} increased more than dose-proportionally. Elimination from plasma was multiphasic and the accumulation ratios for C_{max} and AUC_{tau} were 0.88 to 1.22 and 1.04 to 1.37, respectively. Trough values indicated that steady-state is achieved by Day 3 to Day 5. The single-dose C_{max} and AUC of DCV increased dose-proportionally.

Table 29. Pharmacokinetic parameters of DCV after multiple oral doses (Foreign healthy adult subjects)

Dose	N	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	CL/F (mL/min)	$t_{1/2}$[a] (h)
1 mg QD	6	16.0 (22)	125 (22)	133 (26)	12.7 ± 3.9
10 mg QD	6	257 (29)	2454 (29)	67.9 (41)	13.1 ± 2.6
30 mg QD	5	734 (30)	6275 (39)	79.7 (44)	14.9 ± 4.1
60 mg QD	6	1582 (37)	15,666 (47)	63.8 (39)	13.9 ± 3.7

Geometric mean (CV%)
a) Mean ± SD

4.(ii).A.(2).1).(c) Mass balance study in foreign healthy adult subjects (5.3.3.1.3, Study AI444006 [20 to 20])

The pharmacokinetics, metabolites, and routes and rates of excretion of ^{14}C-DCV solution were investigated following a single 25-mg oral dose in foreign healthy adult male subjects (6 subjects included in pharmacokinetic assessment) under fasting conditions. The C_{max} and AUC_{inf} of plasma DCV were 532 ng/mL and 4692 ng·h/mL, respectively, and accounted for 93% and 95%, respectively, of the total plasma radioactivity, which indicated that most of the plasma radioactivity was associated with unchanged DCV. A mean of 94.4% of radioactivity was recovered over 240 hours and 6.6% and 87.7% of radioactivity were recovered in urine and feces, respectively.

116) Although doses up to 100 mg were originally planned to be tested, this study was terminated after doses up to 60 mg were tested, as it was considered that the data needed to continue development of DCV were obtained from other studies.

4.(ii).A.(2).2) Administration of ASV alone

4.(ii).A.(2).2).(a) Phase I single-dose/multiple-dose study in Japanese healthy adult subjects (5.3.3.1.3, Study AI447005 [20 to 20])

The pharmacokinetics of ASV were investigated following single oral doses of 200, 400, 600, 900, or 1200 mg of ASV suspension and 14-day oral administration of ASV hard capsules at 200, 400, or 600 mg BID[117)] in Japanese healthy adult male subjects (48 subjects included in pharmacokinetic assessment [6 subjects/group]) under fasting conditions. The results were as shown in Table 30 and the multiple-dose C_{max} and AUC_{tau} of ASV hard capsules increased almost dose-proportionally. Elimination from plasma was biphasic and the accumulation index for AUC_{tau} was 1.79 to 2.38. Trough values indicated that steady-state is nearly achieved by Day 3 to Day 5.

Table 30. Pharmacokinetic parameters of ASV after single or multiple oral doses (Japanese healthy adult subjects)

	Dose	N	C_{max} (ng/mL)	AUC[a)] (ng·h/mL)	CL/F (L/h)	$t_{1/2}$[b)] (h)
Suspension Single dosing	200 mg	6	68.3 (81)	571 (67)	351 (67)	19.6 ± 8.0
	400 mg	6	812 (47)	2492 (43)	161 (78)	15.4 ± 2.2
	600 mg	6	448 (153)	1871 (102)	321 (58)	17.1 ± 3.0
	900 mg	6	704 (110)	2793 (89)	322 (90)	21.2 ± 11.6
	1200 mg	6	728 (147)	2726 (137)	440 (147)	15.7 ± 7.8
Hard capsules Multiple dosing Day 14	200 mg BID	6	310 (65)	804 (34)	249 (31)	21.8 ± 13.2 c)
	400 mg BID	6	625 (63)	1357 (52)	295 (62)	32.8 ± 15.7 c)
	600 mg BID	6	889 (62)	2230 (54)	269 (48)	32.2 ± 27.9 c)

Geometric mean (CV%)
a) AUC_{inf} for single dosing, AUC_{tau} for multiple dosing, b) Mean ± SD, c) Calculated from the accumulation indexs

4.(ii).A.(2).2).(b) Phase I single-dose and multiple-dose studies in foreign healthy adult subjects (5.3.3.1.1, Study AI447001 [20 to 20]; 5.3.3.1.2, Study AI447003 [20 to 20])

The pharmacokinetics of ASV were investigated following single oral doses of 10, 50, 100, 200, 400, 600, or 1200 mg of ASV suspension in foreign healthy adult male subjects (42 subjects included in pharmacokinetic assessment [6 subjects/group]) under fasting conditions (Study AI447001) and following 14-day oral administration of ASV hard capsules at 10, 50, 100, 200, 400, or 600 mg BID in foreign healthy adult male and female subjects (30 subjects included in pharmacokinetic assessment [6 subjects/group]) under fasting conditions (Study AI447003). The results were as shown in Table 31 and the C_{max} and AUC increased more than dose-proportionally. Elimination from plasma was biphasic. The accumulation indices at doses of 10 to 600 mg were 2.31 to 5.41 based on C_{max} and 1.72 to 5.11 based on AUC_{tau}.

117) Subjects were to be fasted for at least 10 hours before administration in the morning and fasted for about 2 hours before administration in the afternoon.

Table 31. Pharmacokinetic parameters of ASV after single or multiple oral doses (Foreign healthy adult subjects)

	Dose	N	C_{max} (ng/mL)	AUC[a)] (ng·h/mL)	CL/F (L/h)	$t_{1/2}$[b)] (h)
Suspension Single dosing (AI447001)	10 mg	6	0.96 (66)	7.3 (29)	1378 (35)	17.8 ± 12.4
	50 mg	6	9.07 (30)	78.9 (34)	634 (28)	20.3 ± 2.8
	100 mg	6	33.4 (60)	229 (40)	438 (54)	17.4 ± 4.0
	200 mg	6	35.8 (46)	391 (40)	511 (49)	20.3 ± 6.4
	400 mg	6	86.3 (78)	599 (35)	668 (48)	15.6 ± 3.5
	600 mg	6	270 (207)	1242 (186)	483 (94)	15.2 ± 4.7
	1200 mg	6	505 (131)	1945 (107)	617 (62)	13.9 ± 4.1
Hard capsules Multiple dosing Day 14 (AI447003)	10 mg BID	6	1.98 (51)	9.43 (42)	1060 (48)	23.4 ± 6.2
	50 mg BID	6	38.6 (55)	109 (49)	461 (43)	17.4 ± 7.1
	100 mg BID	6	59.7 (75)	258 (40)	388 (31)	19.4 ± 8.4
	200 mg BID	6	87.9 (62)	300 (39)	667 (46)	19.1 ± 4.0
	400 mg BID	5	228 (51)	596 (39)	671 (55)	19.0 ± 3.5
	600 mg BID	6	489 (77)	1257 (57)	477 (67)	21.3 ± 10.4

Geometric mean (CV%)
a) AUC_{inf} for single dosing, AUC_{tau} for multiple dosing, b) Mean ± SD

4.(ii).A.(2).2).(c) Mass balance study in foreign healthy adult subjects (5.3.3.1.4, Study AI447010 [20 to 20)

The pharmacokinetics, metabolism, and routes and rates of excretion of ^{14}C-ASV solution were investigated following a single 200-mg oral dose in foreign healthy adult male subjects (9 subjects included in pharmacokinetic assessment [6 subjects without bile collection; 3 subjects with bile collection]) under fasting conditions. In subjects without bile collection, the C_{max} and AUC_{inf} of plasma ASV were 367 ng/mL and 1087 ng·h/mL, respectively, and accounted for 75% and 22%, respectively, of the total plasma radioactivity.[118)] The $AUC_{0\text{-}24}$ of plasma ASV accounted for approximately 55% of the total plasma radioactivity, showing that the ratio of unchanged ASV to total radioactivity in plasma declined over time. The major metabolite in plasma was BMS-558364 (4.6% of the total plasma radioactivity). Of the total radioactivity administered, 83.9% and 0.24% (mean) were recovered in feces and urine, respectively. ASV represented 7.5% of the radioactivity in feces and the major metabolites were M8 and M12 (14.6% and 8.3%, respectively). In subjects with bile collection, the AUC_{inf} of plasma ASV was 684 ng·h/mL and 73.1%, 8.14%, and 0.19% (mean) of the total radioactivity administered were recovered in feces, bile, and urine, respectively. There were no unique human ASV metabolites detected in plasma or excreta.

4.(ii).A.(2).2).(d) Phase I study in Chinese and Caucasian healthy adult subjects (5.3.3.1.5, Study AI447030 [20 to 20])

A 3-treatment, 3-period, 2-sequence, crossover study was conducted in Chinese and Caucasian healthy adult subjects (32 subjects included in pharmacokinetic assessment [16 subjects/group]) to investigate the pharmacokinetics of ASV softgel capsules after a single 100-mg dose (Treatment A), a single 200-mg dose (Treatment B), and multiple doses of 100 mg BID (Treatment C)[119)] under fasting conditions. The C_{max} values following single doses of 100 mg and 200 mg were 62.5 and 310 ng/mL, respectively, in Chinese subjects and 43.5 and 219 ng/mL, respectively, in Caucasian subjects. The C_{max} was 1.4-fold higher in Chinese subjects than in Caucasian subjects, but other pharmacokinetic parameters were similar. In both Chinese and Caucasian subjects, the ASV exposure increased more than dose-proportionally. The multiple-dose C_{max} values in Chinese and Caucasian subjects were 364 and 192 ng/mL, respectively, and the multiple-dose AUC_{tau} values were 962

118) The t_{max} was 1.75 hours for both ASV and total radioactivity.
119) A 5-day washout period was included between the treatment periods.

and 625 ng·h/mL, respectively.

4.(ii).A.(2).3) Coadministration of DCV and ASV

4.(ii).A.(2).3).(a) US Phase I multiple-dose study in foreign healthy adult subjects (DCV5.3.3.1.5, ASV5.3.3.4.2, Study AI447009 [[redacted] to [redacted]])

DCV capsules (60 mg QD) or ASV hard capsules (600 mg BID) were orally administered to foreign healthy adult male subjects (28 subjects included in pharmacokinetic assessment [14 subjects/group]) and then DCV capsules (30 mg QD) in combination with ASV hard capsules (200 mg BID) were orally administered to subjects in both groups[117] to assess the effects of coadministration of DCV and ASV on their respective pharmacokinetics. The results were as shown in Table 32.

Table 32. Pharmacokinetic parameters after administration of DCV or ASV hard capsules alone or coadministration of DCV and ASV

	DCV			ASV		
	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	C_{min} (ng/mL)	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	C_{min} (ng/mL)
DCV or ASV alone (N = 14)	1496 (24)	12,704 (25)	163 (39)	420 (48)	1063 (58)	11.0 (55)
DCV+ASV[a] (N = 26)	1599 (26)	14,515 (28)	217 (35)	247 (64)	923 (44)	19.3 (28)
DCV+ASV/DCV or ASV alone Adjusted geometric mean ratio [90% CI]	1.07 [0.971, 1.18]	1.20 [1.11, 1.30]	1.33 [1.22, 1.45]	0.581 [0.446, 0.758]	0.868 [0.726, 1.038]	1.76 [1.42, 2.17]

Geometric mean (CV%)
a) Pharmacokinetic parameters of DCV (dose adjusted to 60 mg QD) or ASV (dose adjusted to 600 mg BID)

4.(ii).A.(3) Studies in patients with chronic hepatitis C

4.(ii).A.(3).1) Administration of DCV alone

4.(ii).A.(3).1).(a) Phase I study in foreign patients with chronic hepatitis C (5.3.3.2.1, Study AI444002 [November 2007 to May 2008])

The pharmacokinetics of DCV solution were investigated following single oral doses of 1, 10, or 100 mg in foreign patients with chronic hepatitis C (genotype 1) (16 subjects included in pharmacokinetic assessment [6 subjects in the 1 mg group, 5 subjects each in the 10 and 100 mg groups]). The results were as shown in Table 33.

Table 33. Pharmacokinetic parameters of DCV after single oral doses (Foreign patients with chronic hepatitis C)

Dose	N	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)	CL/F (mL/min)	$t_{1/2}$[a] (h)
1 mg	6	15.7 (56)	129 (49)	129 (48)	9.7 ± 2.7
10 mg	5	178 (52)	1431 (45) [b]	117 (43) [b]	12.1 ± 2.0 [b]
100 mg	5	2417 (27)	29,256 (53)	57.0 (49)	14.0 ± 6.4

Geometric mean (CV%)
a) Mean ± SD, b) N = 4

4.(ii).A.(3).1).(b) Early phase II study in foreign patients with chronic hepatitis C (5.3.3.2.2, Study AI444004 [May 2008 to June 2009])

The pharmacokinetics of DCV capsules were investigated following 14-day oral administration of 1, 10, 30, 60, or 100 mg QD, or 30 mg BID in treatment-naïve foreign patients with chronic hepatitis C (genotype 1) (24 subjects included in pharmacokinetic assessment [4 subjects/group]) under fasting conditions. The results were as shown in Table 35 and the C_{max} and AUC increased dose-proportionally.

Table 34. Pharmacokinetic parameters of DCV after multiple oral doses (Foreign patients with chronic hepatitis C)

Dose	Day of measurement (Day)	N	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	CL/F (mL/min)	$t_{1/2}$[a)] (h)
1 mg QD	1	4	15.7 (48)	112 (54)	-	-
	14	4	10.4 (76)	92.0 (80)	181 (52)	11.7 ± 2.2
10 mg QD	1	4	160 (41)	1114 (38)	-	-
	14	4	154 (49)	1332 (46)	125 (52)	14.3 ± 3.8
30 mg QD	1	4	483 (25)	3529 (19)	-	-
	14	4	556 (38)	4391 (27)	114 (25)	13.0 ± 2.0
60 mg QD	1	4	1409 (13)	10,692 (20)	-	-
	14	4	1726 (21)	15,121 (35)	66.1 (29)	12.8 ± 1.2
30 mg BID	1	4	564 (26)	3307 (36)	-	-
	14	4	832 (37)	5432 (35)	92.1 (35)	13.0 ± 3.7
100 mg QD	1	4	1961 (21)	15,136 (19)	-	-
	14	4	1854 (26)	17,593 (15)	94.7 (15)	15.2 ± 3.4

Geometric mean (CV%)
a) Mean ± SD

4.(ii).A.(3).2) Administration of ASV alone

4.(ii).A.(3).2).(a) Phase I/II study in foreign patients with chronic hepatitis C (5.3.3.2.1, Study AI447002 [January 2008 to July 2008])

The pharmacokinetics of ASV suspension were investigated following single oral doses of 10, 50, 200, or 600 mg in foreign patients with chronic hepatitis C (genotype 1) (20 subjects included in pharmacokinetic assessment [5 subjects/group]). The C_{max} and $AUC_{0\text{-}24}$ of plasma ASV increased almost dose-proportionally. The exposures in patients tended to be similar to or slightly higher than the exposures in healthy subjects after the same single doses of ASV suspension (Table 31). The t_{max} (median) and $t_{1/2}$ (mean) were 2.5 to 4 and 15 to 22 hours, respectively, which were similar to those in healthy subjects.

4.(ii).A.(3).2).(b) Early phase II study in foreign patients with chronic hepatitis C (5.3.3.2.2, Study AI447004 [January 2009 to December 2009])

The pharmacokinetics of ASV hard capsules were investigated following 3-day oral administration of 200, 400, or 600 mg BID in treatment-naïve foreign patients with chronic hepatitis C (genotype 1) (12 subjects included in pharmacokinetic assessment [4 subjects/group]) under fasting conditions. The results were as shown in Table 35. The increases in the C_{max} and AUC_{tau} on Day 3 were more than dose-proportional and the exposures were higher on Day 3 than on Day 1 at all dose levels. The exposures in patients tended to be higher than the exposures in healthy subjects after multiple-dose administration of ASV hard capsules at the same dose levels (Table 31). The t_{max} (median) was 2.25 to 3.50 hours, which was similar to that in healthy subjects.

Table 35. Pharmacokinetic parameters of ASV after multiple oral doses (Foreign patients with chronic hepatitis C)

Dose	Day of measurement (Day)	N	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)
200 mg BID	1	4	109 (64)	407 (41)
	3	4	207 (67)	1032 (53)
400 mg BID	1	4	322 (149)	1113 (121)
	3	4	566 (97)	1895 (93)
600 mg BID	1	4	547 (125)	1760 (115)
	3	4	3491 (55)	10,956 (68)

Geometric mean (CV%)

4.(ii).A.(3).3) Coadministration of DCV and ASV

4.(ii).A.(3).3).(a) Phase II study in Japanese patients with chronic hepatitis C (5.3.5.2.2, Study AI447017 [April 2010 to May 2012])

The pharmacokinetics following 24-week oral administration of DCV tablets at 60 mg QD plus ASV tablets at 600 mg BID or 200 mg BID in IFN null-responder (null-responder)[120] and IFN-ineligible-naïve[121] or IFN-intolerant[122] (IFN-ineligible-naïve/intolerant) Japanese patients with chronic hepatitis C (genotype 1) (20 subjects included in pharmacokinetic assessment [10 subjects/group]) were investigated. The results were as shown in Table 36.

Table 36. Pharmacokinetic parameters after multiple oral doses of DCV plus ASV (Japanese patients with chronic hepatitis C)

ASV dose	Day of measurement (Day)	N	DCV				ASV			
			C_{max} (ng/mL)	C_{min} (ng/mL)	t_{max} [a] (h)	AUC_{tau} (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	t_{max} [a] (h)	AUC_{tau} (ng·h/mL)
600 mg BID	1	10	1084 (25)	375 (23)	3.0 [1.0, 8.0]	13,840 (19)	10,075 (29)	516 (117)	4.0 [1.9, 8.0]	38,670 (23)
	14	10	1213 (45)	280 (109)	4.0 [1.0, 8.0]	13,547 (73)	5520 (75)	204 (291)	3.9 [b] [1.9, 8.0]	20,517 (158)
200 mg BID	1	10	1194 (22)	352 (36)	3.9 [1.9, 4.1]	14,366 (26)	459 (63)	76.6 (132)	4.0 [1.9, 7.6]	2093 (55)
	14	10	1535 (31)	379 (63)	3.8 [1.0, 8.1]	17,078 (54)	711 (83)	65.7 (163)	4.0 [2.0, 7.3]	2947 (93)

Geometric mean (CV%)
a) Median [range], b) N = 9

4.(ii).A.(3).3).(b) Phase III study in Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis (5.3.5.2.1, Study AI447026 [January 2012 to April 2013])

The pharmacokinetics following multiple oral administration of DCV tablets at 60 mg QD plus ASV softgel capsules at 100 mg BID in IFN non-responder (non-responder)[123] and IFN-ineligible-naïve[124]/intolerant[122] Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis (genotype 1b) (20 subjects/group included in pharmacokinetic assessment) were investigated. The results were as shown in Table 37.

120) Patients with <2 log_{10} IU/mL decline in HCV RNA from baseline following ≥12 weeks of treatment with PegIFNα/RBV.
121) Patients ineligible for IFN-based therapies due to age or medical conditions such as current or prior depression, anemia, myelosuppression, diabetes, hypertension, cardiovascular disorder, and renal impairment and not scheduled to receive IFN-based therapy for the next 12 months.
122) Patients who previously received IFN-based therapy for <12 weeks and then discontinued from therapy due to toxicities associated with IFN or RBV.
123) Patients who failed to achieve undetectable HCV RNA following ≥12 weeks of treatment with PegIFNα/RBV or IFNβ/RBV.
124) Patients with anemia (hemoglobin, <12.0 g/dL and ≥8.5 g/dL), neutropenia (neutrophil count, <1500/mm^3 and ≥750 mm^3), thrombocytopenia (platelet count, <120,000/mm^3 and ≥50,000/mm^3), depression (a diagnosis of mild to moderate, stable depression by a psychiatrist), or other medical conditions requiring medication (hypertension, diabetes, autoimmune disease, thyroid dysfunction, etc.) or the elderly (patients who were 65 to 75 years and considered IFN-ineligible based on health status, laboratory findings, or comorbidities etc.) who were not scheduled to receive IFN-based therapy for the next 12 months.

Table 37. Pharmacokinetic parameters after multiple oral doses of DCV plus ASV (Day 14)
(Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis)

Subject populations	N	DCV				ASV			
		C_{max} (ng/mL)	C_{min} (ng/mL)	t_{max} a) (h)	AUC_{tau} (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	t_{max} a) (h)	AUC_{tau} (ng·h/mL)
Non-responder	20	986 (37)	199 (43)	2.0 [1.0, 4.0]	9830 (33)	710 (98)	31.8 (74)	2.0 [0.5, 4.0]	2421 (63)
IFN-ineligible-naïve/ intolerant	20	1260 (35)	322 (90)	2.0 [1.0, 8.0]	14,353 (55)	590 (93)	30.8 (101)	2.0 [0.9, 4.0]	1918 (98)
Overall population	40	1115 (37)	253 (90)	2.0 [1.0, 8.0]	11,878 (55)	647 (95)	31.3 (90)	2.0 [0.5, 4.0]	2155 (80)

Geometric mean (CV%)
a) Median [range]

4.(ii).A.(3).3).(c) PPK and E-R analyses of clinical studies in Japanese patients with chronic hepatitis C (5.3.3.5.2, 5.3.3.5.3)

Using plasma DCV concentrations (336 subjects; 3801 sampling points) obtained from 4 clinical studies in Japanese patients with chronic hepatitis C (genotype 1) (Study AI444021, Study AI444022,[125)] Study AI447017, Study AI447026), PPK analysis of DCV was performed (NONMEM ver.7.2). The pharmacokinetics of DCV were appropriately described by a linear 1-compartment model with first-order absorption and baseline creatinine clearance (CL_{cr}), treatment, and gender for CL/F and baseline body weight for apparent volume of distribution (V/F) were selected as covariates in the final model,[126)] but it has been discussed that these covariates have no clinically significant influence.

Using plasma ASV concentrations (265 subjects; 2626 sampling points) obtained from 2 clinical studies in Japanese patients with chronic hepatitis C (genotype 1b) (Study AI447017 and Study AI447026), PPK analysis of ASV was performed (NONMEM ver.7.2). The pharmacokinetics of ASV were appropriately described by a linear 1-compartment model with first-order absorption and with respect to the covariates included in the final model,[127)] the CL/F in cirrhotic patients (Child-Pugh A) was estimated to be 65% of that in non-cirrhotic patients and the F of the softgel capsule formulation was estimated to be 137% of that of the tablet formulation, suggesting that these covariates potentially influence the ASV exposure.

E-R analyses of DCV and ASV were conducted, using the data from 265 subjects in 2 clinical studies in Japanese patients with chronic hepatitis C (genotype 1b) (Study AI447017 and Study AI447026), to investigate the relationship between DCV and ASV exposures (steady-state average plasma concentration [C_{avgss}] and steady-state AUC [AUC_{ss}]), efficacy (sustained virologic response [SVR] 24 and SVR12 rates[128)]) and safety events[129)].

125) In Study AI444021 and Study AI444022, triple therapy with DCV/PegIFNα/RBV was used.

126) The effects of gender, age, baseline CL_{cr}, baseline body weight, baseline and on-treatment ALT and AST values, patient type (non-responder and IFN-ineligible-naïve/intolerant), treatment (PegIFNα/RBV or DCV/ASV combination therapy), presence or absence of cirrhosis (non-cirrhotics or cirrhotics [Child-Pugh A]) on CL/F and the effect of baseline body weight on V/F were evaluated as covariates in the final model.

127) The effects of gender, age, baseline CL_{cr}, baseline body weight, baseline and on-treatment ALT and AST values, patient type (non-responder and IFN-ineligible-naïve/intolerant), presence or absence of cirrhosis (non-cirrhotics or cirrhotics [Child-Pugh A]), dosage form (tablet or softgel capsule), and OATP1B1 haplotype on CL/F and the effects of baseline body weight and dosage form on V/F were evaluated as covariates in the final model.

128) SVR24 rate: proportion of subjects with HCV RNA <LLOQ at post-treatment Week 24, SVR12 rate: proportion of subjects with HCV RNA <LLOQ at post-treatment Week 12

129) C_{avgss} was selected for the efficacy measure and AUC_{ss} for the safety measure. C_{avgss}, equivalent to AUC_{ss}, was selected because a parameter indicative of total exposure during the dosing interval like AUC_{ss}/C_{avgss} rather than the plasma concentration at a specific time point (C_{maxss} or C_{minss}) was considered appropriate for investigating the relationship with anti-viral effect, taking account of the duration of treatment, the site of action (the liver), and preferential distribution into the liver. For safety, the incidence and severity of ALT and AST elevations associated with ASV tablets tended to be increased at 600 mg QD or 600 mg BID than at 200 mg BID in a foreign phase II study (Study AI447016), suggesting that these events are related to ASV exposure. In addition, ALT and AST elevations occurred mostly after Week 4 in Japanese and foreign phase II studies (AI447017, AI447016, AI447011). Thus, these events were considered more likely to be associated with AUC rather than C_{max}, and AUC_{ss} was selected.

The relationship between the C_{avgss} values of DCV and ASV and efficacy was analyzed using a logistic regression model and among the covariates evaluated,[130] baseline NS5A Y93H resistance mutation was identified as a covariate in the final model, suggesting the possibility that therapeutic effect is reduced in subjects with the Y93H resistance mutation than in subjects without the resistance mutation at the same exposures. Although an investigation of the relationship between the AUC_{ss} values of DCV and ASV and safety events[131] suggested that ASV exposure rather than DCV exposure is highly related to safety events, as the number of subjects with events was limited and the distribution of the AUC_{ss} values of ASV overlapped between subjects with and without events, it has been discussed that a definitive conclusion could not be drawn.

4.(ii).A.(3).3).(d) Phase II study in foreign patients with chronic hepatitis C (5.3.5.2.3, Study AI447011 [December 2009 to September 2012])

The pharmacokinetics following multiple oral administration of DCV tablets (60 mg QD) plus ASV tablets (200 mg QD, 200 mg BID, or 600 mg BID) in null-responder[120] foreign patients with chronic hepatitis C (genotype 1) (34 subjects included in pharmacokinetic assessment [10-12 subjects/group]) were investigated. The results were as shown in Table 38.

Table 38. Pharmacokinetic parameters after administration of DCV + ASV (Day 14) (Foreign patients with chronic hepatitis C)

ASV dose	N	DCV				ASV			
		C_{max} (ng/mL)	C_{min} (ng/mL)	t_{max} [a] (h)	AUC_{tau} (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	t_{max} [a] (h)	AUC_{tau} (ng·h/mL)
200 mg QD	12	1363 (24)	266 (110)	1.0 [0.9, 4.0]	15,078 (30)	242 (67)	12.5 (100)	4.0 [2.0, 8.0]	1782 (46)
200 mg BID	12 [b]	1095 (35)	292 (113)	2.0 [1.0, 24]	13,133 (47)	325 (99)	49.6 (254)	4.0 [0.0, 5.0]	1784 (98)
600 mg BID	10	1025 (33)	208 (91)	2.0 [0.0, 24]	10,800 (32)	1781 (87)	94.3 (82)	2.0 [2.0, 4.0]	6755 (77)

Geometric mean (CV%)
a) Median [range], b) N = 11 for ASV

4.(ii).A.(4) Intrinsic factor pharmacokinetic studies

4.(ii).A.(4).1) Administration of DCV alone

4.(ii).A.(4).1).(a) Pharmacokinetic study in subjects with hepatic impairment (5.3.3.3.1, Study AI444013 [[redacted] 20[redacted] to [redacted] 20[redacted]])

Pharmacokinetic parameters were determined following a single oral dose of DCV tablets 30 mg in foreign healthy adult subjects (12 subjects included in pharmacokinetic assessment) and foreign subjects with hepatic impairment[132] (6 subjects each with mild, moderate, and severe hepatic impairment included in pharmacokinetic assessment). The results were as shown in Table 39 and although the C_{max} and AUC were lower in subjects with hepatic impairment compared with healthy adult subjects, there was no relationship between the exposure and the degree of hepatic impairment based on Child-Pugh classification.

130) Age, baseline body weight, gender, baseline ALT, baseline CL_{cr}, IL28B gene single nucleotide polymorphism (rs1279860), baseline NS5A Y93H resistance mutation, baseline viral load, patient type (non-responder and IFN-ineligible-naïve/intolerant), presence or absence of cirrhosis (non-cirrhotics or Child-Pugh A), study (Study AI447017 or Study AI447026), and OATP1B1 haplotype were evaluated as covariates.

131) ALT increased, AST increased, and total bilirubin increased, pyrexia, and eosinophilia were selected as important safety events. Since the incidence of safety events was low, a model analysis was not performed and the relationship between safety events and exposure was investigated using plots.

132) Stratified by Child-Pugh classification (class A, mild; class B, moderate; class C, severe).

Table 39. Pharmacokinetic parameters after a single oral dose of DCV tablets in healthy adult subjects or subjects with hepatic impairment

	N	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)	CL/F (L/h)	$t_{1/2}$ a) (h)	Adjusted geometric mean ratio [90% CI] b)	
						C_{max}	AUC_{inf}
Healthy adult subjects	12	698 (30)	7286 (25)	69 (29)	12.4 ± 2.2	-	-
Subjects with mild hepatic impairment	6	380 (44)	4174 (43)	120 (85)	12.3 ± 2.5	0.545 [0.380, 0.781]	0.573 [0.400, 0.820]
Subjects with moderate hepatic impairment	6	382 (23)	4550 (39)	110 (47)	15.0 ± 4.6	0.548 [0.430, 0.698]	0.624 [0.470, 0.830]
Subjects with severe hepatic impairment	6	317 (65)	4649 (78)	108 (78)	17.2 ± 10.6	0.454 [0.301, 0.685]	0.638 [0.397, 1.025]

Geometric mean (CV%)
a) Mean ± SD, b) Ratio to healthy adult subjects

4.(ii).A.(4).1).(b) Pharmacokinetic study in subjects with renal impairment (5.3.3.3.2, Study AI444063 [[redacted]])

The pharmacokinetics of DCV tablets following a single 60-mg oral dose were studied in foreign healthy adult subjects[133] (11 subjects included in pharmacokinetic assessment) and foreign subjects with renal impairment[134] (10 subjects with end-stage renal disease, 5 subjects with moderate renal impairment, and 6 subjects with severe renal impairment included in pharmacokinetic assessment). A regression analysis was performed, excluding subjects with end-stage renal disease. As a result, the adjusted geometric mean ratios of total DCV (free and protein-bound DCV) AUC_{inf} [90% CIs] for renally impaired subjects with CL_{cr} of 60, 30, and 15 mL/min vs. healthy adult subjects (CL_{cr} ≥90 mL/min) were 1.26 [1.14, 1.40], 1.60 [1.30, 1.96], and 1.80 [1.39, 2.32], respectively, and the adjusted geometric mean ratios of free DCV AUC_{inf} [90% CIs] were 1.18 [1.07, 1.30], 1.39 [1.14, 1.70], and 1.51 [1.18, 1.94], respectively. The adjusted geometric mean ratio of total DCV AUC_{inf} for subjects with end-stage renal disease on hemodialysis vs. healthy adult subjects [90% CI] was 1.27 [0.992, 1.62] and the adjusted geometric mean ratio of free DCV AUC_{inf} [90% CI] was 1.20 [0.903, 1.60]. The CL/F values in healthy adult subjects, subjects with end-stage renal disease, subjects with moderate renal impairment, and subjects with severe renal impairment were 89.2, 70.1, 40.3, and 45.6 mL/min, respectively.

4.(ii).A.(4).2) Administration of ASV alone

4.(ii).A.(4).2).(a) Pharmacokinetic study in subjects with hepatic impairment (5.3.3.3.1, Study AI447012 [[redacted]])

Pharmacokinetic parameters following 7-day oral administration of ASV hard capsules at 200 mg BID in foreign healthy adult subjects (12 subjects included in pharmacokinetic assessment) and foreign subjects with hepatic impairment[132] (6 subjects with mild hepatic impairment, 6 subjects with moderate hepatic impairment, and 4 subjects with severe hepatic impairment included in pharmacokinetic assessment) were as shown in Table 40. Compared with healthy adult subjects, the exposure was lower in subjects with mild hepatic impairment and higher in subjects with moderate or severe hepatic impairment. The lower exposure in subjects with mild hepatic impairment has been explained as follows: the coefficients of variation for C_{max} and AUC were 75% and 45%, respectively, and there was a large variability in pharmacokinetic parameters, and there are no differences in the pharmacokinetics of ASV between subjects with mild hepatic impairment and healthy adult subjects.

133) Cockcroft-Gault-formula-estimated CL_{cr} ≥90 mL/min

134) Subjects with renal impairment were enrolled based on glomerular filtration rate (GFR) estimated by the MDRD equation (subjects with end-stage renal disease on hemodialysis [GFR estimated by the MDRD equation, <15 mL/min/1.73 m^2], subjects with moderate renal impairment [GFR estimated by the MDRD equation, 30-59 mL/min/1.73 m^2], subjects with severe renal impairment [GFR estimated by the MDRD equation, 15-29 mL/min/1.73 m^2]) and the primary analysis was an analysis based on CL_{cr} estimated by the Cockcroft-Gault equation.

Table 40. Pharmacokinetic parameters after multiple oral doses of ASV in healthy adult subjects or subjects with hepatic impairment (Day 7)

	N	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	CL/F (L/h)	$t_{1/2}$ [a)] (h)	Adjusted geometric mean ratio [90% CI] [b)]	
						C_{max}	AUC_{tau}
Healthy adult subjects	12	233 (65)	824 (47)	243 (38)	16.8 ± 2.3	-	-
Subjects with mild hepatic impairment	6	136 (75)	654 (45)	306 (43)	15.3 ± 1.8	0.581 [0.345, 0.977]	0.793 [0.546, 1.15]
Subjects with moderate hepatic impairment	6	1175 (63)	8100 (61)	24.7 (49)	12.6 ± 2.1	5.03 [2.99, 8.47]	9.83 [6.76, 14.3]
Subjects with severe hepatic impairment	4	5352 (25)	26,447 (20)	7.56 (22)	9.9 ± 1.7	22.9 [12.6, 41.8]	32.1 [20.8, 49.4]

Geometric mean (CV%)
a) Mean ± SD, b) Ratio to healthy adult subjects

4.(ii).A.(4).2).(b) Pharmacokinetic study in subjects with renal impairment (5.3.3.3.2, Study AI447033 [[redacted] to [redacted]])

The pharmacokinetics of ASV softgel capsules following 7-day oral administration of 100 mg BID were studied in foreign healthy adult subjects[135)] and foreign subjects with renal impairment[136)] (12 subjects/group included in pharmacokinetic assessment). The adjusted geometric mean ratios of C_{max} and AUC_{tau} for subjects with renal impairment to those for healthy adult subjects [90% CI] were 1.23 [0.72, 2.12] and 0.85 [0.60, 1.21], respectively. The CL/F values in healthy adult subjects and subjects with renal impairment were 163 and 206 L/h, respectively.

4.(ii).A.(5) Drug interaction studies

4.(ii).A.(5).1) Interactions between DCV and coadministered drugs (5.3.1.1.1, Study AI444009 [[redacted]]; 5.3.3.4.1, Study AI444005 [[redacted]]; 5.3.3.4.2, Study AI444008 [[redacted]]; 5.3.3.4.3, Study AI444012 [[redacted]]; 5.3.3.4.4, Study AI444020 [[redacted]]; 5.3.3.4.5, Study AI444024 [[redacted]]; 5.3.3.4.6, Study AI444027 [[redacted]]; 5.3.3.4.7, Study AI444032 [[redacted]]; 5.3.3.4.8, Study AI444033 [[redacted]]; 5.3.3.4.9, Study AI444034 [[redacted]]; 5.3.3.4.10, Study AI444054 [[redacted]]; 5.3.3.4.11, Study AI444065 [[redacted]]; 5.3.3.4.12, Study AI444084 [[redacted]]; Reference data 5.3.3.4.15, Study AI444064 [[redacted]]; Reference data 5.3.3.1.7, Study AI444067 [[redacted]]; Reference data 5.3.3.1.8, Study TMC435HPC1005 [[redacted]])

In order to evaluate drug interactions of DCV, 16 clinical studies were conducted in Japan or overseas. The geometric mean ratios of the pharmacokinetic parameters of DCV and coadministered drugs (combination vs. alone) [90% CIs] are shown in Table 41 and Table 42, respectively.

135) CL_{cr} estimated using the Cockcroft-Gault formula >90 mL/min
136) Subjects with end-stage renal disease on or not on hemodialysis (GFR estimated by the MDRD equation, <15 mL/min/1.73 m^2)

Table 41. Effect of coadministered drug on pharmacokinetic parameters of DCV

Coadministered drug	Dosage regimen		N	Adjusted geometric mean ratio [90% CI]	
	Coadministered drug	DCV		C_{max}	AUC
Ketoconazole	400 mg single dose	10 mg single dose Capsule	14	1.57 [1.31, 1.88]	3.00 [2.62, 3.44]
Rifampicin	600 mg single dose 12 hours after administration of DCV	60 mg single dose Tablet	14	0.438 [0.399, 0.481]	0.212 [0.193, 0.233]
Famotidine	40 mg single dose	60 mg single dose Tablet	18	0.557 [0.461, 0.674]	0.815 [0.695, 0.956]
Omeprazole	40 mg single dose	60 mg single dose Tablet	12	0.643 [0.536, 0.771]	0.840 [0.732, 0.963]
Atazanavir/Ritonavir	300/100 mg QD	20 mg QD [a)] Tablet	14	1.35 [1.24, 1.47]	2.10 [1.95, 2.26]
Tenofovir	300 mg QD	60 mg QD Tablet	20	1.06 [0.977, 1.15]	1.10 [1.01, 1.21]
Efavirenz	600 mg QD	120 mg QD[a)] Tablet	15	0.834 [0.756, 0.920]	0.684 [0.603, 0.777]
Cyclosporine	400 mg single dose	60 mg single dose Tablet	14	1.04 [0.936, 1.15]	1.40 [1.29, 1.53]
Tacrolimus	5 mg single dose	60 mg single dose Tablet	14	1.07 [1.02, 1.12]	1.05 [1.03, 1.07]
Escitalopram	10 mg QD	60 mg QD Tablet	15	1.14 [0.977, 1.32]	1.12 [1.01, 1.26]
Telaprevir	500 mg BID	20 mg QD Tablet	15	1.46 [1.28, 1.66]	2.32 [2.06, 2.62]
	750 mg TID	20 mg QD [a)] Tablet	15	1.22 [1.04, 1.44]	2.15 [1.87, 2.48]
Simeprevir	150 mg QD	60 mg QD Tablet	17	1.50 [1.39, 1.62]	1.96 [1.84, 2.10]

a) DCV dose is adjusted assuming that the pharmacokinetic profile of DCV is linear.

Table 42. Effect of DCV on pharmacokinetic parameters of coadministered drug

Coadministered drug	Dosage regimen		N	Adjusted geometric mean ratio [90% CI]	
	Coadministered drug	DCV		C_{max}	AUC
Midazolam	5 mg single dose	60 mg single dose Capsule	18	0.954 [0.879, 1.04]	0.873 [0.828, 0.921]
Rosuvastatin	10 mg single dose	60 mg single dose Tablet	22	2.04 [1.83, 2.26]	1.58 [1.44, 1.74]
Ethinylestradiol [a)]	35 μg QD	60 mg QD Tablet	20	1.11 [1.02, 1.20]	1.01 [0.951, 1.07]
Norgestimate [a)]	0.180/0.215/0.250 mg QD			1.06 [b)] [0.988, 1.14]	1.12 [b)] [1.06, 1.17]
Digoxin	0.125 mg QD	60 mg QD Tablet	15	1.65 [1.52, 1.80]	1.27 [1.20, 1.34]
Tenofovir	300 mg QD	60 mg QD Tablet	20	0.952 [0.890, 1.02]	1.10 [1.05, 1.15]
Cyclosporine	400 mg single dose	60 mg single dose Tablet	14	0.962 [0.908, 1.02]	1.03 [0.966, 1.09]
Tacrolimus	5 mg single dose	60 mg single dose Tablet	14	1.05 [0.904, 1.23]	0.996 [0.877, 1.13]
Escitalopram	10 mg QD	60 mg QD Tablet	15	0.997 [0.921, 1.08]	1.05 [1.02, 1.08]
Methadone	40-120 mg QD [c)]	60 mg QD Tablet	14	1.09 [0.988, 1.21]	1.11 [0.968, 1.26]
Telaprevir	500 mg BID	20 mg QD Tablet	15	0.938 [0.843, 1.04]	1.01 [0.89, 1.14]
	750 mg TID	20 mg QD Tablet	14	0.990 [0.953, 1.03]	1.02 [0.953, 1.09]
Simeprevir	150 mg QD	60 mg QD Tablet	24	1.39 [1.27, 1.52]	1.44 [1.32, 1.56]

a) Combination product of ethinylestradiol and norgestimate
b) Norelgestromin concentrations were measured.
c) Dose adjusted to 40 mg for calculations of summary statistics for total methadone.

4.(ii).A.(5).2) Interactions between ASV and coadministered drugs (5.3.3.3.1, Study AI447001 [[redacted] to [redacted]]; 5.3.3.4.1, Study AI447007 [[redacted] to [redacted]]; 5.3.3.4.3, Study AI447015 [[redacted] to [redacted]]; 5.3.3.4.4, Study AI447014 [[redacted] to [redacted]]; 5.3.3.4.5, Study AI447018 [[redacted] to [redacted]]; 5.3.3.4.6, Study AI447019 [[redacted] to [redacted]]; 5.3.3.4.7, Study AI447020 [[redacted] to [redacted]]; 5.3.3.4.8, Study AI447021 [[redacted] to [redacted]]; 5.3.3.4.9, Study AI447032 [[redacted] to [redacted]]; Reference data 5.3.3.4.12, Study AI447038 [[redacted] to [redacted]])

In order to evaluate drug interactions of ASV, 10 foreign clinical studies were conducted. The geometric mean ratios of the pharmacokinetic parameters of ASV and coadministered drugs (coadministration vs. alone) [90% CIs] are shown in Table 43 and Table 44, respectively.

Table 43. Effect of coadministered drug on pharmacokinetic parameters of ASV

Coadministered drug	Dosage regimen		N	Adjusted geometric mean ratio [90% CI]	
	Coadministered drug	ASV		C_{max}	AUC
Ritonavir	100 mg BID	10 mg single dose Suspension	6	5.22 [2.83, 9.61]	4.81 [4.01, 5.77]
Ketoconazole	200 mg BID	200 mg BID Tablet	19	6.92 [5.92, 8.09]	9.15 [8.33, 10.0]
Rifampicin	600 mg single dose	200 mg single dose Tablet	20	21.1 [14.3, 31.2]	14.8 [11.2, 19.5]
	600 mg QD	600 mg BID Tablet	20	0.950 [0.602, 1.50]	0.785 [0.564, 1.09]
Escitalopram	10 mg QD	100 mg BID Softgel capsule	16	0.874 [0.651, 1.18]	0.922 [0.758, 1.12]
Sertraline	50 mg QD	100 mg BID Softgel capsule	18	0.944 [0.698, 1.28]	0.882 [0.704, 1.11]

Table 44. Effect of ASV on pharmacokinetic parameters of coadministered drug

Coadministered drug	Dosage regimen		N	Adjusted geometric mean ratio [90% CI]	
	Coadministered drug	ASV		C_{max}	AUC
Rosuvastatin	10 mg single dose	200 mg BID Tablet	20	1.95 [1.47, 2.58]	1.41 [1.26, 1.57]
Ethinylestradiol [a)]	35 µg QD	600 mg BID Tablet	17	0.754 [0.668, 0.850]	0.722 [0.667, 0.781]
Norgestimate [a)]	0.180/0.215/0.250 mg QD			0.707 [c)] [0.647, 0.772]	0.661 [c)] [0.624, 0.699]
Midazolam [b)]	5 mg single dose	200 mg BID Tablet	19	0.794 [0.725, 0.869]	0.712 [0.673, 0.753]
Losartan [b)]	25 mg single dose		18	1.63 [1.35, 1.97]	0.891 [0.812, 0.978]
Omeprazole [b)]	40 mg single dose		18	0.957 [0.790, 1.16]	0.804 [e)] [0.691, 0.936]
Dextromethorphan [b)]	30 mg single dose		17	2.72 [2.10, 3.53]	3.94 [f)] [3.09, 5.03]
Caffeine [b)]	200 mg single dose		19	0.951 [0.907, 0.998]	0.957 [0.886, 1.04]
Digoxin	0.5 mg single dose	200 mg BID Tablet	16	1.09 [0.968, 1.22]	1.30 [1.21, 1.40]
Escitalopram	10 mg QD	100 mg BID Softgel capsule	16	0.971 [0.921, 1.02]	0.945 [0.911, 0.981]
Sertraline	50 mg QD	100 mg BID Softgel capsule	18	0.805 [0.669, 0.969]	0.793 [0.669, 0.939]
Methadone	40-120 mg QD [d)]	100 mg BID Softgel capsule	15	0.995 [0.887, 1.12]	0.939 [0.841, 1.05]

a) Combination product of ethinylestradiol and norgestimate
b) Midazolam, losartan, omeprazole, dextromethorphan, and caffeine were administered as a cocktail.
c) Norelgestromin concentrations were measured.
d) Dose adjusted to 40 mg for calculations of summary statistics for total methadone.
e) N = 15
f) N = 16

4.(ii).A.(5).3) Interactions of DCV and ASV with coadministered drugs (Reference data 5.3.3.4.11, Study AI447040 [[redacted] to [redacted]]; Reference data 5.3.3.4.10, Study AI447039 [[redacted] to [redacted]])

In order to evaluate drug interactions with DCV and ASV, 2 foreign clinical studies were conducted. The

geometric mean ratios of the pharmacokinetic parameters of drugs coadministered with DCV tablets and ASV softgel capsules to those of each of the coadministered drugs alone [90% CIs] areshown in Table 45.

Table 45. Effect of DCV and ASV on the pharmacokinetic parameters of coadministered drug

Coadministered drug	Dosage regimen			N	Adjusted geometric mean ratio [90% CI]	
	Coadministered drug	DCV	ASV		C_{max}	AUC
Digoxin	0.25 mg single dose	60 mg QD	100 mg BID	16	1.77 [1.50, 2.07]	1.29 [1.20, 1.39]
Ethinylestradiol [a)]	30 µg QD	60 mg QD	100 mg BID	36	0.925 [0.861, 0.994]	0.858 [0.826, 0.892]
Norethindrone acetate [a)]	1.5 mg QD			37	0.926 [0.852, 1.01]	1.02 [0.938, 1.11]

a) Combination product of ethinylestradiol and norethindrone acetate

4.(ii).A.(6) QT/QTc studies

4.(ii).A.(6).1) Administration of DCV alone (5.3.4.1.1, Study AI444023 [[redacted] to [redacted]])

A 4-treatment, 4-period, crossover study was conducted in 56 foreign healthy adult subjects to evaluate the effects of single oral doses of placebo, DCV tablets 60 mg or 180 mg, and moxifloxacin 400 mg as a positive control on the QT/QTc interval.[137)] The largest mean difference in change from baseline in QTcF (ΔQTcF) between DCV and placebo [90% CI] was 0.68 [-1.06, 2.42] msec at 16 hours post-dose for the DCV 60 mg group and 1.10 [-0.63, 2.82] msec at 16 hours post-dose for the DCV 180 mg group. It has been explained that since the largest mean difference in ΔQTcF between DCV and placebo was below 10 msec, as defined by the ICH E14 criteria, and the upper bound of the 90% confidence interval for the mean difference in ΔQTcF between DCV and placebo was below 5 msec at all time points, DCV has no effects on the QT/QTc interval. The mean difference in ΔQTcF between moxifloxacin and placebo [90% CI] was 10.76 [9.03, 12.49] msec at 3 hours post-dose and ranged from 4.81 to 10.76 msec at all time points.

4.(ii).A.(6).2) Administration of ASV alone (5.3.4.1.1, Study AI447025 [[redacted] to [redacted]])

A nested crossover study was conducted in 120 foreign healthy adult subjects to evaluate the effects of placebo or ASV softgel capsules orally administered at 300 mg BID for 10 days and a single oral dose of moxifloxacin 400 mg as a positive control on the QT/QTc interval.[138)] A linear mixed-effect model was used to model ΔQTcF at Days 3 and 10 post-dose time points for ECG extraction[139)] and the largest time-matched mean difference in ΔQTcF between ASV and placebo [90% CI] was 1.53 [-1.63, 4.69] msec at 12 hours post-dose on Day 10. It has been explained that since at all ECG measurement time points on Days 3 and 10 of ASV treatment, the upper bound of the two-sided 90% confidence interval for the mean difference in ΔQTcF between ASV and placebo was below 10 msec, as defined by the ICH E14 criteria, ASV has no effects on the QT/QTc interval. The lower bound of the 90% confidence interval for the mean difference in ΔQTcF between moxifloxacin and placebo was 7.69 to 8.68 msec.

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137) At least a 3-day washout period was included between the treatment periods.

138) The study was conducted with 3 parallel treatment sequences (A, BP, PB) and the treatment sequences were as shown below:
In sequence A, subjects received ASV placebo BID on Day 1, a single dose of moxifloxacin placebo on Day 2, ASV softgel capsules 300 mg BID on Days 3 to 12, and a single dose of moxifloxacin placebo on Day 13.
In sequence BP, subjects received ASV placebo BID on Day 1, a single dose of moxifloxacin 400 mg on Day 2, ASV placebo BID on Days 3 to 12, and a single dose of moxifloxacin placebo on Day 13.
In sequence PB, subjects received ASV placebo BID on Day 1, a single dose of moxifloxacin placebo on Day 2, ASV placebo BID on Days 3 to 12, and a single dose of moxifloxacin 400 mg on Day 13.

139) Treatment (ASV or placebo), time, and treatment-by-time interaction were included as fixed effects and time-matched baseline value was included as a covariate.

4.(ii).B Outline of the review

4.(ii).B.(1) Racial differences in ASV exposure and the need for dose adjustment

PMDA asked the applicant to explain the cause for higher ASV exposure in Japanese subjects than in Caucasian subjects in Japanese phase II (Study AI447017) and foreign phase II (Study AI447011) studies [see "4.(ii).A.(3).3) Combination of DCV and ASV"].

The applicant explained as follows:

Non-clinical and clinical studies suggested that OATP contributes predominantly to the hepatic uptake of ASV [see "3.(ii).A.(10) Pharmacokinetic drug interactions (ASV)" and "4.(ii).A.(5) Drug interaction studies"]. It has been reported that the exposure, even after adjusted for body weight and OATP1B1 haplotype, was higher in Asians than in Caucasians in a study on racial differences in the pharmacokinetics of OATP1B1 substrates. The intrinsic ethnic variability in the activity of OATP1B1 was considered associated with racial differences in the pharmacokinetics of OATP1B1 substrates.[140)] Although there are also reports on differences in activity among alleles or differences in allele frequencies, it has been reported that the observed pharmacokinetic differences cannot be explained only by these differences.[141)]

Although the major enzymes involved in the metabolism of ASV are CYP3A4 and CYP3A5, a clinically significant genetic variation in the activity of CYP3A4 is not known. It is known that there are differences in the activity of CYP3A5 between Caucasians and Black/African Americans, but no differences in the pharmacokinetics of ASV were observed between Caucasians and Black/African Americans.[142)] Thus, it was considered that these enzymes are unlikely to be associated with the racial differences in the pharmacokinetics of ASV.

No other genetic factors that can explain the racial differences in the pharmacokinetics of ASV have been identified.

Based on the above, though genetic variation in OATP is considered associated with the racial differences in the pharmacokinetics of ASV, the possibility that factors other than OATP are also associated with the racial differences cannot be ruled out.

PMDA asked the applicant to explain the relationship between the plasma exposure of ASV and the occurrence of adverse events and the need for dose adjustment for Japanese patients.

The applicant explained as follows:

The incidence and severity of ALT and AST elevations tended to be increased with ASV tablets 600 mg QD and 600 mg BID compared with 200 mg BID in a foreign phase II study (Study AI447016), suggesting that the ASV

140) Tomita Y, et al. *Clin Pharmacol Ther*. 2013;94(1):37-51.
141) Niemi M, et al. *Pharmacogenetics*. 2004;14(7):429-440.
142) Xie HG, et al. *Pharmacogenomics*. 2004;5(3):243-272.

exposure is possibly related to adverse events of ALT and AST elevations. Using the data from Japanese and foreign phase II studies (Study AI447017, Study AI447016, Study AI447011), the relative risk of Grade 2 or higher liver function-related adverse events[143)] was assessed. As a result, the estimated relative risk of Grade 2 or higher liver function-related adverse events for Japanese and foreign patients treated with ASV tablets 200 mg BID compared to those treated with placebo were 1.09 and 1.06, respectively, and no increased risk of liver function-related adverse events was observed. The submitted study data have suggested that the hepatic uptake of ASV is mediated by 2 transporters while ASV is also taken up by the liver via a process not mediated by transporters [see "3.(ii).A.(10).2) Characterization of ASV as a potential substrate for drug transporters"]. Although genetic variation in OATP was considered possibly associated with the racial differences in the plasma exposure of ASV, the low activity of one of the hepatic uptake transporters may be complemented by another mechanism that mediates hepatic uptake. In spite of higher plasma exposure of ASV in Japanese subjects than in foreign subjects, no increased risk of liver function-related adverse events was observed, which was considered attributable to similar levels of ASV exposure in the liver.

Among study drug-related adverse events observed in Japanese phase II (Study AI447017) and phase III (Study AI447026) studies, the relationship of pyrexia/eosinophilia with the AUC_{ss} was investigated, taking account of the incidence and clinical importance. Subjects with pyrexia or eosinophilia had 37% or 45% higher AUC_{ss} of ASV (median) than those without pyrexia or eosinophilia, respectively. Although this result suggested a relationship between the ASV exposure and these events, the cause is not clear as there was a large variability in the pharmacokinetics of ASV and the distribution of AUC_{ss} values overlapped between subjects with and without adverse events. However, the incidences of Grade 3 or 4 study drug-related adverse events observed during the treatment period, other than liver function-related adverse events, were all ≤1% in Japanese clinical studies. There were no major differences in the incidence of adverse events between ASV tablets 600 mg BID (sentinel cohort) producing high ASV exposure and 200 mg BID (additional cohort) in Study AI447017 [see "4.(iii).A.(1) Japanese phase II study in Japanese patients with chronic hepatitis C"]. Most adverse events were of Grade 1 or 2, and there were no safety problems in these studies.

Based on the above, no dose adjustment is required.

PMDA considers as follows:
Though no factors other than OATP have been identified, it is understood that OATP is possibly associated with racial differences in ASV exposure. Although a relationship between the plasma exposure of ASV and adverse events of pyrexia and eosinophilia cannot be denied, as the incidences of Grade 3 or 4 study drug-related adverse events were not high and there were no particular safety problems even at 600 mg BID, the applicant's explanation that no dose adjustment is required for Japanese patients is acceptable.

4.(ii).B.(2) Use in patients with hepatic impairment
The applicant explained ASV exposure in patients with hepatic impairment as follows:

143) AST, ALT, or total bilirubin elevations

In a pharmacokinetic study in subjects with hepatic impairment [see "4.(ii).A.(4).2).(a) Pharmacokinetic study in subjects with hepatic impairment"], the ASV exposure was increased markedly in subjects with moderate hepatic impairment (Child-Pugh B) and subjects with severe hepatic impairment (Child-Pugh C) compared to healthy adult subjects (5-fold and 23-fold increases in C_{max}, respectively; 10-fold and 32-fold increases in AUC, respectively). ASV is metabolized primarily via CYP3A and according to literature reports, while the CL of a CYP3A substrate in subjects with mild hepatic impairment was similar to that in healthy adult subjects, the CL values in subjects with moderate to severe hepatic impairment were approximately 75% and 85% lower, respectively, than that in healthy adult subjects,[144)] and the increases in ASV exposure are considered associated with decreases in metabolic CL. Based on the above, it was concluded that ASV should be contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C) and patients with decompensated liver disease.

Based on PPK analysis of ASV in Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis, the CL/F in cirrhotic patients (Child-Pugh A) was estimated to be 0.65-fold that in noncirrhotic patients. Although this means that the AUC of ASV is increased approximately 1.54-fold in cirrhotic patients compared with noncirrhotic patients, the safety profile, the incidence of adverse events, and efficacy were similar, regardless of the presence or absence of compensated cirrhosis (Child-Pugh A), in the Japanese phase III study (Study AI447026) [see "4.(iii).B.(3).2) Use in patients with liver cirrhosis"].

Based on the above, ASV has been contraindicated in patients with moderate or severe (Child-Pugh B or C) hepatic impairment and patients with decompensated liver disease, but no precautionary statements concerning the use of ASV in patients with Child-Pugh A compensated cirrhosis should be necessary.

PMDA considers as follows:
It is appropriate to contraindicate ASV in patients with moderate or severe hepatic impairment and patients with decompensated liver disease. Although the data on the efficacy and safety of DCV + ASV dual therapy in Japanese patients with compensated cirrhosis (Child-Pugh A) are limited, as the results from the Japanese phase III study (Study AI447026) have raised no particular efficacy or safety concerns, the applicant's explanation that no precautionary statements are necessary is acceptable. However, it is necessary to continue to collect post-marketing information on efficacy and safety in patients with compensated cirrhosis and provide the obtained information to healthcare providers in clinical settings. The proposed indications will be discussed in "4.(iii).B.(3) Indications".

4.(ii).B.(3) Drug interactions

PMDA asked the applicant to explain the possibility that DCV + ASV dual therapy exhibits stronger interactions with other drugs potentially coadministered in clinical practice in Japan than DCV or ASV alone exhibits with other drugs and the need for precautionary statements.

144) Albarmawi A, et al. *British J Clin Pharmacol*. 2013;77(1):160-169.

The applicant explained as follows:
As effects on CYP or transporters, DCV and ASV both inhibit P-gp and OATP. Thus, DCV + ASV dual therapy potentially exhibits stronger interactions with P-gp or OATP substrates than DCV or ASV alone. When DCV or ASV was coadministered with digoxin, which is a substrate of P-gp, coadministration with DCV alone resulted in 1.65- and 1.27-fold increases in the C_{max} and AUC_{inf} of digoxin, respectively, and coadministration with ASV alone resulted in 1.09- and 1.30-fold increases, respectively. Based on these results, digoxin has been listed in the "Precautions for coadministration" section of the package inserts for DCV and ASV. As digoxin has a narrow therapeutic window, a drug interaction study of the combination of DCV and ASV with digoxin was conducted. As a result, coadministration with DCV and ASV resulted in 1.77- and 1.29-fold increases in the C_{max} and AUC_{inf} of digoxin, respectively. As there were no major differences from the results of coadministration of DCV or ASV alone with digoxin, no new precautionary statements should be necessary for the combination of DCV and ASV.

When rosuvastatin, a substrate of OATP, was coadministered with DCV or ASV, coadministration with DCV alone resulted in 2.04- and 1.58-fold increases in the C_{max} and AUC_{inf} of rosuvastatin, respectively, and coadministration with ASV alone resulted in 1.95- and 1.41-fold increases, respectively. Based on these results, drugs that are substrates of OATP have been listed in the "Precautions for coadministration" section of the package inserts for DCV and ASV. Although no drug interaction studies of the combination of DCV and ASV with OATP substrates have been performed, no new precautionary statements should be necessary for the combination of DCV and ASV, as there have been no reports that drugs that are substrates of OATP have a narrow therapeutic window.

Although DCV and ASV have no common properties in terms of CYP-mediated interactions, a drug interaction study using the clinical doses of DCV and ASV was conducted, as a drug interaction study with ASV alone was conducted at a dose higher than the clinical dose and oral contraceptives are expected to be used concomitantly in clinical settings. When ASV tablets 600 mg BID was coadministered with oral contraceptives (ethinylestradiol and norgestimate), the exposures of the oral contraceptives were decreased by approximately 20% to 30%. Coadministration with DCV tablets 60 mg alone did not affect the exposures of the oral contraceptives. When DCV tablets 60 mg QD plus ASV softgel capsules 100 mg BID were coadministered with oral contraceptives (ethinylestradiol and norethindrone), the 90% confidence intervals for the ratios of C_{max} and AUC_{tau} of the oral contraceptives were contained within the range of 0.8 to 1.25. The different effects on the exposures of oral contraceptives between coadministration with ASV alone and with DCV + ASV are considered associated with dose-dependent CYP3A4 induction by ASV and no precautionary statements concerning coadministration with oral contraceptives should be necessary for the clinical dose of ASV (ASV softgel capsules 100 mg BID).

PMDA considers as follows:
Based on the results from drug interaction studies with DCV alone, ASV alone, and the combination of DCV and ASV, the applicant's explanation that no additional precautionary statements concerning drug interactions of the combination of DCV and ASV are necessary is acceptable. It is necessary to continue to collect post-

marketing information on potential interactions of DCV + ASV with coadministered drugs and appropriately provide the obtained information to healthcare providers in clinical settings.

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

The results from 3 studies (1 Japanese phase II study, 1 Japanese phase III study, 1 foreign phase II study) were submitted in the application as the evaluation data on the efficacy and safety of the DCV + ASV dual therapy. As the reference data, the results from 6 studies (2 Japanese phase II studies and 4 foreign phase II studies) were submitted. Summaries of clinical studies submitted as the evaluation data are as shown in Table 46. Doses of DCV are expressed in terms of Daclatasvir.

Table 46. Clinical studies (Evaluation data)

	Phase	Study population		Objectives	No. of subjects	Dosage regimen
Japan	II	Patients with chronic hepatitis C genotype 1 (Study AI447017)	Null-responder (Sentinel cohort)	Efficacy Safety	10	ASV tablets 600 mg BID plus DCV tablets 60 mg QD for 24 weeks
			Null-responder (Additional cohort)		11	ASV tablets 200 mg BID plus DCV tablets 60 mg QD for 24 weeks
			IFN-ineligible-naïve/ intolerant		22	ASV tablets 200 mg BID plus DCV tablets 60 mg QD for 24 weeks
	III	Patients with chronic hepatitis C genotype 1b, or HCV genotype 1b with compensated cirrhosis (Study AI447026)	Non-responder	Efficacy Safety	87	ASV softgel capsules 100 mg BID plus DCV tablets 60 mg QD for 24 weeks
			IFN-ineligible-naïve/ intolerant		135	
Overseas	II	Patients with chronic hepatitis C genotype 1 (Study AI447011)	Null-responder	Efficacy Safety Pharmacokinetics Pharmacodynamics	Group A: 11	ASV tablets 600 mg BID plus DCV tablets 60 mg QD for 24 weeks
					Group A1: 18	ASV tablets 200 mg BID plus DCV tablets 60 mg QD for 24 weeks
					Group A2: 20	ASV tablets 200 mg QD plus DCV tablets 60 mg QD for 24 weeks
					Group B: 10	ASV tablets 600 mg BID plus DCV tablets 60 mg QD with PegIFNα/RBV for 24 weeks
					Group B1: 20	ASV tablets 200 mg BID plus DCV tablets 60 mg QD with PegIFNα/RBV for 24 weeks
					Group B2: 21	ASV tablets 200 mg QD plus DCV tablets 60 mg QD with PegIFNα/RBV for 24 weeks

4.(iii).A.(1) Japanese phase II study in Japanese patients with chronic hepatitis C (5.3.5.2.2, Study AI447017 [April 2010 to May 2012])

An open-label, uncontrolled study was conducted at 4 centers in Japan to evaluate the efficacy and safety of DCV + ASV dual therapy in null-responder[120)] and IFN-ineligible-naïve[121)]/intolerant[122)] patients with chronic hepatitis C (genotype 1) (target sample size of 40: 10 null-responders [sentinel cohort], 10 null-responders [additional cohort], 20 IFN-ineligible-naïve/intolerant patients).

Null-responders (sentinel cohort) were to be orally given ASV tablets 600 mg[145)] BID plus DCV tablets 60 mg QD for 24 weeks. Null-responders (additional cohort) and IFN-ineligible-naïve/intolerant patients were to be orally given ASV tablets 200 mg BID plus DCV tablets 60 mg QD for 24 weeks.

145) As a foreign phase II study in foreign patients with chronic hepatitis C (Study AI447016) suggested that ASV tablets 600 mg increased the incidence of ALT and AST elevations, the dose was to be reduced to 200 mg BID during the study.

Of 49 subjects enrolled in the study, 43 subjects excluding 6 subjects who did not receive study drug (all due to deviations from the inclusion criteria) (10 null-responders [sentinel cohort], 11 null-responders [additional cohort], 22 IFN-ineligible-naïve/intolerant patients) were included in the efficacy and safety populations.

The primary endpoint of SVR12[146)] rate was 90.0% (9 of 10 subjects) in null-responders (sentinel cohort), 90.9% (10 of 11 subjects) in null-responders (additional cohort), and 63.6% (14 of 22 subjects) in IFN-ineligible-naïve/intolerant patients.

The incidence of adverse events (including abnormal laboratory changes) was 93.0% (40 of 43 subjects) and the incidence of adverse drug reactions[147)] (including abnormal laboratory changes) was 93.0% (40 of 43 subjects). Adverse events reported by ≥5% of all subjects and adverse drug reactions were as shown in Table 47.

Table 47. Adverse events reported by ≥5% of all subjects and adverse drug reactions

Event term	Adverse events				Adverse drug reactions			
	Total	Null-responder (Sentinel cohort)	Null-responder (Additional cohort)	IFN-ineligible-naïve /intolerant	Total	Null-responder (Sentinel cohort)	Null-responder (Additional cohort)	IFN-ineligible-naïve /intolerant
Number of subjects	43	10	11	22	43	10	11	22
Any event	40 (93.0)	10 (100)	11 (100)	19 (86.4)	40 (93.0)	10 (100)	11 (100)	19 (86.4)
Eosinophilia	5 (11.6)	1 (10.0)	0	4 (18.2)	5 (11.6)	1 (10.0)	0	4 (18.2)
Abdominal discomfort	5 (11.6)	2 (20.0)	1 (9.1)	2 (9.1)	4 (9.3)	2 (20.0)	1 (9.1)	1 (4.5)
Nausea	3 (7.0)	0	1 (9.1)	2 (9.1)	3 (7.0)	0	1 (9.1)	2 (9.1)
Malaise	5 (11.6)	1 (10.0)	1 (9.1)	3 (13.6)	5 (11.6)	1 (10.0)	1 (9.1)	3 (13.6)
Pyrexia	8 (18.6)	2 (20.0)	1 (9.1)	5 (22.7)	8 (18.6)	2 (20.0)	1 (9.1)	5 (22.7)
Nasopharyngitis	14 (32.6)	2 (20.0)	4 (36.4)	8 (36.4)	8 (18.6)	1 (10.0)	2 (18.2)	5 (22.7)
ALT increased	12 (27.9)	2 (20.0)	4 (36.4)	6 (27.3)	12 (27.9)	2 (20.0)	4 (36.4)	6 (27.3)
AST increased	10 (23.3)	2 (20.0)	4 (36.4)	4 (18.2)	10 (23.3)	2 (20.0)	4 (36.4)	4 (18.2)
Decreased appetite	3 (7.0)	0	0	3 (13.6)	3 (7.0)	0	0	3 (13.6)
Back pain	4 (9.3)	1 (10.0)	2 (18.2)	1 (4.5)	2 (4.7)	1 (10.0)	0	1 (4.5)
Headache	14 (32.6)	3 (30.0)	5 (45.5)	6 (27.3)	13 (30.2)	2 (20.0)	5 (45.5)	6 (27.3)
Pruritus	3 (7.0)	0	1 (9.1)	2 (9.1)	2 (4.7)	0	1 (9.1)	1 (4.5)
Rash	3 (7.0)	0	1 (9.1)	2 (9.1)	3 (7.0)	0	1 (9.1)	2 (9.1)

n (%)

No deaths were reported. Serious adverse events occurred in 5 subjects (pyrexia [3 subjects]; gastroenteritis, blood bilirubin increased, ALT increased, AST increased, and hypochondriasis, 1 subject each [some subjects had more than one serious adverse event]) and gastroenteritis was assessed as unrelated to study drug and the outcome was reported as "resolved" for all events. Adverse events leading to discontinuation occurred in 4

146) HCV RNA < LLOQ (15 IU/mL) at 12 weeks posttreatment
147) Adverse events assessed by the investigator as "related to" study drug

subjects (blood bilirubin increased, AST increased, lymphopenia, and ALT increased, 1 subject each) and all the events were assessed as related to study drug, but their outcomes were reported as "resolved". Lymphopenia occurred in a subject who was considered to have responded inadequately to the combination of DCV and ASV and received additional treatment with PegIFNα/RBV.

4.(iii).A.(2) Japanese phase III study in Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis (5.3.5.2.1, Study AI447026 [January 2012 to June 2013])

An open-label, uncontrolled study was conducted at 24 centers in Japan to evaluate the efficacy and safety of DCV + ASV dual therapy in non-responder[123)] and IFN-ineligible-naïve[124)]/IFN-intolerant[122)] patients with chronic hepatitis C, or HCV with compensated cirrhosis[148)] (genotype 1b) (target number of cases of 200: 80 non-responders, 120 IFN-ineligible-naïve/intolerant patients).

ASV softgel capsules 100 mg BID plus DCV tablets 60 mg QD were to be orally administered for 24 weeks.

Of 259 subjects enrolled in the study, 222 subjects excluding 37 subjects who did not receive study drug (34 subjects due to deviations from the inclusion criteria, 3 subjects due to consent withdrawal) (87 non-responders, 135 IFN-ineligible-naïve/intolerant patients) were included in the efficacy and safety populations.

The primary endpoint of SVR24 rate [95% CI] was 80.5 [72.1, 88.8]% in non-responders and 87.4 [81.8, 93.0]% in IFN-ineligible-naïve/intolerant patients.

The incidence of adverse events (including abnormal laboratory changes) was 86.5% (192 of 222 subjects) and the incidence of adverse drug reactions[147)] (including abnormal laboratory changes) was 57.7% (128 of 222 subjects). Adverse events reported by ≥5% of all subjects and adverse drug reactions were as shown in Table 48.

Table 48. Adverse events reported by ≥5% of all subjects and adverse drug reactions

Event term	Adverse events			Adverse drug reactions		
	Total	Non-responder	IFN-ineligible-naïve /intolerant	Total	Non-responder	IFN-ineligible-naïve /intolerant
Number of subjects	222	87	135	222	87	135
Any event	192 (86.5)	74 (85.1)	118 (87.4)	128 (57.7)	51 (58.6)	77 (57.0)
Nasopharyngitis	67 (30.2)	27 (31.0)	40 (29.6)	6 (2.7)	3 (3.4)	3 (2.2)
Diarrhoea	22 (9.9)	10 (11.5)	12 (8.9)	14 (6.3)	7 (8.0)	7 (5.2)
Nausea	12 (5.4)	6 (6.9)	6 (4.4)	9 (4.1)	6 (6.9)	3 (2.2)
ALT increased	35 (15.8)	11 (12.6)	24 (17.8)	35 (15.8)	11 (12.6)	24 (17.8)
AST increased	28 (12.6)	10 (11.5)	18 (13.3)	28 (12.6)	10 (11.5)	18 (13.3)
Headache	35 (15.8)	17 (19.5)	18 (13.3)	22 (9.9)	12 (13.8)	10 (7.4)
Pyrexia	27 (12.2)	15 (17.2)	12 (8.9)	24 (10.8)	13 (14.9)	11 (8.1)
Arthralgia	13 (5.9)	7 (8.0)	6 (4.4)	6 (2.7)	5 (5.7)	1 (0.7)

n (%)
Adverse events occurring during rescue therapy (DCV, ASV, and PegIFNα/RBV) are not included.

148) The diagnosis of liver cirrhosis was made by liver biopsy or laparoscopy and for patients who did not undergo liver biopsy or laparoscopy, the following formula was used and those with a positive value were diagnosed with liver cirrhosis and those with a negative value were diagnosed with chronic hepatitis. Compensated cirrhosis was defined by Child-Pugh classification and Child-Pugh A patients were enrolled. About 10% (about 12 IFN-ineligible-naïve/intolerant patients, about 8 non-responders) of the study population had to be cirrhotic.
$Z = 0.124 \times (\gamma\text{-globulin (\%)}) + 0.001 \times (\text{hyaluronate}) (\mu g/L) - 0.075 \times (\text{platelet} (\times 10^4 \text{ counts per } mm^3)) - 0.413 \times \text{gender (male,1; female, 2)} - 2.005$

No deaths were reported. Serious adverse events occurred in 13 subjects (burns second degree, ALT increased, AST increased, blood bilirubin increased, CRP increased, oesophageal varices haemorrhage, herpes zoster, periarthritis, schizoaffective disorder, myasthenia gravis, myocardial infarction, pyrexia, appendicitis, pyelonephritis, basal cell carcinoma, and hepatocellular carcinoma, 1 subject each [some subjects had more than one serious adverse events]) and ALT increased, AST increased, blood bilirubin increased, CRP increased, myasthenia gravis, and pyrexia were assessed as related to study drug and their outcomes were all reported as resolved. Adverse events leading to discontinuation occurred in 11 subjects (ALT increased and AST increased, 10 subjects each; blood bilirubin increased [3 subjects]; CRP increased, eosinophilia, atypical mycobacteriosis, and myasthenia gravis, 1 subject each [some subjects had more than one adverse event leading to discontinuation]) and all events were assessed as related to study drug and their outcomes were all reported as resolved.

4.(iii).A.(3) Foreign phase II study in foreign patients with chronic hepatitis C (5.3.5.2.3, Study AI447011 [started on December [redacted], 2009, ongoing (database lock date of [redacted] [redacted], 20[redacted])])

A randomized, open-label, parallel-group study was conducted at a total of 20 centers in 3 countries (the US, France, Puerto Rico) to evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of combination therapy with DCV and ASV alone or with PegIFNα/RBV in null-responder[120)] patients with chronic hepatitis C (genotype 1[149)]) (target sample size of 100: 10 subjects in Group A in the sentinel cohort, 20 subjects in Group A1 in the additional cohort, 20 subjects in Group A2 in the additional cohort, 10 subjects in Group B in the sentinel cohort, 20 subjects in Group B1 in the additional cohort, 20 subjects in Group B2 in the additional cohort).

ASV tablets 600 mg[150)] BID (Group A in the sentinel cohort), ASV tablets 200 mg BID (Group A1 in the additional cohort), or ASV tablets 200 mg QD (Group A2 in the additional cohort) plus DCV tablets 60 mg QD were to be orally administered for 24 weeks. ASV tablets 600 mg PO (orally) BID (Group B in the sentinel cohort), ASV tablets 200 mg PO BID (Group B1 in the additional cohort), or ASV tablets 200 mg PO QD (Group B2 in the additional cohort) plus DCV tablets 60 mg PO QD in combination with PegIFNα/RBV[151)] were to be administered for 24 weeks.

In the sentinel cohort, 21 randomized subjects treated with study drug (11 subjects in Group A, 10 subjects in Group B) were included in the efficacy and safety populations. In Groups A1 and A2 in the additional cohort, 38 randomized subjects treated with study drug (18 subjects in Group A1, 20 subjects in Group A2) were included in the efficacy and safety populations. In Groups B1 and B2 in the additional cohort, 41 randomized subjects treated with study drug (20 subjects in Group B1, 21 subjects in Group B2) were included in the efficacy and safety populations.

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149) In Group A in the sentinel cohort, SVR24 rate was lower in subjects infected with HCV genotype 1a compared with subjects infected with HCV genotype 1b. Thus, patients infected with HCV genotype 1b only were included in the additional cohort.

150) As of September 21, 2010, the dose of ASV was to be reduced from 600 mg BID to 200 mg BID for all subjects on study (Protocol amendment, Version 6).

151) PegIFNα-2a 180 μg was to be subcutaneously administered once weekly and RBV 400 mg (body weight ≤75kg) or 600 mg (body weight >75 kg) after breakfast and RBV 600 mg after evening meal were to be administered.

In the sentinel cohort, the primary endpoints of the proportions of subjects achieving successful response[152)] and RVR[153)] and SVR12 rate were 90.0% (9 of 10 subjects), 63.6% (7 of 11 subjects), and 36.4% (4 of 11 subjects), respectively, in Group A and 81.8% (9 of 11 subjects), 60.0% (6 of 10 subjects), and 100% (10 of 10 subjects), respectively, in Group B. In the additional cohort, the primary endpoint of SVR12 rate was 77.8% (14 of 18 subjects) in Group A1, 65.0% (13 of 20 subjects) in Group A2, 95.0% (19 of 20 subjects) in Group B1, and 95.2% (20 of 21 subjects) in Group B2.

The incidences of adverse events (including abnormal laboratory changes) were 90.9% (10 of 11 subjects) in Group A in the sentinel cohort, 100% (10 of 10 subjects) in Group B in the sentinel cohort, 94.4% (17 of 18 subjects) in Group A1 in the additional cohort, 100% (20 of 20 subjects) in Group A2 in the additional cohort, 100% (20 of 20 subjects) in Group B1 in the additional cohort, and 100% (21 of 21 subjects) in Group B2 in the additional cohort. Adverse events reported by ≥2 subjects in any group were as shown in Table 49.

Table 49. Adverse events reported by ≥2 subjects in any group

Event term	Sentinel cohort		Additional cohort			
	Group A	Group B	Group A1	Group A2	Group B1	Group B2
N	N = 11	N = 10	N = 18	N = 20	N = 20	N = 21
Any event	10 (90.9)	10 (100)	17 (94.4)	20 (100)	20 (100)	21 (100)
Anaemia	0	2 (20.0)	0	0	5 (25.0)	3 (14.3)
Neutropenia	0	3 (30.0)	0	0	3 (15.0)	2 (9.5)
Chalazion	0	0	0	0	2 (10.0)	1 (4.8)
Vision blurred	1 (9.1)	0	1 (5.6)	0	1 (5.0)	3 (14.3)
Visual acuity reduced	0	0	0	0	2 (10.0)	1 (4.8)
Abdominal distension	1 (9.1)	0	2 (11.1)	1 (5.0)	0	0
Abdominal pain	0	2 (20.0)	2 (11.1)	1 (5.0)	3 (15.0)	4 (19.0)
Abdominal pain upper	0	2 (20.0)	1 (5.6)	0	0	0
Anal fissure	0	0	0	0	2 (10.0)	0
Aphthous stomatitis	0	0	0	1 (5.0)	0	2 (9.5)
Constipation	0	2 (20.0)	1 (5.6)	0	2 (10.0)	1 (4.8)
Diarrhoea	7 (63.6)	7 (70.0)	5 (27.8)	6 (30.0)	9 (45.0)	7 (33.3)
Dyspepsia	0	2 (20.0)	1 (5.6)	0	0	2 (9.5)
Gastrooesophageal reflux disease	0	2 (20.0)	3 (16.7)	0	0	1 (4.8)
Haemorrhoids	0	1 (10.0)	1 (5.6)	0	4 (20.0)	1 (4.8)
Nausea	1 (9.1)	5 (50.0)	3 (16.7)	3 (15.0)	7 (35.0)	3 (14.3)
Vomiting	0	2 (20.0)	0	0	1 (5.0)	2 (9.5)
Asthenia	0	1 (10.0)	3 (16.7)	4 (20.0)	6 (30.0)	12 (57.1)
Chills	3 (27.3)	1 (10.0)	0	1 (5.0)	4 (20.0)	4 (19.0)
Fatigue	5 (45.5)	7 (70.0)	5 (27.8)	2 (10.0)	8 (40.0)	5 (23.8)
Influenza like illness	0	2 (20.0)	2 (11.1)	2 (10.0)	4 (20.0)	7 (33.3)
Injection site erythema	0	0	0	0	1 (5.0)	2 (9.5)
Injection site haematoma	0	1 (10.0)	0	0	0	2 (9.5)
Irritability	1 (9.1)	2 (20.0)	2 (11.1)	0	7 (35.0)	6 (28.6)
Pain	1 (9.1)	2 (20.0)	0	3 (15.0)	1 (5.0)	1 (4.8)
Pyrexia	3 (27.3)	1 (10.0)	0	0	1 (5.0)	3 (14.3)
Xerosis	0	0	0	1 (5.0)	0	2 (9.5)
Bronchitis	0	1 (10.0)	0	2 (10.0)	0	0
Influenza	0	0	0	2 (10.0)	0	1 (4.8)
Nasopharyngitis	0	0	0	2 (10.0)	0	2 (9.5)
Sinusitis	2 (18.2)	1 (10.0)	0	2 (10.0)	0	0
Rhinitis	0	0	1 (5.6)	0	0	2 (9.5)
Urinary tract infection	1 (9.1)	2 (20.0)	2 (1.1)	1 (5.0)	2 (10.0)	2 (9.5)
Weight increased	0	0	0	2 (10.0)	0	0
Weight decreased	0	1 (10.0)	0	0	0	2 (9.5)
Decreased appetite	1 (9.1)	1 (10.0)	1 (5.6)	1 (5.0)	2 (10.0)	3 (14.3)
Arthralgia	0	2 (20.0)	3 (16.7)	0	6 (30.0)	4 (19.0)
Back pain	0	0	2 (11.1)	3 (15.0)	0	2 (9.5)
Muscle spasms	0	0	1 (5.6)	2 (10.0)	3 (15.0)	1 (4.8)
Myalgia	1 (9.1)	1 (10.0)	4 (22.2)	1 (5.0)	2 (10.0)	8 (38.1)
Pain in extremity	0	0	0	2 (10.0)	0	0

152) HCV RNA < LLOQ (10 IU/mL) or a decrease from baseline in plasma HCV RNA ≥2 $\log_{10}$ IU/mL at Week 2 and no rebound.
153) HCV RNA < LLOQ (10 IU/mL) at Week 4

Event term	Sentinel cohort		Additional cohort			
	Group A	Group B	Group A1	Group A2	Group B1	Group B2
N	N = 11	N = 10	N = 18	N = 20	N = 20	N = 21
Any event	10 (90.9)	10 (100)	17 (94.4)	20 (100)	20 (100)	21 (100)
Tendonitis	0	0	1 (5.6)	2 (10.0)	1 (5.0)	0
Dizziness	1 (9.1)	2 (20.0)	0	0	3 (15.0)	0
Headache	5 (45.5)	5 (50.0)	8 (44.4)	8 (40.0)	12 (60.0)	9 (42.9)
Anxiety	0	1 (10.0)	1 (5.6)	1 (5.0)	3 (15.0)	2 (9.5)
Depression	0	1 (10.0)	0	2 (10.0)	2 (10.0)	0
Insomnia	1 (9.1)	3 (30.0)	3 (16.7)	3 (15.0)	9 (45.0)	3 (14.3)
Cough	2 (18.2)	2 (20.0)	3 (16.7)	0	3 (15.0)	4 (19.0)
Dyspnoea	0	2 (20.0)	1 (5.6)	0	3 (15.0)	6 (28.6)
Alopecia	0	1 (10.0)	0	1 (5.0)	6 (30.0)	8 (38.1)
Dermatitis	0	2 (20.0)	0	0	0	0
Dry skin	0	3 (30.0)	3 (16.7)	0	5 (25.0)	5 (23.8)
Eczema	0	1 (10.0)	0	0	1 (5.0)	2 (9.5)
Erythema	0	0	0	1 (5.0)	1 (5.0)	2 (9.5)
Pruritus	1 (9.1)	1 (10.0)	3 (16.7)	1 (5.0)	3 (15.0)	8 (38.1)
Rash	0	0	2 (11.1)	1 (5.0)	4 (20.0)	7 (33.0)

n (%)
Adverse events occurring during rescue therapy (DCV, ASV, and PegIFNα/RBV) are not included.

No deaths were reported. Serious adverse events occurred in 1 subject in Group A1 (panic attack), 2 subjects in Group A2 (forearm fracture and prostate cancer, 1 subject each), and 3 subjects in Group B1 (squamous cell carcinoma, accidental overdose of DCV, and overdose of DCV, 1 subject each) and accidental overdose of DCV in Group B1 was considered related to study drug, but their outcomes were reported as resolved for all events. No adverse events leading to discontinuation were reported.

4.(iii).B Outline of the review

4.(iii).B.(1) Efficacy

As a result of the following reviews, PMDA concluded as follows:

The efficacy of the DCV + ASV dual therapy was demonstrated, but it is necessary to provide the information on the efficacy of the DCV + ASV dual therapy in patients infected with HCV genotype 1a to healthcare providers in clinical setting. The information on the association between resistance mutations and efficacy should be also provided to them and drug resistance testing prior to the start of the DCV + ASV dual therapy should be recommended.

The above conclusions by PMDA will be finalized, taking account of comments from the expert advisors.

4.(iii).B.(1).1) Study design

The applicant explained the appropriateness of including only IFN-ineligible-naïve/intolerant and non-responder Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis (genotype 1b) in a Japanese phase III study (Study AI447026) and of employing an uncontrolled design as follows:

When Study AI447026 was initiated (January 2012), triple therapy with PegIFNα-2b/RBV and telaprevir (PegIFNα/RBV/telaprevir triple therapy) for treatment-naïve patients with chronic hepatitis C had been approved (approved on September 26, 2011) and a high SVR24 rate even in relapsers after previous therapy had been reported. On the other hand, no therapy was expected to achieve SVR in IFN-ineligible-naïve/intolerant patients. Although PegIFNα/RBV/telaprevir triple therapy was considered a therapeutic option

for non-responders, the SVR24 rate with this therapy [95% CI] had been reported to be 34.4 [18.6, 53.2]%[154] and no therapy was expected to be sufficiently effective.

Based on the above, it was decided to limit the target population for earlier clinical studies to IFN-ineligible-naïve/intolerant and non-responder patients with chronic hepatitis C with unmet medical need. A Japanese phase III study in treatment-naïve patients with chronic hepatitis C (Study AI447031) is currently ongoing.

With respect to the inclusion of a control group, it is very rare that patients with chronic hepatitis C, or HCV with compensated cirrhosis spontaneously clear HCV, there was no therapeutic option likely to achieve SVR in IFN-ineligible-naïve/intolerant patients, and a Japanese phase II study (Study AI447017) showed that the SVR24 rate with the combination of DCV and ASV [95% CI] was 90.9 [58.7, 99.8]% in null-responders. Taking account of the results from clinical studies with PegIFNα/RBV/telaprevir triple therapy in non-responders, it was considered difficult to conduct a comparative study using patients treated with PegIFNα/RBV/telaprevir triple therapy etc., as a control group.

Based on the above, it was appropriate that the Japanese phase III study (Study AI447026) employed an open-label, uncontrolled design.

PMDA considers as follows:
It is very rare that patients with chronic hepatitis C, or HCV with compensated cirrhosis spontaneously clear the virus and there was no therapeutic option likely to achieve SVR in IFN-ineligible-naïve/intolerant patients. Taking account of the results from clinical studies with PegIFNα/RBV/telaprevir triple therapy in non-responders and expected SVR24 rates with the combination of DCV and ASV, it was unavoidable not to include a control group, in terms of the feasibility of the clinical study.

4.(iii).B.(1).2) Results of efficacy assessment

The applicant explained the efficacy of the DCV + ASV dual therapy in non-responders and IFN-ineligible-naïve/intolerant patients as follows:
In the Japanese phase III study (Study AI447026), the SVR24 rate [95% CI] was 80.5 [72.1, 88.8]% in non-responders, which was higher than the SVR24 threshold predefined based on the results of PegIFNα/RBV/telaprevir triple therapy (45%), and the SVR24 rate [95% CI] was 87.4 [81.8, 93.0]% in IFN-ineligible-naïve/intolerant patients, which was higher than the SVR24 threshold predefined based on clinical usefulness (30%). Therefore, the efficacy of the DCV + ASV dual therapy in these patient populations was demonstrated.

Virologic responses in subgroups defined by baseline demographics and disease characteristics were as shown in Table 50, which were largely similar except for the subgroups of subjects with mutations in the NS5A region (Y93H and L31M/V).

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[154] Application dossier for Telavic Tablets 250 mg (2.7.3. Summary of Clinical Efficacy)

Table 50. SVR24 rates in subgroups (Japanese phase III study: Study AI447026)

Baseline demographics and disease characteristics		SVR24 rate		
		Total N = 222	Non-responder N = 87	IFN-ineligible-naïve /intolerant N = 135
Age	< 65 years	108/133 (81.2)	47/60 (78.3)	61/73 (83.6)
	≥ 65 years	80/89 (89.9)	23/27 (85.2)	57/62 (91.9)
Gender	Male	64/77 (83.1)	32/39 (82.1)	32/38 (84.2)
	Female	124/145 (85.5)	38/48 (79.2)	86/97 (88.7)
HCV RNA	≥ 800,000 IU/mL	157/189 (83.1)	64/80 (80.0)	93/109 (85.3)
	< 800,000 IU/mL	31/33 (93.9)	6/7 (85.7)	25/26 (96.2)
Degree of fibrosis	Chronic hepatitis	168/200 (84.0)	60/76 (78.9)	108/124 (87.1)
	Compensated cirrhosis (Child-Pugh A)	20/22 (90.9)	10/11 (90.9)	10/11 (90.9)
Baseline ALT	Grade 0	67/84 (79.8)	16/24 (66.7)	51/60 (85.0)
	Grade 1-4 [a)]	121/138 (87.7)	54/63 (85.7)	67/75 (89.3)
Prior IFN therapy	IFN-ineligible-naïve	85/100 (85.0)	—	85/100 (85.0)
	IFN-intolerant	33/35 (94.3)	—	33/35 (94.3)
Type of response to prior IFN therapy	Null responder	39/48 (81.3)	39/48 (81.3)	—
	Partial responder [b)]	28/36 (77.8)	28/36 (77.8)	—
IL-28B rs12979860 genotype	CC	93/110 (84.5)	14/16 (87.5)	79/94 (84.0)
	CT	90/106 (84.9)	52/66 (78.8)	38/40 (95.0)
	TT	5/6 (83.3)	4/5 (80.0)	1/1 (100)
IL-28B rs8099917 genotype	GG	5/6 (83.3)	4/5 (80.0)	1/1 (100)
	GT	86/102 (84.3)	49/63 (77.8)	37/39 (94.9)
	TT	95/112 (84.8)	17/19 (89.5)	78/93 (83.9)
NS5A resistance mutation Y93H [c)]	Yes	13/30 (43.3)	3/9 (33.3)	10/21 (47.6)
	No	168/184 (91.3)	66/77 (85.7)	102/107 (95.3)
NS5A resistance mutation L31M/V [c)]	Yes	2/8 (25.0)	1/6 (16.7)	1/2 (50.0)
	No	179/206 (86.9)	68/80 (85.0)	111/126 (88.1)
Body weight	< 50kg	59/65 (90.8)	17/20 (85.0)	42/45 (93.3)
	≥ 50kg and < 60kg	66/82 (80.5)	23/30 (76.7)	43/52 (82.7)
	≥ 60kg and < 70kg	45/54 (83.3)	22/28 (78.6)	23/26 (88.5)
	≥ 70kg	18/21 (85.7)	8/9 (88.9)	10/12 (83.3)
BMI	≤ 25kg/m^2	151/179 (84.4)	60/74 (81.1)	91/105 (86.7)
	> 25kg/m^2 and ≤ 30kg/m^2	31/36 (86.1)	9/12 (75.0)	22/24 (91.7)
	> 30kg/m^2	6/7 (85.7)	1/1 (100)	5/6 (83.3)

n (%)

a) Graded by Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0 of Division of Autoimmune Immunodeficiency Disorders (DAIDS).

b) After ≥12 weeks of treatment with PegIFNα/RBV or IFN β/RBV, patients who did not achieve HCV RNA < LLOQ (undetectable) while they had a decrease from baseline in HCV RNA ≥2 $\log_{10}$ IU/mL.

c) Excluding 8 patients with no amino acid sequence information at baseline (7 IFN-ineligible-naïve/intolerant patients, 1 non-responder).

PMDA considers as follows:

Although the Japanese phase III study (Study AI447026) did not include verifying the hypothesis on efficacy in the non-responder and IFN-ineligible-naïve/intolerant patient populations, DCV + ASV dual therapy is of clinical significance because no other effective therapy was available for these patient populations as shown in "4.(iii).B.(1).1) Study design", and the observed SVR24 rates were 80.5 [72.1, 88.8]% and 87.4 [81.8, 93.0]%, respectively.

Therefore, a certain level of efficacy of the DCV + ASV dual therapy can be expected in non-responder and IFN-ineligible-naïve/intolerant patients.

4.(iii).B.(1).3) Efficacy in patients with chronic hepatitis C genotype 1a

PMDA asked the applicant to explain the efficacy of the DCV + ASV dual therapy in patients with HCV genotype 1a.

The applicant explained as follows:

In a foreign early phase II study (Study AI447011), the SVR24 rate was 22.2% (2 of 9 subjects) in 9 genotype 1a null-responders treated with DCV in combination with ASV, which was lower than 100% (2 of 2 subjects) in genotype 1b null-responders. However, the number of subjects in this study was very limited and efficacy in genotype 1a patients is unknown. Based on the results from this study, it was decided to develop the DCV + ASV dual therapy only for patients infected with HCV genotype 1b.

PMDA considers as follows:

With respect to efficacy in patients infected with HCV genotype 1a in Japanese clinical studies, there are no adequate evaluable data and the efficacy of the DCV + ASV dual therapy in HCV genotype 1a patients is unknown. Genotype to be specified in the indication statement will be discussed in "4.(iii).B.(3) Indications".

4.(iii).B.(1).4) Influence of viral mutations on efficacy

The applicant explained the association between the efficacy of the DCV + ASV dual therapy and resistance mutations as follows:

Genotypic analysis was performed using samples from 255 subjects receiving DCV + ASV in the Japanese phase III study (Study AI447026) and a Japanese phase II study (Study AI447017, additional cohort) and 18 subjects receiving DCV + ASV in a foreign phase II study (Study AI447011, Group A1). SVR24 rate by major baseline NS5A or NS3 polymorphism was as shown in Table 51.

Table 51. SVR24 rate by baseline polymorphism (Pooled analysis of Studies AI447026, AI447017, and AI447011)

	Subjects with SVR24/Subjects with polymorphism [SVR24 rate (%)]	Subjects with SVR24/Subjects without polymorphism [SVR24 rate (%)]
No. of subjects evaluated for NS5A polymorphisms	N = 264	
Q54E/G/H/L/N/V/Y	82/99 (82.8)	139/165 (84.2)
Y93F/H/S	18/41 (43.9)	203/223 (91.0)
R30K/L/Q	30/35 (85.7)	191/229 (83.4)
Q62A/E/H/K/L/N/P/R/S/V	21/22 (95.5)	200/242 (82.6)
L28/M/V	15/19 (78.9)	206/245 (84.1)
P58A/L/S/T	17/20 (85.0)	204/244 (83.6)
A92E/P/T/V	15/19 (78.9)	206/245 (84.1)
L31F/M/V	3/10 (30.0)	218/254 (85.8)
No. of subjects evaluated for NS3 polymorphisms	N = 271	
S122C/G/N/T	73/86 (84.9)	154/185 (83.2)
Q80L/K	26/33 (78.8)	201/238 (84.5)
T54S	5/6 (83.3)	222/265 (83.8)
D168E	1/2 (50.0)	226/269 (84.0)
N77A/S	2/2 (100)	215/269 (79.9)
F169L	2/2 (100)	215/269 (79.9)

n (%)

Pooled analysis of Studies AI447026, AI447017, and AI447011 included 273 subjects.

Based on a pooled analysis of Studies AI447026, AI447017, and AI447011, the results of resistance analysis at failure were available for 43 of 45 subjects with virologic failure and NS5A Y93H/N was detected in 40 subjects (93.0%), NS5A L31F/I/M/V in 39 subjects (90.7%), and NS3 D168A/E/N/T/V/Y in 37 subjects (86.0%). The numbers of subjects with these resistance mutations detected at failure by baseline polymorphism associated with drug resistance were as shown in Table 52. Both NS5A and NS3 resistance mutations were detected at

treatment failure in 37 subjects (86.0%).

Table 52. Drug resistance mutations in subjects with virologic failure (Pooled analysis of Studies AI447026, AI447017, and AI447011)

	Baseline polymorphism associated with drug resistance		
	NS5A region		NS3 region
	Y93H/N (N = 39)	L31F/I/M/V (N = 10)	D168E (N = 2)
Resistance mutation at failure			
NS5A Y93H/N	23 (59.0)	7 (70.0)	1 (50.0)
NS5A L31F/I/M/V	21 (53.8)	7 (70.0)	1 (50.0)
NS3 D168A/E/N/T/V/Y	20 (51.3)	6 (60.0)	1 (50.0)

n (%)

Based on a pooled analysis of Studies AI447026, AI447017, and AI447011, the SVR24 rates were 41.0% in subjects with baseline Y93H (16 of 39 subjects) and 22.2% in subjects with baseline L31M/V (2 of 9 subjects). An analysis was conducted to identify factors associated with failure to achieve SVR24 and the results were as shown in Table 53.

Table 53. Factors associated with failure to achieve SVR24 (Pooled analysis of Studies AI447026, AI447017, and AI447011)

	Odds ratio [95% CI]
Liver cirrhosis: Yes vs. No	0.57 [0.10, 3.26]
Cohort: IFN-ineligible-naïve/intolerant vs. Non-responder	0.87 [0.33, 2.27]
IL28B SNP: non-CC vs. CC	1.58 [0.57, 4.38]
HCV RNA level: <800 kIU/mL vs. ≥800 kIU/mL	0.43 [0.08, 2.31]
Gender: Male vs. Female	0.82 [0.35, 1.87]
Y93H: Yes vs. No	17.81 [7.17, 44.25]
L31M/V: Yes vs. No	26.81 [4.61, 155.7]

IL28B SNP: IL28B rs12979860
Calculated using a multivariate logistic regression model.
Odds ratio relative to the category on the right for each factor in the table.

Based on the above, the SVR24 rate was lower in subjects with baseline Y93H or L31M/V than in subjects without these polymorphisms, which indicated that Y93H and L31M/V are predictors of treatment failure. However, as the proportion of subjects with baseline Y93H or L31M/V was not high and even these patients achieved a certain level of SVR24 rate, DCV + ASV dual therapy can be recommended. It is not essential to perform drug resistance testing before the initiation of therapy.

PMDA considers as follows:

The proportion of patients with chronic hepatitis C, or HCV with compensated cirrhosis who had baseline NS5A polymorphisms associated with drug resistance was low and though the combination of DCV and ASV was effective for some patients, the SVR24 rate was lower in the patient population with these polymorphisms before the initiation of DCV + ASV dual therapy compared to the patient population without these polymorphisms. According to the results of resistance analysis of subjects with treatment failure after administration of DCV + ASV, NS3 and NS5A resistance-associated variants were detected at a high frequency and many of the subjects had baseline polymorphisms associated with drug resistance. Thus, it is necessary to provide the information on baseline polymorphisms associated with drug resistance obtained from Japanese and foreign clinical studies and the information on efficacy in these patient populations and recommend that physicians perform drug resistance testing.

4.(iii).B.(2) Safety

PMDA reviewed the safety data on the combination of DCV and ASV, mainly from a Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026) and concluded as follows:
It is necessary to caution about abnormal liver function tests (LFTs) associated with DCV + ASV dual therapy. It is also necessary to adequately provide information on abnormal LFT results and hypersensitivity reactions and then continue to collect post-marketing information.

However, as long as monitoring and management of adverse events are conducted and appropriate actions such as interruption/discontinuation of treatment are taken with an adequate understanding of the safety profile of the combination of DCV and ASV under the supervision of physicians with adequate knowledge and experience in the treatment of viral liver disease, patients with chronic hepatitis C and patients with HCV with compensated cirrhosis can be treated with DCV + ASV.

The above conclusions by PMDA will be finalized, taking account of comments from the expert advisors.

4.(iii).B.(2).1) Safety profile

Regarding the safety of the combination of DCV and ASV, summary of safety and adverse events (including abnormal laboratory changes) reported by ≥5% of all subjects in a Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026) were as shown in Table 54 and Table 55, respectively. A pooled safety analysis of the Japanese clinical studies included 255 subjects treated with dual combination therapy with DCV tablets 60 mg QD plus ASV softgel capsules 100 mg BID or ASV tablets 200 mg BID (the proposed dosage regimen).

Table 54. Summary of safety (Pooled analysis of Studies AI447017 and AI447026)

	Non-responder	IFN-ineligible-naïve/intolerant	Total
	N = 98	N = 157	N = 255
All adverse events	85 (86.7)	137 (87.3)	222 (87.1)
Grade 3 or 4 adverse events	11 (11.2)	32 (20.4)	43 (16.9)
Fatal serious adverse events	0	0	0
Serious adverse events	4 (4.1)	12 (7.6)	16 (6.3)
Adverse events leading to treatment discontinuation	3 (3.1)	10 (6.4)	13 (5.1)
Adverse events leading to treatment interruption	2 (2.0)	7 (4.5)	9 (3.5)

n (%)

Table 55. Adverse events reported by ≥5% of all subjects (Pooled analysis of Studies AI447017 and AI447026)

Event term	Non-responder	IFN-ineligible-naïve/intolerant	Total
	N = 98	N = 157	N = 255
Nasopharyngitis	31 (31.6)	48 (30.6)	79 (31.0)
Headache	22 (22.4)	24 (15.3)	46 (18.0)
ALT increased	15 (15.3)	30 (19.1)	45 (17.6)
AST increased	14 (14.3)	22 (14.0)	36 (14.1)
Pyrexia	16 (16.3)	17 (10.8)	33 (12.9)
Diarrhoea	11 (11.2)	14 (8.9)	25 (9.8)
Nausea	7 (7.1)	8 (5.1)	15 (5.9)
Eosinophilia	3 (3.1)	12 (7.6)	15 (5.9)
Malaise	6 (6.1)	8 (5.1)	14 (5.5)
Constipation	4 (4.1)	9 (5.7)	13 (5.1)
Arthralgia	7 (7.1)	6 (3.8)	13 (5.1)

n (%)

Based on the summary of safety, PMDA considers that as long as monitoring and management of adverse events are conducted and appropriate actions such as interruption/discontinuation of treatment are taken with an adequate understanding of the safety profile of the combination of DCV and ASV under the supervision of physicians with adequate knowledge and experience in the treatment of viral liver disease, coadministration of DCV and ASV is tolerable. However, as ALT increased and AST increased occurred at a relatively high frequency and accounted for the majority of adverse events leading to treatment discontinuation (12 of 13 subjects), it was decided to review the details of abnormal LFTs associated with the combination of DCV and ASV in the section below.

4.(iii).B.(2).2) Abnormal LFTs

PMDA asked the applicant to explain abnormal LFTs associated with the combination of DCV and ASV.

The applicant explained as follows:

Based on a pooled analysis of a Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026) (255 subjects treated with the proposed dosage regimen), the incidences of ALT, AST, and blood bilirubin increased as abnormal LFTs were summarized and the results were as shown in Table 56.

Table 56. Summary of abnormal LFTs

	Total	ALT increased	AST increased	Blood bilirubin increased
All adverse events	50 (19.6)	45 (17.6)	36 (14.1)	11 (4.3)
Grade 3 or 4 adverse events	22 (8.6)	21 (8.2)	15 (5.9)	2 (0.8)
Serious adverse events	1 (0.4)	1 (0.4)	1 (0.4)	1 (0.4)
Adverse events leading to discontinuation	12 (4.7)	11 (4.3)	11 (4.3)	3 (1.2)
Adverse events leading to interruption	3 (1.2)	2 (0.8)	2 (0.8)	1 (0.4)

n (%)

There were no subjects with abnormal LFTs leading to hepatic failure or death.

The median time to the first onset of Grade 3 or 4 ALT, AST, or blood bilirubin elevation as an adverse event [range] was 90.5 [27, 169] days and there was no consistent trend in the time to onset. In all subjects, all adverse events resolved after completion or discontinuation of study drug and the median time to resolution [range] was 22.5 [2, 70] days. In the Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026), treatment was allowed to be interrupted for up to 7 days in the event of adverse events. Three subjects resumed treatment after interruption due to abnormal LFTs, but all of them relapsed after treatment resumption and treatment lasted only for ≤7 days.

Concerning abnormal LFTs associated with the combination of DCV and ASV, the association between Grade 3 or 4 ALT elevation and baseline demographics and disease characteristics of patients (age, gender, body weight, cirrhotic or non-cirrhotic, baseline ALT value, prior IFN therapy and response to IFN, HCV RNA level at baseline) was analyzed and the results were as shown in Table 57. Similar results were obtained also for Grade 3 or 4 AST elevation. Although there were limitations to the analysis due to major differences in the number of subjects between the subgroups and low incidences of abnormal LFTs, the incidences of Grade 3 or

4 ALT and AST elevations were almost comparable between the subgroups.

Table 57. Incidence of Grade 3 or 4 ALT elevation by subgroup (Pooled analysis of Studies AI447017 and AI447026)

Baseline demographics and disease characteristics		Non-responder	IFN-ineligible-naïve/ intolerant	Total
		N = 98	N = 157	N = 255
Age	< 65 years	4/68 (5.9)	7/83 (8.4)	11/151 (7.3)
	≥ 65 years	2/30 (6.7)	7/74 (9.5)	9/104 (8.7)
Gender	Male	3/43 (7.0)	5/44 (11.4)	8/87 (9.2)
	Female	3/55 (5.5)	9/113 (8.0)	12/168 (7.1)
HCV RNA	≥ 800,000 IU/mL	5/91 (5.5)	10/128 (7.8)	15/219 (6.9)
	< 800,000 IU/mL	1/7 (14.3)	4/29 (13.8)	5/36 (13.9)
Body weight	< 60 kg	4/59 (6.8)	9/114 (7.9)	13/173 (7.5)
	≥ 60 kg	2/39 (5.1)	5/43 (11.6)	7/82 (8.5)
Degree of fibrosis	Chronic hepatitis	6/87 (6.9)	13/146 (8.9)	19/233 (8.2)
	Compensated cirrhosis (Child-Pugh A)	0/11 (0)	1/11 (9.1)	1/22 (4.6)
Baseline ALT	Grade 0	2/27 (7.4)	7/73 (9.6)	9/100 (9.0)
	Grade 1-4 [a)]	4/71 (5.6)	7/84 (8.3)	11/155 (7.1)
Prior IFN therapy	IFN-ineligible-naïve	—	12/118 (10.2)	12/118 (10.2)
	IFN-intolerant	—	2/39 (5.1)	2/39 (5.1)
Type of response to prior IFN therapy	Null responder	4/59 (6.8)	—	4/59 (6.8)
	Partial responder	1/36 (2.8)	—	1/36 (2.8)

n (%)
a) Graded by Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, Division of Autoimmune Immunodeficiency Disorders (DAIDS)

PMDA asked the applicant to explain monitoring of LFTs during treatment with DCV + ASV and actions to be taken in the event of abnormal LFTs and the need for a precautionary statement in the package insert.

The applicant explained as follows:
The protocols for a Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026) required that laboratory tests including LFTs be performed at least every 2 weeks for the first 12 weeks of treatment and every 4 weeks thereafter and that in the event of abnormal LFTs,[155)] the test be repeated within 2 weeks to observe how the level is changing. The protocol for Study AI447017 defined the criteria for stopping treatment due to abnormal LFTs[156)] and 2 subjects who met at least one of the criteria discontinued treatment. The protocol for Study AI447026 defined 5 criteria for stopping treatment due to abnormal LFTs[157)] and among 9 subjects who met at least one of the criteria, 8 subjects discontinued treatment and 1 subject interrupted treatment.[158)]

In the Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026), the background of subjects with abnormal LFTs leading to treatment discontinuation (12 subjects), the time to treatment discontinuation, LFT values at discontinuation, and actions taken were as listed in Table 58.

155) AST, ALT, or total bilirubin exceeding the baseline level and the upper limit of laboratory reference range

156) (1) Decompensated cirrhosis was confirmed (Child-Pugh class B or C, Child-Pugh score of >6). (2) The following condition lasted for ≥1 week: >2-fold increase in ALT from baseline and ALT exceeding 5 times the upper limit of laboratory reference range plus total bilirubin exceeding 2 times the upper limit of laboratory reference range or INR exceeding 2 times the upper limit of laboratory reference range. (3) Grade 4 adverse event related to study drug.

157) (1) >2-fold increase in ALT from baseline and ALT exceeding 5 times the upper limit of laboratory reference range plus total bilirubin exceeding 2 times the upper limit of laboratory reference range or INR exceeding 2 times the upper limit of laboratory reference range. (2) Signs of decompensated cirrhosis (Child-Pugh class B or C, Child-Pugh score of >6). (3) Grade 4 adverse event related to study drug. (4) ALT exceeding 10 times the upper limit of laboratory reference range. (5) A fever of ≥38.7°C, eosinophilia ($\geq 1.5 \times 10^3/\mu L$), and ALT and AST elevations exceeding 5 times the upper limit of laboratory reference range were observed simultaneously and there were no findings of acute viral infection, bacterial infection, or parasitic infection.

158) This subject had a Child-Pugh score of >6 transiently while suffering from pyelonephritis, but no signs of decompensated cirrhosis were observed and treatment was resumed.

Table 58. Summary of subjects with abnormal LFTs leading to treatment discontinuation

Age	Gender	Time to onset *	Time to treatment discontinuation *	Duration (days)	LFT values at discontinuation			Criteria for stopping treatment	Action taken
					AST (U/L)	ALT (U/L)	Total bilirubin (μmol/L)		
7[redacted]	F	63	84	50	416	341	32.5	Met	Treatment discontinued
5[redacted]	M	29	29	46	408	609	54.7	Met	Treatment discontinued Prednisolone administered
6[redacted]	M	153	160	29	219	203	51.3	Unmet	Treatment discontinued
7[redacted]	M	100	106	22	196	445	22.2	Met	Treatment discontinued
7[redacted]	M	69	90	40	141	235	54.7	Met	Treatment discontinued
6[redacted]	M	27	34	20	222	213	18.8	Unmet	Treatment discontinued
5[redacted]	F	99	120	64	332	558	22.2	Met	Treatment discontinued
6[redacted]	F	29	35	36	306	323	17.1	Met	Treatment discontinued
5[redacted]	F	82	82	22	298	382	13.7	Met	Treatment discontinued
7[redacted]	M	57	71	43	510	527	26.8	Met	Treatment discontinued
7[redacted]	F	71	80	78	447	548	30.8	Met	Treatment discontinued
6[redacted]	F	32	32	29	356	348	13.7	Met	Treatment discontinued Glycyrrhizic acid Ursodeoxycholic acid

*: Days of treatment (days)

Among 12 subjects who discontinued treatment, 10 subjects met the criteria (Grade 4 ALT elevation or Grade 4 AST elevation related to study drug and ALT exceeding 10 times the upper limit of laboratory reference range) without signs of decompensated cirrhosis. Excluding 1 subject treated with prednisolone and 1 subject treated with glycyrrhizic acid and ursodeoxycholic acid, 10 subjects just discontinued study treatment and were followed up and abnormal LFTs resolved in all subjects.

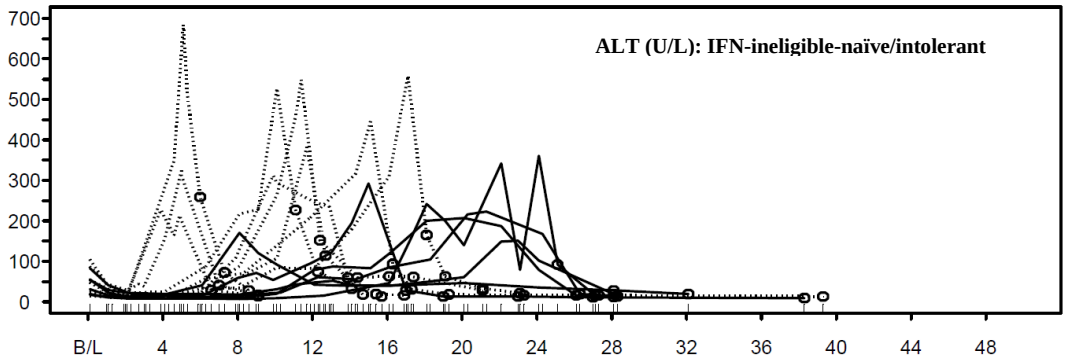
Based on the above, DCV and ASV can be administered safely by appropriate management in accordance with important precautions described in the package insert (LFTs should be performed at least every 4 weeks and in the event of a deterioration of the liver function, the tests should be performed more frequently and appropriate actions such as discontinuation of treatment, should be taken.).

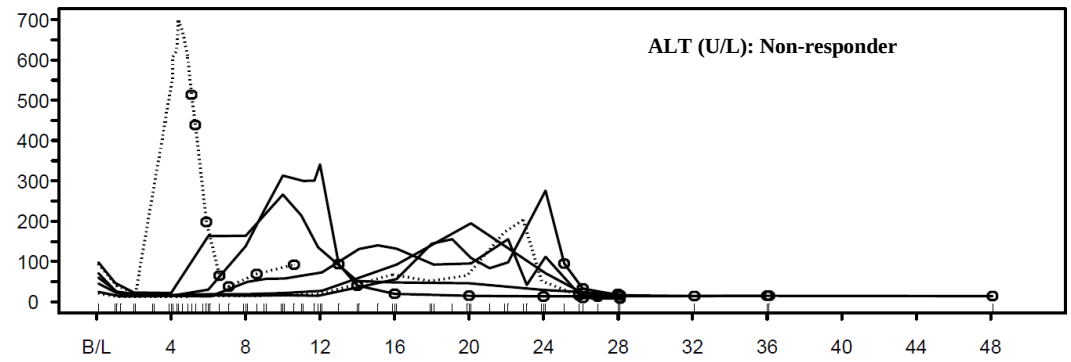
In the Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026), subjects who experienced Grade 3 or worse abnormal LFTs but were able to continue study treatment were as shown in Table 59. In the Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026), ALT and AST over time in subjects with abnormal LFTs leading to treatment discontinuation and subjects who experienced Grade 3 or worse abnormal LFTs but were able to continue study treatment were as shown in Figure 3.

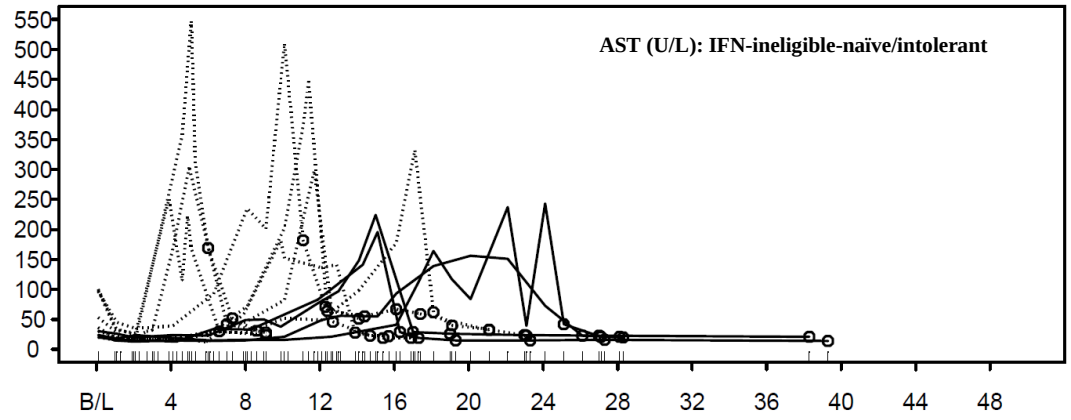
Table 59. Subjects who experienced Grade 3 or worse abnormal LFTs, but were able to continue study treatment

Age	Gender	Adverse event	Time to onset *	Time to peak levels *	Maximum LFT values			Duration (days)
					AST (U/L)	ALT (U/L)	Total bilirubin (µmol/L)	
6[redacted]	M	ALT increased AST increased	127 155	169 169	243	361	23.9	64 36
4[redacted]	F	ALT increased	141	141	109	195	22.2	43
6[redacted]	M	ALT increased	42	70	89	266	13.7	127
5[redacted]	F	ALT increased	106	155	159	155	37.6	78
5[redacted]	F	ALT increased AST increased	127 141	141 141	156	207	18.8	57 43
4[redacted]	F	ALT increased	142	149	134	223	18.8	43
6[redacted]	F	ALT increased	161	161	115	151	13.3	37
7[redacted]	F	ALT increased	57	57	151	170	15.4	141
7[redacted]	F	ALT increased AST increased	105 105	105 105	224	292	27.4	15 15
5[redacted]	M	ALT increased	169	169	132	276	29.1	15

*: Days of treatment (days)







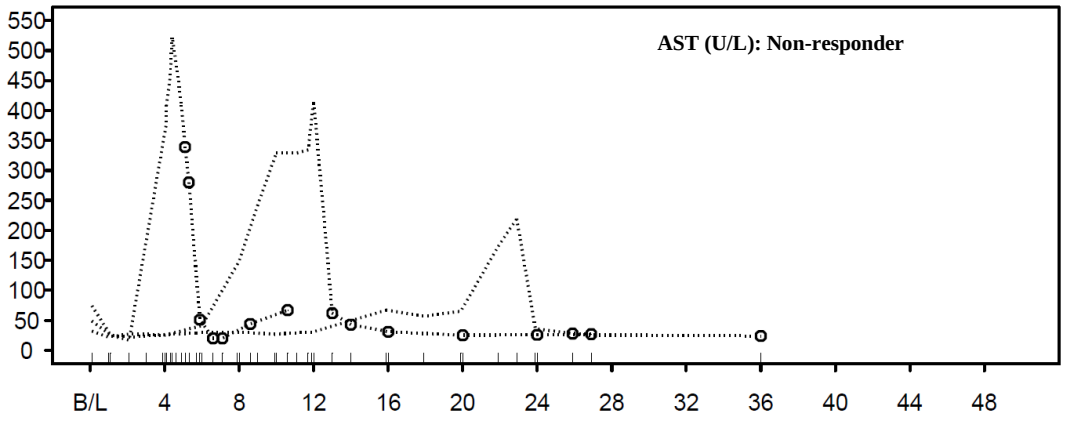


Figure 3. ALT and AST over time (vertical axis, U/L; horizontal axis, weeks of treatment)
B/L, baseline; Solid line, subjects who continued treatment;
Dashed line, subjects who discontinued treatment (Circles in the figure indicate measurements after treatment discontinuation)

PMDA considers as follows:

As long as monitoring and management of LFTs are conducted and appropriate actions such as interruption/discontinuation of treatment are taken with an adequate understanding of the possibility that abnormal LFTs may occur following coadministration of DCV and ASV, abnormal LFTs are tolerable. While, in Japanese clinical studies, no subjects had abnormal LFTs leading to hepatic failure and there were subjects who had Grade 3 abnormal LFTs but were able to continue treatment, patients with abnormal LFTs leading to treatment discontinuation were reported; it is not necessarily easy to decide whether or not to continue treatment; there was no trend towards the occurrence of abnormal LFTs in specific patients or at specific timing; and it takes long time to recover after treatment discontinuation etc. Thus, it is necessary to watch for the possible occurrence of abnormal LFTs, regardless of patient background, and to provide information on the incidences of abnormal LFTs in clinical studies to healthcare providers in clinical settings. Also after the market launch, as required by the protocols for Japanese clinical studies (laboratory tests including LFTs should be performed at least every 2 weeks for the first 12 weeks of treatment and every 4 weeks thereafter and in the event of abnormal LFTs,[155)] the test should be repeated within 2 weeks to observe how the level is changing.), LFTs should be monitored closely and then whether or not to continue treatment should be decided comprehensively by assessing liver function in the event of abnormal LFTs etc. Therefore, it is necessary to appropriately provide information on the safety of DCV + ASV dual therapy and the criteria for stopping treatment using materials etc., so that appropriate actions can be taken in accordance with the monitoring procedures for abnormal LFTs and the criteria for stopping treatment as specified for Japanese clinical studies, under the supervision of physicians with adequate knowledge and experience in the treatment of viral liver disease. It is also necessary to collect post-marketing information on the occurrence of abnormal LFTs.

4.(iii).B.(2).3) Hypersensitivity reaction

As the protocol was amended to add measures against a possible hypersensitivity reaction while the Japanese phase III study (Study AI447026) was ongoing, PMDA asked for the applicant's explanation.

The applicant explained as follows:

Since 1 subject had a series of clinical symptoms suspected of a hypersensitivity reaction (pyrexia, eosinophilia, abnormal LFTs, etc.) in the Japanese phase III study (Study AI447026), the protocol was amended during the study based on the literature on DRESS syndrome[159)] and a hypersensitivity reaction was defined as the occurrence of pyrexia (≥38.7°C), eosinophilia (≥1.5×10^9 cells/L), and abnormal LFTs (AST and ALT ≥5 times the upper limit of laboratory reference range) to collect information. As a result, no hypersensitivity reactions were observed in subjects treated with DCV + ASV dual therapy in Japanese and foreign clinical studies.[160)]

On the other hand, the number of subjects with at least two of pyrexia (≥37.7°C), eosinophilia (≥6% of white blood cells), or abnormal LFTs (AST and ALT ≥5 times the upper limit of laboratory reference range) and their clinical course in a Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026) were as shown in Table 60.

Table 60. Number of subjects with at least two of pyrexia, eosinophilia, or abnormal LFTs

	N (%)	Day of onset	Duration	Treatment discontinued	Treatment interrupted	Outcome
Pyrexia, eosinophilia, and abnormal LFTs	2 (0.8)	Day 15-29	10-46 days	N = 2	N = 1	All subjects recovered
Pyrexia and eosinophilia	18 (7.1)	Day 1-43	1-57 days	N = 2 [a)]	N = 1	All subjects recovered
Eosinophilia and abnormal LFTs	4 (1.6)	Day 9-69	20-43 days	N = 4	N = 0	All subjects recovered

No subjects had pyrexia and abnormal LFTs.
a) One subject discontinued due to viral breakthrough.

As described above, a hypersensitivity reaction as defined during the study did not occur following the DCV + ASV dual therapy, whereas there were subjects with at least 2 of the symptoms of a hypersensitivity reaction. Pyrexia, eosinophilia, or abnormal LFTs have been listed in the package insert and no additional precautionary statement about hypersensitivity reactions is needed at present.

PMDA considers as follows:

Among the symptoms of a hypersensitivity reaction, pyrexia and eosinophilia occurred more commonly, but many of the subjects were able to continue treatment. However, as findings suspected of a hypersensitivity reaction were observed in Japanese clinical studies, it is necessary to appropriately provide information on the occurrence of the symptoms of a hypersensitivity reaction to healthcare providers in clinical settings.

159) Patrice C, et al. *Am J Med*. 2011;124:588-597.

160) The subject with suspected hypersensitivity reaction in the Japanese phase III study (Study AI447026) had no skin symptoms and no lymphadenopathy and the subject's condition was not diagnosed as drug-induced hypersensitivity syndrome.

4.(iii).B.(3) Indications

Based on the reviews in "4.(iii).B.(1) Efficacy" and "4.(iii).B.(2) Safety" and the following reviews, PMDA concluded that the appropriate indications are as follows.

Improvement of viraemia in either of the following patients with chronic hepatitis C serogroup 1 (genotype 1), or chronic hepatitis C serogroup 1 with compensated cirrhosis: (1) patients who are treatment-naïve and ineligible for, or who are intolerant of interferon monotherapy or combination therapy with ribavirin, or (2) patients who have failed to respond to interferon-based therapy.

The above conclusion by PMDA will be finalized, taking account of the comments from the expert advisors.

4.(iii).B.(3).1) Genotype

PMDA asked the applicant to explain the appropriateness of the DCV + ASV dual therapy being indicated for patients infected with HCV genotype 1.

The applicant explained as follows:

A Japanese phase II study (Study AI447017) and the Japanese phase III study (Study AI447026) showed that the DCV + ASV dual therapy achieved high SVR24 rates in IFN-ineligible-naïve/intolerant and non-responder patients with HCV genotype 1b (87.4% [118 of 135 subjects] and 80.5% [70 of 87 subjects], respectively). In a foreign early phase II study (Study AI447011), the SVR24 rate was lower in genotype 1a null-responders compared with genotype 1b null-responders [see "4.(iii).B.(1).3) Efficacy in patients with chronic hepatitis C genotype 1a"].

Based on the above results and the fact that most of Japanese patients infected with HCV genotype 1 have genotype 1b (98%-99%), it is appropriate that the DCV + ASV dual therapy be indicated for patients infected with HCV "serogroup 1 (genotype 1)".

PMDA considers as follows:

Although the combination of DCV and ASV can be indicated for patients infected with HCV genotype 1 based on the applicant's explanation, it is necessary to provide the information on clinical data from genotype 1a patients to healthcare providers in clinical settings and to inform them that the efficacy of the DCV + ASV dual therapy in HCV genotype 1a patients has not been established, as the efficacy of the DCV + ASV dual therapy in genotype 1a HCV patients has not been demonstrated. It is also necessary to collect information on the efficacy and safety of the DCV + ASV dual therapy in HCV genotype 1a patients via post-marketing surveillance.

4.(iii).B.(3).2) Use in patients with liver cirrhosis

The applicant explained the efficacy and safety of the DCV + ASV dual therapy in patients with liver cirrhosis

as follows:

In the Japanese phase III study (Study AI447026), the SVR24 rate was 90.9% (20 of 22 subjects) in patients with compensated cirrhosis, which was similar to 84.0% (168 of 200 subjects) in patients with chronic hepatitis. Adverse events (including abnormal laboratory changes) reported by ≥5% of patients with compensated cirrhosis or patients with chronic hepatitis were as shown in Table 61 and the safety profile and the incidence of adverse events in patients with compensated cirrhosis were similar to those in patients with chronic hepatitis.

Table 61. Adverse events reported by ≥5% of subjects (Study AI447026)

	Patients with compensated cirrhosis	Patients with chronic hepatitis
	N = 22	N = 200
Any adverse event	17 (77.3)	175 (87.5)
Nasopharyngitis	7 (31.8)	60 (30.0)
ALT increased	2 (9.1)	33 (16.5)
Headache	1 (4.5)	34 (17.0)
AST increased	1 (4.5)	27 (13.5)
Pyrexia	3 (13.6)	24 (12.0)
Diarrhoea	1 (4.5)	21 (10.5)
Arthralgia	2 (9.1)	11 (5.5)
Nausea	1 (4.5)	11 (5.5)
Eosinophilia	0	11 (5.5)
Malaise	2 (9.1)	8 (4.0)
Rash	0	10 (5.0)
Blood bilirubin increased	0	10 (5.0)
Abdominal discomfort	2 (9.1)	5 (2.5)
Anaemia	2 (9.1)	3 (1.5)
Weight decreased	2 (9.1)	0

n (%)

Based on the above, the DCV + ASV dual therapy showed a favorable safety and tolerability profile also in patients with compensated cirrhosis and is expected to be highly efficacious in those with compensated cirrhosis as in those with chronic hepatitis.

On the other hand, as the ASV exposure is increased in decompensated cirrhosis patients with moderate or severe hepatic impairment (Child-Pugh class B or C) [see "4.(ii).B.(2) Use in patients with hepatic impairment"], the package insert will advise that a diagnosis of chronic hepatitis or compensated cirrhosis be confirmed by histology or liver function, platelet count, etc., and ASV will be contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh class B or C) and the package insert will caution against the use of the DCV + ASV dual therapy in these patients.

PMDA considers as follows:

The DCV + ASV dual therapy can be used in patients with compensated cirrhosis, as the combination achieved a certain level of SVR24 rate in patients with compensated cirrhosis enrolled in the Japanese phase III study (Study AI447026) and there were no particular safety concerns. However, the number of subjects with compensated cirrhosis treated with DCV + ASV dual therapy is very limited; patients with compensated cirrhosis compared with patients with chronic hepatitis are at increased risk of hepatic failure due to impaired liver function; and it is not necessarily easy to differentiate compensated cirrhosis from decompensated cirrhosis. Therefore, it is necessary to appropriately provide information on the liver function of patients enrolled in clinical studies (Child-Pugh class) etc., and then appropriately advise (using materials etc.) that physicians with adequate knowledge and experience in the treatment of viral liver disease carefully select appropriate patients

and that patients with liver cirrhosis be closely monitored by more frequent blood tests and imaging. It is also necessary to collect information via post-marketing surveillance, focusing on efficacy and safety in patients with liver cirrhosis.

4.(iii).B.(3).3) Use in patients who failed prior therapy with an interferon-based regimen containing HCV NS3/4A protease inhibitor

PMDA asked the applicant to explain the efficacy of DCV + ASV dual therapy in patients who were intolerant to or failed prior therapy with an interferon-based regimen containing HCV NS3/4A protease inhibitor (telaprevir or simeprevir).

The applicant explained as follows:

It has been reported that the major resistance mutations detected in the Japanese phase III study are as follows: V36A/C/M, T54A, R155K, A156S, and T54S+A156S[161)] in patients treated with triple therapy containing telaprevir; single D168V and mixtures of mutations at D168 or multiple mutations including mutations at D168[162)] in patients treated with triple therapy containing simeprevir. In non-clinical studies, among these NS3 mutations, R155K, A156S, and D168V were associated with resistance to ASV, but other mutations did not significantly affect the activity of ASV [see "3.(i).A.(4).1).(e) Resistance"].

No clinical studies with the combination of DCV and ASV in patients who failed prior therapy with an interferon-based regimen containing HCV NS3/4A protease inhibitor have been conducted and taking account of the potential risk of cross-resistance, retreatment of HCV with the combination of DCV and ASV in this patient population cannot be recommended.

On the other hand, given that there are patients who have to discontinue treatment due to adverse events characteristic of therapy with an interferon-based regimen containing HCV NS3/4A protease inhibitor, that the *in vitro* activities of ASV against R155K, A156S, and D168V were higher than those of telaprevir or simeprevir, and that the combination of DCV and ASV exhibited additive or synergistic activity against the control genotype 1b replicon, the possibility of retreatment of HCV with DCV + ASV dual therapy can be considered according to the patient's condition for those who discontinued telaprevir or simeprevir due to adverse events early after the start of therapy with an interferon-based regimen containing HCV NS3/4A protease inhibitor.

PMDA considers as follows:

Given that the efficacy and safety of the DCV + ASV dual therapy in patients previously treated with triple therapy containing HCV NS3/4A protease inhibitor have not been studied and that some of the resistance mutations that emerged during triple therapy containing HCV NS3/4A protease inhibitor are cross-resistant to ASV, retreatment cannot be recommended. Although the applicant explained that the *in vitro* activities of ASV against R155K, A156V, and D168V were higher than those of telaprevir or simeprevir, the EC_{50} values of ASV against R155K, A156V, and D168V were lower than those of telaprevir or simeprevir, but indicated an apparent

161) Application dossier for Telavic Tablets 250 mg (telaprevir) CTD 2.7.3
162) Application dossier for Sovriad capsules 100 mg (simeprevir) CTD 2.7.3

trend towards resistance and the relationship between the antiviral activity and efficacy of ASV is unknown. Therefore, it cannot be concluded to be effective based only on comparison of *in vitro* activity which suggested that the effectiveness of ASV can be expected.

However, based on the following point, the possibility of the retreatment efficacy with the combination of DCV and ASV even in patients previously treated with triple therapy containing HCV NS3/4A protease inhibitor cannot be denied.

● It has been confirmed that depending on type of telaprevir- or simeprevir-resistance mutations, susceptibility to ASV is not reduced.

For patients who discontinued treatment for safety problems considered associated with HCV NS3/4A protease inhibitor or PegIFN/RBV, retreatment with the combination of DCV and ASV may be considered.

Based on the above, the term, interferon-based therapy, should be used in the indication statement to describe prior therapy so that IFN-based therapy may or may not contain a protease inhibitor. Meanwhile, it is important to inform that there is some cross-resistance between ASV and similar drugs and to advise that the efficacy and safety of the DCV + ASV dual therapy in patients previously treated with therapy containing a protease inhibitor have not been studied etc., and then to ensure that physicians with adequate knowledge and experience in the treatment of viral liver disease decide whether or not to use DCV + ASV dual therapy in patients who failed prior therapy. If the combination of DCV and ASV is administered to patients previously treated with triple therapy containing HCV NS3/4A protease inhibitor, it is necessary to collect baseline demographics and disease characteristics of patients and efficacy and safety information via post-marketing surveillance and provide the obtained information to medical practice.

4.(iii).B.(3).4) Definition of IFN-ineligible or -intolerant patients

PMDA asked the applicant to explain the need for calling attention to the criteria for IFN-ineligible or -intolerant patients.

The applicant explained as follows:

In a Japanese phase II study (Study AI447017), the criteria for IFN-ineligible patients were defined as follows: patients difficult to be treated with IFN-based therapies due to medical reasons (age or concurrent or prior depression, anemia, myelosuppression, diabetes, hypertension, cardiovascular disorder, or renal impairment) and not scheduled to receive IFN-based therapy for the next 12 months. In the Japanese phase III study (Study AI447026), the criteria for IFN-ineligible patients were defined more objectively for cytopenia, [163)]

[163)] Anemia (hemoglobin at screening <12.0 g/dL), neutropenia (neutrophil count at screening <1500/mm^3), or thrombocytopenia (platelet count at screening <120,000/mm^3)

depression,[164] other comorbidities,[165] and age.[166] On the other hand, the criteria for IFN-intolerant patients were defined as follows: patients who previously received IFN-based therapy for <12 weeks and discontinued from therapy due to adverse drug reactions associated with IFN or RBV. These criteria were established based on the current treatment guidelines for chronic hepatitis C, IFN package insert, and medical experts' opinions, and IFN-ineligible or -intolerant patients in these studies are not considered substantially different from those considered IFN-ineligible or -intolerant in actual medical practice. Therefore, there is no need for calling special attention to the criteria for IFN-ineligible or -intolerant patients, but the information will be provided using healthcare professional-directed materials.

Another Japanese phase III study (Study AI447031) is currently ongoing in treatment-naïve patients with chronic hepatitis C and patients with chronic hepatitis C who have relapsed after prior therapy.

PMDA considers as follows:
The following applicant's explanation is understood: the criteria established for the Japanese phase III study (Study AI447026) are not substantially different from the criteria that would be used in medical practice. However, the information on patients included in Japanese clinical studies should be provided to healthcare providers in clinical settings because there are no clear criteria for IFN eligibility and such information is useful for considering whether or not to use the DCV + ASV dual therapy. As the efficacy and safety of the DCV + ASV dual therapy in treatment-naïve patients with chronic hepatitis C, or HCV with compensated cirrhosis and those who relapsed after prior therapy have not been established and its use in these patients cannot be recommended at present, it is important for the applicant to make efforts to promote proper use of DCV and ASV in clinical settings.

4.(iii).B.(4) Dosage and administration

Based on the reviews provided in the subsequent sections, PMDA concluded as described below:
The following proposed dosage and administration statement for DCV is acceptable: the usual adult dosage is 60 mg of Daclatasvir orally administered once daily; Daklinza should be used in combination with Asunaprevir for a duration of 24 weeks. The following proposed dosage and administration statement for ASV is also acceptable: the usual adult dosage is 100 mg of Asunaprevir orally administered twice daily; Sunvepra should be used in combination with Daclatasvir Hydrochloride for a duration of 24 weeks. The information on the criteria for discontinuation or interruption of DCV or ASV etc., should be provided using the package insert, information leaflet, etc.

The above conclusions by PMDA will be finalized, taking account of the comments from the expert advisors.

4.(iii).B.(4).1) Dosage and administration of DCV

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[164] Patients diagnosed with depression by psychiatrists and judged to be IFN-ineligible by the investigator. Patients with mild to moderate, stable depression at screening were eligible for inclusion in the study.

[165] Patients with current or prior hypertension, diabetes, autoimmune disease, or thyroid dysfunction etc., requiring medication who were judged to be IFN-ineligible by the investigator. Patients with mild or moderate, stable conditions were eligible for inclusion in the study. Patients with severe or unstable (poor control etc., in spite of medication therapy) conditions were excluded.

[166] Elderly (≥65 years) patients judged to be IFN-ineligible by the investigator based on health status, laboratory findings, or co-morbidities etc.

The applicant explained the rationale for dosage and administration of DCV as follows:
In a foreign phase II study with DCV tablets 20 mg or 60 mg QD (Study AI444010),[167] the SVR24 rates were 37.5% (27 of 72 subjects) in the placebo + PegIFNα/RBV group, 59.2% (87 of 147 subjects) in the DCV 20 mg + PegIFNα/RBV group, and 59.6% (87 of 146 subjects) in the DCV 60 mg + pegIFNα/RBV group; the safety profiles in the three groups were similar; and no dose response relationship between 20 mg and 60 mg QD was observed.

Using the E-R model, efficacy in the subject population with disease characteristics associated with poor response to IFN (genotype 1a viral infection, high viral load at baseline [≥7.75 $\log_{10}$ IU/mL], liver cirrhosis, non-CC IL28B polymorphism) was predicted.[168] As a result, the cEVR[169] rate was predicted to be 2% to 5% higher at 60 mg compared to 20 mg.

Based on the above results, the 60 mg QD regimen of DCV tablets was selected for Japanese and foreign phase II studies of the combination of DCV and ASV (Study AI447011 and Study AI447017), and DCV tablets 60 mg QD in combination with ASV tablets 200 mg BID showed high efficacy and favorable safety profile. Also in the Japanese phase III study (Study AI447026), DCV tablets 60 mg QD in combination with ASV softgel capsules 100 mg BID showed high efficacy and favorable safety profile.

Based on the above, the 60 mg QD regimen of DCV tablets was selected for coadministration with ASV.

PMDA considers that there is no particular problem with the proposed 60 mg QD regimen of DCV.

4.(iii).B.(4).2) Dosage and administration of ASV

The applicant explained the rationale for dosage and administration of ASV as follows:
In a foreign dose-finding study of ASV (Study AI447016),[170] the dose of ASV for coadministration with PegIFNα-2a/RBV was determined. Since the incidences of AST and ALT elevations were higher with ASV tablets 600 mg QD or 600 mg BID compared with 200 mg BID[171] and the antiviral activity (the primary endpoint of the eRVR[172] rate) was comparable, the protocol was amended to use ASV tablets 200 mg BID in combination with DCV tablets 60 mg QD during the ongoing Japanese and foreign phase II studies of the combination of DCV and ASV (Study AI447017 and Study AI447011). The results of a relative BA study [see "4.(i).A.(2).1) Relative BA and food effect studies"] indicated that ASV softgel capsules 100 mg BID provides comparable plasma exposure to ASV tablets 200 mg BID and the Japanese phase III study of ASV softgel capsules 100 mg BID in combination with DCV tablets 60 mg QD (Study AI447026) demonstrated the efficacy and safety of the

167) Treatment-naïve patients with chronic hepatitis C genotype 1 or 4 received DCV tablets 20 mg, 60 mg, or placebo QD in combination with PegIFNα-2a/RBV.
168) In order to assess the E-R relationship in patients considered treatment-refractory, a logistic regression model for anti-viral effect as a function of DCV C_{avgss} included HCV subgenotype, viral load at baseline, the presence or absence of cirrhosis, and IL28B polymorphism as covariates.
169) HCV RNA <LLOQ (undetectable) at Week 12.
170) Treatment-naïve patients with chronic hepatitis C genotype 1 or 4 received ASV tablets 200 mg BID, 600 mg BID, 600 mg QD, or placebo in combination with PegIFNα-2a/RBV.
171) The incidences of Grade 3 or worse laboratory abnormalities at 200 mg BID, 600 mg QD, and 600 mg BID were 0% (0 of 12 subjects), 16.7% (2 of 12 subjects), and 8.3% (1 of 12 subjects), respectively, for both parameters.
172) HCV RNA <LLOQ (undetectable) at both Weeks 4 and 12.

combination of DCV and ASV in patients with chronic hepatitis C, or HCV with compensated cirrhosis.

Based on the above, the 100 mg BID regimen of ASV softgel capsules was selected for coadministration with DCV.

PMDA considers as follows:
In a Japanese phase II study (Study AI447017), following coadministration of ASV tablets 600 mg BID and DCV tablets 60 mg QD in 10 Japanese subjects in the sentinel cohort, the incidences of AST increased and ALT increased were both 20% (2 of 10 subjects), which were not different from those in the Japanese phase III study using ASV softgel capsules 100 mg BID (Study AI447026). Thus, it is not clear whether or not dose adjustment from ASV tablets 600 mg BID to ASV tablets 200 mg BID was optimal. However, the Japanese phase III study (Study AI447026) demonstrated the usefulness of DCV + ASV dual therapy in Japanese patients with chronic hepatitis C, or HCV with compensated cirrhosis and its safety is also acceptable. Therefore, the proposed 100 mg BID regimen of ASV softgel capsules for coadministration with DCV is acceptable.

4.(iii).B.(5) Clinical positioning
The applicant explained the clinical positioning of the DCV + ASV dual therapy in non-responder and IFN-ineligible-naïve/intolerant patients with chronic hepatitis C, or HCV with compensated cirrhosis as follows:
According to the Japanese guidelines,[2)] for non-responders, the indication should be determined based on age and the degree of fibrosis and triple therapy with PegIFN/RBV/simeprevir is recommended if appropriate. Although its therapeutic effect in IFN-naïve patients and relapsers after prior therapy is better than that of PegIFN/RBV combination therapy, which has been standard of care, the SVR24 rate in non-responders has been reported to be 38.5% to 50.9%, which is not sufficient. In addition, triple therapy with PegIFN/RBV/simeprevir has the following problems: patients have to attend hospital every week to receive PegIFN; dose reduction or discontinuation of PegIFN or RBV may be required according to the patient's condition; and there are many patients who are difficult to be treated due to adverse drug reactions. Patients with anaemia, neutropenia, thrombocytopenia, depression, or other comorbidities and elderly patients are not eligible for current IFN-based antiviral therapies and their options are liver protection therapy, long-term administration of low-dose PegIFN, etc. In light of the above situation, no effective therapy is available and the development of an effective, new treatment associated with fewer adverse drug reactions is needed for patients with chronic hepatitis C, especially those who are difficult to be treated with IFN and IFN-nonresponders. The results of the Japanese phase III study (Study AI447026) showed that the DCV + ASV dual therapy achieved a high SVR24 rate and that this all-oral regimen was easy for administration and well-tolerated [see "4.(iii).B.(1) Efficacy and 4.(iii).B.(2) Safety"]. Moreover, as high effectiveness is expected, regardless of age or the degree of fibrosis [see "4.(iii).B.(1) Efficacy"], the DCV + ASV dual therapy will become the first-line treatment for these patients.

PMDA considers as follows:
While the Japanese phase III study (Study AI447026) was conducted as an open-label, uncontrolled study and there are limitations in evaluation, the DCV + ASV dual therapy demonstrated a high SVR24 rate. Although there are safety issues to be noted, such as the emergence of resistant virus and abnormal LFTs, the combination

of DCV and ASV is useful for non-responder and IFN-ineligible-naïve/intolerant patients with chronic hepatitis C, or HCV with compensated cirrhosis, as long as monitoring and management of adverse events are conducted and appropriate actions such as interruption/discontinuation of treatment are taken with an adequate understanding of the safety profile of the DCV + ASV dual therapy under the supervision of physicians with adequate knowledge and experience in the treatment of viral liver disease. Based on the above, the DCV + ASV dual therapy will become a therapeutic option for non-responders and can offer a therapeutic opportunity also for IFN-ineligible-naïve/intolerant patients.

4.(iii).B.(6) Post-marketing investigations

The applicant plans to conduct the following post-marketing surveillance study.

[Drug use-results survey]

● Objectives: collecting safety and efficacy information in routine clinical settings and evaluating safety and efficacy

● Target number of patients: 3000 (including 300 patients with HCV with compensated cirrhosis)

【Basis for sample size determination】

The total target enrollment is 3000 patients, which provides a 95% probability of detecting at least one case of an unknown adverse drug reaction with an incidence of 0.1%. As about 10% of the Japanese study population enrolled were patients with HCV with compensated cirrhosis, the target number of patients with HCV with compensated cirrhosis in this survey is also 300 (10%).

● Survey period: 2 years and 6 months

PMDA considers that it is necessary to collect the following post-marketing information.

● Association between efficacy and resistance mutations at baseline

● Occurrence of abnormal LFTs

● Efficacy and safety in patients with compensated cirrhosis

● Efficacy and safety in patients infected with HCV genotype 1a

● Efficacy and safety in patients previously treated with triple therapy containing HCV NS3/4A protease inhibitor

The above conclusions by PMDA will be finalized, taking account of comments from the expert advisors.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.2.1, 5.3.5.2.2). As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

IV. Overall Evaluation

Based on the submitted data, the efficacy of the DCV + ASV dual therapy in non-responder and IFN-ineligible-naïve/intolerant patients with chronic hepatitis C, or HCV with compensated cirrhosis (genotype 1) can be expected and its safety is acceptable. However, as resistance mutations are considered associated with efficacy; abnormal LFTs and hypersensitivity reactions may occur; there is limited clinical experience in patients with compensated cirrhosis; and there are patient populations in whom experience with DCV + ASV is limited, it is necessary to continue to collect the following information via post-marketing surveillance.

- Association between efficacy and resistance mutations
- Occurrence of abnormal LFTs
- Efficacy and safety in patients with compensated cirrhosis
- Efficacy and safety in patients infected with HCV genotype 1a
- Efficacy and safety in patients previously treated with triple therapy containing HCV NS3/4A protease inhibitor

The proposed products (Daclatasvir Hydrochloride and Asunaprevir) may be approved if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

Review Report (2)

June 5, 2014

I. Products Submitted for Registration

[Brand name]	(a) Daklinza Tablets 60 mg
	(b) Sunvepra Capsules 100 mg
[Non-proprietary name]	(a) Daclatasvir Hydrochloride
	(b) Asunaprevir
[Applicant]	Bristol-Myers K.K.
[Date of application]	October 29, 2013

II. Content of the Review

The Expert Discussion and subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below. The expert advisors for the Expert Discussion were nominated based on their declarations etc., concerning the products submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc., by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

PMDA's conclusions described in the Review Report (1) were largely supported at the Expert Discussion and PMDA conducted an additional review of the following points and took necessary actions.

(1) Efficacy

PMDA's conclusions on the efficacy of the DCV + ASV dual therapy [see "Review Report (1), II.4.(iii).B.(1) Efficacy"] were agreed and the expert advisors made the following comments on the influence of viral mutations on efficacy:

● As the SVR24 rates in subjects with baseline NS5A Y93H or L31M/V were extremely low, resistance analysis should be performed prior to treatment and the DCV + ASV dual therapy should not be used if a resistance-associated variant (RAV) is present. NS5A RAV together with NS3 RAV (at D168) were detected in subjects without SVR at the time of failure and there is a concern that drugs with a similar mechanism of action become ineffective.

● As resistance analyses and the information on RAVs can become important elements for selecting therapy or predicting therapeutic response, it is recommended that drug resistance testing be performed prior to the start of treatment.

● Although the presence or absence of RAVs is important information, this test is not reimbursable under the National Health Insurance system and in practice, the testing costs are expected to be covered by research grants or paid out-of-pocket by patients. Given that the DCV + ASV dual therapy achieved 30% to 40% efficacy rates even in patients with NS5A RAVs, this therapy can be used if approved drugs cannot be used or are likely to exhibit low efficacy. Taking account of these situations, drug resistance testing before the start of treatment

is not mandatory.

Based on the above comments from the expert advisors, PMDA conducted the following reviews, which were agreed by the expert advisors:
Whether or not the DCV + ASV dual therapy should be used needs to be decided appropriately, based on the information obtained during its development. Thus, the currently available information on the association between resistance mutations and SVR should be provided to healthcare providers in clinical settings. Then, given that the DCV + ASV dual therapy may achieve SVR even in patients with NS5A RAVs who cannot receive IFN-based therapy or who have failed to respond to IFN-based therapy, the DCV + ASV dual therapy should not be prohibited in patients with RAVs and physicians with adequate knowledge and experience in the treatment of viral liver disease should decide whether or not to use DCV and ASV, after being well informed of the results of clinical studies.

Based on the above, PMDA instructed the applicant to include the following statement in the precautions of indications section of the package insert and the applicant accepted it.

> Physicians with adequate knowledge and experience in the treatment of viral liver disease should decide whether or not to use DCV and ASV, after being well informed of the results of clinical studies (see "OTHER PRECAUTIONS" and "CLINICAL STUDIES").

The indication statement concerning the use in IFN-ineligible-naïve/intolerant patients will be modified as "(1) patients who are treatment-naïve and ineligible for, or who are intolerant of interferon-based therapy".

(2) Draft drug risk management plan
PMDA's conclusions on post-marketing surveillance [see "Review Report (1), II.4.(iii).B.(6) Post-marketing investigations"] were supported by the expert advisors and the following comment was added:
● As hepatic dysfunction occurred commonly and hepatic dysfunction leading to treatment discontinuation was also reported in clinical studies, careful monitoring is needed after the market launch. Patients with compensated cirrhosis should be treated with DCV and ASV more carefully.

Based on the comment from the expert advisor, PMDA considers that in relation to the use in patients with compensated cirrhosis, Child Pugh classes should also be recorded and the following information should be collected via post-marketing drug-use results survey.
● Association between efficacy and resistance mutations at baseline
● Occurrence of abnormal LFTs
● Efficacy and safety in patients with compensated cirrhosis
● Efficacy and safety in patients infected with HCV genotype 1a
● Efficacy and safety in patients previously treated with triple therapy containing HCV NS3/4A protease inhibitor

As discussed in "(1) Efficacy", the information on NS5A RAVs at baseline is considered important, and drug resistance testing should be performed at a central laboratory in the post-marketing surveillance study so that the same assay procedures, the same assay conditions, etc., are used and the medical institutions should be informed of the test results of their patients as soon as possible and upon completion of examining "the association between efficacy and resistance mutations at baseline" become available, this information should be provided to healthcare providers in clinical settings.

PMDA instructed the applicant to consider the above points and the applicant accepted it.

Based on the above discussion, PMDA concluded that the safety specification and efficacy concerns as shown in Table 62 should be included in the current draft drug risk management plan and that additional pharmacovigilance activities and risk minimization activities as shown in Table 63 should be performed. An outline of drug-use results survey plan was submitted as shown in Table 64.

Table 62. Safety specification and efficacy concerns of draft drug risk management plan

Safety specification		
Important identified risks	Important potential risks	Important missing information
[DCV and ASV] · Hepatic dysfunction	[DCV] · Hematotoxicity [ASV] None	[DCV and ASV] None
Efficacy concerns		
[DCV and ASV] · Efficacy in routine clinical settings · Emergence of drug resistance		

Table 63. Summary of additional pharmacovigilance activities and risk minimization activities in draft drug risk management plan

Additional pharmacovigilance activities	Additional risk minimization activities
[DCV and ASV] · Early post-marketing phase vigilance (EPPV) · Drug use-results survey	[DCV and ASV] · Development and distribution of healthcare professional-directed materials · EPPV

Table 64. Outline of draft post-marketing surveillance plan

Drug use-results survey	
Objective	To confirm the safety and efficacy of the combination of DCV and ASV in routine clinical settings
Survey method	Central registration system
Patients to be surveyed	Patients with chronic hepatitis C or patients with HCV with compensated cirrhosis
Survey period (Observation period)	2 years and 6 months (Safety, 28 weeks; Efficacy, 48 weeks)
Planned number of cases	3000 patients (including 300 patients with HCV with compensated cirrhosis)
Main information to be collected	Hepatic dysfunction, Emergence of drug resistance

III. Overall Evaluation

As a result of the above review, PMDA concludes that the products may be approved after modifying the indication and dosage and administration statements as shown below. As DCV and ASV are drugs with a new active ingredient, the re-examination period is 8 years, the drug substance and the drug product are both classified as powerful drugs, and the product is not classified as a biological product or a specified biological product, for both DCV and ASV.

[Indications]	Improvement of viraemia in either of the following patients with chronic hepatitis C serogroup 1 (genotype 1), or chronic hepatitis C serogroup 1 with compensated cirrhosis: (1) patients who are treatment-naïve and ineligible for, or who are intolerant to interferon-based therapy, or (2) patients who have failed to respond to interferon-based therapy.
[Dosage and administration]	[Daklinza Tablets 60 mg] The usual adult dosage is 60 mg of Daclatasvir orally administered once daily. Daklinza should be used in combination with Asunaprevir for a duration of 24 weeks. [Sunvepra Capsules 100 mg] The usual adult dosage is 100 mg of Asunaprevir orally administered twice daily. Sunvepra should be used in combination with Daclatasvir Hydrochloride for a duration of 24 weeks.