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List of abbreviations:
HCV, hepatitis C virus; SVR, sustained virological response; ACTG, AIDS Clinical Trials Group; ALT, alanine aminotransferase; AUC, area under the plasma concentration versus time curve; C_max, observed maximum plasma concentration; PI, protease inhibitor; AUC_0-∞, area under the curve from time 0 to infinity; C_{12h}, plasma concentration 12 hours after dosing; C_{trough}, trough concentration; danoprevir/r, oral danoprevir plus oral ritonavir; placebo/r, oral danoprevir placebo plus oral ritonavir; BID, twice daily; QD, once daily; LC-MS/MS, liquid chromatography with tandem mass spectrometric detection; AUC_{0–τ,ss}, steady-state area under the curve over the dosing interval; C_{max,ss}, observed maximum plasma concentration at steady state; C_{trough,ss}, observed trough concentration at steady state; EC_{50}: effective concentration at which a 50% reduction in replication is observed, T_{max,ss} = time to maximum plasma concentration at steady state

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Abstract (Word count: 250 words)

Background and Aims
Danoprevir (RG7227; ITMN-191) is a potent inhibitor of the HCV NS3/4A serine protease. The aims of this double-blind, placebo-controlled, multiple-ascending dose phase Ib study were to evaluate safety, tolerability, antiviral activity, resistance and pharmacokinetics of once- and twice-daily danoprevir in the presence of low-dose ritonavir (danoprevir/r) and in combination with peginterferon alfa-2a (40KD)/ribavirin in treatment-naive HCV genotype 1 patients.

Methods
Thirty eligible patients were enrolled into 3 cohorts and treated with danoprevir/r or placebo/r all in combination with peginterferon alfa-2a (40KD)/ribavirin for 15 days. Cohort 1 received danoprevir/r at 100/100mg twice daily; Cohort 2 200/100mg once daily; and Cohort 3 200/100mg twice daily.

Results
The median reductions in HCV RNA from baseline after 14 days of treatment (day 15) were –5.1, –4.8 and –4.6 log_{10} IU/mL in Cohorts 1, 2 and 3, respectively, and –2.7 log_{10} in placebo/r and peginterferon alfa-2a (40KD)/ribavirin recipients. Viral breakthrough was not observed in any patient. On day 15 HCV RNA was undetectable (<15 IU/mL) in 6/9 (67%), 4/8 (50%) and 8/8 (100%) patients in
Cohorts 1, 2 and 3 respectively. When co-administered with low dose ritonavir, danoprevir concentrations reached steady state between 6 to 10 days of dosing. Danoprevir exposures increased more than dose proportionally between 100/100 mg and 200/100 mg. Danoprevir/r plus peginterferon alfa-2a (40KD)/ribavirin was well-tolerated with no safety-related discontinuations.

**Conclusions**

Danoprevir/r plus peginterferon alfa-2a (40KD)/ribavirin provides profound and robust reductions in serum HCV RNA, at substantially lower systemic exposures compared to those observed with higher doses of danoprevir in the absence of ritonavir. These results support further studies of danoprevir/r.

**Keywords**: hepatitis C; protease inhibitor; virological response; danoprevir; ritonavir
Introduction

Approximately half of patients infected with genotype 1 hepatitis C virus (HCV) achieve a sustained virological response (SVR) after treatment with the combination of pegylated interferon plus ribavirin for 48 weeks, the current standard of care for chronic HCV infection [1]. Thus, new drugs are required to improve treatment efficacy.

Several classes of direct acting antiviral agents are under clinical development with the primary objective of increasing SVR rates in genotype 1 HCV infection. Danoprevir (RG7227; ITMN-191) is a potent, selective, macrocyclic inhibitor of the HCV NS3/4A serine protease [2,3]. When administered orally to patients with chronic hepatitis C at doses up to 900 mg twice daily in combination with peginterferon alfa-2a (40KD) plus ribavirin for 15 days, the median reduction in serum HCV RNA level was 5.3 log_{10} and no patient experienced viral breakthrough [4]. Treatment of patients with the 900 mg twice daily dose in an ongoing phase IIb study has been discontinued because of asymptomatic AIDS Clinical Trials Group (ACTG) Grade 4 alanine aminotransferase (ALT) elevations (>10 times above the upper limit of normal) documented in three out of 194 patients [5]. Higher danoprevir systemic exposure (area under the plasma concentration versus time curve [AUC] and observed maximum plasma concentration [C_{max}]) was subsequently shown to be associated with higher probability of ALT elevations [6].
Ritonavir, an HIV-1 protease inhibitor (PI), is currently used as a pharmacokinetic enhancer at low doses ranging from 100 to 400 mg/day in combination with other HIV PIs [7]. Although ritonavir displays a mixed inhibition and induction effect on the cytochrome P450 3A (CYP3A), its inhibitory effect is predominant at steady state [8]. As a result, when co-administered with a low sub-therapeutic dose of ritonavir, the pharmacokinetics of HIV PIs that are substrates of CYP3A are significantly enhanced, allowing for simplification of the dosing regimen of most HIV PIs, including a reduction in the dosing frequency and/or a decrease in the number of tablets or capsules needed to maintain therapeutic drug concentrations [7].

Since danoprevir is a substrate of CYP3A, low-dose ritonavir also has the potential to enhance the pharmacokinetic profile of danoprevir. Such changes have been demonstrated in healthy volunteers who received danoprevir 100 mg single dose before and after administration of ritonavir 100 mg twice daily for 10 days. Danoprevir area under the curve from time 0 to infinity (AUC₀–∞), C_max, and plasma concentration 12 hours after dosing (C₁₂h) were increased by approximately 5-fold, 3-fold, and 42-fold, respectively, compared with danoprevir alone [9]. Given the substantial effect of low-dose ritonavir on danoprevir C₁₂h, which is equivalent to trough concentration (Cₜrough) for a twice-daily regimen, a reduced danoprevir dose and overall exposure (AUC and C_max) could be explored while still maintaining danoprevir concentrations above the efficacy threshold.
The objectives of this trial were to evaluate the safety, tolerability, antiviral activity, and pharmacokinetics of once- and twice-daily reduced danoprevir doses in the presence of low-dose ritonavir (danoprevir/r) and in combination with peginterferon alfa-2a (40KD) plus ribavirin in patients with chronic hepatitis C genotype 1 infection.

Materials and methods

Study design

This randomized, placebo-controlled, and partially blinded study was conducted between August and December 2009 at 4 study centers in New Zealand, France, and Poland. The protocol was approved by the research ethics board at each participating institution. The research was conducted in accordance with the Declaration of Helsinki and tenets of Good Clinical Practice. All patients provided written informed consent before undergoing any study-specific assessments or procedures.

Thirty eligible patients were assigned to 1 of 3 cohorts in which they were randomized (within each cohort) to 15 days of treatment with either oral danoprevir plus oral ritonavir (danoprevir/r), or oral danoprevir placebo plus oral ritonavir (placebo/r) as follows: danoprevir 100 mg twice daily plus ritonavir 100 mg twice daily (danoprevir/r 100/100 mg BID), or placebo/r 0/100 mg BID (Cohort 1); danoprevir/r 200/100 mg once daily (QD), or placebo/r 0/100 mg QD (Cohort
2); danoprevir/r 200/100 mg BID, or placebo/r 0/100 mg BID (Cohort 3). The computerized randomization list was generated by the sponsor and maintained by a third party. Randomization was managed through a centralized interactive voice and web response system. All patients received concurrent treatment with subcutaneous peginterferon alfa-2a (40KD) (PEGASYS®, Roche, Basel, Switzerland) 180 µg once weekly plus oral ribavirin 1000 mg/day (bodyweight <75 kg) or 1200 mg/day (bodyweight ≥75 kg). After completion of the 15 day experimental phase of the trial, patients continued treatment with peginterferon alfa-2a (40KD) plus ribavirin for a total of 48 weeks as recommended in contemporary treatment guidelines for chronic hepatitis C [1]. All oral medications (danoprevir or placebo, ritonavir, ribavirin) were administered together with approximately 240 mL of water within 30 mins of the start of breakfast (morning dose) or within 30 min of a snack or meal (evening dose).

Patients were confined in the clinical study unit on 2 separate occasions: from the day before dosing started until the morning of study day 2, and again from the evening of study day 13 to the morning of study day 16. From study days 3 to 12, patients returned each morning for observed dosing of study medications. The evening doses of medications from study days 3 to 12 were self-administered at home. Patients returned to the clinical study unit 14 days after dosing for follow-up visit safety assessments. Following the completion of study drug treatment on day 15, patients had the option to continue receiving treatment of peginterferon alfa-2a and ribavirin for HCV treatment at the discretion of their treating physician.
outside of the study protocol, as required by local regulations. Treatment outcomes after cessation of danoprevir/r or placebo/r are not captured and hence not reported.

Study population
HCV treatment-naive adult patients aged 18 to 65 years with chronic hepatitis C genotype 1 infection, an HCV RNA level ≥1 x 10^5 IU/mL, a body mass index between 18 and 35 kg/m^2 and without evidence of liver cirrhosis on a liver biopsy or non-invasive procedure (e.g. Fibroscan) obtained within the preceding 24 months were eligible for the trial.

Patients were ineligible for the trial if they had decompensated liver disease; impaired liver function (indicated by a history of ascites, hepatic encephalopathy, hepatocellular carcinoma or bleeding oesophageal varices); chronic liver disease attributed to a cause other than HCV; or serological evidence of hepatitis B virus or HIV infection.

Patients were also ineligible if they had an increased risk of anemia; a clinically significant medical condition such as cardiovascular or cerebrovascular disease, chronic pulmonary disease, poorly controlled thyroid function, diabetes mellitus requiring medication, ophthalmic disorders related to diabetes or hypertension, or diseases associated with alterations in immune function; or a history of clinically significant psychiatric disease, a history of excessive alcohol consumption
(defined as more than 2 standard drinks per day within the previous 3 months), or a history of drug abuse within the last year.

Other exclusion criteria were serum ALT level >5 times the upper limit of normal, creatinine clearance <50 mL/min, haemoglobin <120 g/L (if female) or <130 g/L (if male), an absolute neutrophil count $\leq 1.5 \times 10^9$/L, platelet count $\leq 100 \times 10^9$/L, or serum albumin level <35 g/L.

Female patients of child-bearing potential and male partners of potentially fertile women were required to use 2 forms of non-hormonal contraception during treatment and for 6 months after the end of treatment. Pregnant and lactating women and male partners of pregnant women were not eligible.

Due to the potential for drug–drug interactions with either danoprevir or ritonavir, patients were not eligible for the study if they had any recent use or anticipated need for drugs, herbal preparations or nutrients known to inhibit or induce CYP enzymes, or were substrates of CYP3A or CYP2C9 with a narrow therapeutic index (including oral contraceptives, steroids, antacids, H-2 blockers or proton-pump inhibitors). Systemic immunosuppressive drugs, cytotoxic or chemotherapeutic agents, radiation therapy, oral or inhaled corticosteroids, or topical class 1 and 2 steroids, were also prohibited during this study. Use of paracetamol (acetaminophen) at dosages of $\leq 4$ g/day was permitted. Other
concomitant medications were prohibited during the study, unless required to manage treatment-emergent adverse events.

Participants were required to abstain from strenuous exercise such as weight lifting or aerobics within 72 h of receipt of the first dose of study drug and throughout treatment. Alcohol consumption was prohibited at least 48 hours before dosing and up through 7 days after the last dose of study drug.

**Pharmacokinetic assessments**

Blood samples for determination of danoprevir concentrations were collected prior to drug intake and at 0.5, 1, 2, 3, 4, 6, 8, and 12 h post-dose for all regimens and at 24 h post-dose (for the QD regimen) on days 1 and 14. Morning pre-dose blood samples were collected on days 2, 3, 6, 9, and 12.

Blood samples were drawn by direct venipuncture in a forearm vein using a Vacuette (Greiner-Bio One, Monroe, NC) tube containing potassium EDTA anticoagulant. Plasma was extracted after centrifugation within 60 min of sample collection and stored at −20°C until being shipped to the analytical site for sample analysis. Measurements of danoprevir in plasma were performed using a validated and specific liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) assay by Covance Laboratories Inc. (Madison, WI). Danoprevir and the internal standard, ARRY-333802, were extracted from human plasma by solid-phase extraction. After evaporation under nitrogen, the residue
was reconstituted prior to analysis by LC-MS/MS. Results were calculated using peak area ratios of analyte to internal standard. Calibration curves for danoprevir in human plasma ranged from 10 to 10 000 pg/mL and were generated using a weighted (1/x2) linear least-squares regression. Back-calculated concentrations for all calibration curve points and the results for the calibration standards, quality control samples and danoprevir samples were evaluated. The analytical runs for this study met acceptance criteria.

Danoprevir pharmacokinetic parameters including steady-state area under the curve over the dosing interval (AUC\textsubscript{0–τ,ss}), observed maximum plasma concentration at steady state (C\textsubscript{max,ss}), observed trough concentration at steady state (C\textsubscript{trough,ss}), and time to C\textsubscript{max,ss} (T\textsubscript{max,ss}) were estimated by non-compartmental methods using WinNonlin (v5.2.2 Pharsight Corporation, Mountain View, CA).

**HCV RNA measurements**

Blood samples for determination of HCV RNA levels were collected pre-dose and at 4 and 12 h post-dose on day 1, and before administration of the morning dose on days 2, 3, 6, 9, 12, 14, 15, 16, 17, and 28. Serum HCV RNA levels were determined by real-time PCR assay (COBAS® Ampliprep/COBAS® TaqMan® HCV test; detection limit 15 IU/mL, Roche Diagnostics North America, Indianapolis, IN).
**Drug resistance monitoring**

Blood samples were collected on days 3, 6, 9, 12, 14, 15, 16, 17, and 28 to evaluate the potential for danoprevir resistance development.

The criteria for sample selection for resistance monitoring (through population sequencing and drug susceptibility phenotypic analysis) were as follows: samples at baseline were sequenced for all patients spanning the complete NS3/4A coding region. Based on viral load kinetics the following samples were also studied: 1) the first sample after viral breakthrough (≥1 log IU/mL increase in HCV RNA above nadir on at least 2 consecutive measurements); 2) end of treatment sample in non-responders (<0.5 log IU/mL decrease in viral load from nadir); 3) end-of-treatment sample in partial response patients (an initial viral load decline ≥ 0.5 log IU/mL from baseline followed by stabilization or an HCV RNA level ≥1000 IU/mL at the end of treatment with danoprevir). Clonal analysis was performed if viral breakthrough, partial or non response was observed and population sequence did not detect resistance mutations or if mixtures of resistance mutations were observed by population sequence.

Amplification of the entire NS3/4A coding region was performed through reverse transcription and nested PCR. Sequencing was performed using primers covering both DNA strands using the ABI 3730 xl DNA Analyzer. Sequences were analyzed using Sequencher and VNTI software programs. The IUPAC
(International Union of Pure and Applied Chemistry) nucleotide ambiguity code was used to call nucleotide positions where more than one base was observed (mixture of nucleotides). The sensitivity of this methodology allows the detection of minority variants at a frequency of ~20%.

Phenotypic characterization of samples was to be performed by transferring the NS3 protease coding sequence amplified from baseline and corresponding on-treatment samples from patients experiencing viral load rebound, non-response or partial response into a replicon shuttle vector and testing susceptibility to danoprevir in vitro. Reduced susceptibility was to be expressed as the fold-change in the EC$_{50}$ of the on-treatment sample compared with the baseline sample.

**Safety assessments**

Patients were monitored for safety and tolerability at regular intervals from the start of study drug dosing through a follow-up visit 2 weeks after completion of study drug dosing. Safety assessments included physical examination, clinical laboratory tests, ECGs measurements and monitoring of adverse events.

**Statistical analysis**

Sample sizes for each cohort were determined by practical considerations, and no formal hypothesis testing was planned. All patients who received at least 1 dose of the study medication, whether prematurely withdrawn from the study or
not, were included in the safety analysis. Adverse events, vital signs, laboratory tests, HCV RNA, and pharmacokinetics were descriptively compared across the various treatment groups. The study was not powered to detect the difference between danoprevir/r and placebo/r and therefore no statistical comparison was performed.

Results
A total of 30 patients were treated in this study and divided into 3 cohorts. 25 patients were assigned to 1 of the 3 danoprevir/r dosing cohorts, and 5 were assigned to placebo/r. Baseline characteristics are described in Table 1.

HCV RNA levels
Individual HCV RNA levels during the 2-week treatment of danoprevir/r or placebo/r plus peginterferon alfa-2a (40KD)/ribavirin are shown in Figure 1. The earliest timepoint for undetectable HCV RNA was observed at day 3 in each dose level. Between 63% and 67% of patients treated with twice-daily danoprevir/r plus peginterferon alfa-2a (40KD)/ribavirin had undetectable HCV RNA (<15 IU/mL) by day 9 of treatment. All 8 patients (100%) treated with the highest dose of danoprevir/r (200/100 mg BID) had undetectable HCV RNA levels (<15 IU/mL) on day 15, as compared with 6 of 9 (67%) patients treated with the lower BID regimen (100/100 mg) and 4 of 8 (50%) of those treated with the QD regimen (200/100 mg) (Figures 3 and 4, Table 2).
HCV RNA levels decreased by $-4.6$ to $-5.1 \log_{10} \text{IU/mL}$ between baseline and day 15 in the 3 danoprevir/r plus peginterferon alfa-2a (40KD)/ribavirin cohorts, compared to only a $-2.7 \log_{10} \text{IU/mL}$ reduction in the cohort treated with peginterferon alfa-2a (40KD)/ribavirin in combination with placebo/r (Figures 1 and 2, Table 2).

All patients experienced a continuous viral load decline. Viral breakthrough; partial response or non-response was not observed in any patient during treatment with danoprevir/r plus peginterferon alfa-2a (40KD)/ribavirin (Figure 1); thus, resistance analysis was performed through population sequencing of baseline samples for all patients, with no danoprevir resistance mutations observed in any patient. No further analysis of on-treatment samples was performed.

**Pharmacokinetics**

Following oral administration of danoprevir/r with food, danoprevir median $T_{\text{max}}$ ranged between 2.0 and 3.0 hours. In the presence of low-dose ritonavir, danoprevir concentrations increased during the first 2 to 3 days of dosing and then gradually declined toward steady state after 6 to 10 days of dosing (data not shown), reflecting the mixed inhibition/induction effect of ritonavir on CYP3A.

Danoprevir steady-state pharmacokinetic parameters are summarized in Table 3. Danoprevir exposures increased more than dose proportionally between 100/100 mg and 200/100 mg. Doubling the dose of danoprevir (100/100 mg BID to
200/100 mg BID) resulted in an approximately 4- to 5-fold increase in exposure. The terminal elimination phase of the plasma concentration-time profiles was not adequately characterized and therefore terminal elimination half life ($t_{1/2}$) cannot be appropriately determined.

Exposure-response relationships were evaluated by fitting $E_{\text{max}}$-type models via non-linear regression. No significant relationship between danoprevir exposure parameters and various measures of antiviral activity were identified (data not shown).

**Safety**

Safety and tolerability data are presented in Table 4. There were no treatment-related discontinuations, withdrawals, or dose reductions of danoprevir/r. Only one serious adverse event was reported during the study. This patient had been assigned to the danoprevir/r 100/100 mg BID and experienced altered mood during the follow-up period 2 wks after the last dose of danoprevir/r while receiving ongoing treatment with peginterferon alfa-2a (40 KD) plus ribavirin. The patient required overnight hospitalization.

Mean serum ALT values decreased during treatment in all treatment groups. No patient in any of the 3 danoprevir/r cohorts experienced Grade 3 or 4 ALT elevations.
Adverse events were generally mild in severity and similar in nature to those associated with treatment with peginterferon alfa-2a (40KD) plus ribavirin.

Discussion
This study demonstrates that danoprevir/r in combination with SOC provides significant reductions in serum HCV RNA levels at much lower overall exposures (AUC and C\text{max}) than with danoprevir alone. Danoprevir/r in combination with peginterferon alfa-2a (40 KD) plus ribavirin has a promising safety profile and was well tolerated in patients infected with genotype 1 HCV.

Danoprevir/r regimens provided potent antiviral activity. Indeed, in each of the 3 danoprevir/r cohorts, a higher proportion of patients had undetectable HCV RNA levels after 14 days of treatment (50–100%) than in a historical control group treated with danoprevir 900 mg BID (14%) [4]. Similarly, the time taken to achieve undetectable HCV RNA for all three danoprevir/r cohorts in this study (day 9 for BID regimens and day 12 for QD regimen) appears to be faster than in the historical 900 mg BID group (day 15) [4]. It should be noted that the comparison to historical 900 mg BID data is limited by two major factors: 1) a small sample size and 2) the different lower limit of detection for the HCV RNA assay (9.3 IU/mL for historical vs 15 IU/mL for this study).

The addition of an investigational HCV protease inhibitor (telaprevir) to peginterferon alfa-2a (40KD)/ribavirin significantly increased both the early on-
treatment virological response rate at week 4 (90% vs. 15% with peginterferon alfa-2a (40KD)/ribavirin alone) and the SVR rate (75% vs. 45% with peginterferon alfa-2a (40KD)/ribavirin alone) in genotype 1 patients enrolled in phase II clinical trials [10–12]. The rate and magnitude of reduction in serum HCV RNA levels with all danoprevir/r regimens plus peginterferon alfa-2a (40KD)/ribavirin in the present study appeared to be at least comparable with a telaprevir-based triple therapy regimen in the same dosing duration [13]. On day 15 of telaprevir plus peginterferon alfa-2a/ribavirin treatment, 3 of 12 (25%) patients had undetectable HCV RNA (<10 IU/mL, Roche Taqman Assay). Whereas in the present study 6 of 9 (67%) patients treated with danoprevir/r 100/100 mg BID plus peginterferon alfa-2a/ribavirin had undetectable HCV RNA (<15 IU/mL) at the same time-point. The addition of an HCV protease inhibitor to standard therapy can potentially provide a much more effective therapeutic regimen for patients with genotype 1 infection.

Although low dose ritonavir is commonly used as a pharmacokinetic enhancer for HIV protease inhibitors, this pharmacological strategy to optimize pharmacokinetics of HCV protease inhibitors that are CYP3A substrates has only been recently explored. Following 2-week treatment of narlaprevir/r 400/200 mg BID plus Peg-IFN alfa-2b in eight treatment-naive patients, four patients (50%) had HCV RNA < 25 IU/mL [14]. In comparison, 2-week treatment of danoprevir/r 100/100 mg BID and 200/100 mg BID plus peginterferon alpha-2a/ribavirin
resulted in 67% (6/9) and 100% (8/8) of patients with undetectable HCV RNA (<15 IU/mL).

Naturally occurring resistant mutations to HCV protease inhibitors may be present in a small number of treatment-naïve individuals with genotype 1 HCV infection[15] and after the initiation of protease inhibitor monotherapy, viral breakthrough occurred within 1 to 2 weeks [16,17]. The emergence of protease-inhibitor resistant variants correlating to viral breakthrough has also been described in patients receiving the combination of a protease inhibitor plus peginterferon alfa-2a (40KD)/ribavirin [10,11]. However, in the present study, viral breakthrough was not observed in any patient during treatment with danoprevir/r in combination with peginterferon alfa-2a (40KD)/ribavirin.

Certain adverse events have been observed during therapy with HCV investigational protease inhibitors (for example rash with telaprevir [10–12]; anemia with boceprevir [18]). Notably, severe cardiotoxicity resulted in the discontinuation of ciluprevir, the first drug in the class to be studied in humans [19]. When administered for 15 days in combination with peginterferon alfa-2a (40KD)/ribavirin in 25 patients, danoprevir/r has a promising safety profile and was generally well tolerated. There was no evidence of cardiotoxicity or hepatotoxicity, and the incidence of individual adverse events was low.
Danoprevir 900 mg BID regimen in an ongoing phase II study has been discontinued because of asymptomatic Grade 4 ALT elevations. High exposure to danoprevir was associated with Grade 3 and 4 elevations in ALT, whilst no Grade 4 ALT elevations were observed for AUC ≤ 782 ng•hr/mL [6]. Given that all three danoprevir/r regimens had substantially lower mean AUC values than 782 ng•hr/mL, the probability of ALT elevations in patients treated with danoprevir/r regimens in future clinical studies is expected to be substantially reduced or eliminated.

Liver exposure is considered important for the efficacy of a drug meant to disrupt HCV replication. The liver-to-plasma AUC ratios in monkeys and rats are approximately 10-fold and 127-fold, respectively. [3] The difference in the liver-to-plasma ratios between species could be attributed to differences in plasma protein binding. Based on the plasma protein binding of 99.9% in rats, 92.5% in monkeys, and 97.9% in humans [20] the estimated human liver-to-plasma ratios for danoprevir is approximately 43-fold using simple linear regression. Estimated danoprevir C\textsubscript{trough,ss} values in the liver for danoprevir/r 100/100 mg BID, 200/100 mg QD, and 200/100 mg BID are approximately 1.5-fold, 0.7-fold, and 10-fold above the replicon assay EC\textsubscript{90} value of 14 nM (10.2 ng/mL).[3] It should be emphasized that the efficacious C\textsubscript{trough,ss} value for danoprevir has not been established. The lack of exposure-response relationship and the potent antiviral activity associated with all three danoprevir/r regimens suggests that a lower C\textsubscript{trough,ss} may be sufficient. However, additional pharmacokinetics and
pharmacodynamics data (e.g., efficacy, resistance) from phase II and/or III studies may help to elucidate the efficacious $C_{\text{trough,ss}}$ value.

The use of low dose ritonavir as a pharmacokinetic enhancer is faced with the challenges associated with potential drug-drug interactions. Experience in the treatment of HIV shows that drug-drug interactions associated with the use of ritonavir-boosted HIV protease inhibitors are manageable [21]. The available data and knowledge on those interactions would be valuable for use of danoprevir/r in the treatment of HCV.

In conclusion, this study confirms that the use of low dose danoprevir plus low dose ritonavir to reduce the overall danoprevir exposure is a feasible therapeutic strategy and, in particular, that danoprevir/r regimens are safe and well tolerated, when administered together with peginterferon alfa-2a (40KD) plus ribavirin for 14 days. Danoprevir/r regimens show potent viral suppression with no evidence of danoprevir resistance up to 15 days of administration in treatment-naive patients. Furthermore, reduction of danoprevir exposure by using a combination of low dose danoprevir and low dose ritonavir is expected to significantly reduce the probability of ALT elevations. The excellent antiviral activity and tolerability of all danoprevir/r regimens in this study support ongoing and future clinical trials of danoprevir/r in patients with genotype 1 HCV infection.
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References


20. Roche: data on file

# Tables

## Table 1. Baseline characteristics

<table>
<thead>
<tr>
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<th>Placebo/r (pooled from 3 cohorts)</th>
<th>Danoprevir/r</th>
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<tr>
<td></td>
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<td>100mg/100mg BID</td>
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<tr>
<td>No. of patients</td>
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<td>9</td>
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<tr>
<td>Median HCV RNA level, IU/mL</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>HCV genotype 1a/1b</td>
<td>4/1</td>
<td>9/0</td>
</tr>
<tr>
<td>Mean ALT, IU/mL ± SD</td>
<td>160 ± 85</td>
<td>121 ± 62</td>
</tr>
<tr>
<td>Mean AST, IU ± SD</td>
<td>70 ± 47</td>
<td>50 ± 21</td>
</tr>
<tr>
<td>Total bilirubin, µmol/L ± SD</td>
<td>7 ± 3</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Serum albumin, g/L ± SD</td>
<td>43 ± 1</td>
<td>44 ± 1</td>
</tr>
<tr>
<td>Mean platelet count, x 10⁹/L ± SD</td>
<td>216 ± 52</td>
<td>213 ± 35</td>
</tr>
<tr>
<td>Mean haemoglobin, g/L ± SD</td>
<td>157 ± 9</td>
<td>158 ± 14</td>
</tr>
<tr>
<td>Creatinine, µmol/L ± SD</td>
<td>76 ± 9</td>
<td>76 ± 16</td>
</tr>
</tbody>
</table>
SD, standard deviation; BID, twice daily; QD, once daily
### Table 2. Summary of virological response

<table>
<thead>
<tr>
<th>Regimen plus peginterferon alfa-2a (40KD) and ribavirin</th>
<th>Median HCV RNA level, $\log_{10}$ IU/mL</th>
<th>Patients with undetectable HCV RNA (&lt;15 IU/mL), n (%)</th>
<th>Patients with viral rebound, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo/r (N=5)$^{a}$</td>
<td>6.5</td>
<td>–2.7</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Danoprevir/r</td>
<td>6.5</td>
<td>–5.1</td>
<td>6 (67)</td>
</tr>
<tr>
<td>100mg/100mg BID (N=9)</td>
<td>6.5</td>
<td>–4.8</td>
<td>4 (50)</td>
</tr>
<tr>
<td>200mg/100mg BID (N=8)</td>
<td>5.8</td>
<td>–4.6</td>
<td>8 (100)</td>
</tr>
</tbody>
</table>

$^{a}$ Pooled from 3 cohorts
Table 3  Mean (SD) of danoprevir steady-state pharmacokinetic parameters for Danoprevir/r regimens in treatment-naive patients with CHC genotype 1

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>DNV/r 100/100 mg BID (n=9)</th>
<th>DNV/r 200/100 mg QD (n=8)</th>
<th>DNV/r 200/100 mg BID (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{τ,ss}$ (ng·h/mL)</td>
<td>87.0 (42.9)</td>
<td>460 (557)</td>
<td>420 (269)</td>
</tr>
<tr>
<td>C$_{\text{max,ss}}$ (ng/mL)</td>
<td>40.1 (32.9)</td>
<td>125 (149)</td>
<td>141 (88.9)</td>
</tr>
<tr>
<td>T$_{\text{max,ss}}$ (h)</td>
<td>2.00 (1.00 – 4.00)*</td>
<td>3.00 (2.00 – 4.00)*</td>
<td>3.00 (0.50 – 3.00)*</td>
</tr>
<tr>
<td>C$_{\text{trough,ss}}$ (ng/mL)</td>
<td>0.352 (0.247)</td>
<td>0.168 (0.132)</td>
<td>2.42 (3.10)</td>
</tr>
</tbody>
</table>

* Median (range)

Abbreviations: AUC$_{0–τ,ss}$ = area under the curve over the dosing interval at steady-state; C$_{\text{max,ss}}$ = observed maximum plasma concentration at steady state; C$_{\text{trough,ss}}$ = observed trough concentration at steady state; SD = standard deviation; T$_{\text{max,ss}}$ = time of maximum plasma concentration at steady state.
Table 4. Summary of adverse events

<table>
<thead>
<tr>
<th></th>
<th>Placebo/r (pooled from 3 cohorts)</th>
<th>Danoprevir/r 100mg/100mg BID</th>
<th>200mg/100mg QD</th>
<th>200mg/100mg BID</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>5</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Discontinuation of treatment for AEs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serious AEs, n</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AEs, n</td>
<td>21</td>
<td>73</td>
<td>57</td>
<td>24</td>
</tr>
<tr>
<td>Patients with at least 1 AE, n (%)</td>
<td>5 (100)</td>
<td>9 (100)</td>
<td>8 (100)</td>
<td>7 (87)</td>
</tr>
<tr>
<td>Mean AEs/patient</td>
<td>4.2</td>
<td>8.1</td>
<td>7.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Treatment-emergent AEs, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>4 (80)</td>
<td>5 (56)</td>
<td>5 (63)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>2 (40)</td>
<td>4 (44)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>1 (11)</td>
<td>0</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (60)</td>
<td>4 (44)</td>
<td>0</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (40)</td>
<td>1 (11)</td>
<td>1 (13)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
<td>3 (33)</td>
<td>3 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (20)</td>
<td>2 (22)</td>
<td>3 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Lethargy</td>
<td>1 (20)</td>
<td>3 (33)</td>
<td>2 (25)</td>
<td>0</td>
</tr>
<tr>
<td>Irritability</td>
<td>0</td>
<td>3 (33)</td>
<td>2 (25)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1 (20)</td>
<td>3 (33)</td>
<td>0</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>1 (20)</td>
<td>3 (33)</td>
<td>1 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Flu-like illness</td>
<td>0</td>
<td>0</td>
<td>3 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Eye pain</td>
<td>0</td>
<td>1 (11)</td>
<td>3 (38)</td>
<td>2 (25)</td>
</tr>
</tbody>
</table>

Mean maximum change in laboratory parameters ± SD (day of maximum)
<table>
<thead>
<tr>
<th>Change</th>
<th>ALT, IU/mL</th>
<th>Platelet count, x $10^9$/L</th>
<th>Haemoglobin, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–129 ± 85 (day 28)</td>
<td>–49 ± 21 (day 16)</td>
<td>–29 ± 12 (day 28)</td>
</tr>
<tr>
<td></td>
<td>–73 ± 54 (day 16)</td>
<td>–37 ± 32 (day 3)</td>
<td>–40 ± 16 (day 28)</td>
</tr>
<tr>
<td></td>
<td>–44 ± 31 (day 16)</td>
<td>–48 ± 35 (day 16)</td>
<td>–33 ± 14 (day 28)</td>
</tr>
<tr>
<td></td>
<td>–39 ± 36 (day 16)</td>
<td>–90 ± 72 (day 28)</td>
<td>–25 ± 18 (day 28)</td>
</tr>
</tbody>
</table>

SD, standard deviation; BID, twice daily; QD, once daily

a. Reported by at least three patients in one treatment group.
b. Maximum mean change reported between baseline and day 28 of treatment and follow-up
Figure Legends

**Figure 1.** Individual HCV RNA during 15-day treatment of danoprevir/r plus peginterferon alfa-2a (40KD) and ribavirin

**Figure 2.** Median HCV RNA during 15-day treatment of danoprevir/r plus peginterferon alfa-2a (40KD) and ribavirin

**Figure 3.** Proportion of patients with undetectable HCV RNA at each study visit
Danoprevir/r 100/100 mg BID (n=9)
Danoprevir/r 200/100 mg QD (n=8)
Danoprevir/r 200/100 mg BID (n=8)