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Characterization of vaniprevir, a hepatitis C virus NS3/4A protease inhibitor, in patients with HCV genotype 1 infection: Safety, antiviral activity, resistance, and pharmacokinetics

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ABSTRACT

Vaniprevir is a competitive inhibitor of the hepatitis C virus (HCV) NS3/4A protease that has potent anti-HCV activity in preclinical models. This placebo-controlled dose-ranging study assessed the safety, tolerability, and antiviral efficacy of vaniprevir monotherapy in patients with genotype 1 chronic HCV infection. Treatment-naive and treatment-experienced non-cirrhotic adult patients with baseline HCV RNA >10^6 IU/ml were randomized to receive placebo or vaniprevir at doses of 125 mg *qd*, 600 mg *qd*, 25 mg *bid*, 75 mg *bid*, 250 mg *bid*, 500 mg *bid*, and 700 mg *bid* for 8 days. Forty patients (82.5% male, 75% genotype 1a) received at least one dose of placebo or vaniprevir. After 1 week of vaniprevir, the decrease in HCV RNA from baseline ranged from 1.8 to 4.6 $\log_{10}$ IU/ml across all treatment groups, and there was a greater than dose-proportional increase in vaniprevir exposure at doses above 75 mg *bid*. The most commonly reported drug-related adverse events (AEs) were diarrhea ($n = 5$) and nausea ($n = 5$). No pattern of laboratory or ECG abnormalities was observed, all AEs resolved during the study, and there were no discontinuations due to AEs. No serious AEs were reported. Resistance-associated amino acid variants were identified at positions R155 and D168 in patients infected with genotype 1a virus. Vaniprevir monotherapy demonstrated potent antiviral activity in patients with chronic genotype 1 HCV infection, and was generally well tolerated with no serious AEs or discontinuations due to AEs. Further development of vaniprevir, including studies in combination with other anti-HCV agents, is ongoing.

Abbreviations: AE, adverse event; AUC, area under the concentration-time curve; *bid*, twice daily; BMI, body mass index; BUN, blood urea nitrogen; CI, confidence interval; C_{max}, maximum concentration; C_{trough}, trough concentration; CRU, clinical research unit; DAA, direct-acting antiviral; EC_{50}, half-maximal effective concentration; ECG, electrocardiogram; GMR, geometric mean ratio; GT, genotype; HCV, hepatitis C virus; IC_{50}, half-maximal inhibitory concentration; IRF-3, interferon regulatory factor 3; LLoQ, lower limit of quantitation; MRL, Merck Research Laboratories; NS3/4A, nonstructural protein 3/4A; PCR, polymerase chain reaction; PD, pharmacodynamic(s); PEG-IFN, pegylated interferon; PI, protease inhibitor; PK, pharmacokinetic(s); *qd*, once daily; RAVs, resistance-associated amino acid variants; RBV, ribavirin; SAE, serious adverse event; SEAP, secreted alkaline phosphatase; SVR, sustained viral response; $t_{1/2}$, terminal half-life; T_{max}, maximum time to reach C_{max}; ULN, upper limit of normal.

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1. Introduction

An estimated 3% of the world's population is infected with the hepatitis C virus (HCV), equivalent to 170–200 million patients (Report of a World Health Organization Consultation, 1999). Given the asymptomatic nature of early infection and the slow progression to severe liver disease, it is expected that the prevalence of HCV will peak over the coming decades (Davis et al., 2003; El-Serag, 2004). The former standard-of-care treatment consisted of peginterferon (PEG-IFN) and ribavirin (RBV) which yielded sustained virologic response (SVR) rates of approximately 40% in patients with genotype (GT) 1 HCV infection (McHutchison et al., 2009). More recently, inhibitors of the HCV non-structural (NS) 3/4A serine protease have been shown to improve rates of SVR to 66–79% in treatment-naive patients with GT1 HCV infection (Bacon et al., 2011; Poordad et al., 2011).

The NS3/4A protein contains both serine protease activity and RNA helicase activity essential for viral replication. NS3/4A protease activity is also implicated in viral inhibition of innate immunity, by interfering with the interferon regulatory factor 3 (IRF-3) signaling pathway, which plays an important role in activating intracellular antiviral defense. Therefore, inhibition of NS3/4A effectively reduces viral polyprotein processing and may rapidly restore cellular innate immune responses (De Francesco et al., 2003; Goudreau and Llinàs-Brunet, 2005).

Vaniprevir is a competitive inhibitor of the HCV NS3/4A protease that has shown antiviral efficacy when administered orally to HCV-infected chimpanzees (Olsen et al., 2011). The purpose of this study was to assess the safety, tolerability, pharmacokinetics (PK), and antiviral efficacy of multiple doses of vaniprevir in patients with GT1 HCV infection.

2. Materials and methods

2.1. Study design

This was a double-blind, randomized, placebo-controlled trial (ClinicalTrials.gov identifier: NCT00704184), conducted in accordance with the principles of Good Clinical Practice and approved by the appropriate institutional review boards and regulatory agencies. Patient safety was overseen by an external Data Monitoring Committee, and informed consent was documented prior to enrollment. This study was funded by Merck & Co., Inc.

Participants were admitted to the Clinical Research Unit for the first 36 h of dosing. At study initiation, patients were assigned to receive either placebo ($n = 4$) or vaniprevir ($n = 21$) at doses of 75 mg *bid*, 250 mg *bid*, 500 mg *bid*, or 600 mg *qd*. Prior to randomization, a protocol amendment added 2 vaniprevir treatment groups (25 mg *bid* and 125 mg *qd*; $n = 8$ each). A second amendment after initial randomization added the vaniprevir 700 mg *bid* ($n = 6$) and placebo ($n = 1$) treatment groups. All patients were treated for 8 days and then followed for 14 days after the last dose. Patients received treatment orally with or without food except on days 1 and 8 when PK sampling required fasting before the morning dose.

2.2. Study population

Patients aged 18–55 years with a body mass index $\geqslant$18.5 to $\leqslant$36 kg/m^2 and chronic, compensated, GT1 HCV infection were enrolled. All patients had a baseline HCV RNA >10^6 IU/ml and no evidence of cirrhosis or bridging fibrosis (according to biopsy within 3 years of screening). Patients also had laboratory values within pre-specified criteria at study entry. Patients previously treated with approved HCV therapy or with a direct-acting antiviral for HCV, or with chronic HBV or HIV infection were excluded. Initially, women of childbearing potential were excluded, but a post-randomization amendment allowed enrollment based on supportive preclinical data.

2.3. Randomization procedures

In stage 1, patients were randomized to receive either placebo or vaniprevir at doses of 25 mg *bid*, 75 mg *bid*, 250 mg *bid*, 500 mg *bid*, 125 mg *qd*, or 600 mg *qd*. In stage 1.1, patients were randomized to receive either placebo or vaniprevir 700 mg *bid*. Vaniprevir and matching placebo were identical in size and appearance, and the number of capsules administered was the same regardless of treatment group. A computer-generated centralized randomization schedule was employed, with numbers assigned sequentially by blinded Merck personnel.

2.4. Clinical supplies

All clinical supplies were manufactured by Merck & Co., Inc., provided in a blinded fashion, and stored between 2 and 8 °C.

2.5. Assessments

Primary assessments included safety, tolerability, and efficacy throughout the 8-day study. Exploratory outcomes included the relationship between dose and antiviral activity, plasma PK profile, PK-pharmacodynamic (PD) association, viral resistance profiles, and effect of dose on antiviral activity. Medication adherence was assessed using diary cards.

2.5.1. Efficacy measurements

Blood samples for determination of HCV RNA levels were collected at screening (Roche Cobas TaqMan v2.0; lower limit of detection, 10 IU/ml; lower limit of quantification [LLoQ], 25 IU/ml) to determine eligibility. For efficacy analyses, blood samples were collected pre-dose and up to 36 h post-dose on days 1 and 8; pre-dose on days 3, 4, and 5; and daily on days 10, 12, 14, 15, and 22.

2.5.2. Safety measurements

Safety and tolerability were monitored via assessment of adverse events (AEs) and measurement of vital signs, physical examinations, electrocardiograms, and standard safety laboratory tests.

2.5.3. Pharmacokinetic measurements

Blood samples were obtained on days 1, 2, 3, 4, 5, and 8. Vaniprevir trough concentrations (C_{trough}) were assessed on days 2, 3, 4, and 5. PK parameters included area under the plasma concentration versus time curve ($AUC_{0-12\ h}$ for twice-daily dosing and $AUC_{0-24\ h}$ for once-daily dosing), maximum plasma concentration (C_{max}), trough plasma concentration (C_{trough}, $C_{12\ h}$ for *bid* and $C_{24\ h}$ for *qd*), time to C_{max} (T_{max}), apparent terminal half-life ($t_{1/2}$), and accumulation ratio, as appropriate. Accumulation was determined by taking the ratio of the PK parameter value (i.e. AUC, C_{max}, C_{trough}) on day 8 to that on day 1.

Determination of vaniprevir plasma concentrations involved isolation of the analyte and internal standard using 96-well liquid–liquid extraction, followed by high-performance liquid chromatography–tandem mass spectrometry analysis. The LLoQ for the assay was 1 ng/ml (1.32 nM) and the linear calibration range was 1–1000 ng/ml.

2.6. Viral resistance

2.6.1. Population sequencing

Blood samples drawn before and after dosing were analyzed for resistance-associated amino acid variants (RAVs). Putative RAVs were identified as amino acid changes in the HCV protease conferring resistance in preclinical replicon selection experiments and in chimpanzee studies, and causing decreased sensitivity to vaniprevir in cell-based SEAP HCV protease assays following their insertion into prototypical GT1a (H77, Genbank NC_004102) and 1b (Con1, Genebank AJ238799) sequences (Ludmerer et al., 2008; Olsen et al., 2011).

The resistance-sequencing assay had a detection limit of 1000 IU/ml for both GT1a and GT1b samples. Samples were selected for analysis at baseline, at the first sample after the 7-day dosing period with a viral load of >1000 IU/ml, and at day 21. Population sequences were aligned to either H77 or Con1 for GT1a and 1b, respectively. For resistance analysis, 8 independent polymerase chain reactions (PCR) were attempted for each sample and a maximum of 4 PCR products were directly sequenced (population sequencing).

2.6.2. HCV-secreted alkaline phosphatase (SEAP) protease assays

The reporter construct has been described previously (Pacini et al., 2004). GT1a H77 and Con1b versions of NS3/4A protease were cloned into the mammalian expression vector pCDNA3.1 (Invitrogen) via HindIII and EcoRI sites. Desired variants of these prototype proteases were generated by site-directed mutagenesis (Quik Change II kit, Agilent). The mutant proteases were confirmed by standard sequencing and large-scale plasmid preparations were performed (Qiagen Plasmid Maxi Kit). The SEAP assay was conducted as previously described (Ludmerer et al., 2008) with the following modifications. Following preparation of transfection mixtures, samples were mixed and added to 96-well plates containing Huh-7 cells using a Velocity Bravo2 robot (Agilent, USA). Each compound was titrated with 3-fold dilutions over 8 points and was added in 100 μl of compound/transfection mixture. Titrations were performed in triplicate and dimethyl sulfoxide was used as a vehicle control.

2.7. Statistical analyses

The safety analysis included all patients who received at least 1 dose of study medication. AEs were categorized as follows: (1) at least 1 AE; (2) drug-related AEs; (3) serious AEs (SAEs); and (4) serious and drug-related AEs. In stage 1 of the study, if an AE occurred at a rate of 1% or 10%, then the chance of observing at least 1 AE of this type among 5 patients receiving that dose was 4.9% or 40.9%, respectively. If no given AE was observed in any of the 5 patients at a given dose, then with 80% confidence, the true incidence of the AE at that dose was $\leqslant$36.9%. Change in HCV RNA ($\log_{10}$) from baseline was also summarized for each dose regimen.

Change from baseline in $\log_{10}$ HCV RNA on day 8, slope of HCV RNA decrease during the viral clearance phase, was plotted against the PK parameters (i.e. AUC, C_{max}, $C_{12\ h}$, and $C_{24\ h}$) to visually assess any PK/PD relationships. Correlations between PK and PD were also assessed via Pearson (parametric) correlation coefficient (r) and Spearman (nonparametric) rank correlation coefficient. A negative Pearson or Spearman correlation indicates that an increase in a PK parameter leads to an increase in antiviral effect; a positive correlation indicates that an increase in a PK parameter leads to a decrease in antiviral effect; zero correlation indicates no evidence of association. This analysis was conducted only for *bid* dosing regimens, because limited data were available from the *qd* dose groups.

3. Results

3.1. Baseline characteristics and duration of therapy

This study was conducted at 12 centers in Germany and the United States between July 2007 and August 2008. A total of 165 patients were screened, of whom 40 were randomized, and 39 patients completed 8 days of therapy and 14 days of follow-up (Fig. 1). The most common reasons for screen failure were: regular user of illicit drugs or a history of drug use within 1 year of the study, HCV RNA levels below the minimum required at baseline, and liver biopsy within 3 years of study start unavailable. The majority of the patients (75.0%) had GT1a HCV, and the baseline mean plasma HCV RNA level was 6.7 $\log_{10}$ IU/ml, with values ranging between 4.8 and 7.8 $\log_{10}$ IU/ml (Table 1). Most patients received treatment per protocol; however, 5 patients had minor deviations from the per-protocol dosing regimen as reported on diary cards. Mean duration of vaniprevir therapy was 7.9 days (range 4–8 days).

3.2. Efficacy

In total, 39 patients were included in the efficacy analyses: one patient receiving vaniprevir 75 mg *bid* was excluded because of noncompliance. By day 3, there was an average decrease from baseline HCV RNA of >3 $\log_{10}$ IU/ml in all vaniprevir treatment groups except the 25-mg-*bid* group; on day 8 the average decrease in HCV RNA exceeded 2 $\log_{10}$ IU/ml in all but the 25-mg-*bid* and 125-mg-*qd* groups (Fig. 2, Table 2). Consistent decreases in HCV RNA of up to 4 $\log_{10}$ IU/ml were seen in patients receiving vaniprevir 500 mg *bid*, 600 mg *qd*, and 700 mg *bid* on days 3 through 8, suggesting that reduction in HCV RNA tended to increase at higher doses with both *qd* and *bid* vaniprevir regimens.

3.3. Safety

The overall incidence of AEs was similar for vaniprevir and placebo, with 23 of 35 (65.7%) vaniprevir recipients and 3 of 5 (60.0%) placebo recipients reporting $\geqslant$1 AEs. The incidence of drug-related AEs was higher in the vaniprevir treatment groups compared with placebo (42.9% *vs.* 20.0%) (Supplementary Table 1). The most commonly reported drug-related AEs in the vaniprevir groups were diarrhea and nausea. The highest incidence of drug-related AEs occurred in the vaniprevir 250-mg-*bid* treatment group (83.3%), while no drug-related AEs were reported in the vaniprevir 600-mg-*qd* group. All drug-related AEs were either mild or moderate in severity, and there was no apparent relationship between the frequency or intensity of AEs and the dose of vaniprevir.

Three patients (vaniprevir 25 mg *bid*, $n = 1$; vaniprevir 500 mg *bid*, $n = 2$) experienced blood creatinine increases, but none was considered vaniprevir-related or reported as an AE. In 2 patients receiving 500 mg *bid* vaniprevir, increases in creatinine were sporadic, not associated with increases in blood urea nitrogen (BUN), and resolved spontaneously within one day without need for dose adjustment or discontinuation. The maximum creatinine values in these 2 patients were 1.4 mg/dl (normal range of 0.5–1.2 mg/dl) on day 4 of dosing (baseline of 1.0 mg/dl) and 2.4 mg/dl on day 4 of dosing (baseline of 0.9 mg/dl), respectively. The estimated glomerular filtration rates (eGFR) were >60 and 35 ml/min/1.73 m^2, respectively, at the peak serum creatinine values. The third patient, receiving vaniprevir 25 mg *bid*, had a serum creatinine of 1.3 mg/dl (baseline of 0.9 mg/dl) one day after dosing was completed. The serum creatinine slowly increased to 1.7 mg/dl on day 16, off therapy. Maximum BUN value was 23 mg/dl, which remained within normal limits of 4–24 mg/dl. Per verbal report

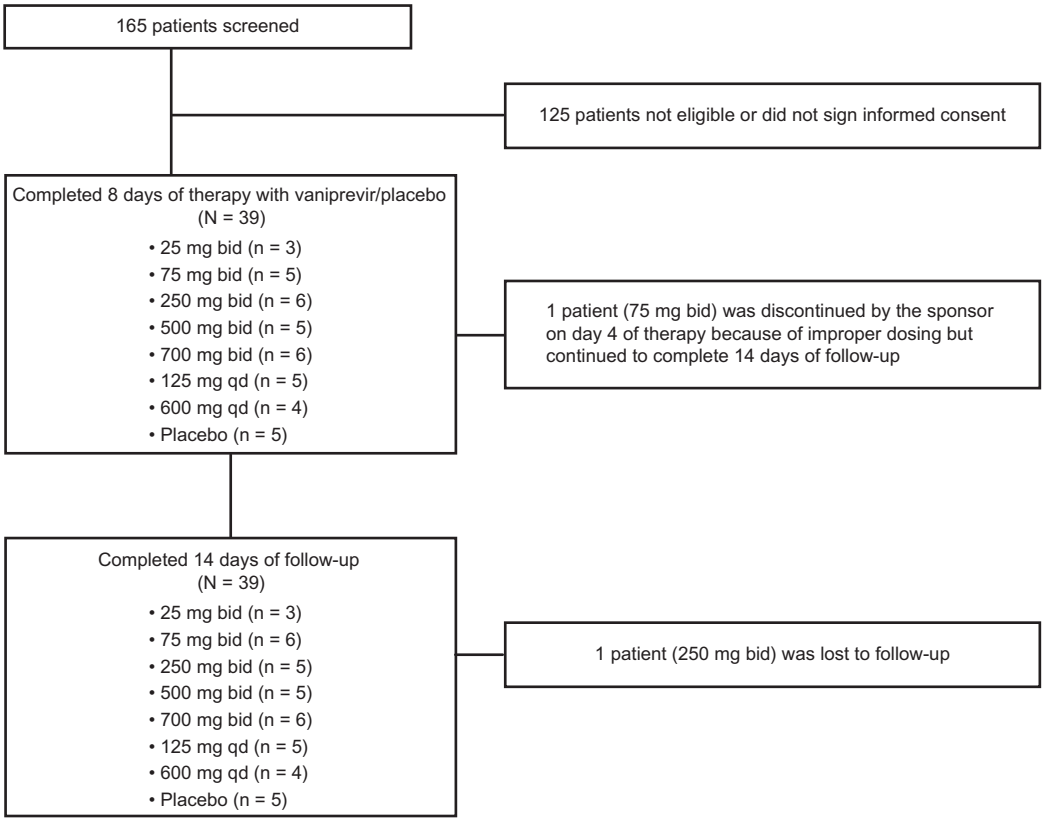

Fig. 1. Patient disposition.

Table 1
Baseline patient characteristics.

	Vaniprevir								
	25 mg *bid* (*n* = 3)	75 mg *bid* (*n* = 6)	250 mg *bid* (*n* = 6)	500 mg *bid* (*n* = 5)	700 mg *bid* (*n* = 6)	125 mg *qd* (*n* = 5)	600 mg *qd* (*n* = 4)	Placebo (*n* = 5)	Total (*n* = 40)
Gender, n (%)									
Male	3	6 (100.0)	3 (50.0)	5 (100.0)	5 (83.3)	3 (60.0)	4 (100.0)	4 (80.0)	33 (82.5)
Female	0 (0.0)	0 (0.0)	3 (50.0)	0 (0.0)	1 (16.7)	2 (40.0)	0 (0.0)	1 (20.0)	7 (17.5)
Race, n (%)									
White	0 (0.0)	2 (33.3)	5 (83.3)	1 (20.0)	3 (50.0)	2 (40.0)	0 (0.0)	1 (20.0)	14 (35.0)
Black	2 (66.7)	3 (50.0)	1 (16.7)	3 (60.0)	1 (16.7)	3 (60.0)	3 (75.0)	3 (60.0)	19 (47.5)
Hispanic	1 (33.3)	1 (16.7)	0 (0.0)	1 (20.0)	2 (33.3)	0 (0.0)	1 (25.0)	1 (20.0)	7 (17.5)
Age (years)									
Mean (SD)	47.7 (2.5)	46.8 (4.0)	46.3 (7.1)	49.2 (5.6)	42.3 (9.6)	46.4 (6.8)	41.0 (14.4)	46.2 (5.9)	45.7 (7.4)
Median	48.0	47.5	47.0	48.0	41.0	50.0	45.5	46.0	48.0
Range	45–50	40–51	38–54	41–55	28–53	38–52	21–52	40–54	21–55
Genotype, n (%)									
Genotype 1	1 (33.3)	1 (16.7)	0 (0.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	4 (10.0)
Genotype 1a	1 (33.3)	5 (83.3)	5 (83.3)	4 (80.0)	5 (83.3)	2 (40.0)	4 (100.0)	4 (80.0)	30 (75.0)
Genotype 1b	1 (33.3)	0 (0.0)	1 (16.7)	0 (0.0)	1 (16.7)	2 (40.0)	0 (0.0)	1 (20.0)	6 (15.0)
Plasma HCV RNA (log_{10} IU/ml)									
Mean (SD)	7.0 (0.4)	6.7 (0.2)	6.8 (0.4)	6.6 (0.4)	6.7 (0.4)	6.6 (0.5)	7.1 (0.5)	6.3 (0.9)	6.7 (0.5)
Median	7.0	6.7	6.8	6.7	6.7	6.6	6.9	6.6	6.7
Range	6.6–7.4	6.4–7.1	6.3–7.3	6.1–7.2	6.2–7.4	5.9–7.1	6.6–7.8	4.8–6.9	4.8–7.8

n = Number of patients in each group.

from the investigator, the patient's anti-hypertensive diuretic medications were being adjusted during the study period and may have contributed to the increase in creatinine. The eGFR was 53 ml/min/1.73 m^2 at the peak serum creatinine value. All three patients remained asymptomatic.

All laboratory AEs resolved by day 22 of the study. No SAEs were reported, and there were no discontinuations due to AEs.

3.4. Pharmacokinetics

Vaniprevir was rapidly absorbed, with median plasma T_{max} ranging from 1.0 to 3.0 h (Supplementary Table 2). The apparent $t_{1/2}$ was 4–9 h across all doses. Vaniprevir accumulation was seen with *bid* but not *qd* regimens. Following *bid* dosing, the $AUC_{0-12\ h}$

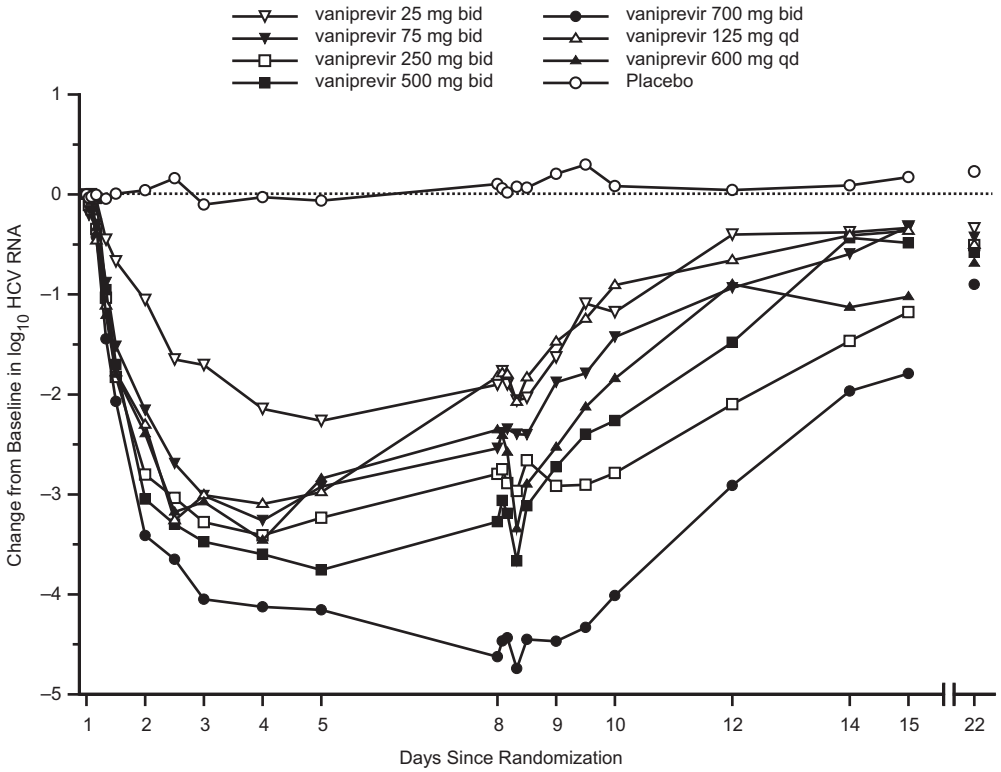

Fig. 2. Mean change from baseline in $\log_{10}$ HCV RNA over time by treatment group (stage 1 and stage 1.1).

and C_{max} accumulation ratios ranged from 1.3 to 1.8, with steady state achieved by day 2. Following *qd* dosing, the $AUC_{0-24\,h}$ and C_{max} accumulation ratios ranged from 0.88 to 1.2, consistent with the relatively short terminal half-life of vaniprevir. Mean AUC and C_{max} values appeared to increase more than dose proportionally between 75-mg- and 700-mg-*bid* doses. The interpatient variability for AUC, C_{max}, and C_{trough} appeared to be high (i.e. greater than 30% coefficient of variation) within each dose.

Based on PK/PD correlation analysis, day 8 dose, AUC, C_{max}, and C_{trough} were similarly predictive of viral response with Spearman Rank Correlations of −0.784, −0.766, −0.755, and −0.714, respectively. Similar results were found when Pearson's correlation coefficients were used (data not shown).

3.5. Viral resistance

RAVs were detected in patients with GT1a infection but not with GT1b infection. In patients with GT1a infection, RAVs were not detected at baseline; however, several RAVs were detected in viral sequences isolated at the end of the vaniprevir dosing period (days 7–13) (Supplementary Table 3). Of the variants detected, RAVs at position R155 were observed most frequently. Changes at D168 were also observed, as was a single variant at A156. RAVs were detected in all patients with GT1a infection receiving vaniprevir doses above 75 mg *bid*, and also in patients in both *qd* arms. Only the R155K variant was identified at day 21 (14 days after vaniprevir dosing), possibly reflecting the increased fitness of this variant compared with the other RAVs. The R155, A156, and D168 variants all produced substantial increases in half-maximal inhibitory concentration of vaniprevir in the SEAP HCV protease assay (Table 3).

4. Discussion

Despite the small sample size, vaniprevir demonstrated robust antiviral activity given the substantial reduction in plasma HCV RNA from baseline. In all vaniprevir groups there was an average decrease of $\geqslant 2\,\log_{10}$ IU/ml in HCV RNA relative to baseline at $\geqslant 1$ time point during the treatment period. However, doses greater than 25 mg were associated with a more rapid initial decline in HCV RNA and a greater nadir decline from baseline compared with the 25-mg group. The most rapid decrease in HCV RNA and the greatest reduction from baseline was observed in the 700-mg-*bid* dose group. Additionally, a rebound in HCV RNA levels during the 8-day dosing period was observed for some patients in all vaniprevir dose groups, except the 700-mg-*bid* group. These data indicate an association between increasing dose of vaniprevir and improved antiviral activity. There was no difference in post-treatment HCV RNA kinetics during return to baseline levels across the vaniprevir dose groups.

Vaniprevir was generally well tolerated over the 8-day dosing period. There were no clinically meaningful differences between treatment groups regarding the incidence of AEs or laboratory test results, and no SAEs or discontinuations due to AEs. Changes in laboratory values were reported infrequently; however, an increase from baseline serum creatinine was observed in 3 patients receiving vaniprevir. These cases were not thought to be of clinical significance. Since the completion of this study, larger studies of vaniprevir in approximately 700 patients who received vaniprevir in combination with PEG-IFN/RBV have not found an association between vaniprevir and nephrotoxicity (Lawitz et al., 2011; Manns et al., 2012).

There was a more-than-dose-proportional increase in vaniprevir exposure at doses greater than 75 mg *bid*; however, this was not associated with an increase in AEs or laboratory changes. There was a general association between increasing vaniprevir exposure and improved antiviral activity, but the specific PK results were

Table 2
Analysis of change from baseline in $\log_{10}$ HCV RNA (IU/ml) by visit time point.

Visit time point	Treatment	*n*	Baseline mean	Change from baseline by day		Treatment (versus placebo)	
				Mean (SD)	95% CI[b]	Difference[a] 95% CI	*P*[c]
Day 1 hour 12	Vaniprevir 25 mg *bid*	3	6.98	−0.67 (0.55)	(−2.02, 0.69)	−0.68 (−1.94, 0.58)	0.1578
	Vaniprevir 75 mg *bid*	4	6.63	−1.52 (0.30)	(−2.00, −1.04)	−1.53 (−1.98, −1.08)	0.0005
	Vaniprevir 250 mg *bid*	6	6.76	−1.82 (0.40)	(−2.25, −1.40)	−1.83 (−2.26, −1.41)	0.0000
	Vaniprevir 500 mg *bid*	5	6.63	−1.70 (0.18)	(−1.93, −1.47)	−1.71 (−1.97, −1.45)	0.0000
	Vaniprevir 700 mg *bid*	6	6.70	−2.07 (0.32)	(−2.40, −1.74)	−2.08 (−2.43, −1.73)	0.0000
	Vaniprevir 125 mg *qd*	5	6.57	−1.78 (0.12)	(−1.93, −1.63)	−1.79 (−2.01, −1.57)	0.0000
	Vaniprevir 600 mg *qd*	4	7.07	−1.80 (0.42)	(−2.46, −1.14)	−1.81 (−2.43, −1.18)	0.0015
	Placebo	5	6.27	0.01 (0.17)	(−0.21, 0.22)		
Day 3	Vaniprevir 25 mg *bid*	3	6.98	−1.71 (0.91)	(−3.98, 0.57)	−1.60 (−3.70, 0.49)	0.0845
	Vaniprevir 75 mg *bid*	4	6.63	−3.01 (0.29)	(−3.47, −2.55)	−2.91 (−3.39, −2.43)	0.0000
	Vaniprevir 250 mg *bid*	6	6.76	−3.28 (0.57)	(−3.88, −2.68)	−3.18 (−3.80, −2.55)	0.0000
	Vaniprevir 500 mg *bid*	5	6.63	−3.47 (0.24)	(−3.78, −3.17)	−3.37 (−3.79, −2.96)	0.0000
	Vaniprevir 700 mg *bid*	6	6.70	−4.05 (0.38)	(−4.44, −3.65)	−3.95 (−4.42, −3.48)	0.0000
	Vaniprevir 125 mg *qd*	5	6.57	−3.01 (0.74)	(−3.92, −2.09)	−2.91 (−3.81, −2.01)	0.0003
	Vaniprevir 600 mg *qd*	4	7.07	−3.07 (0.88)	(−4.48, −1.67)	−2.97 (−4.32, −1.63)	0.0043
	Placebo	5	6.27	−0.10 (0.31)	(−0.49, 0.29)		
Day 8 hour 0	Vaniprevir 25 mg *bid*	3	6.98	−1.90 (0.40)	(−2.89, −0.92)	−2.01 (−2.87, −1.14)	0.0070
	Vaniprevir 75 mg *bid*	5	6.73	−2.54 (0.69)	(−3.40, −1.68)	−2.64 (−3.49, −1.80)	0.0007
	Vaniprevir 250 mg *bid*	6	6.76	−2.80 (0.96)	(−3.80, −1.79)	−2.90 (−3.90, −1.90)	0.0005
	Vaniprevir 500 mg *bid*	5	6.63	−3.27 (0.98)	(−4.49, −2.05)	−3.38 (−4.59, −2.17)	0.0013
	Vaniprevir 700 mg *bid*	6	6.70	−4.62 (0.39)	(−5.03, −4.21)	−4.73 (−5.15, −4.31)	0.0000
	Vaniprevir 125 mg *qd*	5	6.57	−1.82 (0.61)	(−2.58, −1.06)	−1.93 (−2.68, −1.18)	0.0014
	Vaniprevir 600 mg *qd*	4	7.07	−2.35 (0.21)	(−2.69, −2.02)	−2.46 (−2.78, −2.13)	0.0000
	Placebo	5	6.27	0.11 (0.18)	(−0.12, 0.33)		
Day 22	Vaniprevir 25 mg *bid*	3	6.98	−0.34 (0.26)	(−0.99, 0.31)	−0.57 (−1.08, −0.06)	0.0369
	Vaniprevir 75 mg *bid*	5	6.73	−0.43 (0.36)	(−0.87, 0.02)	−0.66 (−1.11, −0.20)	0.0112
	Vaniprevir 250 mg *bid*	5	6.72	−0.51 (0.92)	(−1.65, 0.63)	−0.74 (−1.86, 0.39)	0.1480
	Vaniprevir 500 mg *bid*	5	6.63	−0.58 (0.59)	(−1.31, 0.15)	−0.81 (−1.53, −0.09)	0.0338
	Vaniprevir 700 mg *bid*	6	6.70	−0.90 (0.73)	(−1.67, −0.13)	−1.13 (−1.90, −0.36)	0.0114
	Vaniprevir 125 mg *qd*	5	6.57	−0.51 (0.27)	(−0.84, −0.17)	−0.73 (−1.11, −0.36)	0.0021
	Vaniprevir 600 mg *qd*	4	7.07	−0.69 (0.93)	(−2.16, 0.79)	−0.92 −2.35, 0.52)	0.1404
	Placebo	5	6.27	0.23 (0.24)	(−0.07, 0.53)		

One patient in the vaniprevir 250-mg-*bid* group consumed both am and pm dose on the morning of day 1.
Two patients in the vaniprevir 700-mg-*bid* group had HCV RNA levels below the limit of quantification (25 IU/ml) on day 8. For computational purposes, the value of 24 was used.
SD, standard deviation; CI, confidence interval.
[a] A negative value means the treatment is better than placebo.
[b] The *t* distribution was used to compute all confidence intervals.
[c] *P* are for descriptive purposes only.

variable and no strong association between HCV RNA decline and a single PK parameter was identified. The PK/PD correlation analysis suggests a potential disconnect between plasma PK and efficacy, because PK did not account for additional variability beyond dose. Despite a terminal plasma half-life of <9 h, the antiviral activity with *qd* dosing was similar to that of the twice-daily regimens, suggesting that vaniprevir may have clinical utility administered once or twice daily.

RAVs were observed in most patients receiving vaniprevir at doses >25 mg *bid*, most commonly at positions R155 and D168. Variants at these loci have decreased susceptibility to vaniprevir in *in vitro* assays (Liverton et al., 2010). The lack of viral rebound in the 700-mg-*bid* dose group suggests that hepatic vaniprevir concentrations achieved during the 8-day dosing period at this dose may suffice to inhibit HCV replication of RAV-containing viruses. This is also consistent with the emergence of RAVs during the post-treatment period after vaniprevir dosing was completed.

RAVs were not identified at baseline, but the population sequencing methods employed probably lacked the sensitivity to detect low levels of pre-existing resistant variants. RAVs were identified in patients infected with GT1a virus. RAVs were not identified in GT1b patients; however, few GT1b patients were enrolled ($n = 6$), limiting the conclusions that can be drawn regarding selection of resistant variants in GT1b by vaniprevir. Clonal and allele-specific PCR analysis of the RAVs identified in this study is described elsewhere (Barnard et al., 2009). RAVs were detected during and up to 21 days following the vaniprevir dosing period indicating that, like other DAA agents, vaniprevir should be used in combination with other agents in order to suppress and potentially eradicate HCV infection.

Table 3
Summary of the magnitude of resistance conferred by vaniprevir RAVs identified in this study.

Variant	EC_{50} (nM)	Fold-change in IC_{50}
H77	7.7	N/A
R155G	1392.0	181.8
R155K	342.0	44.7
R155N	304.8	39.8
R155S	557.2	72.8
R155T	268.7	35.1
D168V	1485.0	194.0
D168G	422.4	55.2
D168A	746.2	97.5
A156V	1151	150.3

In conclusion, this phase 1b trial demonstrated that vaniprevir has potent antiviral activity and is generally well tolerated in patients with GT1 HCV infection. Vaniprevir has the potential to form the basis for a convenient once- or twice-daily therapy for chronic

HCV infection in combination with other antiviral agents. Although the trial was small and vaniprevir was administered as monotherapy, the data support further study in GT1 HCV-infected patients. Further trials using vaniprevir are ongoing to better define the role of vaniprevir in combination HCV therapy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2013.05.015.

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