Original article

IDX184 in combination with pegylated interferon-α2a and ribavirin for 2 weeks in treatment-naive patients with chronic hepatitis C

Jacob Lalezari[1], Terry Box[2], William O'Riordan[3], Purvi Mehra[3], Tuan Nguyen[4], Fred Poordad[5], Edwin DeJesus[6], Paul Kwo[7], Eliot Godofsky[8], Shannon Lawrence[1], Gloria Dubuc-Patrick[9], Jie Chen[9], Joseph McCarville[9], Keith Pietropaolo[9], Xiao-Jian Zhou[9], John Sullivan-Bólyai[9], Douglas Mayers[9]*

[1]Quest Clinical Research, San Francisco, CA, USA
[2]University of Utah School of Medicine, Salt Lake City, UT, USA
[3]e Study Site, San Diego, CA, USA
[4]Alvarado Hospital Medical Center, San Diego, CA, USA
[5]Cedars-Sinai Medical Center, Los Angeles, CA, USA
[6]Orlando Immunology Center, Orlando, FL, USA
[7]Indiana University, Indianapolis, IN, USA
[8]University Hepatitis Center at Bach & Godofsky, Sarasota, FL, USA
[9]Idenix Pharmaceuticals, Inc., Cambridge, MA, USA

*Corresponding author e-mail: drjay@questclinical.com

Background: IDX184 is a liver-targeted nucleotide prodrug that selectively inhibits HCV NS5B polymerase.
Methods: This randomized, double-blind, placebo-controlled, ascending-dose study investigated the antiviral activity, safety and pharmacokinetics of IDX184 plus pegylated interferon-α2a and ribavirin (P/R) in treatment-naive patients with genotype-1 HCV. A total of 81 patients with baseline HCV RNA≥5 $\log_{10}$ IU/ml, alanine aminotransferase ≤3× upper limit of normal and compensated liver disease were dosed. Sequential cohorts of 20 patients, randomized 16:4 (active:placebo), received IDX184 for 14 days at rising daily doses of 50, 100, 150 or 200 mg in combination with P/R for 14 days.
Results: At the end of triple dosing, HCV RNA changes from baseline (mean ±SD $\log_{10}$) and proportion of patients achieving undetectable viral load (<15 IU/ml) based on the efficacy-evaluable population were -2.7 ±1.3 (13%), -4.0 ±1.7 (50%), -4.2 ±1.9 (50%), -4.1 ±1.2 (40%), -4.3 ±1.5 (29%) and -3.7 ±1.2 (25%) for the 50 mg once daily, 50 mg twice daily, 100 mg once daily, 150 mg once daily, 100 mg twice daily and 200 mg once daily IDX184 doses, respectively. P/R alone resulted in a reduction of -1.5 ±1.3 $\log_{10}$ with only 6% of patients with undetectable viral load. Patients with genotypes-1a or -1b responded similarly. No viral breakthrough or resistance associated with IDX184 was observed. Anti-HCV activity of IDX184 correlated with plasma exposure of its nucleoside metabolite 2'-methylguanosine. Most adverse events were mild or moderate in severity and were consistent with those associated with P/R. The most common adverse events were fatigue and headache.
Conclusions: IDX184 in combination with P/R for 14 days was well tolerated and demonstrated greater antiviral activity with more patients achieving undetectable viral load than P/R.

Introduction

The recent approval of direct-acting antiviral agents (DAAs), two NS3/4A protease inhibitors of the HCV, has marked the beginning of a new era for the treatment of chronic HCV infection whose only treatment option had been thus far limited to the combination of pegylated interferon (PEG-IFN) and ribavirin (P/R) [1,2].

Treatment of HCV with P/R is characterized by low sustained virological response (SVR) rates, particularly in treatment-naive patients with genotype-1 infection, long duration and poor tolerability, which makes P/R unsuitable for patients with advanced liver disease or other concurrent medical conditions [3,4]. The addition of HCV protease inhibitors to P/R has significantly

improved SVR rates and shortened treatment duration for many patients [1,2]. However, these first-generation protease inhibitors brought additional safety concerns, such as severe skin reaction and/or additional anaemia to an already difficult-to-tolerate regimen which continues to be contraindicated for large numbers of patients [1,2]. Furthermore, protease inhibitors have a low genetic barrier to resistance, which results in high rates of resistance mutations associated with treatment failure, potentially limiting treatment options with additional protease inhibitors [5,6]. Thus, there is an urgent need for simple and better-tolerated anti-HCV therapies consisting preferably of all oral drugs, which could ultimately eliminate interferon and/or ribavirin.

Among the various classes of HCV DAAs developed to date, including HCV NS3/4A protease inhibitors, NS5A inhibitors and non-nucleoside NS5B polymerase inhibitors, nucleoside HCV NS5B polymerase inhibitors have the highest genetic barrier to resistance and are pan-genotypic [5–8]. They have the potential to be an integral part of future interferon-free DAA combination regimens.

IDX184 is a liver-targeted nucleotide prodrug that leads to high concentrations of the active 5′-triphosphate (TP) in hepatocytes of 2′-methylguanosine (2′-MeG), a potent and specific HCV polymerase inhibitor. Liver targeting may result in a favourable therapeutic index by increasing the levels of the active 2′-MeG-TP in liver cells while lowering systemic exposure to the nucleoside, potentially minimizing the risk for systemic side effects [9].

Clinical data from a single-ascending-dose, first-in-human study and a 3-day proof-of-concept trial using doses up to 100 mg/day show that IDX184 was well-tolerated and exhibited dose-related anti-HCV activity in treatment-naive patients infected with genotype-1 HCV [10,11]. Systemic concentrations of IDX184 and 2′-MeG were low, consistent with liver-targeting. The overall pharmacokinetic profile of IDX184 supported once-daily dosing in HCV-infected patients [10].

The objective of this study was to evaluate the antiviral activity, safety and pharmacokinetics of IDX184 following multiple-ascending-doses up to 200 mg/day administered once or twice daily for 14 days in combination with P/R in patients with genotype-1 chronic HCV.

Methods

Study design and organization

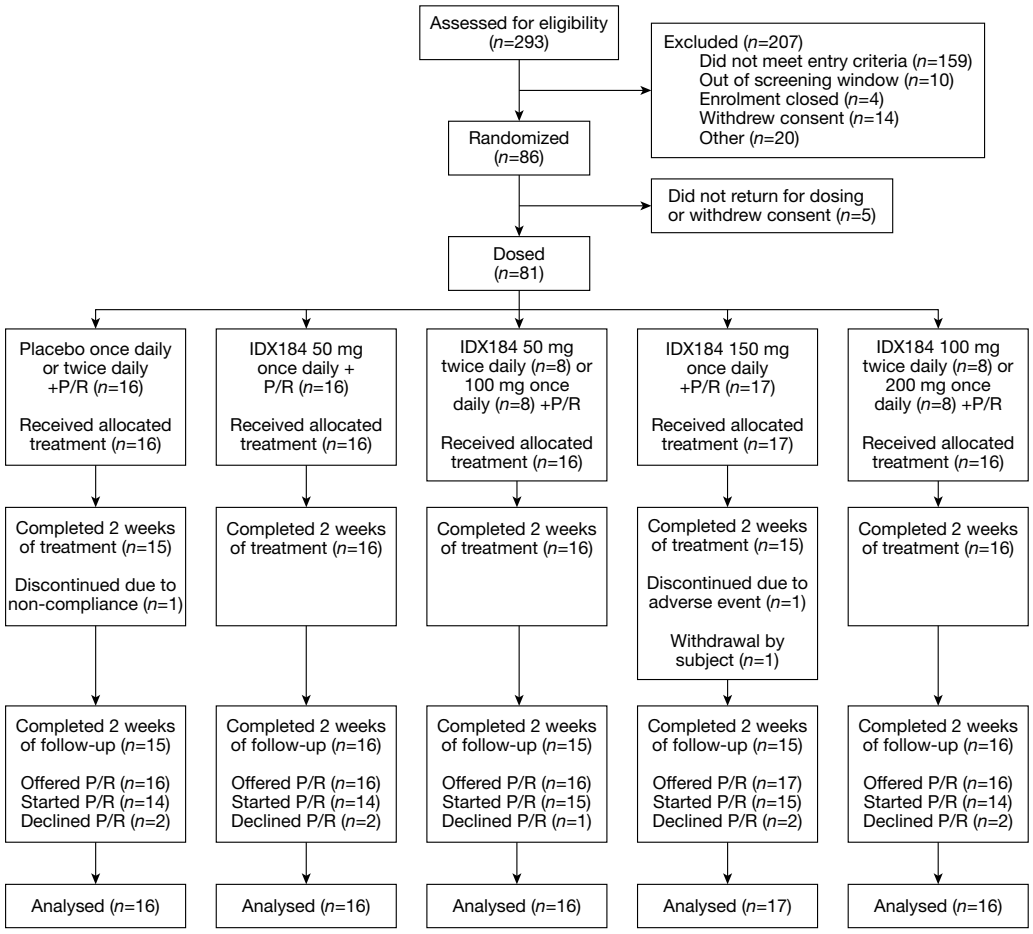
This was a multicentre, randomized, double-blind, placebo-controlled, sequential-cohort, ascending-dose study of IDX184 in combination with PEG-IFN-α2a and ribavirin for 14 days, followed by P/R alone for an additional 14 days in treatment-naive patients with genotype-1 chronic HCV. Sequential cohorts of 20 patients, randomized 16:4 (active:placebo), were administered total daily doses of 50 mg, 100 mg, 150 mg or 200 mg of IDX184. Half the patients in the 100 and 200 mg per day cohorts were administered the total dose once daily; the other half received the total dose as twice daily doses of 50 mg or 100 mg, respectively. IDX184 and matching placebo were supplied as white opaque capsules (manufactured by Patheon Pharmaceuticals, Inc. for Idenix Pharmaceuticals, Inc.). A 50 mg capsule was provided. PEG-IFN-α2a (180 mg single-use, pre-filled syringes, Hoffman–La Roche, Inc., Nutley, NJ, USA) was administered subcutaneously at a dose of 180 μg once per week and ribavirin (200 mg capsules, Zydus Pharmaceuticals, Pennington, NJ, USA) was administered orally at a dose of 1,000–1,200 mg (weight-based) daily, per the approved product labelling. After all patients in a cohort completed all 21 days, safety data were reviewed by an independent safety committee before escalating to the next, higher-dose cohort. Upon completion of the 28-day study, patients were offered P/R treatment for up to 48 weeks. Written informed consent was obtained from all patients. This study was approved by the institutional review boards of the trial centres and conducted from November 2009 to July 2010 in accordance with Good Clinical Practice procedures and the principles of the Declaration of Helsinki, with authorization from regulatory authorities.

Inclusion and exclusion criteria

Major inclusion criteria included: male or female patients 18–65 years old; documented clinical history compatible with chronic hepatitis C, including positive anti-HCV antibody or presence of HCV RNA in the plasma for at least 6 months and liver biopsy within 24 months with histology consistent with chronic hepatitis C infection; HCV genotype-1, plasma HCV RNA≥5 $\log_{10}$ IU/ml; all patients agreed to use double-barrier birth control (such as a condom plus spermicide) from screening through at least 6 months after the last dose of the study drug.

Major exclusion criteria included: pregnancy or breastfeeding; body mass index >35 kg/m^2; co-infection with HBV or HIV; history or evidence of decompensated liver disease; prior clinical or histological evidence of cirrhosis (Metavir 4 or Ishak 6); alanine aminotransferase (ALT) or aspartate aminotransferase level >3.0× upper limit of normal; history of hepatocellular carcinoma or findings suggestive of possible hepatocellular carcinoma; one or more additional known primary or secondary causes of liver disease, other than hepatitis C; previous antiviral treatment for HCV; current abuse of alcohol or illicit drugs; current use of any major inhibitor or inducer of cytochrome P450 3A4 or any other investigational drugs within 30 days of dosing; or other clinically significant diseases

Figure 1. Patient disposition



P/R, pegylated interferon-α2a/ribavirin.

that, in the opinion of the investigator, would jeopardize the safety of the patient or affect the validity of the study results.

Viral assessments

The primary efficacy end point of this study was the change in plasma HCV RNA level at week 2 from baseline. Secondary efficacy end points included change in plasma HCV RNA level at week 4 from baseline, proportion of patients with undetectable plasma HCV RNA at week 2 and week 4, proportion of patients experiencing virological breakthrough during the 2-week period of triple combination treatment, defined as 1 $\log_{10}$ increase in plasma HCV RNA from nadir on two consecutive measurements, or last available measurement, or reappearance of HCV RNA in plasma on two consecutive measurements, or last available measurement in a patient who became plasma HCV RNA undetectable. Secondary efficacy end points also included ALT change at week 2 and week 4 from baseline. The primary efficacy analyses were performed on the efficacy-evaluable population using data as-observed with the placebo-treated group comprising all placebo patients pooled across cohorts.

The change in plasma HCV RNA level from baseline was obtained on days 1, 4, 8, 11 and 15 for triple dosing and on days 21 and 28 during follow-up. Plasma HCV RNA was determined by a validated real-time (RT) PCR assay (COBAS® AmpliPrep/

COBAS® Taqman HCV Test) with a quantitative dynamic range of 43–69,000,000 IU/ml and a lower limit of detection of 15 IU/ml.

A baseline DNA sample was collected from each patient to determine the genotype (C/C, C/T or T/T) of the rs12979860 single nucleotide polymorphism near the human IL28B gene [12].

NS5B sequence analyses

Baseline population sequencing of the entire HCV NS5B region was performed using plasma samples obtained from all patients. NS5B population sequencing was also performed using samples from all patients with viral load ≥1,000 IU/ml at the completion of the 14-day triple dosing and at study completion on day 28. RNA was isolated from clinical samples using the Total Nucleic Acid isolation kit (Roche Applied Science, Indianapolis, IN, USA). The NS5B domain was converted into circular DNA by the Roche Transcriptor kit and amplified by nested PCR using the Expand High Fidelity kit A (Roche Applied Science) mixed base was called if the lowest signal exceeded 25% of the highest signal.

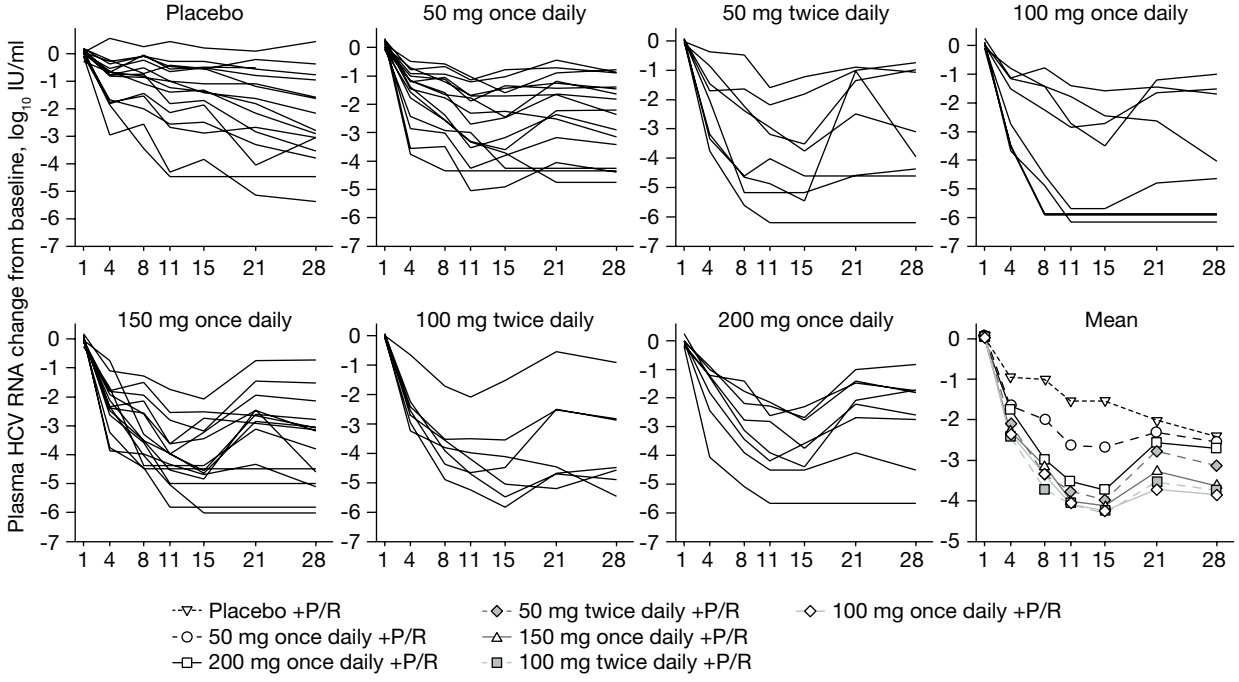
Pharmacokinetics and drug interaction

Intensive plasma pharmacokinetic assessments were performed over a period of 8 h after dosing on day 1 (IDX184 only) and day 11 (IDX184 and ribavirin) with additional pre-dose sampling for trough level monitoring. IDX184 plus P/R versus P/R alone allowed for assessing potential effects of IDX184 on the pharmacokinetics of ribavirin. Plasma concentrations of IDX184, 2′-MeG [10] and ribavirin were measured using validated liquid chromatography/mass spectrometry/mass spectrometry methodologies. Plasma pharmacokinetic parameters included maximum drug concentration (C_{max}), time to C_{max} (T_{max}), pre-dose trough concentration (C_t) for 2′-MeG, area under the plasma concentration-time curve (AUC) over the dosing interval for 2′-MeG and ribavirin and from time 0 to infinity for IDX184, and observed half-life ($t_{1/2}$) over the sampling period for IDX184 and 2′-MeG.

Safety assessments

Safety evaluation consisted of collecting all adverse events and serious adverse events, as well as regular

Figure 2. Individual and mean changes from baseline in plasma HCV RNA (log_{10} IU/ml) over time (days 1–14: IDX184 +P/R; beyond day 14: P/R)



P/R, pegylated interferon-α2a/ribavirin.

Table 1. Demographic and baseline characteristics

Baseline characteristics	Placebo +P/R	IDX184+P/R 50 mg once daily	50 mg twice daily	100 mg once daily	150 mg once daily	100 mg twice daily	200 mg once daily	All IDX184
n	16	16	8	8	17	8	8	65
Mean age, years (SD)	48.1 (8.6)	51.3 (9.8)	49.8 (11)	47.8 (12)	47.6 (12)	49.0 (7.8)	42.0 (12)	48.3 (11)
Male gender, *n* (%)	10 (62.5)	14 (87.5)	5 (62.5)	5 (62.5)	13 (76.5)	4 (50.0)	5 (62.5)	46 (70.8)
Race								
Black, *n* (%)	4 (25.0)	5 (31.3)	3 (37.5)	2 (25.0)	4 (23.5)	2 (25.0)	–	16 (24.6)
White, *n* (%)	11 (68.8)	9 (56.3)	5 (62.5)	5 (62.5)	13 (76.5)	6 (75.0)	7 (87.5)	45 (69.2)
Other, *n* (%)	1 (6.3)	2 (12.5)	–	1 (12.5)	–	–	1 (12.5)	4 (6.2)
Mean BMI, kg/m^2 (SD)	27.8 (2.9)	27.0 (4.1)	29.0 (3.6)	28.7 (4.1)	26.3 (4.1)	27.9 (4.1)	29.4 (4.2)	27.7 (4.0)
Mean HCV RNA, $\log_{10}$ IU/ml (SD)	6.3 (0.5)	6.1 (0.6)	6.3 (0.5)	6.4 (0.6)	6.4 (0.6)	6.5 (0.6)	6.1 (0.5)	6.3 (0.6)
HCV genotype								
1a, *n* (%)	13 (81.3)	13 (81.3)	7 (87.5)	7 (87.5)	16 (94.1)	7 (87.5)	5 (62.5)	55 (84.6)
1b, *n* (%)	3 (18.8)	3 (18.9)	1 (12.5)	1 (12.5)	1 (5.9)	1 (12.5)	3 (37.5)	10 (15.4)
IL28B polymorphism								
C/C, *n* (%)	4 (25.0)	4 (25.0)	1 (12.5)	5 (62.5)	5 (29.4)	2 (25.0)	1 (12.5)	18 (27.7)
C/T, *n* (%)	8 (50.0)	8 (50.0)	6 (75.0)	2 (25.0)	4 (23.5)	4 (50.0)	5 (62.5)	29 (44.6)
T/T, *n* (%)	2 (12.5)	3 (18.8)	1 (12.5)	1 (12.5)	2 (11.8)	2 (25.0)	–	9 (13.8)
NA, *n* (%)	2 (12.5)	1 (6.2)	–	–	6 (35.3)	–	2 (25.0)	9 (13.8)

BMI, body mass index; NA, not applicable as IL28B genotype status not available; P/R, pegylated interferon-α2a.

monitoring of haematology, blood chemistry, urinalysis, vital signs, 12-lead electrocardiogram and physical examinations. Concomitant medications were also collected.

Statistical analyses

With a sample size of 16 IDX184-treated patients per cohort, there was approximately an 80% chance to observe an adverse event with a rate of 10% and to detect a 1.4 $\log_{10}$/ml difference between an IDX184-treated and the pooled (*n*=16) placebo cohorts. Safety, antiviral activity and pharmacokinetic parameters were summarized for each cohort using descriptive statistics. Safety and antiviral data from placebo (P/R alone) patients were pooled and summarized. Quantitative pharmacokinetic–pharmacodynamic relationships were explored between antiviral activity at the end of triple dosing and trough concentrations of 2′-MeG using a maximum effect (E_{max}) modelling approach. Potential effects of IDX184 on the plasma C_{max} and area under the curve $(AUC)_t$ of ribavirin were analysed for each dose of IDX184, as well as pooled data. Additional exploratory analyses by stepwise and logistic regression were performed to identify pharmacokinetic and baseline predictors of viral response. The baseline characteristics included gender, weight, body mass index, pretreatment HCV RNA, HCV genotype-1 subtypes, IL28B genotype, pretreatment ALT, race, liver histology and investigational site. All statistical analyses were performed using SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics and disposition

As shown in Figure 1, in total, 86 treatment-naive patients with genotype-1 chronic HCV infection were randomized; of whom, 81 received at least one dose of the study drugs and, therefore, constituted the safety population. Individuals were not cirrhotic as confirmed by liver biopsy: of the 81 individuals, the majority (76) had F0–F2 fibrosis and only 5 had F3 fibrosis (Metavir score for fibrosis staging). The demographics and other baseline characteristics across the cohorts were comparable. Patients were predominately male, Caucasian and infected with genotype-1a HCV. Table 1 summarizes the baseline characteristics.

Of the 81 dosed patients, 78 completed 14 days of triple dosing (including placebo) and constituted the efficacy-evaluable population. They were included in the 2-week antiviral activity and pharmacokinetic analyses.

A total of three treated patients were excluded from the 2-week efficacy and pharmacokinetic analyses: one patient in the 150 mg cohort and one patient in the 100 mg twice daily cohort had <80% compliance with the study drug regimen; a third patient in the 150 mg cohort discontinued prior to the completion of triple dosing as a result of a serious adverse event of acute cholecystitis (see *Safety and tolerability*). An additional patient who received placebo discontinued the study after day 14 for poor compliance.

Table 2. HCV RNA change from baseline and number/proportion of patients with undetectable viral load (<15 IU/ml)

IDX184 dose, mg/day	*n*	Mean HCV RNA reduction, $\log_{10}$ IU/ml ±SD (minimum, maximum)		HCV RNA undetectable (<15 IU/ml), *n* (%)	
		Week 2	Week 4	Week 2	Week 4
Placebo	16	-1.5 ±1.3 (-4.5, 0.2)	-2.4 ±1.6 (-5.4, 0.4)	1 (6)	1 (6)
50 Once daily	16	-2.7 ±1.3 (-4.9, -0.8)	-2.5 ±1.4 (-4.7, -0.8)	2 (13)	3 (19)
50 Twice daily	8	-4.0 ±1.7 (-6.2, -1.2)	-3.1 ±2.0 (-6.2, -0.7)	4 (50)	2 (25)
100 Once daily	8	-4.2 ±1.9 (-6.2, -1.6)	-3.9 ±2.2 (-6.2, -1.0)	4 (50)	3 (38)
150 Once daily	15	-4.1 ±1.2 (-6.0, -2.1)	-3.6 ±1.5 (-6.0, -0.7)	6 (40)	4 (27)
100 Twice daily	7	-4.3 ±1.5 (-5.8, -1.5)	-3.7 ±1.6 (-5.5, -0.9)	2 (29)	1 (14)
200 Once daily	8	-3.7 ±1.2 (-5.7, -2.3)	-2.7 ±1.6 (-5.7, -0.8)	2 (25)	2 (25)

Plasma pharmacokinetics and drug interaction

The mean (SD) steady-state plasma concentration versus time courses of IDX184 and 2′-MeG and summary pharmacokinetic parameters are shown in Additional files 1 and 2, respectively. Plasma exposures of both entities were dose related. Parent IDX184 was rapidly cleared from plasma and no accumulation was observed over time. For the same daily dose, twice daily and once daily dosing had comparable 2′-MeG exposure. Steady-state plasma concentrations of 2′-MeG had minimum fluctuations over the dose interval. In fact, stable 2′-MeG trough concentrations were reached by day 3. Mean ±SD steady-state trough 2′-MeG concentrations were 2.55 ±2.01, 7.34 ±4.96, 7.15 ±7.28, 7.47 ±4.72, 12.8 ±8.46 and 9.69 ±6.42 for the 50 mg once daily, 50 mg twice daily, 100 mg once daily, 150 mg once daily, 100 mg twice daily and 200 mg once daily doses of IDX184, respectively (Additional file 2).

Plasma exposure of ribavirin was not affected by co-administered IDX184. Geometric mean C_{max} and AUC over dosing interval of ribavirin in the presence versus absence of IDX184 were 2.1 μg/ml versus 2.3 μg/ml and 13.4 μg/ml×h versus 14.0 μg/ml×h, respectively.

Anti-HCV activity and ALT response

Individual and mean changes over time of plasma HCV RNA from baseline are shown in Figure 2. Antiviral response at week 2 increased rapidly with IDX184 dose, reaching a plateau of approximately -4 $\log_{10}$ with doses ≥100 mg/day, compared with P/R alone with only -1.5 $\log_{10}$ (Table 2). During the week following completion of IDX184 dosing, plasma HCV RNA transiently increased and then decreased through day 28 in the IDX184 plus P/R groups. During the 14-day follow-up period, IDX184-containing groups consistently maintained a numerically better antiviral activity than the P/R alone group (Table 2).

The number and proportion of patients who achieved undetectable levels of plasma HCV RNA at week 2 was greater in the triple dosing cohorts compared with P/R alone. As summarized in Table 2, amongst the IDX184 plus P/R dose cohorts, the 100 mg once daily dose had the greatest PCR undetectable rate of 50% and 38% at week 2 and week 4, respectively, compared with P/R alone with only one patient (6%) achieving PCR-negative viral load throughout the study. For those who had undetectable viral load at week 2, time to reach HCV RNA negativity was also shorter in the IDX184 groups: approximately one-third (7/20) of patients in the IDX184 groups achieved HCV RNA negativity within the first 8 days of dosing compared with none in the P/R only group.

During triple dosing, none of the patients experienced virological breakthrough, defined as a confirmed 1 $\log_{10}$ increase in HCV RNA from nadir or the confirmed reappearance of HCV RNA in a patient who had become HCV RNA undetectable.

Serum ALT levels also decreased from baseline (Figure 3). Upon completion of the 14-day triple treatment, mean decreases from baseline ALT were greater in the IDX184 treatment groups compared with the P/R alone group.

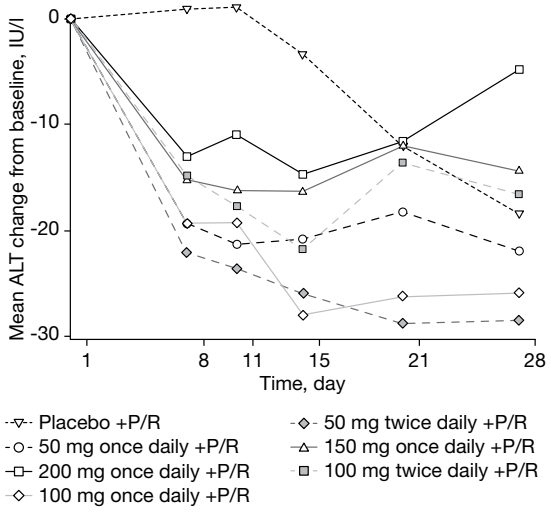
Predictors of anti-HCV activity

Among all the potential factors examined, only IDX184 dose (P=0.002), trough plasma concentration of 2′-MeG (P<0.0001) and IL28B genotype status (P<0.0001) were consistently identified as significant covariates of antiviral response at week 2. Although the number of patients was small, sub-genotypes (1a and 1b) did not appear to differentiate antiviral response.

NS5B sequence analyses

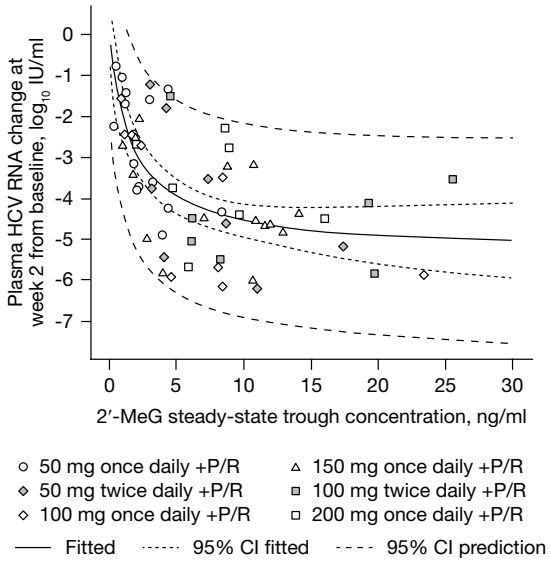
Samples from 24 IDX184-treated and 14 placebo (P/R only) patients were available at end of treatment for comparison with baseline NS5B population sequences. The S282T substitution, the hallmark mutation associated with *in vitro* resistance to IDX184 and the 2′-C-methylribonucleoside class of HCV polymerase inhibitors, was not detected in any sample at any time point by population sequencing [9,13]. Similarly, no other significant genotypic changes (n>1) were identified between pre- and post-treatment samples or between IDX184- and placebo-treated patients.

Figure 3. Mean changes from baseline in serum ALT (days 1–14: IDX184+P/R; beyond day 14: P/R)



ALT, alanine aminotransferase; P/R, pegylated interferon-α2a/ribavirin.

Figure 4. E_{max} modelling of dose-response



E_{max}, maximum effect; P/R, pegylated interferon-α2a/ribavirin.

Pharmacokinetic–pharmacodynamic analyses

The dose-relatedness of antiviral response was further evaluated by fitting an E_{max} model to the mean individual steady-state trough concentration (C_t) of 2′-MeG and viral load reduction at week 2 from baseline. Figure 4 shows the fitted E_{max} curve overlapped the actual data for virological response at week 2 as a function of the 2′-MeG C_τ. This relationship indicated that virological response at week 2 increased rapidly with C_t up to approximately 5 ng/ml and started approaching the maximum antiviral activity with C_t>10 ng/ml. The estimates (95% CI) for the maximum viral load reduction at week 2 and the 2′-MeG C_τ that resulted in 50% of maximum effect were -5.3 $\log_{10}$ (-6.1,-4.5) and 1.7 ng/ml (0.7, 2.7), respectively.

Safety and tolerability

Adverse events reported during the triple-dosing phase and the follow-up period were consistent with those typically observed with P/R. As summarized in Table 3, fatigue and headache were the most common adverse events across treatment groups. The incidence and severity of other adverse events across treatment groups were comparable. The majority of the patients (91% in the IDX184 groups and 75% in the placebo group) experienced adverse events that were either mild or moderate in intensity.

There were three serious adverse events, of which one occurred during triple dosing and two occurred during the 14-day follow-up period with P/R alone. One patient in the 150 mg IDX184 once-daily group experienced acute cholecystitis on day 12 during triple dosing and discontinued. One patient in the 50 mg IDX184 once-daily group experienced pancreatitis attributed to gallstones on the last day of the follow-up period. One patient in the placebo group (P/R alone) was hospitalized for agitation near the end of the follow-up period. The investigator considered the event possibly related to PEG-IFN.

Newly occurring or worsening graded laboratory abnormalities during triple dosing and follow-up were comparable across treatment groups, with no discernible relationship to IDX184 dose. Grade 3 and 4 laboratory abnormalities were rare, with the majority being neutrophil and haemoglobin decreases, which are commonly associated with P/R. Other laboratory parameters were stable over time for all treatment groups. No clinically meaningful changes were observed in vital sign measurements, physical examination findings or ECG parameters.

Discussion

Nucleoside and nucleotide anti-HCV agents are pangenotypic and have a high genetic barrier to resistance [5,6]. This class of DAAs, therefore, represents a potential key component in an all-oral regimen for the treatment of HCV infection. Early nucleoside analogues had limited clinical activity primarily because of low levels of active triphosphate in the liver cells [14]. Recent

Table 3. Number of patients with adverse events during the 14-day triple dosing and the 14-day follow-up period regardless of attributability[a]

		IDX184+P/R					
Preferred term	Placebo +P/R (*n*=16)	50 mg once daily (*n*=16)	50 mg twice daily (*n*=8)	100 mg once daily (*n*=8)	150 mg once daily (*n*=17)	100 mg twice daily (*n*=8)	200 mg once daily (*n*=8)
Fatigue, *n* (%)	9 (56.3)	9 (56.3)	4 (50.0)	3 (37.5)	9 (52.9)	7 (87.5)	4 (50.0)
Headache, *n* (%)	7 (43.8)	2 (12.5)	5 (62.5)	2 (25.0)	9 (52.9)	4 (50.0)	5 (62.5)
Nausea, *n* (%)	3 (18.8)	4 (25.0)	4 (50.0)	1 (12.5)	5 (29.4)	5 (62.5)	3 (37.5)
Myalgia, *n* (%)	5 (31.3)	7 (43.8)	2 (25.0)	3 (37.5)	2 (11.8)	2 (25.0)	3 (37.5)
Chills, *n* (%)	5 (31.3)	4 (25.0)	2 (25.0)	2 (25.0)	2 (11.8)	3 (37.5)	2 (25.0)
Insomnia, *n* (%)	5 (31.3)	1 (6.3)	1 (12.5)	2 (25.0)	3 (17.6)	2 (25.0)	1 (12.5)
Pain, *n* (%)	3 (18.8)	1 (6.3)	2 (25.0)	–	4 (23.5)	2 (25.0)	3 (37.5)
Irritability, *n* (%)	4 (25.0)	1 (6.3)	1 (12.5)	4 (50.0)	2 (11.8)	1 (12.5)	1 (12.5)
Neutropenia, *n* (%)	4 (25.0)	2 (12.5)	1 (12.5)	1 (12.5)	1 (5.9)	1 (12.5)	–
Diarrhoea, *n* (%)	2 (12.5)	–	2 (25.0)	2 (25.0)	1 (5.9)	2 (25.0)	–
Pyrexia, *n* (%)	–	1 (6.3)	1 (12.5)	2 (25.0)	3 (17.6)	1 (12.5)	1 (12.5)
Decreased appetite, *n* (%)	3 (18.8)	1 (6.3)	–	1 (12.5)	1 (5.9)	2 (25.0)	–
Pruritus, *n* (%)	2 (12.5)	2 (12.5)	–	–	–	2 (25.0)	2 (25.0)
Depression, *n* (%)	3 (18.8)	–	1 (12.5)	–	–	1 (12.5)	2 (25.0)
Injection site erythema, *n* (%)	–	2 (12.5)	–	–	3 (17.6)	–	1 (12.5)
Disturbance in attention, *n* (%)	–	1 (6.3)	–	1 (12.5)	–	2 (25.0)	1 (12.5)
Malaise, *n* (%)	–	–	2 (25.0)	2 (25.0)	–	1 (12.5)	–
Vomiting, *n* (%)	–	1 (6.3)	2 (25.0)	–	1 (5.9)	1 (12.5)	–
Gingival bleeding, *n* (%)	–	–	–	–	–	2 (25.0)	–
Gingivitis, *n* (%)	–	–	–	–	–	2 (25.0)	–

[a]>15% in any group. P/R, pegylated interferon-α2a.

developments in the field of anti-HCV nucleosides/nucleotides have been centred on liver targeting and have produced several nucleotide prodrugs with promising activity in early clinical trials [15–17].

IDX184 is a liver-targeted prodrug of the nucleotide 2′-MeG-MP. *In vitro* and *in vivo* data showed enhanced formation of the active triphosphate in the liver with increased anti-HCV activity [18,19]. Clinical data demonstrated low systemic exposure, favourable safety profile and significant anti-HCV activity with low doses of IDX184 for 3 days [10,11] and prompted this longer-term study in combination with P/R.

In this study, patients who received P/R alone for 2 weeks had HCV RNA reduction consistent with previously reported data [20,21]. IDX184 exhibited potent antiviral activity when combined with P/R: at week 2, the lowest daily dose of 50 mg produced approximately 1 $\log_{10}$ more viral load reduction than P/R alone. Antiviral activity increased with IDX184 dose and reached a plateau of approximately 4 $\log_{10}$ at total daily doses of 100 mg and above. In the week following discontinuation of IDX184, although patients were maintained on continued P/R, mean viral loads in the IDX184 groups transiently increased, presumably as a result of the withdrawal of antiviral drug pressure exerted by IDX184, before decreasing again at rates comparable with P/R but with up to 1.5 $\log_{10}$ more viral load reduction than P/R alone at week 4. The number and proportion of patients who achieved undetectable levels of HCV RNA were consistent with the degree of plasma HCV RNA reduction and were higher with triple dosing than P/R alone. For those who achieved undetectable levels of HCV RNA by week 2, time to HCV RNA negativity was shorter in the IDX184 groups than the P/R alone group.

Although antiviral activity analysis was performed using the efficacy-evaluable population, which excluded data from three individuals receiving triple dosing who had poor compliance or discontinued prior to reaching week 2, intent-to-treat analysis showed similar antiviral activity and proportion of individual with undetectable levels of HCV RNA at week 2 (data not shown, D Mayers, Idenix Pharmaceuticals).

Exploratory analyses were performed to identify predictors of antiviral activity. IDX184 dose/exposure and IL28B genotype status were the only significant predictors of antiviral response at week 2. Twice-daily dosing did not appear to result in better antiviral activity than once-daily dosing. Similar to the previous proof-of-concept study [11], the majority of patients enrolled in the current study had genotype-1a infection. Although the number of patients was small, descriptively, antiviral

response was similar between patients with genotype-1a and -1b infection. As was previously observed in HCV-infected patients with 3 days of dosing [11], a similar pharmacokinetic–pharmacodynamic relationship was also delineated by E_{max} modelling analysis: higher steady-state 2′-MeG trough concentrations resulted in larger HCV viral load decrease at week 2.

Pharmacokinetic data showed that plasma IDX184 and 2′-MeG exposures were, in general, dose-related and low, consistent with previous results in HCV-infected patients [11]. Although plasma exposures of IDX184 and 2′-MeG were somewhat lower in HCV-infected than healthy individuals [10,11], these differences did not appear to result from differences in metabolic drug disposition between HCV-infected and healthy individuals, but rather were likely because of an effect of food that was recently characterized (XJ Zhou, unpublished data). In addition, IDX184 did not affect the pharmacokinetics of co-administered ribavirin.

None of the patients experienced virological breakthrough. The signature S282T mutation, reported to confer resistance to the 2′-C-methylribonucleoside class of HCV polymerase inhibitors in *in vitro* studies [9], was not detected as a pre-existing variant nor was it observed in any sample following treatment with IDX184 or placebo. The changes observed by NS5B sequencing primarily involved changes in the composition of amino acid mixtures, and all changes appeared to be random. These viral genotypic observations, together with the absence of virological breakthrough, suggest that resistance to IDX184 did not emerge in this trial.

Overall, adverse events were consistent with those that would be expected with administration of a drug combination, including P/R. Fatigue and headache were most common and occurred in all treatment groups. Serum ALT levels improved during the 14-day IDX184 treatment period and the improvement persisted in most IDX184 groups. The trend was similar for aspartate aminotransferase (data not shown).

In summary, at doses of 50–200 mg/day in combination with P/R for 14 days in treatment-naive patients with genotype-1 HCV infection, IDX184 produced more profound anti-HCV activity with a higher proportion of patients achieving undetectable plasma HCV RNA than P/R alone. The adverse event profile of IDX184 in combination with P/R was consistent with the safety profile of P/R alone. There were no clinically significant patterns of ECG or other laboratory abnormalities. The antiviral activity, safety and pharmacokinetic data from this 14-day study in combination with P/R supported a 3-month clinical trial of IDX184 in combination with P/R in treatment-naive, genotype-1 HCV-infected patients [22].

Acknowledgements

The authors wish to thank the HCV-infected patients, who generously agreed to participate in this clinical study. We also thank the study coordinators, nurses and staff at clinical study sites.

The investigators who participated in the study include: D Asmuth, University of California Davis, Sacramento, CA, USA; T Box, University of Utah School of Medicine, Salt Lake City, UT, USA; E DeJesus, Orlando Immunology Center, Orlando, FL, USA; A Di Bisceglie, Saint Louis University, Saint Louis, MO, USA; E Godofsky, University Hepatitis Center at Bach & Godofsky, Sarasota, FL, USA; P Kwo, Indiana University, Indianapolis, IN, USA; J Lalezari, Quest Clinical Research, San Francisco, CA, USA; P Mehra eStudy Site, Chula Vista, CA, USA; F Mendes, VA Medical Center Miami, Miami, FL, USA; A Muir, Duke University Medical Center, Durham, NC, USA; T Nguyen, San Diego, CA, USA; W O'Riordan, eStudy Site, LaMesa, CA, USA; F Poordad, Cedars-Sinai Medical Center, Los Angeles, CA, USA; J Poulos, Cumberland Research Associates, LLC, Fayetteville, NC, USA; H Vargas, Mayo Clinic Arizona, Phoenix, AZ, USA.

Disclosure statement

This study was supported by Idenix Pharmaceuticals, Inc., Cambridge, MA, USA. JL, TB, WO, PM, TN, FP, ED, PK, EG and SL are clinical investigators contracted by Idenix Pharmaceuticals, Inc. for the conduct of the study. GD-P, JC, JM, KP, X-JZ, JS-B and DM are employees of Idenix Pharmaceuticals, Inc.

Additional files

Additional file 1: A figure depicting the mean (SD) steady-state plasma concentration–time profiles of IDX184 and the nucleoside metabolite 2′-MeG in patients with genotype-1 HCV infection can be found at http://www.intmedpress.com/uploads/documents/2754_Lalezari_Additional_file_1.pdf

Additional file 2: A table summarizing the steady-state plasma pharmacokinetic parameters of IDX184 and the nucleoside metabolite 2′-MeG in patients with genotype-1 HCV infection can be found at http://www.intmedpress.com/uploads/documents/2754_Lalezari_Additional_file_2.pdf

References

1. Victrelis (boceprevir). *Package insert* 2012. Merck, Whitehouse Station, NJ, USA.
2. Incivek (telaprevir). *Package insert* 2012. Vertex Pharmaceuticals, Cambridge, MA, USA.

3. Manns MP, McHutchison JG, Gordon SC, *et al*. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358:**958–965.
4. Fried MW, Shiffman ML, Reddy KR, *et al*. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347:**975–982.
5. McCown MF, Rajyaguru S, Le Pogam S, *et al*. The hepatitis C virus replicon presents a higher barrier to resistance to nucleoside analogs than to nonnucleoside polymerase or protease inhibitors. *Antimicrob Agents Chemother* 2008; **52:**1604–1612.
6. Sarrazin C, Zeuzem S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. *Gastroenterology* 2010; **138:**447–462.
7. Thompson A, Patel K, Tillman H, McHutchison JG. Directly acting antivirals for the treatment of patients with hepatitis C infection: a clinical development update addressing key future challenges. *J Hepatol* 2009; **50:**184–194.
8. Rodríguez-Torres M. On the cusp of change: new therapeutic modalities for HCV. *Ann Hepatol* 2010; **9 Suppl:**123–131.
9. Cretton-Scott E, Perigaud C, Peyrottes S, *et al*. *In vitro* antiviral activity and pharmacology of IDX184, a novel and potent inhibitor of HCV replication. *J Hepatol* 2008; **48 Suppl 2:**S220.
10. Zhou XJ, Pietropaolo K, Chen J, Khan S, Sullivan-Bólyai J, Mayers D. Safety and pharmacokinetics of IDX184, a liver-targeted nucleotide polymerase inhibitor of hepatitis C virus, in healthy subjects. *Antimicrob Agents Chemother* 2011; **55:**76–81.
11. Lalezari J, Asmuth D, Casiró A, *et al*. Short-term monotherapy with IDX184, a liver-targeted nucleotide polymerase inhibitor, in patients with chronic hepatitis C infection. *Antimicrob Agents Chemother* 2012; **56:**6372–6378.
12. Ge D, Fellay J, Thompson AJ, *et al*. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461:**399–401.
13. Le Pogam S, Jiang WR, Leveque V, *et al*. *In vitro* selected Con1 subgenomic replicons resistant to 2′-C-Methyl-Cytidine or to R1479 show lack of cross resistance. *Virology* 2006; **351:**349–359.
14. Godofsky E, Afdhal N, Rustgi V, *et al*. First clinical results for a novel antiviral treatment for hepatitis C: a Phase I/II dose escalation trial assessing tolerance, pharmacokinetics, and antiviral activity of NM283. *J Hepatol* 2004; **40 Suppl 1:**35.
15. Nelson DR, Lalezari J, Lawitz E, *et al*. Once-daily PSI-7977 plus PEG-IFN/RBV in HCV GT1: 98% rapid virologic response, complete early virologic response: the PROTON study. *J Hepatol* 2011; **54 Suppl 1:**S544.
16. Rodriguez-Torres M, Lawitz E, Denning J, *et al*. PSI-352938, a novel purine nucleotide analog, exhibits potent antiviral activity and no evidence of resistance in patients with HCV genotype 1 over 7 days. *J Hepatol* 2011; **54 Suppl 1:**S488.
17. Rodriguez-Torres M, Lawitz E, Hazan L, *et al*. Antiviral activity and safety of INX-08189, a nucleotide polymerase inhibitor following 7-days of oral therapy in naive genotype-1 HCV patients. *Hepatology* 2011; **54 Suppl 1:**535A.
18. Standring DN, Lanford R, Cretton-Scott E, *et al*. Potent antiviral activity of second generation nucleoside inhibitors, IDX102 and IDX184, in HCV-infected chimpanzees. *J Hepatol* 2008; **48 Suppl 2:**S30.
19. Standring DN, Lanford R, Li B, *et al*. Antiviral activity of the liver targeted nucleotide HCV polymerase inhibitor IDX184 correlates with trough serum levels of the nucleoside metabolite in HCV-infected chimpanzees. *J Hepatol* 2009; **50 Suppl 1:**S37.
20. Herrmann E, Lee JH, Marinos G, Modi M, Zeuzem S. Effect of ribavirin on hepatitis C viral kinetics in patients treated with pegylated interferon. *Hepatology* 2003; **37:**1351–1358.
21. Hézode C, Forestier N, Dusheiko G, *et al*. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360:**1839–1850.
22. Lawitz E, Box TD, Pruitt R, *et al*. High rates of rapid virologic response (RVR) and complete early virologic response (cEVR) with IDX184, pegylated interferon and ribavirin in genotype 1 HCV-infected subjects: interim results. *63th Annual Meeting of the American Association for the Study of liver diseases*. 9–13 November 2012, Boston, MA, USA. Abstract 1748.

Accepted 10 January 2013; published online 25 February 2013