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Clinical trial: the efficacy and safety of oral PF-03491390, a pancaspase inhibitor – a randomized placebo-controlled study in patients with chronic hepatitis C

M. L. SHIFFMAN*, P. POCKROS†, J. G. MCHUTCHISON‡, E. R. SCHIFF§, M. MORRIS¶ & G. BURGESS¶

*McGuire Research Institute, McGuire Veterans Administration Medical Center, Richmond, VA, USA; †Division of Gastroenterology and Hepatology, Scripps Clinic, La Jolla, CA, USA; ‡Division of Gastroenterology and Duke Clinical Research Institute, Duke University, Durham, NC, USA; §Division of Hepatology, Center for Liver Diseases, University of Miami School of Medicine, Miami, FL, USA; ¶Pfizer Ltd, Sandwich, UK

Correspondence to:
Dr M. L. Shiffman, Liver Institute of Virginia, Bon Secours Health System, Mary Immaculate Hospital – Medical Pavilion, Suite 313, 2 Bernadine Drive, Newport News, VA 23602, USA.
E-mail: mlshiffman@gmail.com

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SUMMARY

Background
Elevated serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reflect hepatocellular injury in patients with chronic hepatitis C virus (HCV). Increased apoptosis and activated caspases are present in these patients. PF-03491390 inhibits multiple caspases and lowers serum AST and ALT levels in patients with chronic liver diseases.

Aim
To determine if treatment with an oral pancaspase inhibitor could reduce serum AST and ALT in patients with HCV.

Methods
Double-blind, randomized, placebo-controlled, parallel-dose study in 204 patients treated with placebo or PF-03491390 (5, 25 or 50 mg) orally twice daily (b.d.) for up to 12 weeks. Serum AST and ALT were monitored weekly.

Results
Significant reductions in serum AST and ALT were observed within 1 week of initiating PF-03491390 in all treatment groups ($P < 0.0001$). These reductions in AST and ALT were maintained throughout the 12 week treatment period and returned to baseline levels when PF-03491390 was discontinued. Increasing the dose did not further lower AST or ALT. The most frequently reported adverse events were headache and fatigue.

Conclusion
PF-03491390 significantly reduced serum AST and ALT levels in patients with chronic HCV, and was well tolerated over 12 weeks.

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INTRODUCTION

Hepatitis C virus (HCV) causes hepatic inflammation and leads to progressive liver fibrosis, cirrhosis and hepatocellular carcinoma.[1] The current standard of care for chronic HCV is peginterferon and ribavirin and this yields a sustained virologic response (SVR) in approximately 40% of patients with genotype 1[2–4] and 70–80% of patients with genotypes 2 and 3.[2, 3, 5] Preliminary studies have demonstrated that the addition of a protease inhibitor to peginterferon and ribavirin can increase SVR in patients with HCV genotype 1 into the 60–70% range.[6, 7] Thus, many patients with chronic HCV will require alternative therapies to prevent disease progression.

Apoptosis is a highly regulated programmed form of cell death that occurs during normal development, normal tissue turnover and in numerous diseases, including hepatitis.[8, 9] The process occurs through activation of specific molecular pathways that are regulated via a complex network of proteins and endogenous inhibitors.[10] Caspases are key mediators of apoptosis. They are a family of 14 intracellular cysteine-dependent aspartate-specific proteases which can be controlled upstream by factors that lead to their activation or downstream by inhibitors that prevent them from acting on their substrates.[11] The role of apoptosis in patients with chronic HCV infection is uncertain. Apoptosis of hepatocytes may promote liver injury and fibrosis, whereas apoptosis of collagen-producing activated hepatic stellate cells may limit hepatic fibrosis.[8] Increased levels of apoptotic cells have been observed in the liver of patients with chronic HCV infection[12] and the number of apoptotic cells present in liver biopsies has been shown to correlate with the grade of inflammation.[13] Nonresponders to interferon treatment have lower serum caspase activity compared to patients who become HCV RNA undetectable during treatment.[14]

PF-03491390 (formerly called IDN-6556) is an irreversible, specific, broad-spectrum caspase inhibitor, which blocks the function of caspases 1, 2, 3, 6, 7, 8 and 9.[15, 16] It has been shown to have activity *in vitro* and in animal models of liver disease where apoptosis is thought to contribute to pathogenesis.[16–18] In a previous study conducted in patients with various forms of chronic liver disease and elevated serum aminotransferases, 2 weeks of treatment with PF-03491390 was associated with a significant reduction in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[19–21] The objectives of this study were to expand upon these observations specifically in patients with chronic HCV and to determine if PF-03491390 had any effect on serum HCV RNA level.

PATIENTS AND METHODS

Study design and treatment schedule

This was a phase II, randomized, multicentre, placebo-controlled, parallel group, dose–response study in patients with chronic HCV infection conducted at 15 sites in the US, as detailed in the Acknowledgements. The study included a baseline period of up to 4 weeks, a randomized double-blind treatment period of 10 weeks, an open-label treatment period of 2 weeks, and an observation period of 4 weeks. Patients randomized to and treated in the placebo group for 12 weeks were eligible to enter a 12-week open-label dose escalation treatment phase and then followed for an additional 4 weeks. The total duration of treatment in the protocol for these patients was 29 weeks.

The study sponsor supplied gelatin capsules containing PF-03491390 at doses of 5, 25 or 50 mg and matched placebo. Compliance with drug dosing was assessed by means of pill counts at all visits. The study protocol was approved by the Institutional Review Board and/or Independent Ethics Committee at each participating investigational centre. The study was registered on ClinTrials.gov, ID number: NCT00088140 and conformed to the ethical guidelines of the Declaration of Helsinki including subsequent amendments and clarifications through the end of the study. Informed consent was obtained in writing from all participants before enrolment.

Double-blind treatment period

The duration of the double-blind treatment period was 12 weeks. Eligible patients were randomized (using a fixed block design) into four groups to receive either oral PF-03491390 at 5, 25 or 50 mg twice daily (b.d.) or to matching placebo. The drug was administered orally 30 min before the morning and evening meals. Patients were treated for 10 weeks. If the serum AST and ALT had normalized patients remained on their assigned dose through treatment week 12. However, if serum AST and ALT were not normal the dose of study drug was doubled during weeks 11 and 12. During this period, the investigators, patients and study sponsor

remained blinded to the assigned treatment. Patients could only be unblinded if they developed an adverse event which required that the investigator know the patient had received study drug or placebo.

Open-label treatment period

Patients who received placebo during the double-blind treatment period were given the option to enter a 12-week open-label phase during which they would receive oral PF-03491390 at a dose of 5 mg b.d. If either AST or ALT remained above the normal range the dose of PF-03491390 was increased stepwise every 2 weeks to 10, 25, 50 and 100 mg b.d. Patients in whom the serum AST and ALT levels declined below the upper limit of normal (ULN) were maintained at the dose associated with this biochemical response.

Subject selection and procedures

The study included male and female adults with chronic HCV infection who were previously intolerant to, or failed to achieve an SVR during previous HCV treatment. Serum levels of AST and/or ALT had to be within 1.5–10 times ULN on at least two occasions during the baseline period. Patients had to have a haemoglobin level ≥10 mg/dL, a platelet count ≥75 × 10^9/L and a white blood cell count ≥1.5 x 10^9/L.

Patients were excluded from the study if AST or ALT were >10 × ULN during the baseline period or had evidence of bridging fibrosis or cirrhosis on liver biopsy performed within 2 year of enrolment. Patients were also excluded from the study if they had an alpha-fetoprotein of >50 mg/dL; known or suspected hepatocellular carcinoma; hepatitis B virus and/or human immunodeficiency virus co-infection; renal impairment (creatinine >1.5 × ULN); any other known liver disorder, or a recent history of alcohol or illicit drug abuse.

Endpoints and assessments

The co-primary endpoints were the median change in the absolute levels of AST and ALT from the start of treatment to week 10. Blood samples for the assessment of serum AST and ALT activity were obtained at screening, baseline (weeks 2 and 4), throughout the randomized, placebo, controlled double-blind phase (weeks 1, 2, 5, 8, 10, 12) and during the observation period (weeks 13 and 26). Patients who received placebo during the initial 12 weeks and then entered the open-label dose escalation treatment phase were also assessed at weeks 13, 14, 15, 18, 21, 23 and 25 during treatment and after treatment was stopped at weeks 16 and 29. Adverse events were also recorded at every clinic visit. Additional safety assessments, including clinical laboratory assessments (haematology, clinical chemistry, coagulation panel, alpha-fetoprotein), urinalysis, and liver and abdominal ultrasounds were performed at intervals throughout the study. The clinical laboratory assessments included quantitative HCV RNA testing performed at baseline and weeks 5, 12, 18, 25 and 29. The numbers of patients with an aminotransferase flare (ALT or AST value twice the baseline value while on treatment) or aminotransferase overshoot (ALT or AST values twice the baseline value after discontinuation of treatment) were also recorded.

Statistical analyses

For the double-blind treatment period, absolute changes in AST and ALT levels from baseline were calculated, where the baseline value was the average of the values from the screening visit, the baseline visit (s) and the week 0 (predose on day 1) visit. Pairwise comparisons of the change in aminotransferases between the different treatment groups were performed using a two-sided Wilcoxon–Mann–Whitney test. The course of the aminotransferase activity was analysed by utilizing repeated measurement analysis methods and the pairwise difference between treatments estimated at each visit. The absolute changes in AST and ALT levels from baseline to week 10 were considered the co-primary endpoints. Treatment effects were determined by deriving Hodges–Lehman estimates and 95% confidence intervals for the change in aminotransferases from baseline to week 10. The primary analyses were based on the intention-to-treat population, but a subset of the intention-to-treat population, who received dose escalation at week 10, were also analysed. Missing data were imputed by carrying forward the last available observation. The 5% level of significance, unadjusted for multiple comparisons, was used for all statistical comparisons. The sample size of 50 subjects per treatment arm provided >90% power to detect a clinically meaningful change in AST and ALT. This is sufficient to provide 90% power to detect a treatment effect of 0.7. Analyses were performed using the validated statistical software of SAS (version

8.2, Cary, NC, USA). No formal statistical analyses were performed on data from the open-label treatment period or the safety data.

RESULTS

Patient population

A total of 204 patients met all entry criteria and were randomized to receive either placebo or one of several doses of PF-03491390. The clinical characteristics of these patient groups are summarized in Table 1. The mean age of the patients was 51.4 year, 163/204 (80%) were Caucasian and 27 (13%) African-American, 180 (88%) had previously received interferon or peginterferon with or without ribavirin, 194 (95%) had previous immunotherapy and 71 (35%) had previously received another treatment for chronic HCV. The mean serum AST and ALT levels at baseline were 78.5 and 129.0 IU/mL, respectively. Mean $\log_{10}$ serum HCV-RNA was 6.3 IU/mL. Among the 199 (98%) patients for whom liver biopsies were available, METAVIR fibrosis scores were F0 (no fibrosis) in 22 (11%), F1 (periportal fibrosis) in 78 (38%), F2 (periportal fibrosis with few septa) in 73 (36%) and F3 (septal fibrosis) in 26 (13%). No patients had histologic evidence of cirrhosis. All treatment groups were well matched. Overall compliance was excellent (95% averaged

Table 1. Baseline demographic and disease characteristics

		Double blind			
		Placebo	PF-03491390		
	Open label PF-03491390 Escalating regimen (n = 39)	n = 51	5 mg b.d. (n = 55)	25 mg b.d. (n = 50)	50 mg b.d. (n = 48)
Gender (%)					
Male	25 (64)	30 (59)	38 (69)	34 (68)	27 (56)
Female	14 (36)	21 (41)	17 (31)	16 (32)	21 (44)
Age (years)*	51 (37–69)	51 (37–69)	51 (38–65)	50 (38–60)	53 (40–75)
Body mass index*	29 (20–41)	29 (20–41)	29 (22–41)	29 (20–41)	28 (19–41)
Race (%)					
Caucasian	28 (72)	37 (73)	45 (82)	41 (82)	40 (83)
Black	6 (15)	8 (16)	6 (11)	8 (16)	5 (10)
Hispanic	4 (10)	5 (10)	4 (7)	1 (2)	2 (4)
Asian	1 (3)	1 (2)	0	0	0
Missing	0	0	0	0	1 (2)
HCV genotype† (%)					
1	N/A	51 (100)	52 (96)	44 (88)	44 (92)
2	N/A	0	1 (2)	2 (4)	1 (2)
3	N/A	0	1 (2)	4 (8)	1 (2)
4	N/A	0	0	0	1 (2)
Missing	N/A	0	0	0	1 (2)
METAVIR fibrosis score‡ (%)					
F0	N/A	10 (20)	7 (13)	4 (8)	1 (2)
F1	N/A	19 (37)	14 (25)	22 (44)	23 (48)
F2	N/A	14 (27)	24 (44)	16 (32)	19 (40)
F3	N/A	6 (12)	8 (15)	7 (14)	5 (10)
Missing	N/A	1 (2)	2 (4)	0	0
Not done	N/A	1 (2)	0	1 (2)	0

N/A, not available.
* Values presented are means and range.
† The genotype of HCV was determined for 203 (99.5%) of patients.
‡ Liver biopsies were taken from 199 (98%) of patients. None were classified as F4.

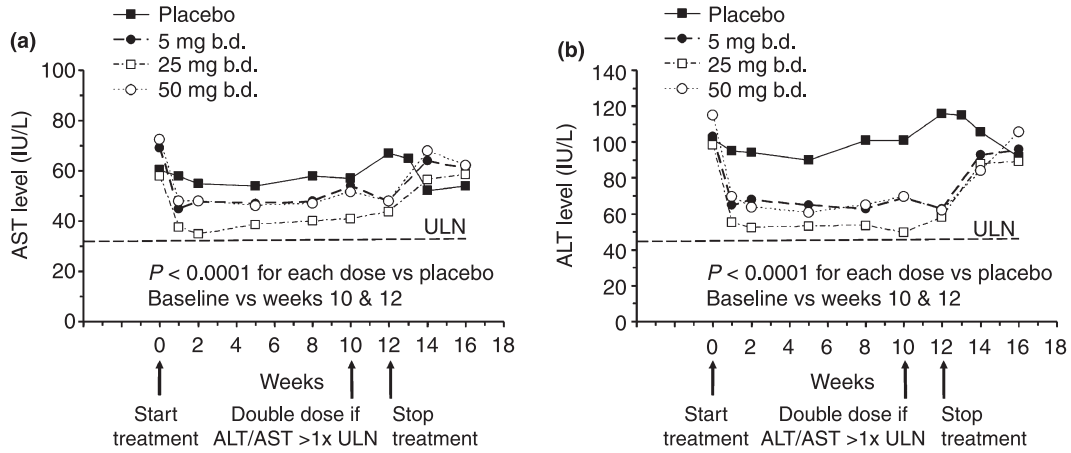

Figure 1. Median absolute levels of (a) aspartate aminotransferase (AST) and (b) alanine aminotransferase (ALT) from the start of treatment to week 16 in all patients.

across all visits during the double-blind treatment period) and comparable in each treatment group.

Effect of treatment on serum liver transaminases

Treatment with PF-03491390 was associated with a decline in both AST and ALT levels. These reductions were apparent as early as 1 week after the initiation of treatment and were maintained throughout the 12-week double-blind treatment period in all three PF-03491390 treatment groups. Serum AST and ALT levels increased back to baseline values after treatment was discontinued (Figure 1). The declines in both AST and ALT observed at week 10 (the primary endpoint) were statistically significant compared with placebo ($P < 0.0001$) for all three doses of PF-03491390. The mean absolute decline in AST and ALT observed in those patients treated with 25 or 50 mg b.d. was somewhat greater than for patients treated with the 5 mg dose.

The per cent decline in AST from baseline to week 10 was 0.9 for placebo and 36%, 36% and 39% for patients treated with 5, 25 and 25 mg respectively. The per cent declines in serum ALT were 5.5%, 36.5%, 42.2% and 46.3% for placebo and each of the treatment groups respectively. The per cent decline in AST and ALT for each dose of PF-03491390 was highly significant ($P < 0.0001$).

At week 10, the dose of PF-03491390 was doubled in those patients who continued to have a value for serum AST and ALT above the limits of normal and

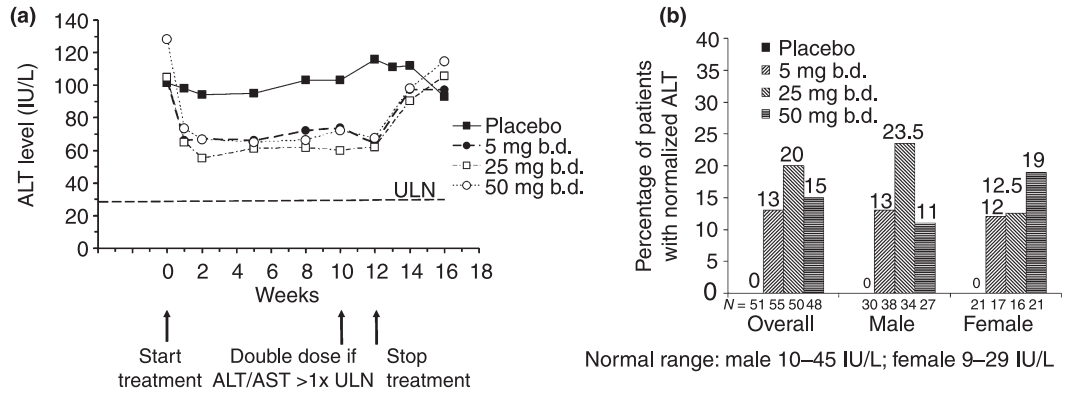

Figure 2. Median absolute levels of (a) alanine aminotransferase (ALT) from the start of treatment to week 16 for patients who failed to normalize serum ALT by week 10. All patients received a higher dose of PF-03491390 between weeks 10 and 12. (b) Per cent of all patients with normal serum ALT by week 12 when treatment was stopped.

had not dropped out of the study. This occurred in 45/53, 38/49 and 42/47 patients in the 5, 25 and 50 mg b.d. groups respectively. None of the patients in the placebo group normalized serum AST or ALT. Figure 2a illustrates absolute levels of ALT in those patients who failed to normalize serum liver transaminases by week 10 and required dose escalation. No apparent change in serum ALT occurred between weeks 10 and 12 following dose escalation. Similar results were observed for serum AST (data not shown). The percentages of all patients with normal ALT levels at weeks 10 and 12 are illustrated in Figure 2b.

The changes in serum aminotransferase levels over time for patients initially treated with placebo and subsequently enrolled into the open-label treatment period broadly resembled those for the PF-03491390 treatment groups during the double-blind treatment period. Reductions in serum AST and ALT were apparent within 1 week after the initiation of active treatment, were maintained throughout the 12-week open-label treatment period and returned to near pre-treatment values after treatment was discontinued. Although dose escalation of PF-03491390 up to 100 mg b.d. occurred in the majority of patients, this did not appear to lower serum aminotransferase levels. During the open-label treatment period, 8–13% of patients had normal serum levels of AST and ALT between weeks 14 and 15.

A total of six patients experienced a flare in serum liver aminotransferases (AST or ALT value twice the baseline value while on treatment) during the 12-week treatment period. Flares in serum AST and ALT during treatment were observed in only two, one and three patients treated with 5, 25 and 50 mg of PF-03491390 respectively. Aminotransferase overshoots (AST or ALT values twice the baseline value after discontinuation of treatment) occurred in five patients treated with placebo, and six, three and one patients treated with the various doses of PF-03491390 respectively. Neither liver histology nor serum HCV RNA level was specifically evaluated at the time of the flair in these patients.

Effect of treatment on serum HCV-RNA

No significant change in the mean serum HCV-RNA level occurred during the double-blind treatment period in either the placebo or PF-03491390 treatment groups (Figure 3). The mean $\log_{10}$ HCV-RNA level for patients in the treatment groups remained constant (6.3 IU/mL at both baseline and treatment week 12).

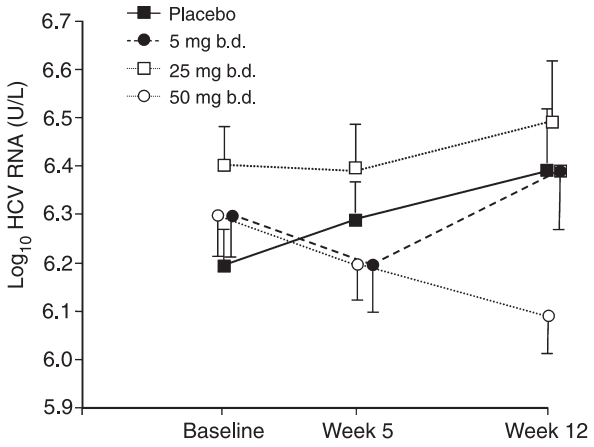

Figure 3. Serum $\log_{10}$ HCV RNA levels at baseline and weeks 5 and 12. Values are given as mean (±s.d.).

The mean $\log_{10}$ HCV RNA level for patients in the placebo group was 6.2 IU/mL at baseline and 6.3 IU/mL after 12 weeks.

Safety

A total of 13 patients withdrew from the study during the randomized, placebo-controlled double-blinded, treatment phase (seven patients in the placebo group, three in the 5 mg group, two from the 0 mg group and one patient receiving 25 mg of PF-03491390). However, only three of these patients withdrew due to an adverse event and only one of these patients was receiving study drug (5 mg b.d.).

Adverse events which occurred during the double-blind and open-label treatment periods are summarized in Table 2. During the double-blind treatment period, a similar number of patients in each treatment group and the placebo group experienced adverse events. The most frequently reported adverse events were headache and fatigue. The majority of adverse events were of mild or moderate severity. However, 19 adverse events in 14 (7%) patients were rated as severe during the double-blind treatment period. The incidence of severe adverse events was greatest in the placebo and PF-03491390 5 mg treatment groups (seven events each) and lowest in the PF-03491390 25 mg treatment group (one event). The majority of adverse events had resolved by the end of the study and the numbers of continuing events were similar for each of the double-blind treatment groups. Serious adverse events were varied and showed no pattern across the

Table 2. Adverse events reported during the double-blind and open-label treatment periods

		PF-03491390		
Double-blind treatment period	Placebo (n = 51)	5 mg b.d. (n = 55)	25 mg b.d. (n = 50)	50 mg b.d. (n = 48)
Adverse events	97	118	99	129
Patients with adverse events	30	42	31	36
Patients with adverse events occurring in >6 PF-03491390 recipients				
Headache	8	6	5	5
Fatigue	4	7	4	7
Nausea	2	6	1	6
Diarrhoea	6	2	2	4
Back pain	1	1	4	5
Upper respiratory tract infection	1	2	2	5
Insomnia	2	4	2	1

Open-label treatment period	PF-03491390 5–100 mg b.d. (n = 39)
Adverse events	34
Patients with adverse events	17
Patients with adverse events occurring in >1 PF-03491390 recipient	
Headache	2
Fatigue	2
Arthralgia	2
Rash	2

treatment groups. No concerning changes in any of the laboratory parameters and no clinically relevant changes in vital signs, electrocardiograms, physical examinations, or in liver ultrasound scans, could be attributed to PF-03491390.

DISCUSSION

This study demonstrated that the pancaspase inhibitor PF-03491390 significantly reduced aminotransferase activity in patients with chronic HCV infection. Overall, AST and ALT declined by 37% and 42% respectively after 12 weeks of treatment and 17% of patients had normalization in serum aminotransferases. The reduction in serum AST and ALT activity occurred within 2 weeks of initiating study drug, was maintained throughout the entire dosing period and returned to baseline values within 2–4 weeks after stopping treatment. No clear dose effect was observed between the range of 5–50 mg b.d. with regards to the lowering and normalization in serum aminotransferase activity. These observations both confirm and extend those made during previous short-term studies of this agent.[19–21] In a 7-day, phase I study in which PF-03491390 was administered intravenously, dose-related decreases in aminotransferase levels were seen in all but one patient.[19] In a 14-day, phase I, double-blind, placebo-controlled, dose-ranging study PF-03491390 at daily doses between 5 and 200 mg administered one to three times daily was associated with a significant decline in AST and ALT in patients with various forms of chronic liver disease. Nearly 80% of the patients in that study had chronic HCV. Reductions in aminotransferase activity have also been observed in patients with non-alcoholic steatohepatitis.[20, 21]

PF-03491390 was well tolerated in this and the previous phase I study.[21] The majority of adverse events were of mild or moderate severity, and just one-third patients who withdrew from the study due to adverse events was receiving PF-03491390. The dose regimens chosen for this study were selected largely on the basis of the 14-day, phase I study in which patients with HCV infection received PF-03491390 at doses ranging from 5 mg once daily to 200 mg b.d. (an 80-fold or 1.4 log dose range).[20, 21] In that study, no clear dose–response relationship was observed and all groups except that treated with the lowest dose of

PF-03491390 had a significant decline in aminotransferase levels. The b.d. dosing appeared to be more effective than once-daily dosing. In the present study, dose escalation during the open-label treatment period (5–100 mg b.d.) had no apparent effect on serum aminotransferases. These observations suggest that the optimal dose of PF-03491390 could be 25 mg b.d. and that the therapeutic index for the drug is quite broad.

This study was not designed to examine the mechanism of action of PF-03491390. However, the reduction in serum aminotransferase activity observed during treatment could be attributable to an anti-apoptotic effect that restricts hepatocyte death and prevents the release of aminotransferases into serum. In individuals with inflammatory liver disorders like chronic HCV, apoptosis appears to occur as a consequence of death receptor signalling and activation of the Fas pathway.[8, 12, 22, 23] PF-03491390 has been shown to block Fas-induced apoptosis *in vitro* and in animal models of liver injury and fibrosis.[16–18] In the bile duct ligated mouse, the administration of PF-03491390 suppressed hepatic apoptosis, inflammation and fibrosis.[17] In a rat model PF-03491390 lowered aminotransferase activity and improved liver function following ischaemia/reperfusion injury.[18] It is therefore likely that the suppression of aminotransferase levels associated with PF-03491390 treatment in patients with chronic HCV infection is, at least in part, the result of a decline in Fas-induced hepatocyte death and a reduction in apoptosis and inflammation. An alternative explanation is that PF-03491390 may block inflammasome mediated caspase-1 inhibition and this would down regulate the production of interleukins and other mediators of inflammation.[24]

In this phase II study, serum levels of aminotransferase activity were utilized as surrogate markers of hepatic inflammation and liver cell injury. In general, the serum level of AST and ALT do increase with the severity of hepatic inflammation in patients with chronic HCV.[25, 26] However, variable degrees of inflammation are also present in HCV patients with persistently normal aminotransferases.[5, 26] Thus, it remains unclear if the decline in serum AST and ALT observed during treatment with PF-03491390 in this study was the result of a decline in hepatic inflammation or simply a biochemical change without significant histologic impact. The only way to confirm that this pancaspase inhibitor reduces hepatic inflammation, apoptosis and affects hepatic fibrosis progression is to compare liver histology obtained from biopsies performed at baseline and after a defined period of treatment. The lack of liver histology can certainly be considered a shortcoming of the present study. However, this was only a preliminary, proof of concept, dose finding study performed to define the optimal dose of PF-03491390 that could suppresses serum liver transaminases long term. In addition, it is unrealistic to request that patients undergo two liver biopsies within 3 month. Assessment of liver histology was therefore not included in the current study. The current data, however, does support the hypothesis that PF-03491390 may improve hepatic inflammation and apoptosis and provides the foundation for a longer randomized, double-blinded, placebo controlled trial of sufficient duration in which liver histology and other non-invasive markers of inflammation and fibrosis should be utilized as the primary and secondary endpoints respectively.

There are several potential concerns when utilizing a pancaspase inhibitor in patients with chronic HCV. One concern is that serum HCV RNA might increase during treatment. This could result from a reduction in hepatocyte turnover and continued replication of HCV in viable hepatocytes. However, no significant impact on the serum level of HCV RNA was observed during 12 weeks of treatment with PF-03491390 in the present study. This suggests that caspase inhibition neither enhances nor suppresses HCV RNA replication.

Another concern is the risk of a biochemical flare or overshoot, which, if severe, could be associated with acute necrosis and hepatic failure particularly in patients with cirrhosis. Marked elevations in serum liver transaminases could result from the normal apoptotic process suddenly escaping caspase inhibition during treatment or when the inhibitor of apoptosis is suddenly removed followed by the simultaneous necrosis of cells which have been waiting to undergo this process. In the two phase I studies, three patients had a temporary increase in aminotransferase activity after discontinuing 7 days of treatment and in the 14-day trial only one patient experienced a temporary aminotransferase overshoot after the treatment was stopped.[19, 21] In the present study, 12 patients experienced an aminotransferase flare, but six (50%) of these occurred in the placebo group. Similarly, 15 patients experienced an aminotransferase overshoot when treatment was discontinued but five (33%) of these also occurred in the placebo group and just one of 10 was observed in the highest dose (50 mg) group. This suggests that

PF-03491390 treatment is not associated with a flare in aminotransferase levels above baseline either during or after treatment. The gradual return of AST and ALT back to baseline levels within 2–4 weeks after discontinuing PF-03491390 suggests synchronized massive apoptosis does not occur either during or after treatment with a pancaspase inhibitor.

Another potential concern when utilizing a caspase inhibitor long term to treat chronic HCV is that this could increase the risk or developing hepatocellular carcinoma. The caspase pathway is well known to play a role in carcinogenesis and deficiencies in this pathway have seen associated with an increased risk for developing certain cancers and resistance to chemotherapy.[27] For this reason, patients with an increased risk for hepatocellular carcinoma, those with cirrhosis or an elevated alpha-fetoprotein level (>50 mg/dL), were excluded from participating in this study. Although no evidence for a carcinogenic effect of PF-03491390 was identified, the present study was not specifically designed and not of sufficient duration or size to assess for this possibility. Future and longer studies of PF-03491390 will require careful monitoring for hepatocellular carcinoma, especially in patients with cirrhosis where the risk of this is already increased.[28]

In conclusion, PF-03491390 significantly reduced serum AST and ALT levels in patients with chronic HCV. These effects occurred within 7 day of initiating treatment and were maintained for as long as patients remained on treatment. Increasing the dose of PF-03491390 in patients who failed to normalize serum liver transaminases did not lead to a further reduction in AST and ALT. PF-03491390 was well tolerated and without significant adverse events. Further studies in larger numbers of patients, for a longer period of time and with histologic endpoints, are now required to determine whether PF-03491390 can impact the natural history of chronic HCV.

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REFERENCES

1 Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436–41.
2 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**: 975–82.
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**: 958–65.
4 McHutchison JG, Lawitz EJ, Shiffman ML, *et al.* Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; **361**: 580–93.
5 Shiffman ML, Suter F, Bacon BR, *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; **357**: 124–34.
6 McHutchison JG, Everson GT, Gordon SC, *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827–38.
7 Kwo P, Lawitz EJ, McCone J, *et al.* HCV Sprint-1: boceprevir plus peginterferon alfa-2b/ribavirin for treatment of genotype 1 chronic hepatitis C in previously untreated patients. *Hepatology* 2008; **48**(supl 4):1027A.
8 Guicciardi ME, Gores GJ. Apoptosis: a mechanism of acute and chronic liver injury. *Gut* 2005; **54**: 1024–33.
9 Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. *Cell Death Differ* 2005; **12**: 942–61.
10 Bantel H, Schulze-Osthoff K. Apoptosis in hepatitis C virus infection. *Cell Death Differ* 2003; **10**(Suppl. 1): S48–58.
11 Callus BA, Vaux DL. Caspase inhibitors: viral, cellular and chemical. *Cell Death Differ* 2007; **14**: 73–8.
12 Pianko S, Patella S, Ostapowicz G, Desmond P, Sievert W. Fas-mediated hepatocyte apoptosis is increased by hepatitis C virus infection and alcohol consumption, and may be associated with hepatic fibrosis: mechanisms of liver cell injury in chronic hepatitis C virus infection. *J Viral Hepat* 2001; **8**: 406–13.
13 Bantel H, Lugering A, Poremba C, *et al.* Caspase activation correlates with the degree of inflammatory liver injury in chronic hepatitis C virus infection. *Hepatology* 2001; **34**: 758–67.
14 Volkmann X, Cornberg M, Wedemeyer H, *et al.* Caspase activation is required for antiviral treatment response in chronic hepatitis C virus infection. *Hepatology* 2006; **43**: 1311–6.
15 Linton SD, Aja T, Armstrong RA, *et al.* First-in-class pan caspase inhibitor developed for the treatment of liver disease. *J Med Chem* 2005; **48**: 6779–82.
16 Hoglen NC, Chen LC, Fisher CD, Hirakawa BP, Groessl T, Contreras PC. Characterization of IDN-6556 (3-{2-(2-tert-butylphenylaminooxalyl)-amino]-propionylamino} -4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid): a liver-targeted. *J Pharmacol Exp Ther* 2004; **309**: 634–40.
17 Canbay A, Feldstein A, Baskin-Bey E, Bronk SF, Gores GJ. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. *J Pharmacol Exp Ther* 2004; **308**: 1191–6.
18 Natori S, Higuchi H, Contreras P, Gores GJ. The caspase inhibitor IDN-6556 prevents caspase activation and apoptosis in sinusoidal endothelial cells during liver preservation injury. *Liver Transpl* 2003; **9**: 278–84.
19 Valentino KL, Gutierrez M, Sanchez R, Winship MJ, Shapiro DA. First clinical trial of a novel capsase inhibitor: anti-apoptotic caspase inhibitor, IDN6556, improves liver enzymes. *J Pharmacol Exp Ther* 2003; **41**: 441–9.
20 Schiff ER, Pockros P, Shiffman ML, *et al.* Oral IDN-6556, an anti-apoptotic caspase inhibitor, lowers aminotransferase levels in HCV patients. *J Hepatol* 2004; **40**(Suppl. 1): 24 (abstract).
21 Pockros PJ, Schiff ER, Shiffman ML, *et al.* Oral IDN-6556, an antiapoptotic caspase inhibitor, may lower aminotransferase activity in patients with chronic hepatitis C. *Hepatology* 2007; **46**: 324–9.
22 Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths? *Hepatology* 2006; **43**(Suppl. 1): S31–44.
23 Elsharkawy AM, Oakley F, Mann DA. The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. *Apoptosis* 2005; **10**: 927–39.
24 Pedra JH, Cassel SL, Sutterwala FS. Sensing pathogens and danger signals by the inflammasome. *Curr Opin Immunol* 2009; **21**: 10–6.
25 Giannini EG, Zaman A, Ceppa P, Mastracci L, Risso D, Testa R. A simple approach to noninvasively identifying significant fibrosis in chronic hepatitis C patients in clinical practice. *J Clin Gastroenterol* 2006; **40**: 521–7.
26 Sanai FM, Benmousa A, Al-Hussaini H, *et al.* Is serum alanine transaminase level a reliable marker of histological disease in chronic hepatitis C infection? *Liver Int* 2008; **28**: 1011–8.
27 Ghavami S, Hashemi M, Ande SR, *et al.* Apoptosis and cancer: mutations within caspase genes. *J Med Genet* 2009; **46**: 497–510.
28 Lok AS, Seeff LB, Morgan TR, *et al.* Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138–48.