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# Alisporivir with peginterferon/ribavirin in patients with chronic hepatitis C genotype 1 infection who failed to respond to or relapsed after prior interferon-based therapy: FUNDAMENTAL, a Phase II trial

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SUMMARY. Alisporivir (ALV) is an oral, investigational host-targeting agent, with pangenotypic activity against hepatitis C virus (HCV). This randomized, double-blind, placebo-controlled, Phase II study explored the efficacy and safety of ALV with peginterferon-α2a/ribavirin (PR) in patients with chronic HCV genotype 1 infection in whom prior PR had failed (43% relapsers, 34% null responders and 23% partial responders). Four-hundred-and-fifty-nine patients were randomized (1:1:1:1) to ALV 600 mg once daily (QD), ALV 800 mg QD, ALV 400 twice daily (BID) or placebo plus PR for 48 weeks. When the global ALV trial programme was put on clinical hold, all patients in this study had received ≥31 weeks of randomized treatment; patients completed 48 weeks on PR alone. All ALV groups demonstrated superior rates of complete early virologic response (cEVR; primary endpoint) *vs* PR alone ($P \leq 0.0131$), with highest cEVR rate seen with ALV 400 mg BID (74% *vs* 36% with PR alone; $P < 0.0001$). Respective SVR12 rates (key secondary endpoint) were 65% *vs* 26% in prior relapsers, 63% *vs* 5% in partial responders and 68% *vs* 3% in null responders. In patients who received >40 weeks of randomized treatment, the SVR12 rate was 89% for ALV 400 mg BID *vs* 30% for PR alone ($P = 0.0053$). Rates of viral breakthrough and relapse were lowest with ALV 400 mg BID. One case of pancreatitis (fully recovered) occurred with ALV/PR. Common AEs were headache, fatigue, anaemia, neutropenia and nausea. Hypertension was infrequent, but more common with ALV. ALV merits further investigation in interferon-free regimens in combination with direct-acting antiviral agents.

*Keywords:* alisporivir, antiviral therapy, genotype 1, hepatitis C virus, host-targeting agent.

Abbreviations: AE, adverse event; ALV, alisporivir; BID, twice daily; BSL, baseline; cEVR, complete early virologic response; DAA, direct-acting antiviral agent; FAS, full analysis set; FDA, US Food and Drug Administration; HCV, hepatitis C virus; IFN, interferon; ITT, intent-to-treat; PR, peginterferon-α2a/ribavirin; QD, once daily; RVR, rapid virologic response; SAE, serious adverse event; SD, standard deviation; SVR12, sustained virologic response at Week 12 post-treatment; SVR24, sustained virologic response at Week 24 post-treatment; SVR, sustained virologic response; TSH, thyroid-stimulating hormone; ULN, upper limit of normal; V, visit; W, week.

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## INTRODUCTION

Hepatitis C virus (HCV) infection imposes a high burden on global healthcare systems [1]. Direct-acting antiviral agents (DAAs) that target viral mechanisms required for replication, such as the NS3/NS4A protease complex or the NS5A phosphoprotein (part of HCV replicase), are now available [2–4]. However, as regional access varies, interferon (IFN) remains the foundation of therapy for chronic HCV genotype 1 infection in many parts of the world. At least 50% of patients with HCV genotype 1 infection fail to achieve sustained virologic response (SVR) with peginterferon-α2a/ribavirin (PR) [5,6], and up to one-third of responders relapse following cessation of PR therapy [7]. Consequently, there remains a need for novel treatments for use in patients with chronic HCV genotype 1 infection who cannot tolerate or fail to respond adequately to current therapeutic approaches. An alternative or complementary approach to treating HCV with DAA combination therapy is to target host cell factors essential for efficient HCV replication.

Alisporivir (ALV) is an oral host-targeting agent that inhibits HCV replication by binding to host cyclophilin A and blocking its peptidyl–prolyl isomerase activity [8,9]. Host cell cyclophilins are essential for efficient HCV replication in hepatocytes. Due to its unique mode of action, ALV has pangenotypic anti-HCV activity, a high genetic barrier to development of resistance and lack of cross-resistance to DAAs [10]. ALV in combination with PR has been shown to have potent and synergistic anti-HCV activity in treatment-naïve patients with HCV genotype 1 and 2/3 infection [11–13].

This paper presents the final results of FUNDAMENTAL, a randomized, double-blind, placebo-controlled, Phase II study undertaken to assess the efficacy and safety of ALV in combination with PR in patients with chronic HCV genotype 1 infection in whom prior PR therapy had failed, including null responders. Efficacy was assessed overall and according to baseline factors known to affect treatment outcome, including response to prior PR therapy, cirrhosis status and *IL28B* rs12979860 polymorphism.

## MATERIALS AND METHODS

### *Patients*

Patients aged 19–69 years with chronic HCV genotype 1 infection and plasma HCV RNA ≥1000 IU/mL who had failed to respond to or had relapsed after prior PR therapy were enrolled at 73 study centres in Europe, North America and the Asia–Pacific region. Patients were classified according to response to prior PR: null nonresponders, treated with PR for ≥12 weeks, did not achieve ≥2 $\log_{10}$ reduction in HCV RNA during the first 12 weeks of treatment and never achieved undetectable HCV RNA; partial nonresponders, treated with PR for ≥24 weeks, achieved ≥2 $\log_{10}$ reduction in HCV RNA during the first 12 weeks of treatment, but did not achieve undetectable HCV RNA; relapsers had undetectable HCV RNA at end of PR treatment, but detectable HCV RNA during post-treatment follow-up. All patients had to have a liver biopsy within 3 years or transient elastography within 6 months of enrolment. Patients with compensated cirrhosis were eligible.

Key exclusion criteria were standard for HCV trials and included nongenotype 1 infection, presence or history of hepatic decompensation and haematological abnormalities, and recent treatment with any anti-HCV drug (Table S1). Due to the potential for drug interactions, concomitant treatment with known substrates or inhibitors of cytochrome P450 3A, P-gp, OATPs, MRP2 or BSEP was not permitted within 2 weeks of study entry.

The study protocol was reviewed and approved by the appropriate Institutional Ethics Committees and health authorities. All patients provided written informed consent.

### *Study design*

This was a multicentre, randomized, double-blind, placebo-controlled, Phase II study (CDEB025A2210; clinicaltrials.gov NCT01183169) conducted between 30 August 2010 and 9 May 2013, consisting of a 48-week treatment period with a planned interim analysis at Week 12 and post-treatment follow-up until Week 72 (Fig. 1). Patients were randomized (1:1:1:1) to receive oral ALV 600 mg once daily (QD), ALV 800 mg QD, ALV 400 mg twice daily (BID) or placebo for 48 weeks. Patients in the QD arms received a loading dose of ALV 600 mg BID for the first week of treatment. The ALV 400 mg BID treatment arm was included following a protocol amendment on 14 December 2010, that is after enrolment into the study had started. Treatment groups were stratified by body mass index (<25 or ≥25 kg/m$^2$), *IL28B* polymorphism at rs12979860 (CC or CT/TT) and response to prior PR therapy. All patients received concomitant PR for 48 weeks (peginterferon-α2a 180 μg/week plus ribavirin 1000 or 1200 mg/day based on body weight). ALV and ribavirin were administered with food. At Week 12, patients with detectable HCV RNA were discontinued from the placebo arm, considered treatment failures and offered retreatment with ALV plus PR. Any patient with detectable HCV RNA after 24 weeks of therapy discontinued treatment as a nonresponder.

On 18 April 2012, the global ALV clinical trial programme was put on partial clinical hold in the United States by the Food and Drug Administration (FDA). At that time, all active patients in this study had received at least 31 weeks of treatment. Patients randomized to ALV-containing regimens were allowed to complete the scheduled 48 weeks of PR treatment.

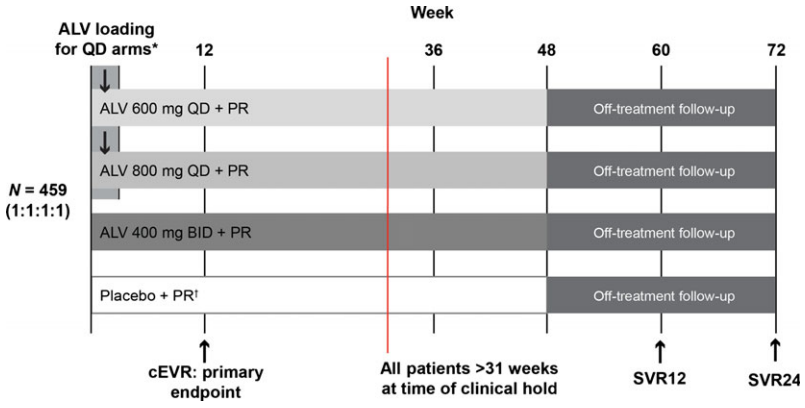


**Fig. 1** FUNDAMENTAL study design. ALV, alisporivir; BID, twice daily; cEVR, complete early virologic response; PR, peginterferon-α2a/ribavirin; QD, once daily; SVR12, sustained virologic response at Week 12 post-treatment; SVR24, sustained virologic response at Week 24 post-treatment. Virologic response defined as HCV RNA <25 IU/mL. *Loading dose of ALV 600 mg BID for 1 week. †Patients who were HCV RNA+ at Week 12 were discontinued from the placebo + PR arm.

### *Study endpoints and assessments*

The primary efficacy endpoint was the proportion of patients achieving undetectable serum HCV RNA after 12 weeks of treatment (complete early virologic response; cEVR). The key secondary efficacy endpoint was the proportion of patients achieving undetectable serum HCV RNA at Week 12 post-treatment (SVR12). Other secondary endpoints included the proportion of patients achieving undetectable serum HCV RNA after 4 weeks of treatment (rapid virologic response; RVR) and at Week 24 post-treatment (SVR24).

Rates of viral breakthrough, viral rebound and relapse were also determined. Viral breakthrough was defined as confirmed increase of HCV RNA ≥1 $\log_{10}$ above the lowest HCV RNA level during treatment on two consecutive visits, or confirmed HCV RNA increase to ≥100 IU/mL after HCV RNA <25 IU/mL during treatment. Viral rebound was defined as confirmed HCV RNA ≥100 IU/mL at two consecutive visits before the end of PR treatment after being <25 IU/mL during or at the end of treatment with ALV; this definition was not applicable to the PR control group because patients did not receive ALV. Relapse was defined as HCV RNA ≥25 IU/mL during post-treatment follow-up after being <25 IU/mL at the end of treatment.

Plasma HCV RNA levels were measured using the Roche COBAS TaqMan HCV assay (v2.0; Roche Molecular Diagnostics, Basel, Switzerland). This assay has a reported lower limit of quantification of 25 IU/mL. HCV genotyping and subtyping were performed using the INNO-LiPA HCV assay (v2.0; Innogenetics N.V., Ghent, Belgium). Population sequencing of HCV NS5A was performed at DDL Diagnostic Laboratories (Rijswijk, The Netherlands) for patients experiencing confirmed on-treatment viral breakthrough or relapse with HCV RNA ≥1000 IU/mL.

Adverse events (AEs), routine laboratory parameters and vital signs were monitored throughout the study.

### *Statistical analysis*

Target sample size was calculated using Fisher's exact test for equal proportions; dropout rates were not taken into consideration. Based on historic data for response rates following retreatment with PR in patients who had failed prior IFN-based therapy [14], it was estimated that 86 randomized patients per treatment arm (344 patients in total) would provide 90% power to establish superiority of all ALV-containing regimens over the PR control at a two-sided 0.05 significance level.

Patient demographics and baseline characteristics were summarized for all randomized patients. Safety was analysed in all patients who received at least one dose of study medication (safety population). Efficacy was analysed in all patients who were randomized after the protocol amendment to include the ALV 400 mg BID treatment arm (full analysis set [FAS]). Differences between groups were compared using standard statistical methods for assessing non-inferiority/equivalence between treatment groups [15]. All tests were adjusted for nonresponder/relapser, *IL28B* rs12979860 polymorphism (CC or CT/TT) and body mass index (<25 kg/m$^2$ or ≥25 kg/m$^2$) status. SVR24 rates were also analysed according to response to prior PR, *IL28B* rs12979860 polymorphism, cirrhosis status and length of exposure to ALV.

## RESULTS

### *Study population*

In all, 459 patients were randomized and 457 treated (Fig. S1). The majority of patients (437; 95%) were randomized after the protocol amendment to include the ALV 400 mg BID treatment arm (FAS). Of the randomized patients, 43% were relapsers, 23% were prior partial

responders and 34% were prior null responders. Patient demographics and baseline disease characteristics, including response to prior PR therapy, were well-balanced between treatment groups (Table 1). Overall, 25% of patients had cirrhosis or bridging fibrosis and 79% had a non-CC *IL28B* genotype.

Fifty-seven per cent of patients completed treatment (Table 2), with the highest rate of completion in the ALV 400 mg BID group (71%). In the placebo arm, 57% of patients were switched in a blinded manner to ALV plus PR after Week 16 due to failure to achieve the efficacy criterion (HCV RNA < limit of quantification) at Week 12. Across treatment arms, the main reasons for discontinuation were unsatisfactory therapeutic effect (11%), AEs (10%) and withdrawal of consent (5%).

### *Efficacy*

All ALV treatment groups demonstrated superior rates of cEVR compared with the PR (placebo) group ($P \leq 0.0131$; Fig. 2a); the highest cEVR rate was seen in the ALV 400 mg BID group (74% *vs* 36% with PR alone; $P < 0.0001$). Because all patients had passed the Week 12 time point at the time of analysis, cEVR was not affected by the FDA clinical hold. Patients receiving ALV 400 mg BID also achieved highest RVR rates (all $P \leq 0.0005$ *vs* placebo; Fig. 2a) and the greatest decline in plasma HCV RNA (Fig. 2b).

All ALV treatment groups maintained higher virologic response rates compared with PR alone during post-treatment follow-up, with the highest rates of post-treatment response seen in the ALV 400 mg BID group (Fig. 2a). The proportion of patients achieving SVR12 was 65% with ALV 400 mg BID compared with 15% with PR alone; all differences in SVR12 rates between the ALV treatment arms and the control group were statistically significant ($P < 0.0001$). All patients who achieved SVR12 also achieved SVR24, except for one patient receiving ALV 600 mg QD who relapsed between Week 60 and Week 72.

The FDA clinical partial hold resulted in patients receiving various durations of ALV therapy (Fig. 2c); however, in patients who received therapy for >40 weeks, the SVR24 rate was 89% with ALV 400 mg BID compared with 30% with PR alone.

SVR24 rates were significantly higher in ALV-treated patients than in those who received PR, regardless of prior treatment response, *IL28B* rs12979860 polymorphism and cirrhosis status (Fig. 3). The effect of ALV and of the 400 mg BID dose was most apparent in the most difficult-to-treat subgroups, such as cirrhotics and prior null responders. In prior relapsers, the SVR24 rate was 65% in the ALV 400 mg BID group compared with 26% in those who received PR alone ($P < 0.0002$). In partial responders, the SVR24 rate was 63% with ALV 400 mg BID compared with 5% with PR alone ($P < 0.001$); respective SVR24 rates in null responders were 68% and 3% ($P < 0.0001$) (Fig. 3a). The SVR24 rate was 52% in patients with cirrhosis who received ALV 400 mg BID with PR; no patient with cirrhosis treated with PR alone achieved SVR24

**Table 1** Patient demographics and baseline disease characteristics

| Characteristic, *n* (%) | ALV 600 mg QD + PR (*N* = 121) | ALV 800 mg QD + PR (*N* = 115) | ALV 400 mg BID + PR (*N* = 109) | Placebo + PR (*N* = 114) | Total (*N* = 459) |
|---|---|---|---|---|---|
| Mean age, years (range) | 50.2 (21–67) | 50.9 (19–69) | 51.0 (20–69) | 50.5 (23–68) | 50.6 (19–69) |
| Male | 63 (52) | 68 (59) | 69 (63) | 78 (68) | 278 (61) |
| Race | | | | | |
| Caucasian | 87 (72) | 89 (77) | 90 (83) | 86 (75) | 352 (77) |
| Asian | 25 (21) | 22 (19) | 13 (12) | 23 (20) | 83 (18) |
| Black | 5 (4) | 1 (1) | 5 (5) | 2 (2) | 13 (3) |
| BMI ≥25 kg/m$^2$ | 73 (60) | 73 (64) | 69 (63) | 75 (66) | 290 (63) |
| HCV RNA ≥800 000 $\log_{10}$ IU/mL | 95 (79) | 98 (85) | 79 (73) | 90 (79) | 362 (79) |
| HCV genotype 1a | 35 (29) | 31 (27) | 36 (33) | 34 (30) | 136 (30) |
| Cirrhosis or bridging fibrosis* | 27 (22) | 35 (30) | 23 (21) | 29 (25) | 114 (25) |
| Response to previous treatment | | | | | |
| Relapser | 48 (40) | 49 (43) | 48 (44) | 54 (47) | 199 (43) |
| Null responder | 48 (40) | 37 (32) | 34 (31) | 36 (32) | 155 (34) |
| Partial responder† | 25 (20) | 29 (25) | 27 (25) | 24 (21) | 105 (23) |
| *IL28B* CT/TT | 94 (78) | 91 (79) | 89 (82) | 90 (79) | 364 (79) |

ALV, alisporivir; BID, twice daily; BMI, body mass index; HCV, hepatitis C virus; PR, peginterferon-α2a/ribavirin; QD, once daily.

*Defined as elasticity score ≥10.8 kPa or Metavir score F3/F4 or Ishak score 5–6.

†Includes nonresponders for whom specification of null or partial response was missing.

**Table 2** Patient disposition and reasons for study discontinuation

| Disposition, *n* (%) | ALV 600 mg QD + PR (*N* = 121) | ALV 800 mg QD + PR (*N* = 115) | ALV 400 mg BID + PR (*N* = 109) | Placebo + PR (*N* = 114) | Total (*N* = 459) |
|---|---|---|---|---|---|
| Completed treatment | 72 (60) | 78 (68) | 77 (71) | 34 (30) | 261 (57) |
| Discontinued treatment | 49 (41) | 37 (32) | 32 (29) | 14 (12) | 132 (29) |
| Switched to ALV | N/A | N/A | N/A | 65 (57) | 65 (14) |
| Reason for discontinuation | | | | | |
| Unsatisfactory therapeutic effect | 25 (21) | 20 (17) | 3 (3) | 4 (4) | 52 (11) |
| Adverse event(s) | 12 (10) | 9 (8) | 18 (17) | 5 (4) | 44 (10) |
| Patient withdrew consent | 7 (6) | 5 (4) | 9 (8) | 3 (3) | 24 (5) |
| Other* | 5 (4) | 3 (3) | 2 (2) | 2 (2) | 12 (3) |

ALV, alisporivir; BID, twice daily; PR, peginterferon-α2a/ribavirin; QD, once daily. For one patient in the placebo + PR arm, end-of-treatment status was not entered into the database before the database was locked; therefore, this patient is not included in the above table or in other summaries dependent on end-of-treatment status.
*Includes abnormal laboratory values (*n* = 5), administrative problems (*n* = 3), protocol deviation (*n* = 2), disease progression (*n* = 1), noncompliance (*n* = 1).

($P < 0.0001$) (Fig. 3b). Response rates in patients with cirrhosis were not analysed according to prior response to PR due to the small numbers of patients in these groups. The SVR24 rate was 65% in patients with non-*CC IL28B* genotypes treated with ALV 400 mg BID and PR compared with 12% in those who received PR alone ($P < 0.0001$) (Fig. 3c).

### *Viral breakthrough, rebound and relapse*

The viral breakthrough rate was lowest in the ALV 400 mg BID group (3% compared with 13% for ALV 600 mg QD, 9% for ALV 800 mg QD and 6% for PR alone). The ALV 400 mg BID group also had the highest exposure to ALV (data not shown). Rates of viral rebound were 3% for ALV 400 mg BID and 4% for ALV 600 mg QD and ALV 800 mg QD. Relapse was documented in 13% of patients who received ALV 400 mg BID compared with 19% of those who received ALV 600 mg QD and 800 mg QD and 17% of those who received PR alone.

The NS5A D320E polymorphism was detected in two patients with population sequencing data at baseline (2 of 404; 0.5%), one of whom experienced virologic failure (ALV 600 mg QD group). Paired baseline and on-treatment NS5A sequencing data were available for 30 patients with viral breakthrough and 16 who relapsed after ≥40 weeks of ALV therapy. 17 of 30 patients had D320E detected at the time of viral breakthrough (8 in the ALV 600 mg QD group, 6 in the ALV 800 mg QD group, 1 in the ALV 400 mg BID group and 2 in the PR control group); D320E was not detected in any of the patients who relapsed. Consistent with a high barrier to resistance, D320E mutant replicons displayed only 3.2- and 4.5-fold reduced susceptibility to ALV compared with wild-type 1a-H77 and 1b-con-1 replicons, respectively (data not shown).

### *Safety*

The overall incidence of AEs was generally similar across all treatment arms, with highest rates seen in the ALV 400 mg BID group (Table 3). Headache, fatigue, anaemia, neutropenia and nausea were the most commonly reported AEs in all groups. AEs more frequent in the ALV arms than with PR control were anaemia, neutropenia, thrombocytopenia, alopecia, hypertension, hyperbilirubinaemia and hypertriglyceridaemia. The majority of AEs were moderate or mild; the incidence of severe AEs was 30% with ALV 400 mg BID, 19% with ALV 600 mg QD, 17% with ALV 800 mg QD and 11% with PR alone. Serious AEs (SAEs) were also more frequent in ALV-treated patients than in those who received PR alone, with the highest incidence in the ALV 400 mg BID group (17%). SAEs occurring in more than a single patient were hypertension, chest pain, anaemia, neutropenia, supraventricular tachycardia, pyrexia, pneumonia, appendicitis, dyspnoea and loss of consciousness (all occurring in 2–4 patients). Blood pressure, electrocardiogram and neurological signs were normal in both patients with loss of consciousness. The small number of patients with individual SAEs did not allow for meaningful comparisons across the treatment groups. The rate of treatment discontinuation due to AEs was 17% with ALV 400 mg BID, 9% with ALV 600 mg QD, 8% with ALV 800 mg QD and 5% with PR alone.

One case of mild acute pancreatitis was reported in this study in a 47-year-old male in the ALV 400 mg BID group. Ankylosing spondylitis was reported in the patient's medical history, but was not active at study entry; no relevant concurrent conditions or concomitant medications were reported. Serum lipase and amylase levels were within normal range until Week 28; serum triglyceride

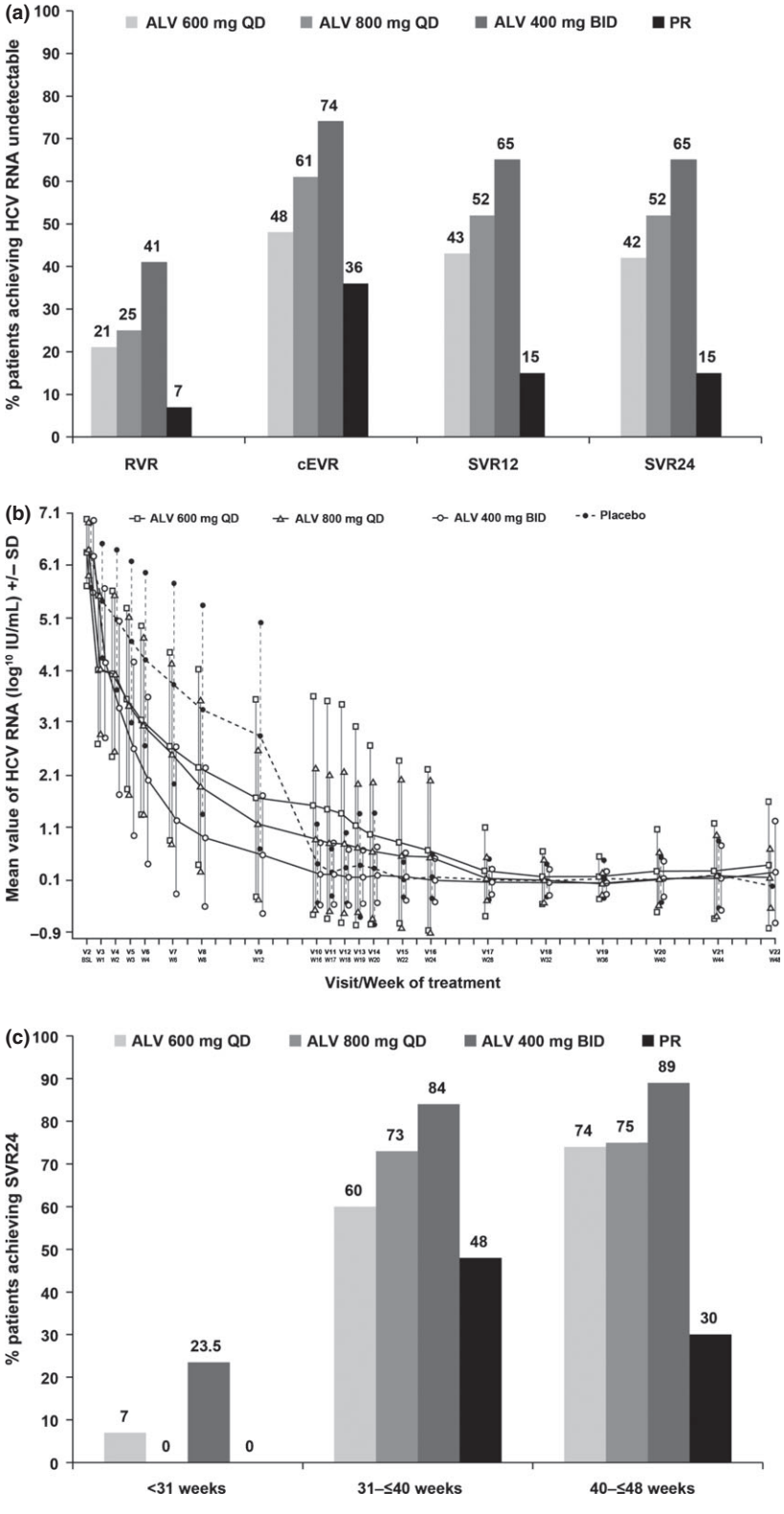


**Fig. 2** Virologic response: (a) Percentage of patients achieving virologic response at Weeks 4, 12, 24, 60 and 72; (b) Mean HCV RNA decrease over time. The drop in HCV RNA level in the PR control arm after Week 12 is an artefact, reflecting the fact that all patients in this arm with HCV RNA > limit of quantification at Week 12 were switched to treatment with ALV plus PR at Week 16; (c) SVR24 rates according to duration of ALV treatment. ALV, alisporivir; BID, twice daily; BSL, baseline; cEVR, complete early virologic response; PR, peginterferon-α2a/ribavirin; QD, once daily; RVR, rapid virologic response; SD, standard deviation; SVR12, sustained virologic response at Week 12 post-treatment; SVR24, sustained virologic response at Week 24 post-treatment; V, visit; W, week.

levels increased above upper limit of normal from Week 2 of treatment, with the highest value reported at Week 20 (6.47 mmol/L). At Week 28, the patient was hospitalized for abdominal pain, nausea and vomiting. Local laboratory results showed serum amylase levels of 2184 mg/dL, and abdominal ultrasonography was suggestive of pancreatitis, which was confirmed with computed tomography scanning. All study drugs were discontinued. The patient was

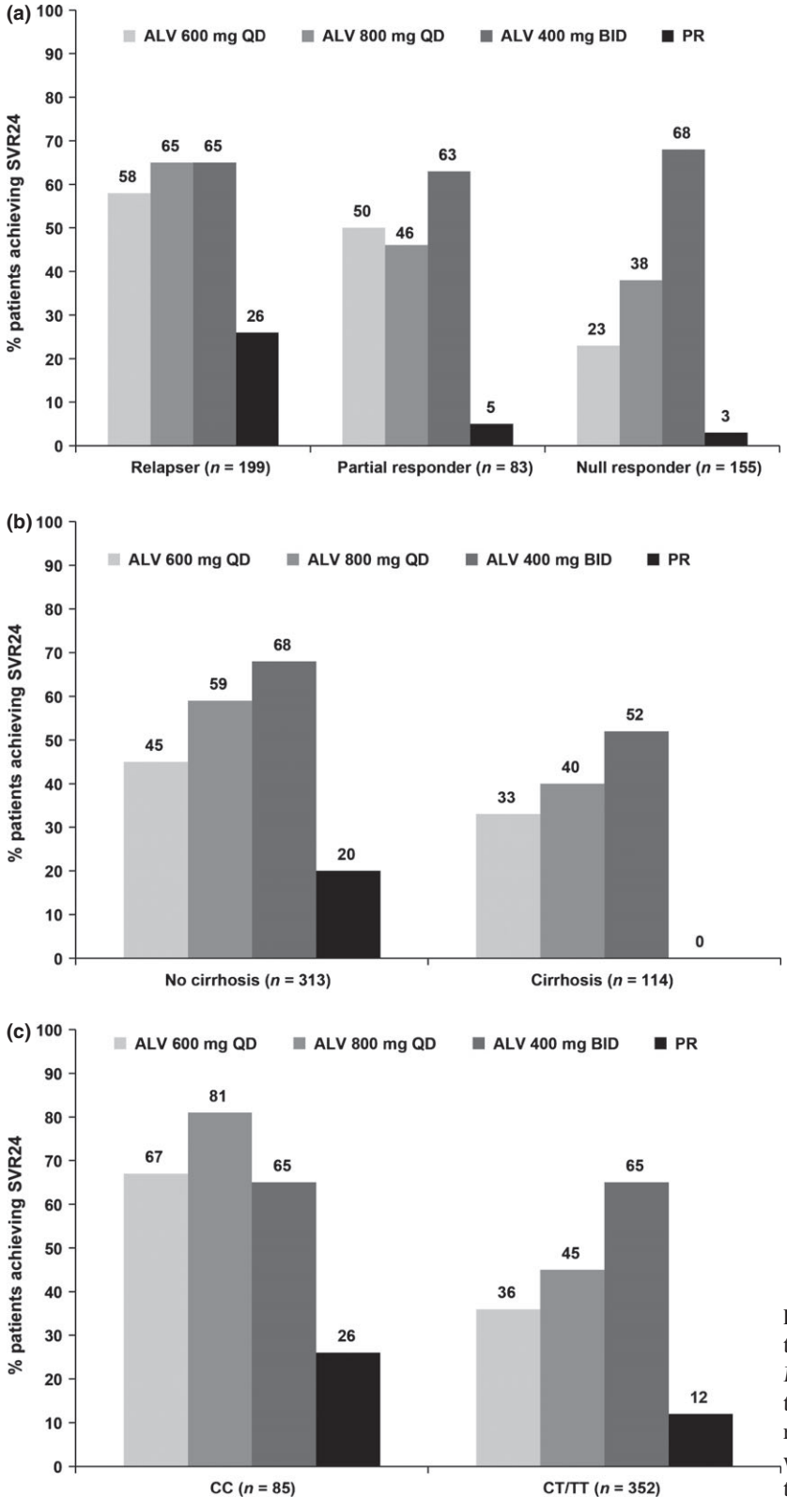


**Fig. 3** SVR24 rates according to (a) prior treatment response; (b) cirrhosis status; (c) *IL28B* genotype. ALV, alisporivir; BID, twice daily; PR, peginterferon-α2a/ribavirin; QD, once daily; SVR24, sustained virologic response at Week 24 post-treatment.

put on a low-fat diet and made a full recovery. Despite the shorter treatment duration (28 weeks), this patient achieved an SVR24.

Two deaths occurred during this study: one patient in the ALV 400 mg BID arm died due to septic shock and multi-organ failure following cellulitis in the lower limb

**Table 3** Incidence of adverse events during any study treatment

| Adverse event, *n* (%) | ALV 600 mg QD + PR (*N* = 120) | ALV 800 mg QD + PR (*N* = 115) | ALV 400 mg BID + PR (*N* = 108) | Placebo + PR (*N* = 114) |
|---|---|---|---|---|
| Serious adverse events | 7 (6) | 11 (10) | 18 (17) | 6 (5) |
| Deaths | 0 (0) | 0 (0) | 1 (1) | 0 (0) |
| Any adverse event | 117 (98) | 108 (94) | 107 (99) | 104 (91) |
| Discontinued study drug due to adverse event | 11 (9) | 9 (8) | 18 (17) | 6 (5) |
| Common adverse events (>25% in any treatment arm) | | | | |
| Headache | 60 (50) | 47 (41) | 38 (35) | 41 (36) |
| Fatigue | 47 (39) | 45 (39) | 45 (42) | 41 (36) |
| Anaemia | 49 (41) | 42 (37) | 52 (48) | 27 (24) |
| Neutropenia | 49 (41) | 40 (35) | 47 (44) | 28 (25) |
| Nausea | 46 (38) | 35 (30) | 51 (47) | 29 (25) |
| Pruritus | 36 (30) | 30 (26) | 32 (30) | 32 (28) |
| Pyrexia | 32 (27) | 34 (30) | 28 (26) | 32 (28) |
| Cough | 38 (32) | 29 (25) | 16 (15) | 29 (25) |
| Insomnia | 35 (29) | 24 (21) | 17 (16) | 25 (22) |
| Decreased appetite | 31 (26) | 23 (20) | 26 (24) | 16 (14) |
| Asthenia | 19 (16) | 30 (26) | 17 (16) | 21 (18) |
| Thrombocytopenia | 24 (20) | 20 (17) | 28 (26) | 4 (4) |
| Hypertension | 21 (18) | 23 (20) | 28 (26) | 2 (2) |
| Hyperbilirubinaemia | 19 (16) | 14 (12) | 36 (33) | 2 (2) |
| Key laboratory abnormalities* | | | | |
| Neutropenia | | | | |
| Grade 3 | 42 (35) | 33 (29) | 41 (38) | 29 (25) |
| Grade 4 | 10 (8) | 6 (5) | 11 (10) | 4 (4) |
| Thrombocytopenia | | | | |
| Grade 3 | 10 (8) | 14 (12) | 20 (19) | 1 (1) |
| Grade 4 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Anaemia | | | | |
| Grade 3 | 7 (6) | 3 (3) | 6 (6) | 1 (1) |
| Grade 4 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hypertriglyceridaemia | | | | |
| Grade 3 | 16 (13) | 21 (18) | 19 (18) | 4 (4) |
| Grade 4 | 5 (4) | 6 (5) | 3 (3) | 2 (2) |
| Hyperbilirubinaemia | | | | |
| Grade 3 | 7 (6) | 7 (6) | 25 (23) | 0 (0) |
| Grade 4 | 1 (1) | 0 (0) | 1 (1) | 0 (0) |

ALV, alisporivir; BID, twice daily; DMID, Modified Division of Microbiology & Infectious Diseases; PR, peginterferon-α2a + ribavirin; QD, once daily; ULN, upper limit of normal. Neutropenia: Grade 3, neutrophils $<0.75 \times 10^9$/L; Grade 4, neutrophils $<0.5 \times 10^9$/L. Thrombocytopenia: Grade 3, platelets $<50 \times 10^9$/L; Grade 4, platelets $<20 \times 10^9$/L. Anaemia: Grade 3, haemoglobin <80 g/L; Grade 4, haemoglobin <65 g/L. Hypertriglyceridaemia: Grade 3, triglyceride >500 mg/dL; Grade 4, triglyceride >1000 mg/dL. Hyperbilirubinaemia: Grade 3, total bilirubin >5 × ULN; Grade 4, total bilirubin >10 × ULN.

*Defined according to DMID Toxicity Tables (v2.0).

during treatment (noncirrhotic, no concomitant neutropenia; blood culture positive for *Staphylococcus aureus*), and one patient in the ALV 800 mg QD arm died due to cardiac failure (noncirrhotic, normal electrocardiogram and no concomitant disease at baseline, underlying cholangiocarcinoma at autopsy) during the follow-up period of 202 days after the last dose of study medication; neither death was considered related to ALV.

Neutropenia and hypertriglyceridaemia were the most common laboratory abnormalities reported as AEs, followed by thrombocytopenia and hyperbilirubinaemia (Table 3). Increases in triglyceride levels were apparent at

Week 1 in all treatment groups and were generally greater in the ALV-containing arms than in the control arm. At Week 16, the mean increase from baseline was 1.7 mmol/L in the ALV 400 mg BID arm compared with 0.6 mmol/L with PR control. Triglyceride levels decreased towards baseline values at the end of treatment in all groups. Hyperbilirubinaemia in ALV-treated patients was transient and reversible and was not associated with alanine aminotransferase elevation.

Consistent with the increased rate of hypertension reported as an AE, vital signs data showed blood pressure elevations in patients treated with ALV plus PR. Most blood pressure elevations were single events. Persistent elevation of either systolic or diastolic blood pressure at ≥2 consecutive visits was observed in only 3–4% of patients in the ALV treatment arms. Only 4/457 (0.9%) patients had elevation of both systolic and diastolic blood pressure at ≥2 consecutive visits: 3 (2.8%) in the ALV 400 mg BID arm and 1 (0.8%) in the ALV 600 mg QD arm.

## DISCUSSION

ALV, a potent cyclophilin inhibitor, is an oral host-targeting agent in development for the treatment of chronic HCV infection with activity against all HCV genotypes, including HCV genotype 3 [10]. In this study, addition of ALV to PR significantly improved rates of on- and post-treatment virologic response in HCV genotype 1-infected patients in whom prior PR therapy had failed, even among those who received a shortened duration of treatment. Overall, SVR24 rates were 42–65% in ALV-treated patients compared with only 15% in those who received PR alone. The highest response rates were seen in patients who received ALV 400 mg BID; the SVR24 rate was 89% in patients who received ALV 400 mg BID with PR for >40 weeks compared with 30% in corresponding patients treated with PR alone. This response rate compares favourably with that seen with the first DAAs licensed in combination with PR, boceprevir and telaprevir [16,17]. SVR24 rates of 61–80% were reported in treatment-experienced patients with HCV infection treated with the recently approved second-generation NS3/A protease simeprevir in combination with PR for 48 weeks [18].

ALV 400 mg BID produced superior rates of virologic response even among the most difficult-to-treat patient groups, such as prior null responders, patients with non-CC *IL28B* genotypes and those with cirrhosis. SVR24 was achieved by 68% of prior null responders who received ALV 400 mg BID with PR compared with only 3% of those who received PR alone. SVR24 rate was 65% in patients with non-CC *IL28B* genotypes treated with ALV 400 mg BID and PR compared with 12% in those who received PR alone. SVR24 rate in patients with cirrhosis was 44% with ALV 400 mg BID with PR; no patient with cirrhosis achieved SVR24 with PR alone.

The lowest rates of viral breakthrough (3%), rebound (3%) and relapse (13%) were seen in the ALV 400 mg BID group. This compared with rates of viral breakthrough and relapse of 6% and 17%, respectively, in patients who received PR alone. However, these results should be interpreted with caution, as PR/placebo treatment was stopped at Week 12 for treatment failures, and later ALV treatment was stopped due to the partial clinical hold, resulting in patients receiving various durations of treatment.

The overall safety profile of ALV in triple therapy with PR in this study was consistent with results from a recent integrated safety analysis, which pooled interim data from 24 weeks of treatment with ALV plus PR from this study together with data from three other large Phase II/III studies [19]. That safety analysis provided the first indication of hypertension as a new safety signal associated with ALV. Results from the present study confirm this finding and show no other new or unexpected safety signals. Most patients (95%) experienced at least one AE, with no difference in the overall incidence of AEs across treatment arms. However, use of ALV in combination with PR appears to exacerbate safety issues known to be associated with PR such as haematological AEs and triglyceride abnormalities. Most of these events did not require study drug discontinuation, and very few were reported as SAEs. Blood pressure elevations were mainly single events; the incidence of persistent hypertension was low (3–4% with either systolic or diastolic elevations and 0.9% with both systolic and diastolic elevations) and showed no dose effects. Hyperbilirubinaemia was transient and reversible and not associated with liver toxicity. Hyperbilirubinaemia with ALV is believed to be due to inhibition of the uptake transporters OATP1B1 and OATP1B3 and the efflux transporter MRP [10]; ALV does not inhibit the enzyme UGT1A1 (glucuronide conjugation).

One case of mild acute pancreatitis was reported in this study in a 47-year-old male receiving ALV 400 mg BID/PR. The patient made a full recovery and achieved rapid virologic response and SVR24 despite having only received 28 weeks of treatment. In the pooled safety analysis [19], pancreatitis was reported in 6 of 1365 patients receiving ALV triple therapy and 2 of 489 patients receiving PR alone (both 0.4%). Another patient in the triple therapy group had elevated amylase and lipase as a component of diabetic ketoacidosis. One patient with a complex presentation and concomitant multi-organ failure died; all others recovered or improved. No consistent relationship to triglyceride elevations was noted in ALV-treated patients. Acute pancreatitis is known to be a rare complication of PR therapy [20–23]. Of note, no cases of pancreatitis were reported in 260 patients treated with IFN-free ALV for at least 6 weeks, with 91 of these receiving IFN-free ALV for up to 24 weeks [24]; this is consistent with preclinical and animal studies, which do not suggest any direct role of ALV in injury to the pancreas (Novartis, data on file).

HCV therapy is moving towards IFN-sparing and IFN-free strategies [25]. This may include explicitly licensed DAA combinations as well as 'off-the-shelf' combinations of oral agents, as seen in early anti-HIV therapy. This approach is already reflected in the recently updated American Association for the Study of Liver Diseases (AASLD)/Infectious Diseases Society of America (IDSA) guidelines for the treatment of HCV [26]. Accordingly, future development of ALV is planned as an IFN-free regimen. Due to its unique mechanism of action, ALV has a high genetic barrier for the development of resistance and lack of cross-resistance to DAAs [10]. As such, ALV appears well-suited for use in IFN-free regimens in combination with HCV protease inhibitors, polymerase inhibitors and/or NS5A inhibitors. The ALV combination regimen with optimum synergistic and pangenotypic anti-HCV efficacy has yet to be clinically identified. ALV has been shown to have additive or synergistic activity with multiple drugs in each of these classes *in vitro* [27]. ALV blocks contact between the cyclophilin A and the domain II of NS5A [10], and recent data have demonstrated particular synergy, *in vitro*, with NS5A inhibitors [27]. Experience in treatment-naïve patients has shown the AE profile of ALV in IFN-free regimens to be markedly superior to that when used in combination with IFN [13,19,24]. High rates of SVR and low rates of relapse have been reported in those treatment-naïve patients with HCV genotype 2 of 3 infection who achieved early HCV clearance (RVR) with IFN-free ALV and RBV therapy [13]. IFN was added to the regimen for non-RVR patients. SVR24 rates were 80–85% in patients assigned to ALV plus RBV treatment arms compared with 58% in those who received PR therapy; respective relapse rates were 8–10% and 25%.

The main limitation of this study was the fact that the FDA partial clinical hold resulted in patients receiving various durations of therapy; only 15% of patients received >40 weeks of triple therapy. This study was not designed to identify the optimum duration of ALV therapy. Results for all subgroups were influenced by the fact that treatment failures, discontinuations due to AEs and impact of stopping rules mainly occurred prior to Week 31, which most likely biased results for the subgroup with ≤31 weeks of ALV therapy. However, it is possible that shorter treatment durations of ALV may be effective when used in combination with other potent anti-HCV therapies.

In summary, ALV, especially with the 400 mg BID regimen, markedly increased responsiveness to PR therapy in HCV genotype 1 nonresponders including prior null nonresponders and cirrhotics. This novel host-targeting agent appears to merit further investigation in IFN-free regimens in combination with DAAs.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1:** CONSORT diagram. ALV, alisporivir; BID, twice daily; cEVR, complete early virologic response; FAS, full analysis set; ITT, intent-to-treat; PR, peginterferon-α2a/ribavirin; QD, once daily.

**Table S1:** Study exclusion criteria.