Safety, tolerability, and anti-fibrotic efficacy of the CBP/β-catenin inhibitor PRI-724 in patients with hepatitis C and B virus-induced liver cirrhosis: An investigator-initiated, open-label, non-randomised, multicentre, phase 1/2a study

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Summary

Background We conducted an exploratory study to assess the safety tolerability, and anti-fibrotic effects of PRI-724, a CBP/β-catenin inhibitor, in patients with hepatitis C virus (HCV)- and hepatitis B virus (HBV)-induced cirrhosis.

Methods This multicentre, open-label, non-randomised, non-placebo-controlled phase 1/2a trial was conducted at three hospitals in Japan. Between July 27, 2018, and July 13, 2021, we enrolled patients with HCV- and HBV-induced cirrhosis classified as Child–Pugh (CP) class A or B. In phase 1, 15 patients received intravenous infusions of PRI-724 at escalating doses of 140, 280, and 380 mg/m^2/4 h twice weekly for 12 weeks. In phase 2a, 12 patients received the recommended PRI-724 dose. The primary endpoints of phases 1 and 2a were the frequency and severity of adverse events and efficacy in treating cirrhosis based on liver biopsy. This study was registered at ClinicalTrials.gov (no. NCT 03620474).

Findings Three patients from phase 1 who received the recommended PRI-724 dose were evaluated to obtain efficacy and safety data in phase 2a. Serious adverse events occurred in three patients, one of which was possibly related to PRI-724. The most common adverse events were diarrhoea and nausea. PRI-724 did not decrease hepatic fibrosis with any statistical significance, either by ordinal scoring or measurement of collagen proportionate area at 12 weeks; however, we observed statistically significant improvements in liver stiffness, Model for End-stage Liver Disease score, and serum albumin level.

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; ALB, albumin; AUC, area under the concentration curve; CBP, CREB-binding protein; C_{max}, maximum concentration; CP, Child Pugh; HAI, histology activity index; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LSM, liver stiffness measure; M2BPGi, MAC-2-binding protein glycosylation isomer; MELD, Model for End-stage Liver Disease; NASH, non-alcoholic steatohepatitis; PT, prothrombin time; SAE, serious adverse event; SVR, sustained virological response; TBIL, total bilirubin; T_{max}, time of maximum observed serum

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Interpretation Intravenous administration of 280 mg/m²/4 h PRI-724 over 12 weeks was preliminarily assessed to be well tolerated; however, further evaluation of anti-fibrotic effects in patients with cirrhosis is warranted.

Funding AMED, Ohara Pharmaceutical

Keywords: PRI-724; Liver cirrhosis; HBV; HCV; Anti-fibrotic drug

Research in context

Evidence before this study

We performed PubMed searches for articles using the search terms "liver cirrhosis", "anti-fibrotic drug", and "β-catenin" to collect clinical trials published from inception to December 2021 (restricted to English language publications). Of the 11 search results, we found one study on the use of β-catenin inhibitors as anti-fibrotic drugs for hepatitis C virus (HCV) patients with liver cirrhosis. This was the only phase 1 study that we conducted previously, and there are no reports of other groups conducting clinical trials of β-catenin inhibitors for liver cirrhosis.

Added value of this study

This study assessed the safety, tolerability, pharmacokinetics, and anti-fibrotic effect of PRI-724, a CREB-binding protein (CBP)/β-catenin inhibitor, in patients with HCV and hepatitis B virus (HBV) cirrhosis. The results showed that a dose of 280 mg/m2/4 h of PRI-724 was preliminarily well tolerated and suggested that the treatment can potentially exert an anti-fibrotic effect in HCV and HBV liver cirrhosis. PRI-724 did not decrease the area of reticular fibres in the hepatic lobule at 12 weeks; however, we observed statistically significant improvements in liver stiffness, Model for End-stage Liver Disease score, and serum albumin level. Furthermore, a change from Child-Pugh (CP) class B to CP class A was reported in two patients (29%, 2/7).

Implications of all evidence available

Liver cirrhosis is the leading cause of morbidity and mortality in developed countries and the 11th most common cause of death in adults worldwide. However, there is currently no anti-fibrotic drug therapy available to treat HCV- and HBV-induced cirrhosis. This phase 1/2a study showed that intravenous administration of 280 mg/m^2/4 h PRI-724 twice weekly over 12 weeks was preliminarily well tolerated by patients with HBV and HCV cirrhosis and resulted in improvements of liver stiffness and fibrosis-related biomarkers in several patients.

Introduction

Liver cirrhosis is the leading cause of morbidity and mortality in developed countries and the 11th most common cause of death in adults worldwide.[1] Liver cirrhosis results from several mechanisms of liver injury (i.e., viral hepatitis, alcohol, and metabolites) that lead to fibrogenesis.[2] Fibrosis is an excessive wound-healing response that occurs in most forms of chronic liver damage and results in the accumulation of excessive extracellular matrix.[3] Fibrosis can progress to cirrhosis because of continuous liver damage, and upon cirrhosis progression from the compensatory to the decompensated phase, it induces complications, such as oesophageal varices, infectious diseases, ascites, and renal dysfunction, resulting in liver failure and subsequent poor prognosis.[1] Additionally, liver cirrhosis causes high rates of hepatocellular carcinoma (HCC), which is currently the second leading cause of cancer deaths worldwide[4] and considered a cancer with a poor prognosis due to recurrence and the lack of effective therapeutic agents.[5] Therefore, there is an urgent need to develop a therapeutic drug for liver cirrhosis that can prevent the onset of liver failure and HCC; however, there is currently no therapeutic drug that has been put into practical use.[6]

Instances of non-alcoholic steatohepatitis (NASH)-induced liver fibrosis and cirrhosis have recently increased, and several relevant clinical trials have been conducted mainly in Europe and the United States, although many have been discontinued in the middle of development.[7-9] One reason is the use of pathological diagnosis using liver biopsy as a method to confirm the anti-fibrotic therapeutic effect.[7] Although long-standing problems associated with sampling error have been noted, it is suggested that long time periods are required to observe the effect of fibre reduction due to the strength of the connections between fibres that have progressed to cirrhosis. Recently, there have been more studies on pre-stage fibrosis (F2-3) than on liver cirrhosis.[10,11] Thus, the development of anti-fibrotic drugs for liver cirrhosis remains a clinically significant challenge. In this study, we focused on Wnt/β-catenin signalling, which is important for fibrogenesis in tissue organs. Wnt/β-catenin signalling is an evolutionarily conserved signalling cascade that plays a crucial role in the regulation of organ development, injury repair. and tissue homeostasis.[12,13] Activated Wnt/β-catenin signalling is implicated in fibrosis of a number of organs, including lung, kidney, skin, and liver.[14-17]

A recent study showed that specific inhibition of the CREB-binding protein (CBP)/β-catenin interaction not only ameliorated but also reversed late-stage fibrotic

injury of the lung and kidney in murine models.[18,19] Additionally, we previously reported that the selective CBP/β-catenin inhibitor PRI-724 exhibits anti-fibrotic effects in murine models of liver fibrosis due to hepatic stellate cell inactivation and macrophage migration.[20,21] Based on these findings, we conducted an investigator-initiated clinical trial (phase 1) to evaluate PRI-724 safety and tolerability in hepatitis C virus (HCV)-positive patients with liver cirrhosis.[22,23] Although the number of treated patients was small, the protocol ensured patient safety, and liver biopsy results from some patients indicated PRI-724-mediated anti-fibrotic effects. However, a limitation of the current treatment protocol is the requirement for continuous administration for 1 week, whereas our experience suggests that infusion for 4 h twice weekly would be optimal.

Therefore, in this phase 1/2a trial of PRI-724, we assessed the overall safety, tolerability, and anti-fibrotic efficacy of escalating doses in a cohort of patients with hepatitis B virus (HBV) and HCV liver cirrhosis. Our data could support further evaluation of PRI-724 as an anti-fibrotic drug for liver cirrhosis.

Methods

Study design and patients

In this multicentre, open-label, non-randomised, non-placebo-controlled phase 1/2a dose-escalation trial, we sequentially enrolled patients with HBV and HCV liver cirrhosis treated at the Cancer and Infectious Diseases Center of Tokyo Metropolitan Komagome Hospital (Tokyo, Japan), Kohnodai Hospital, the National Center for Global Health and Medicine (Chiba, Japan), and Kyushu University Hospital (Fukuoka, Japan). PRI-724 (PubChem database CID: 71509318) and the related information were provided by Prism Biolab (Kanagawa, Japan) and Ohara Pharmaceutical Co., Ltd. (Tokyo, Japan).

The rationale for conducting this study using a non-controlled, non-randomised design is as follows. Improvements in the degree of liver fibrosis have been confirmed in some patients with HCV-induced liver cirrhosis (who have achieved sustained virological response (SVR) following treatment with recently authorised direct-acting antiviral (DAA) therapy) as well as some patients with HBV-induced liver cirrhosis (whose viral load decreased after administration of nucleic acid synthesis inhibitors). However, improvements may take years to achieve, during which time patients are susceptible to complications and cancer.[1,24,25] In particular, it is unlikely that fibrosis will be alleviated spontaneously during a study period of only 3 months with patients who have demonstrated SVR for more than 6 months. It was therefore considered that if a before-and-after comparison shows clear improvement, the efficacy of this investigational product can be inferred with a high degree of probability.

Key inclusion criteria were as follows: age of 20-74 years and diagnosis with HCV- or HBV-induced liver cirrhosis that meets condition I or II presented below while also meeting condition III (I. Serum HCV-RNA positive or HCV-antibody positive [irrespective of viral load or treatment]; II. Serum HBV-DNA positive or HBs-antibody positive [irrespective of viral load or treatment]; III. Diagnosis of liver cirrhosis confirmed by a liver biopsy performed during the screening period [modified HAI fibrosis score of 5 or 6 or Metavir score of F4]). A complete list of inclusion and exclusion criteria is provided in Supplementary material.

Procedures

Patients were enrolled into three cohorts and administered 140, 280, or 380 mg/m^2 PRI-724 for 4 h in phase 1 and 280 mg/m^2 in phase 2a, which was the recommended dose determined in phase 1. PRI-724 was administered for 12 cycles, with each cycle comprising two doses administered each week. A single dose was administered on Day-7 (tolerance: -7 days) before the start of administration in the first cycle. The starting dose was designated as level 1 (140 mg/m^2). After confirming tolerability at Level 1, the dose was escalated to Level 2 (280 mg/m^2) then Level 3 (380 mg/m^2). Three patients were enrolled at each level. A minimum of six patients were enrolled at the dosage level regarded as the recommended dose. Safety and pharmacokinetic evaluations were conducted at this dose level. Blood samples were drawn pre-dose and at 30 min as well as 1, 2, and 4 h after dosing and again at 1, 5, and 20 h after administration. We measured plasma concentrations of PRI-724 and C-82 (an active metabolite of PRI-724) in blood samples obtained from patients during phase 1. Three dose levels (140 mg/m^2/4 h [starting dose], 280 mg/m^2/4 h, and 380 mg/m^2/4 h) were administered to three patients according to the guidelines for determining whether a patient proceeded to the next dose level. Fifteen patients received the recommended dose in phase 2a, during which PRI-724 efficacy and safety were assessed.

We enrolled 27 participants (15 in phase 1 and 12 in phase 2a). Three patients in phase 1 who received 280 mg/m^2 PRI-724 and underwent post-treatment biopsy with written informed consent were evaluated to obtain efficacy and safety data in phase 2a. Each patient was monitored for 28 days after the last day of administration. We recorded adverse events (AEs) and clinical laboratory results throughout the study and graded the AEs according to the Common Terminology Criteria for Adverse Events version 4.0. Investigators regularly assessed safety and tolerability, including serious adverse events (SAEs) and those associated with treatment discontinuation. The registration of this trial after enrolment of the first patient was slightly delayed owing to the COVID-19 pandemic.

Liver biopsy samples taken at screening were used as baseline data. Samples were also taken within 2 weeks

of the final treatment. Three independent pathologists examined all biopsy slides at baseline and 12 weeks in a blinded manner. Biopsy slides were evaluated using the Knodell scoring system, and fibrosis was staged according to the Ishak-modified histology activity index (HAI) grading scale. To evaluate whether PRI-724 exhibited an anti-fibrotic effect, we measured fibrosis in hepatic lobules using standardised computer-assisted image analysis. An independent pathologist blindly selected 10 Sirius Red-stained parenchyma spots in all biopsy samples and automatically calculated the positively stained areas using HistoQuant software (3DHISTECH, Budapest, Hungary).

Outcomes

The primary endpoints of phase 1 were safety, tolerability, and dose-limiting toxicities of multiple escalating doses of PRI-724 when administered via intravenous infusion. Safety was measured by analysing the frequency and severity of AEs. The secondary endpoint was the determination of PRI-724 pharmacokinetics (PK) *in vivo*. Plasma PRI-724 and C-82 concentration-time data were analysed by non-compartmental methods. These included the maximum drug concentration (C_{max}), the time to C_{max} (T_{max}), the terminal half-life ($t_{1/2}$), and the area under the plasma concentration-time curve (AUC).

The primary endpoint of phase 2a was the efficacy of liver cirrhosis treatment, which was assessed by examining changes relative to baseline in the area of liver tissue fibrosis according to liver biopsy at 12 weeks post-treatment. The secondary endpoint was the change relative to baseline in liver stiffness measure (LSM) according to FibroScan (Echosens, Waltham, MA, USA), CP score, Model for End-stage Liver Disease (MELD) score, and modified HAI score at 12 weeks.

Statistical analysis

Sample size calculation. The rationale for enrolling patients in phase 2a was based on the results of a previous study on PRI-724-1101 and the results of a study by D'Ambrosio *et al.* who observed the natural course of patients with SVR.[24] In the PRI-724-1101 study, the fibrotic area ratio (%) of five patients (CP class A: 2 and CP class B: 3) was 4·28±1·72% before administration and 2·21 ± 1·96% after administration. The amount of change was −2·06±1·95% (mean ± SD, respectively). In contrast, D'Ambrosio *et al.* reported the use of data extraction tool WebPlot Digitizer Version 4·0 to extract the original data from the graph summarising the natural history of patients with SVR.[24] The fibrotic area ratio of 35 patients was 9·86±4·50% before SVR but improved to 3·59±3·03% five years after SVR, and the amount of change was −6·26±4·88%. Thirty-eight patients were included in the original paper,[24] but three of them were excluded since their data could not be extracted. Assuming a constant rate of decrease in the fibrotic area, the amount of change due to a natural course of three months after SVR can be calculated as −0·31 ± 0·24%, and the bilateral 95% confidence interval can be calculated as [−0·40%, −0·23%]. Therefore, the amount of change due to drug administration can be expected to be at least −2·06% minus the lower limit of the confidence interval of −0·40% of the amount of change due to natural course, which is about −1·66%. Since this clinical trial was based on a small number of studies, the number of patients enrolled was 15 as the amount of change of this study was conservatively assumed to be −1·60%, the standard deviation to be 2·00%, the significance level to be 5% on both sides, and the power to be 80%. Considering the dropout, 16 patients were considered necessary for evaluating the efficacy of phase 2a.

Analyses of primary and secondary endpoints

All patients were included in the analyses of PRI-724 safety, tolerability, and anti-fibrotic effect. All statistical analyses were descriptive and calculated for each treatment group. Data are expressed as the mean±standard deviation. Differences between groups were analysed using a two-tailed Student's *t*-test, with a $P<0.05$ considered statistically significant. Clinical safety and pharmacokinetic data were included in safety analyses. The primary endpoint of phase 2a was analysed using a mixed model for repeated measures (MMRM), which permitted the analysis of continued treatment results even if some data were missing. We chose to use an MMRM since the subjects enrolled in the study were stable and their rapid deterioration was unlikely, indicating that missing data will likely be due to other reasons, including subjects' social engagements. Since these are independent of the treatment effect of the investigational product, the use of an MMRM will not result in an overestimation. There was no further allowance for missing data. In addition, values outside the permissible range of the evaluation implementation time were treated as missing values and were not included with other evaluation time data. PK analyses were conducted in patients with evaluable PK concentrations using non-compartmental methods with Microsoft Office Excel and WinNonlin v6.1 (Pharsight Corporation, St. Louis, MO, USA).

We performed pre-specified analyses of changes in LSM, CP score, and MELD score from baseline to 12 weeks post-treatment. We also performed a pre-specified secondary analysis of change from baseline in histological scores, which focused on patients with biopsy samples from baseline and 12 weeks after PRI-724 treatment. All analyses were performed using SAS software (v9.4; SAS Institute, Cary, NC, USA).

Data handling procedure. To guarantee the reliability and treatment of all study-related data, the Data Management Officer and staff shall implement quality

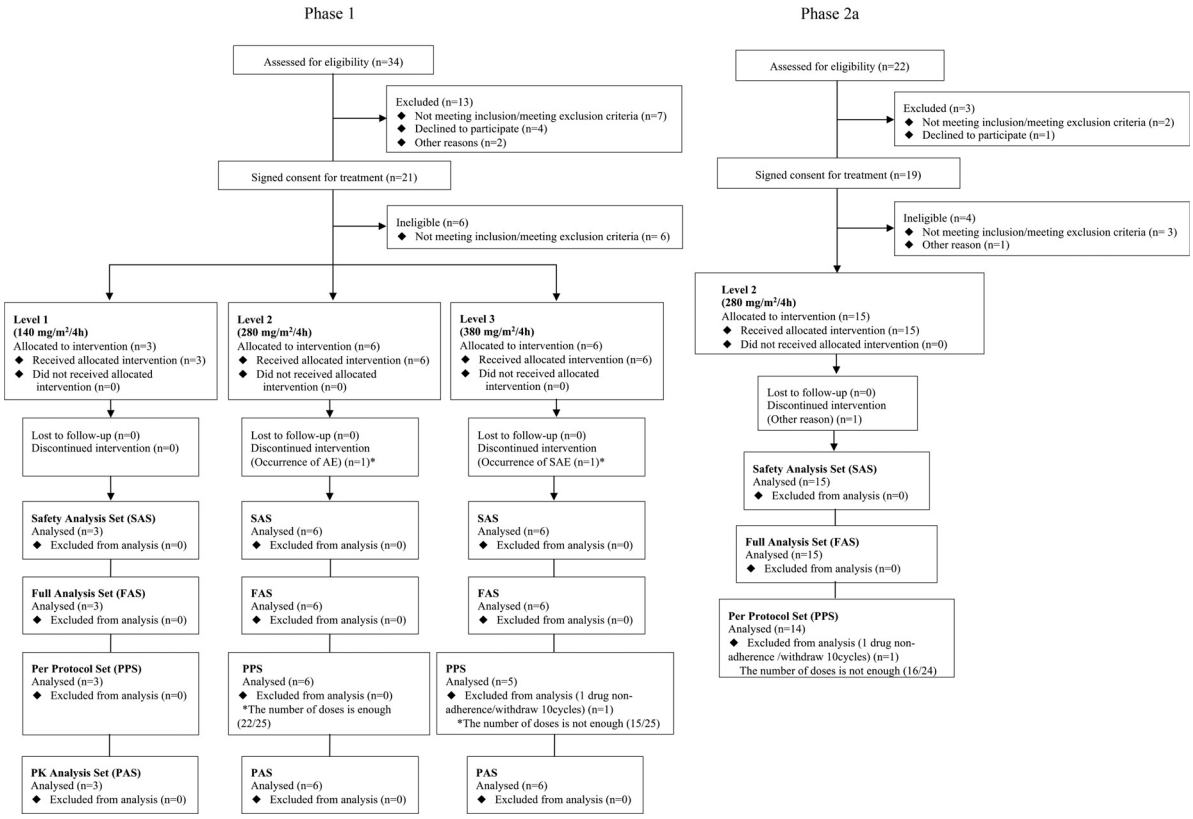

Figure 1. Trial profile (Phase 1/2a). Consort diagram.

control at every level of data handling based on the Procedures for Data Management Operations. The Data Management Officer and staff shall carry out the procedures up to database locking, including EDC system (DATATRAK ONE®)construction, case reports, and data inspection, on the basis of materials including the Data Management Plan for this study. When data management operations have been completed, they shall produce a Data Management Report.

Ethics

Written informed consent was obtained from all patients. The study was approved by the Institutional Review Board of the Komagome Hospital (approval number 18-004) and was performed in compliance with the Good Clinical Practice guidelines, the Declaration of Helsinki, and other regulatory requirements. This study was registered at ClinicalTrials.gov (registration number NCT 03620474).

Role of the funders

The funder of the study was involved in study design but had no role in writing the report or data collection, analysis, or interpretation. The corresponding author had full access to all data in the study and the final responsibility for the decision to submit the study for publication. Japan Agency for Medical Research and Development was responsible for funding the study. Study drug and financial support were provided by Ohara Pharmaceutical.

Results

Between July 27, 2018, and July 13, 2021, we screened 34 patients and enrolled 15 in the phase 1 trial (Figure 1). In this study, 3 patients were enrolled in Level 1, 6 patients in Level 2 (3 of whom were additional patients after administration of 6 patients in Level 3), and 6 patients in Level 3. The safety and PK at Level 1 (140 mg/m^2/4 h) were confirmed in the first 3 patients enrolled, and the transition to Level 2 (280 mg/m^2/4 h) was decided. Once the safety and PK were confirmed in the three patients enrolled in Level 2, they were transitioned to Level 3 (380 mg/m^2/4 h). Three patients were enrolled in Level 3 to confirm their safety and PK, and it was decided to add 3 to Level 3. Serious adverse events (liver dysfunction) occurred in 1 of the 3 patients added. A causal relationship with the investigational drug could not be ruled out. When the transition of liver function of the 12 registered patients up to that point was reconfirmed, 2 other patients experienced a negative effect on liver function (non-serious). Considering

Characteristics	Phase 1 (n = 15)			Phase 2a (n = 15)
	140 mg/m^2/4h (n = 3)	280 mg/m^2/4h (n = 6)	380 mg/m^2/4h (n = 6)	280 mg/m^2/4h
Age (years)	61 (57-66)	62 (56-70)	60 (50-66)	67 (42-73)
Sex				
Men	1 (33%)	4 (67%)	6 (100%)	14 (93%)
Race				
Asian	3 (100%)	6 (100%)	6 (100%)	15 (100%)
HCV	3 (100%)	4 (67%)	3 (50%)	9 (60%)
HCV genotype				
Type 1	3 (100%)	3 (75%)	3 (100%)	4 (44%)
Type 2	0	0	0	3 (33%)
NA	0	1 (25%)	0	2 (22%)
HCV RNA (Mean-log IU/mL)	(-)	*	(-)	(-)
SVR	3 (100%)	3 (75%)	3 (100%)	9 (100%)
Post SVR (months)	27 (16-49)	14 (5-25)	25 (16-39)	18 (5-214)
HBV	0	2 (33%)	3 (50%)	6 (40%)
HBV genotype				
A	–	1 (50%)	0	1 (17%)
C	–	0	0	2 (33%)
NA	–	1 (50%)	3 (100%)	3 (50%)
HBV DNA (Mean-log IU/mL)	–	(-)	**	(-)
CP score - no. (%)				
≤6	1 (33%)	2 (33%)	5 (83%)	8 (53%)
7	2 (67%)	1 (17%)	0	4 (27%)
8	0	3 (50%)	0	1 (7%)
9	0	0	1 (17%)	2 (13%)
≥10	0	0	0	0
MELD score - no. (%)				
≤5	2 (67%)	4 (67%)	3 (50%)	4 (27%)
6-10	1 (33%)	1 (17%)	3 (50%)	7 (47%)
≥11	0	1 (17%)	0	4 (27%)
FibroScan (kPa) - no. (%)				
<10	1 (33%)	0	1 (17%)	0 ***
10-20	1 (33%)	1 (17%)	3 (50%)	6 (43%) ***
>20	1 (33%)	5 (83%)	2 (33%)	8 (57%) ***
HAI score - no. (%)				
<3	0	2 (33%)	3 (50%)	9 (60%)
3-5	2 (67%)	2 (33%)	3 (50%)	5 (33%)
≥6	1 (33%)	2 (33%)	0	1 (7%)
Ascites - no. (%)				
None	3 (100%)	6 (100%)	5 (83%)	13 (87%)
Mild or moderate	0	0	1 (17%)	2 (13%)
Severe	0	0	0	0
Treatment - no. (%)				
No	0	1 (17%)	0	0
Yes	3 (100%)	5 (83%)	6 (100%)	15 (100%)
DAA	2 (67%)	3 (60%)	3 (50%)	6 (40%)
IFN based therapy	1 (33%)	0	0	3 (20%)
NA	0	2 (40%)	3 (50%)	6 (40%)

***Table 1*: Patient characteristics (Phase 1/2a). *Only one patient was HCV-RNA-positive with 5.6 Log IU/mL; all others were negative. **Only one patient was HBV-DNA-positive with < 1.3 Log IU/mL; all others were negative. ***Only one patient in which the characteristics could not be measured because of ascites.**

the importance of "liver dysfunction", it was deemed irresponsible to raise the recommended dose to Level 3, and it was decided to instead add 3 patients to Level 2 (280 mg/m^2/4 h). At Level 2, there were no serious adverse events for which a causal relationship with the investigational drug could not be ruled out in all 6 patients. Level 2 (280 mg/m^2/4 h) was determined to be the recommended dose for the phase 2a stage under consideration from the advice of PK experts.

Thirteen patients were able to complete all 12 cycles, but one patient in the 280 mg/m^2/4 h cohort and one in the 380 mg/m^2/4 h cohort completed only 22 and 15 doses, respectively. By contrast, we screened 22 patients and enrolled 15 in the phase 2a trial, including three patients from phase 1 and subsequently treated with the optimal PRI-724 dose in phase 2a.

Table 1 shows the baseline patient characteristics. There were 10 patients with HCV cirrhosis and five with HBV cirrhosis (CP class A: 8 patients; and CP class B: 7 patients) in the phase 1 trial. Three patients were included in the 380 mg/m^2/4 h dose cohort based on the protocol followed for dose escalation, but one additional patient developed one SAE (liver dysfunction: grade 3) for which a causal relationship could not be ruled out. The dose level was lowered by one step, and three patients were added to the 280 mg/m^2/4 h cohort for safety evaluation. Diarrhoea (grades 1-3), nausea/vomiting (grade 1), and other gastrointestinal symptoms were confirmed as non-SAEs for which a causal relationship could not be ruled out; however, these issues were managed by symptomatic treatment. The 280 mg/m^2/4 h dose was considered safe and tolerable without serious side effects. Safety and pharmacokinetic evaluation from these data resulted in determination of 280 mg/m^2/4 h as the recommended dose by the efficacy and safety evaluation committee in May 2020.

We observed four SAEs among three (one from phase 1 and two from phase 2a) of the 27 patients (Table 2). We concluded that three of the SAEs were unrelated to PRI-724: prolonged hospitalisation due to viral infection (phase 1), anaemia due to liver cirrhosis before PRI-724 treatment, and spontaneous bacterial peritonitis after the end of treatment (phase 2a). One of the four SAEs was possibly related to the PRI-724 (observed in the 380 mg/m^2/4 h cohort). During treatment of all patients, we observed various AEs, including liver dysfunction (15%, 4/27) and gastrointestinal symptoms [diarrhoea (26%, 7/27), nausea (22%, 6/27), vomiting (15%, 4/27), and loss of appetite (22%, 6/27)] (Table 2).

We measured the maximum concentration (C_{max}), area under the concentration curve (AUC) using the last concentration extrapolated based on constant elimination, and time of maximum observed serum (T_{max}) (Table 3). Plasma PRI-724 concentration at the time of a single dose reached the maximum concentration at 1 h to 4 h after initiating administration in all patients (Figure 2a and Supplementary Table 1). Additionally, the T_{max} at levels 1, 2, and 3 (140, 280 and 380 mg/m^2/4 h) were 2·3±1·5 h, 2·2±1·5 h, and 2·0±1·1 h, respectively, the C_{max} was 1150±330 ng/mL, 1710±620 ng/mL, and 3530±800 ng/mL, respectively, and the $AUC_{0\text{-}24\ h}$ was 3740±940 ng·h/mL, 5700±1810 ng·h/mL, and 11,800±2700 ng·h/mL, respectively. At Level 1, we did not calculate the PRI-724 half-life for any of the patients, whereas this was calculated for two of six patients at Level 2, with no mean value obtained. At Level 3, the half-life was calculated for four of six patients, resulting in a mean half-life of 0·474±0·034 h.

Plasma C-82 concentration for a single dose reached the maximum concentration at 2 h or 4 h after initiating administration in all patients (Figure 2b, Supplementary Table 1). The T_{max} at levels 1, 2, and 3 (140, 280, and 380 mg/m^2/4 h) was 3·3±1·2 h, 4·0±0·0 h, and 3·3±1·0 h, respectively, the C_{max} was 1230±250 ng/mL, 2560±690 ng/mL, and 2770±1050 ng/mL, respectively, and the $AUC_{0\text{-}24\ h}$ was 5880±1220 ng·h/mL, 14,400±5100 ng·h/mL, and 14,700±7600 ng·h/mL, respectively (Table 3). There was no significant difference between levels 2 and 3 in these parameters, and we observed no dose-dependent increases in any of the parameters. Moreover, the half-life was calculated at 4·10±0·58 h, 3·85±1·42 h, and 3·35±0·46 h for each level, respectively.

In the phase 2a study, we administered 280 mg/m^2/4 h PRI-724 to 15 patients at three hospitals. Because three of the 15 patients (KOM-017, KOM-018, and KOM-021) were administered the recommended dose of 280 mg/m^2/4 h in phase 1, and a post-treatment liver biopsy was performed with their consent, these three patients were included in the evaluation of the phase 2a results. Of the 15 patients, nine had HCV cirrhosis and six had HBV cirrhosis.

We analysed paired liver biopsy samples obtained from 14 patients (excluding one patient who did not undergo the procedure) before (baseline) and after 12 weeks of PRI-724 administration. To evaluate changes in the reticular fibrotic area (Sirius Red-positive area) in hepatic lobules (the primary endpoint), 10 liver lobule regions were randomly selected using standardised computer-assisted image analysis, and the average value was calculated from the liver fibrosis area ratio at each site. The tissue fibrosis area ratio (mean ± SD [median]) was 0·1472±0·0896 [0·1111]% and 0·1778±0·1141 [0·1600]%, respectively, for a change of 0·0306±0·0854 and with no observed decrease (Table 4). At the same time, the collagen proportionate area of the whole liver tissue was measured, but no statistically significant change was observed between baseline and 12 weeks after PRI-724 administration (Supplementary Figure 1).

We also analysed hepatic necroinflammation and fibrosis, according to the Ishak (modified HAI) scoring system, at 12 weeks after PRI-724 administration in 13

SAEs	Phase 1						Phase 2a (280 mg/m^2/4h)			
	(140 mg/m^2/4h) (*n* = 3)		(280 mg/m^2/4h) (*n* = 6)		(380 mg/m^2/4h) (*n* = 6)		Phase 1/ 2a (*n* = 3)		Phase 2a only (*n* = 12)	
Events	**Grade 1-2**	**Grade 3**	**Grade 1-2**	**Grade 3**	**Grade 1-2**	**Grade 3**	**Grade 1-2**	**Grade 3**	**Grade 1-2**	**Grade 3**
Viral infection	0	0	0	0	0	1 (17%)	0	0	0	0
Spontaneous bacterial peritonitis	0	0	0	0	0	0	0	0	0	1 (8%)
Anaemia	0	0	0	0	0	0	0	0	0	1 (8%)
Hepatic function abnormal	0	0	0	0	0	1 (17%)	0	0	0	0
Common adverse events										
Nasopharyngitis	1 (33%)	0	1 (17%)	0	0	0	0	0	1 (8%)	0
Viral infection	0	0	0	0	0	1 (17%)	0	0	0	0
Spontaneous bacterial peritonitis	0	0	0	0	0	0	0	0	0	1 (8%)
Anaemia	0	0	0	0	0	0	0	0	0	2 (17%)
Hyperuricaemia	0	0	0	0	0	0	0	0	1 (8%)	1 (8%)
Decreased appetite	0	0	2 (33%)	0	1 (17%)	0	1 (33%)	0	3 (25%)	0
Insomnia	0	0	1 (17%)	0	1 (17%)	0	1 (33%)	0	0	0
Headache	0	0	1 (17%)	0	1 (17%)	0	0	0	1 (8%)	0
Hypertension	0	0	1 (17%)	0	0	0	1 (33%)	0	0	1 (8%)
Ascites	0	0	1 (17%)	0	1 (17%)	0	0	0	0	0
Diarrhea	0	0	1 (17%)	1 (17%)	2 (33%)	0	1 (33%)	0	2 (17%)	1 (8%)
Dyspepsia	0	0	1 (17%)	0	0	0	0	0	0	0
Nausea	0	0	2 (33%)	0	2 (33%)	0	1 (33%)	0	2 (17%)	0
Vomiting	0	0	2 (33%)	0	1 (17%)	0	1 (33%)	0	1 (8%)	0
Hepatic function abnormal	1 (33%)	0	1 (17%)	0	0	1 (17%)	0	0	1 (8%)	0
Erythema	0	0	0	0	0	0	0	0	2 (17%)	0
Pruritus	0	0	1 (17%)	0	0	0	0	0	2 (17%)	0
Muscle spasms	0	0	2 (33%)	0	0	0	0	0	0	0
Pyrexia	0	0	0	0	2 (33%)	0	0	0	0	0
Puncture site pain	0	0	1 (17%)	0	0	0	1 (33%)	0	0	0
Laboratory abnormality										
Alanine aminotransferase increased	0	0	0	1 (17%)	0	0	0	0	2 (17%)	1 (8%)
Aspartate aminotransferase increased	0	0	0	1 (17%)	1 (17%)	0	0	0	3 (25%)	0
Blood creatine phosphokinase increased	0	0	0	0	1 (17%)	0	0	0	0	0
Blood creatinine increased	0	0	0	0	0	0	0	0	1 (8%)	0
C-reactive protein increased	0	0	0	0	1 (17%)	0	0	0	0	0

***Table 2*: Serious adverse events, adverse events, and laboratory abnormalities (Phase 1/2a).**

Dose (mg/m^2/4h)		PRI-724 T_{max} (h)	PRI-724 C_{max} (ng/mL)	PRI-724 AUC $_{0-24h}$ (ng•h/mL)	PRI-724 $t_{1/2}$ (h)	C-82 T_{max} (h)	C-82 C_{max} (ng/mL)	C-82 AUC $_{0-24h}$ (ng•h/mL)	C-82 $t_{1/2}$ (h)
140	N	3	3	3	0	3	3	3	3
	Mean	2·3	1150	3740	NC	3·3	1230	5880	4·10
	S.D.	1·5	330	940	NC	1·2	250	1220	0·58
	CV (%)	65·2	28·7	25·1	NC	36·4	20·3	20·7	14·1
	Min	1	888	2790	NC	2	971	4530	3·49
	Max	4	1520	4660	NC	4	1460	6900	4·64
	Median	2·0	1030	3770	NC	4·0	1250	6210	4·17
280	N	6	6	6	2	6	6	6	6
	Mean	2·2	1710	5700	NC	4·0	2560	14400	3·85
	S.D.	1·5	620	1810	NC	0·0	690	5100	1·42
	CV (%)	68·2	36·3	31·8	NC	0·0	27·0	35·4	36·9
	Min	1	757	2650	0·444	4	1570	7860	2·78
	Max	4	2590	7500	0·475	4	3330	19400	6·58
	Median	1·5	1780	5960	0·460	4·0	2840	16600	3·46
380	N	6	6	6	4	6	6	6	6
	Mean	2·0	3530	11800	0·474	3·3	2770	14700	3·35
	S.D.	1·1	800	2700	0·034	1·0	1050	7600	0·46
	CV (%)	55·0	22·7	22·9	7·2	30·3	37·9	51·7	13·7
	Min	1	2590	7940	0·435	2	1300	5820	2·87
	Max	4	4730	16000	0·518	4	4010	25700	4·08
	Median	2·0	3540	11900	0·472	4·0	2740	12700	3·41

***Table 3*: Pharmacokinetic parameters for PRI-724 and C-82 after PRI-724 infusion (Phase 1). The data is shown as mean and SD. NC: Not calculated.**

of the 15 patients (excluding 1 patient who we were unable to evaluate due to poor sample quality and 1 who discontinued treatment). We observed improvements in Ishak fibrosis scores of ≥1 from baseline in three patients (stage 2 improvement: 2 patients; and stage 1 improvement: 1 patient); however, the Ishak fibrosis score was unchanged in six patients, and we confirmed one or more signs of deterioration in four patients (stage 1 deterioration: 3 patients; stage 2 deterioration: 1 patient) (Table 4c and Supplementary Table 2).

Measurement of the HAI score associated with Knodell necroinflammatory score revealed one or more improvements from baseline in four patients (score 2 improvement: 2 patients; and score 1 improvement: 2 patient), five patients remained unchanged, and one or more deteriorations in four patients (score 1 deterioration: 2 patients; and score 2 deterioration: 2 patients). No change was observed in the mean activity score after 12-week PRI-724 administration (Table 4c and Supplementary Table 2).

We also examined changes in CP and MELD scores as secondary endpoints in order to evaluate whether PRI-724 improved liver function. Of the seven patients with CP class B at baseline, two improved to class A, four remained unchanged, and one worsened at 12 weeks after PRI-724 administration. There was no change in mean CP score at 12 weeks after PRI-724 administration. However, in 14 patients (excluding 1 that discontinued treatment), we detected a change of −0·9±1·4 in the MELD score at 12 weeks after PRI-724 administration ($P < 0.05$) (Table 5). Additionally, serum ALB and TBIL levels in 14 patients improved from baseline at some time after PRI-724 administration (Figure 3a).

We observed a change of −4·90±6·51 kPa in LSM at 12 weeks after PRI-724 administration for 13 of 15 patients ($P<0.05$) (Figure 3b, Table 5 and Supplementary Table 3). In particular, we observed a notable decrease in LSM decreased notably in patients with advanced liver stiffness of ≥20 kPa at baseline (Figure 3b). Furthermore, LSM in four of six patients with CP class B decreased by ≥10% relative to baseline.

As exploratory endpoints used to examine efficacy, we measured changes in serum levels of four fibrosis markers [hyaluronic acid, type IV collagen type S, procollagen III peptide, and MAC-2-binding protein glycosylation isomer (M2BPGi)] before and 12 weeks after PRI-724 administration. We observed no difference in mean levels of hyaluronic acid and type IV collagen type S at 12 weeks, whereas those of procollagen III peptide and M2BPGi were lower relative to baseline levels but not statistically significant (Supplementary Figure 2).

We used the FIB-4 index as a post-hoc analysis and observed a statistically significant decrease before and after administration (Supplementary Figure 3).

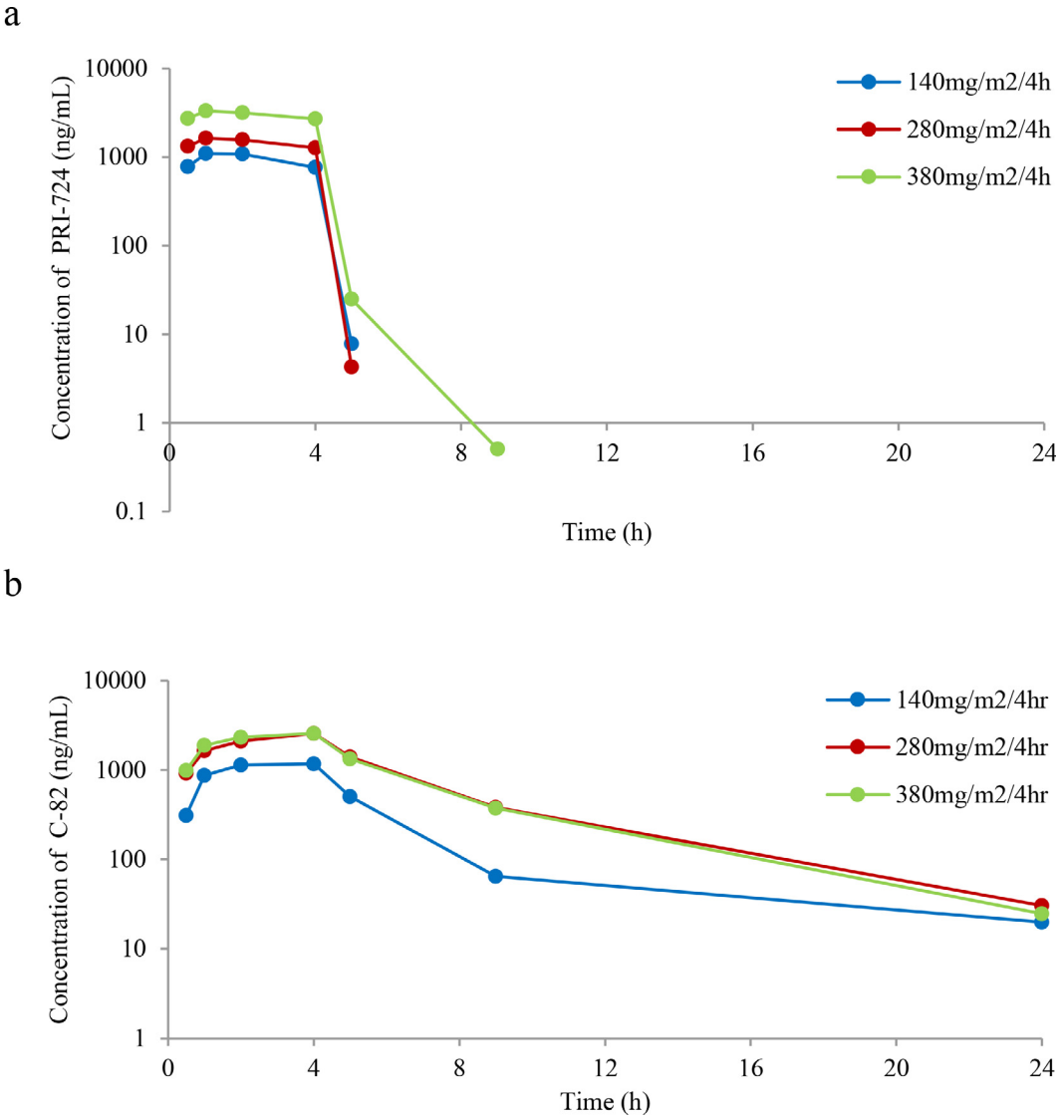

Figure 2. Pharmacokinetics of PRI-724 and C-82 after intravenous infusion of PRI-724 (Phase 1). (a) Mean concentration profiles of PRI-724 in plasma from patients with HCV and HBV liver cirrhosis during intravenous infusion of PRI-724. (b) Mean plasma concentration profiles of C-82, a PRI-724 metabolite, in the same patients.

Discussion

In this study, we evaluated the safety and tolerability of intravenous administration of PRI-724 twice weekly at 4 h intervals to patients with HCV and HBV cirrhosis in a phase 1 study, with 280 mg/m^2/4 h subsequently determined as the recommended dose.

Throughout both trials, the most common AEs and side effects were gastrointestinal symptoms and liver dysfunction events, and two SAEs (viral infection and liver dysfunction) were reported in one Level 3 (380 mg/m^2/4 h) patient during phase 1. Of these SAEs, viral infection was denied a causal relationship with PRI-724, whereas this was not ruled out in the case of liver dysfunction. During phase 2a, spontaneous bacterial peritonitis and anaemia were reported as SAEs in one patient each, with a causal relationship with PRI-724 ruled out in both cases. There were severe cases of liver dysfunction at Level 3 in phase 1; however, liver dysfunction at the recommended dose (Level 2; 280 mg/m^2/4 h) was minor and transient. These results suggest that testing of liver function should be performed before and during treatment, especially in patients with advanced cirrhosis (e.g., CP class B). We considered that the observed cases of liver dysfunction were AEs manageable with careful monitoring.

In the phase 2a study, we administered the recommended dose (280 mg/m^2/4 h) to 15 patients, with efficacy examined in 14 patients. We observed no

Ratio of fibrosis area in liver tissue	Baseline	12 weeks
Number of patients	14	14
Mean ± standard deviation	0·14724554±0·08963210	0·17782102±0·11408882
Median	0·11109065	0·15996835
minimum value	0·0459118	0·0082302
maximum value	0·3564587	0·4782028
Number of patients (change amount)	-	14
Mean ± standard deviation (change amount)	-	0·03057548±0·08544759

Amount of change from baseline*		95% confidence interval		One sample t-test		
Estimated value	Standard error	lower limit	upper limit			
0·03057548	0·02283683	-0·0187605	0·0799114	$p = 0·2036$		
Change in score from baseline		-2	-1	0	1	2
280mg/m^2/4 h	Fibrosis score	2	1	6	3	1
	Necroinflammatory score	2	2	5	2	2

***Table 4*: Histological analysis following PRI-724 treatment (Phase 2a). Changes in (a, b) fibrosis area in hepatic lobules and (c) fibrosis score from baseline to post-treatment after 12 weeks of PRI-724 administration. Necroinflammatory score from baseline to post-treatment after 12 weeks of PRI-724 administration. An independent pathologist blindly selected 10 Sirius Red-stained parenchyma spots in all biopsy samples and automatically calculated the Sirius Red-positive areas using the HistoQuant software. The average of the measured collagen-positive areas at baseline was compared with that at 12 weeks after PRI-724 treatment. Statistical analyses were performed using paired *t*-tests.**

MELD score	Baseline	12 weeks	Paired t-test
Number of patients	15	14	$p = 0·0336$
Mean ± standard deviation	7·7±3·6	7·1±3·3	
Child-Pugh score	Baseline	12 weeks	Paired t-test
Number of patients	15	14	$p = 0·7522$
Mean ± standard deviation	6·4±1·5	6·4±1·7	
Fibroscan (kPa)	Baseline	12 weeks	Paired t-test
Number of patients	13	13	$p = 0·0188$
Mean ± standard deviation	21·45±8·35	16·55±4·47	
Fibroscan (kPa) 20kPa >	Baseline	12 weeks	Paired t-test
Number of patients	8	8	$p = 0·00662$
Mean ± standard deviation	26·78±5·61	18·48±4·55	

***Table 5*: Change in MELD score, CP score, and liver stiffness measure from baseline to post-treatment. Changes in the MELD and CP scores from baseline to post-treatment following 12 weeks of PRI-724 administration (*n* = 14). Change in LSM from baseline to post-treatment following 12 weeks of PRI-724 administration (*n* = 13). The graph (right) shows the results of analysing patients with liver cirrhosis and a baseline LSM of ≥ 20 kPa. Statistical analyses were performed using paired *t*-tests.**

statistically significant changes from baseline at the end of 12 weeks in the primary endpoint or the hepatic lobule fibrosis area ratio. We speculate that the state of fibrosis in the patients in this study differed from that in a previous study (PRI-724-1101) evaluating patients without persistent liver damage due to SVR (HCV) or administration of antiviral drugs (HBV). In fact, the modified HAI scores in the present study showed that intralobular inflammation and portal vein inflammation (stage A) were mostly A0-2 (Supplementary Table 2), whereas most cases in the previous study (PRI-724-1101) were A2-4. One explanation is that all of the patients in the previous study were HCV-positive. Moreover, analysis of liver biopsy data in the present study showed a large variation in the fibrosis area value among patients at the measurement site in the hepatic lobule, making it difficult to compare changes from before and after administration. Although not analysed in this study, immunohistochemical staining of α-SMA in liver tissue has been reported as a method for evaluating fibrosis.[26,27] In the next phase of randomised placebo-controlled studies, we intend to include analysis using α-SMA staining in liver tissue.

Regarding the secondary endpoint, we detected a statistically significant decrease in LSM between baseline and at 12 weeks after PRI-724 administration. Previous

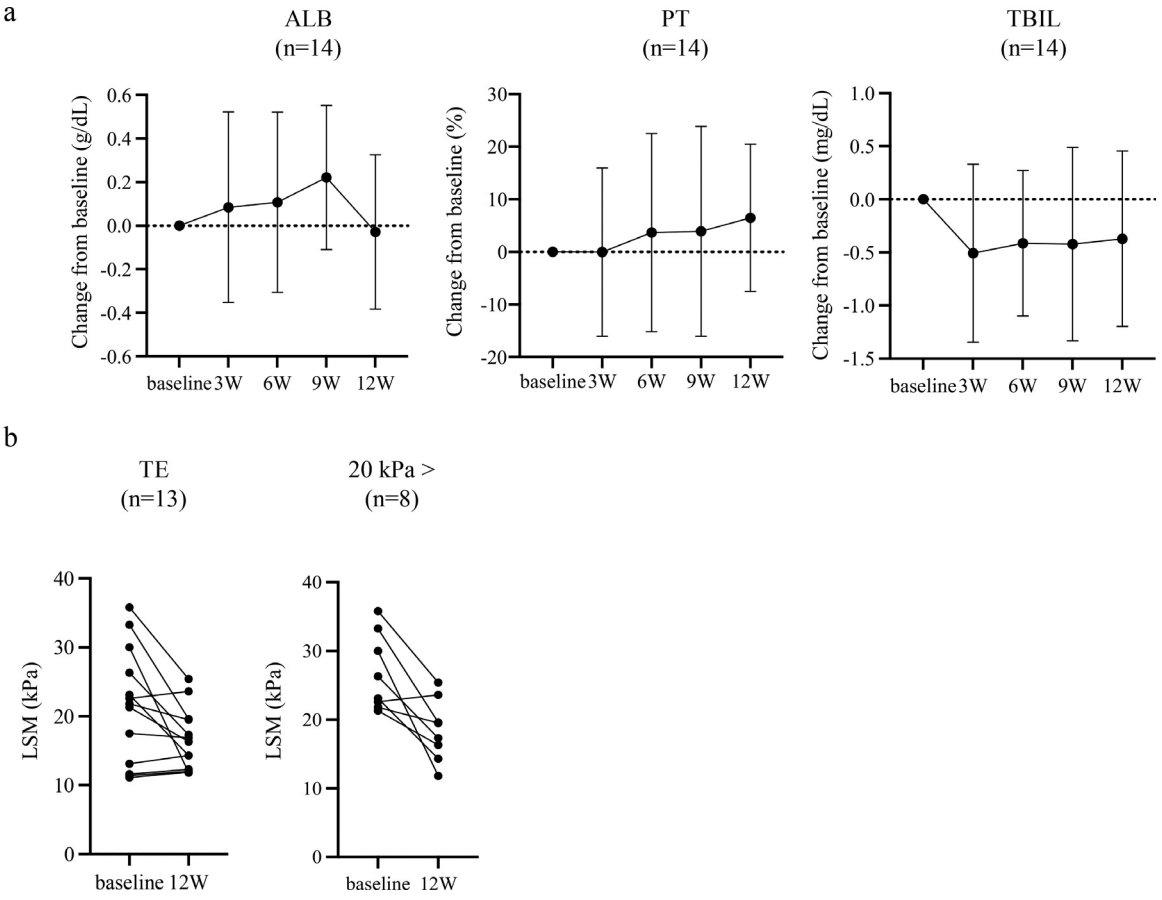

Figure 3. Effects of PRI-724 treatment on liver function and liver stiffness measure (Phase 2a). Changes in (a) serum ALB, PT, and TBIL before, during, and after 12 weeks of PRI-724 administration. (b) Change in LSM from baseline to post-treatment following 12 weeks of PRI-724 administration (n = 13). The graph (right) shows the results of analysing patients with liver cirrhosis and a baseline LSM of ≥ 20 kPa. Statistical analyses were performed using paired t-tests.

reports noted the usefulness of this method for evaluating the degree of fibrosis progression using FibroScan.[28] Liver biopsy has been considered the gold standard for evaluating liver fibrosis; however, the invasiveness of the technique and issues related to sampling errors relegate its use primarily for diagnostic purposes rather than an evaluation of fibrosis.[1,6,7] Additionally, other methods, including those involving a blood test and transient elastography, are currently available for such non-invasive evaluations.[1]

LSM obtained using FibroScan is a simple and widely used method; however, limitations have been identified. In particular, it may be difficult to measure the accuracy of numerical values in cases of hepatitis or cholestasis, patients with a narrow intercostal wall, patients with a thick abdominal wall, or areas with accumulated ascites.[29] However, numerous reports have identified a correlation between fibrosis and liver stiffness, suggesting the utility of LSM for this evaluation. Moreover, LSM in patients with HCV cirrhosis or fibrosis decreases after SVR.[30] In the present study, most patients (94%, 16/17) with HCV cirrhosis were post-SVR patients; however, most treated patients with HCV cirrhosis are more than a year old from SVR, and reports suggest that these patients show a decrease of <1 kPa annually.[6] We consider that the rate of LSM decrease observed in the present study (−4·90 ± 6·51 over 12 weeks) indicated the possibility of an anti-fibrotic effect. Recently, de Franchis *et al.* reported a clinically significant decrease in LSM, which is associated with a substantially reduced risk of decompensation and liver-related mortality. This is defined as a ≥20% decrease in LSM associated with LSM <20 kPa or any decrease in LSM <10 kPa.[31] By applying this definition to our study, 54% (7/13) was judged to be a clinically significant decrease that warrants further investigation.

Regarding the possible use of blood tests for evaluating fibrosis, measurement of changes in serum levels of hyaluronic acid, type IV collagen type S, procollagen III peptide, and M2BPGi as exploratory endpoints revealed no statistically significant decreases in hyaluronic acid

or type IV collagen type S from baseline to 12 weeks after PRI-724 administration, although decreases were observed in procollagen III peptide and M2BPGi levels (Supplementary Figure 2). Recently, the usefulness of the Fibrosis-4, aspartate aminotransferase to platelet ratio index, and enhanced liver fibrosis scores has been reported.[32] Although these scores were not included in the analysis outlined in the protocol for this study, post-hoc analysis revealed statistically significant decreases in the scores (Supplementary Figure 3). Thus, although we could not demonstrate the anti-fibrotic effect of PRI-724 using liver tissue, the results of LSM and blood-based fibrosis markers suggested its potential therapeutic efficacy for treating liver cirrhosis.

Additionally, we identified a statistically significant decrease in MELD score following PRI-724 administration and relative to the baseline score, with this likely associated with improvements in protein-synthesis activities (according to ALB, PT, and TBIL levels).

It is difficult to determine whether the Delta MELD score of -0·9 is meaningful, but it was reported that when DAA was administered to patients with decompensated cirrhosis, it decreased by -0·85 after 12 weeks.[33] This finding is clinically significant because the administration of PRI-724 to patients with liver cirrhosis showed the same improvement as the effect of DAA administration.

Moreover, two of seven patients with CP class B at baseline changed to CP class A following treatment, suggesting that PRI-724 might be effective, even at a limited treatment period of 12 weeks.

Although we observed preliminary therapeutic effects, there remain differences in the liver biopsy results following PRI-724 treatment between the present study and those reported previously (PRI-724-1101).[23] A possible explanation is that the previous study performed continuous administration for 1 week and then intermittently for another 12 weeks. Intravenous administration of PRI-724 results in its rapid metabolism to C-82 and disappearance within minutes.[23] Because a preliminary therapeutic effect was reported at 40 mg/m^2/day, in the present study, we administered the same dose (140 mg/m^2/4 h). Although we expected the same therapeutic effect, the results for the fibrotic area were insufficient. This suggests that the effect of PRI-724 might require maintenance of constant PRI-724 levels in the blood. Therefore, extending the administration period in order to obtain a clinically significant anti-fibrotic effect should be considered.

This study has some limitations. First, the sample size used for the analyses was small. This study did not include a placebo control group; therefore, the accuracy of the therapeutic effect remains questionable. In the next phase of trials, these points will be addressed, and a company-led clinical trial will be planned.

In conclusion, we found that PRI-724 showed potential therapeutic efficacy in patients with liver cirrhosis by evaluating LSM as well as endpoints of liver function, such as the MELD and CP scores. However, we were not able to confirm the effect of PRI-724 in reducing liver tissue fibrosis. The development of anti-fibrotic drugs for treating liver cirrhosis is a highly competitive field owing to the high mortality rate of alcoholic liver cirrhosis among production workers and the expected increase in cases of liver failure and HCC due to increases in the number of NASH patients.[4] To date, bile acid metabolism, galectin-3, and fibroblast growth factor-21 have attracted attention as targets for therapeutic agents to improve fibrosis treatment.[7,34,35] This study aimed to evaluate the anti-fibrotic effect of PRI-724 as a small-molecule inhibitor of CBP/β-catenin signalling. We reported evidence of the anti-fibrotic effect of PRI-724, which highlights the importance of future studies in further evaluating the efficacy of the drug. Based on the results of this clinical trial, we are planning a placebo-controlled, randomised trial to verify the efficacy of PRI-724 for the next phase of HCV/HBV cirrhosis.

Declaration of interests

There are no conflicts of interest.

Contributors

KK was the coordinating principal investigator and led the clinical conduct at the Komagome Hospital, TK was the principal investigator at Kohnodai Hospital, SS was the principal investigator at Kyushu University Hospital. JI, MK and KN were study sub-investigators at Komagome Hospital, and EO at Kyushu University Hospital. KK consider concept of clinical trial, acquired funding, interpret the clinical data and wrote an original draft. TK, SS, EO, II, JK, KT, and TK collected, analysed, and interpreted the data. TK, SS, and EO review and edit the manuscript. MK, KN, and JI investigated and interpreted clinical data. KH, MS, and YI analysed and interpreted the histological data. TO, KI, and TI interpreted the clinical data and organised the safety of the study. JK led the work for the statistical analysis. II contributed to PK analysis. All authors read and approved the final version of the manuscript.

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Data sharing statement

Anonymised data presented in the manuscript or acquired during the course of the clinical trial will be made available upon request to the corresponding author following the publication of the article. The data will be made available in a form that does not deviate from what is accepted by local regulatory authorities with respect to the handling of patient data and that is adherent with the policies of the Tokyo Metropolitan Komagome Hospital.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104069.

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