No apparent trend was noted in relation to SVR12 rates for the selected demographic and baseline characteristics. Analysis of SVR12 rates according to *IL28B* genotype showed no consistent trend (Table 3).

A total of 57 patients (SMV12/PR24/48, $n = 26$; SMV24/PR24/48, $n = 31$) were described as 'failures' (defined as having viral breakthrough or relapse, meeting virologic stopping criteria, and/or having detectable HCV RNA at end of treatment), and 56 of these patients had available NS3 sequence information (unavailable for 1 patient, SMV12/PR24/48 group). Of these patients, 46/56 (82.1 %) had at least one emerging NS3 mutation (positions 36, 43, 54, 80, 122, 138, 155, 156, 168, or 170) at the time of failure despite the absence of baseline polymorphisms. No apparent difference in the rate of emerging NS3 mutations was observed among patients with failure in the SMV12/PR24/48 versus the SMV24/PR24/48 treatment groups (80.0 % and 83.9 %, respectively).

Alanine aminotransferase The proportions of patients whose ALT levels were abnormal at baseline and within normal range ($\leq$40 IU/mL) at the end of treatment were 58.8 % in the SMV12/PR group and 80.0 % in the SMV24/PR group.

CONCERTO-3 (prior relapsers following IFN-based therapy)

Virologic response HCV RNA levels declined rapidly during the first 2 weeks of treatment with simeprevir and were below the LLOQ in all patients for the remainder of the 12-week simeprevir treatment period (Fig. 1). The RVR and cEVR rates were 81.6 % (40/49) and 100 % (48/48), respectively. The rate of SVR12 (primary endpoint) was 95.9 % (47/49), which was higher ($p < 0.0001$) than the 50 % proportion specified in the null hypotheses (Table 2).

The vast majority (95.9 %; 47/49) of patients met the RGT criteria and completed treatment with peginterferon α-2a and RBV at week 24. Of these patients, 95.7 % (45) achieved SVR12.

The rate of SVR24 (95 % CI) was 89.8 % (77.77–96.60) (Table 2).

No apparent trend in SVR12 was noted for selected demographic and baseline characteristics; regardless of gender, age, HCV genotype, prior HCV treatment, or *IL28B* genotype, the SVR12 rate was >85 % for all patient subgroups (Table 3).

Viral breakthrough, viral relapse, or treatment failure There were no viral breakthroughs. Viral relapse was observed in 8.2 % (4/49) of patients: two patients at week 4 and two patients at week 24 of follow-up. No patient met the virologic stopping rule.

Two patients (4.1 %) who did not achieve SVR12 had detectable HCV RNA after 12 weeks of treatment with simeprevir. None of the patients had detectable HCV RNA at the end of treatment or missing data at the SVR12 timepoint.

Alanine aminotransferase The proportion of patients whose ALT level was abnormal at baseline and within normal limits ($\leq$40 IU/mL) at the end of treatment was 73.3 % (11 of 15).

Viral population sequencing Most patients enrolled in Japanese studies were infected with HCV genotype 1b, and only four were infected with genotype 1a (three patients in CONCERTO-2, and one patient in CONCERTO-3).

In CONCERTO-2, there were no notable differences in the position and proportion of baseline polymorphisms between the two treatment groups. The highest prevalence of baseline HCV polymorphisms at NS3 positions was observed at position 170 (39.6 %), followed by 122 (34.9 %), and 80 (11.3 %). Of the 59 simeprevir-treated patients in CONCERTO-2 with treatment failure for any reason and with NS3 sequence information available, 54 (91.5 %) had emerging mutations at positions 80, 122, 155, and/or 168. These mutations were associated with reduced simeprevir activity in vitro at the time of treatment failure. Among these, an emerging D168 V mutation (alone or in combination with other mutations) was the most commonly observed mutation (Supplementary Figure 3). In CONCERTO-3, a higher prevalence of baseline HCV polymorphisms at NS3 positions was observed for polymorphisms at 170 (40.8 %), 122 (36.7 %), and 80 (14.3 %). Three of four patients with viral relapse and without baseline polymorphisms had an emerging D168V mutation at the time of relapse.

Safety

No deaths were reported in either study. AEs are summarized in Table 4. Overall, the incidence of the most common AEs (i.e. occurring in >10 % of patients) was similar between the two studies. In CONCERTO-2, nasopharyngitis and injection-site reaction were considerably more common (>10 %) in the SMV24/PR group compared with the SMV12/PR group (30.2 % vs 18.9 % and 11.3 % vs 22.6 %, respectively). The majority of AEs were grade 1 or 2 in severity. Grade 3/4 AEs were reported for 26.4 % of patients in CONCERTO-2 and 34.7 % of patients in CONCERTO-3. Grade 4 AEs occurred in 6.6 % (7) of patients in CONCERTO-2 and 2.0 % (1) of patients in CONCERTO-3. These AEs were decreased neutrophil count (7 patients) and neutropenia (1 patient); none were classed as serious or led to discontinuation of any study

Table 4 Adverse events occurring in ≥25 % of patients in either study

n (%)	Entire treatment period			Simeprevir/placebo + PR period		
	CONCERTO-2 (N = 106)		CONCERTO-3 (N = 49)	CONCERTO-2 (N = 106)		CONCERTO-3 (N = 49)
	SMV12 + PR24/48 (n = 53)	SMV24 + PR24/48 (n = 53)	SMV12 + PR24/48	SMV12 + PR24/48 (n = 53)	SMV24 + PR24/48 (n = 53)	SMV12 + PR24/48
Any AE	53 (100.0)	52 (98.1)	49 (100)	53 (100.0)	52 (98.1)	49 (100)
Grade 3/4 AE	11 (20.8)	17 (32.1)	17 (34.7)	10 (18.9)	17 (32.1)	11 (22.4)
SAE	2 (3.8)	3 (5.7)	6 (12.2)	0 (0.0)	3 (5.7)	1 (2.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Treatment discontinuation due to AE						
Simeprevir only	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)	1 (2.0)
All treatment[a]	2 (3.8)	2 (3.8)	2 (4.1)	1 (1.9)	2 (3.8)	1 (2.0)
Common AEs[b]						
Pyrexia	33 (62.3)	31 (58.5)	36 (73.5)	32 (60.4)	31 (58.5)	36 (73.5)
White blood cell decreased	33 (62.3)	31 (58.5)	30 (61.2)	30 (56.6)	31 (58.5)	30 (61.2)
Anemia	28 (52.8)	31 (58.5)	22 (44.9)	26 (49.1)	31 (58.5)	20 (40.8)
Neutrophil count decreased	28 (52.8)	28 (52.8)	30 (61.2)	26 (49.1)	28 (52.8)	29 (59.2)
Malaise	30 (56.6)	24 (45.3)	23 (46.9)	30 (56.6)	23 (43.4)	22 (44.9)
Platelet count decreased	27 (50.9)	21 (39.6)	22 (44.9)	26 (49.1)	21 (39.6)	20 (40.8)
Headache	23 (43.4)	23 (43.4)	25 (51.0)	20 (37.7)	23 (43.4)	25 (51.0)
Rash	20 (37.7)	23 (43.4)	16 (32.7)	17 (32.1)	23 (43.4)	14 (28.6)
Alopecia	21 (39.6)	15 (28.3)	17 (34.7)	4 (7.5)	15 (28.3)	5 (10.2)
Pruritus	16 (30.2)	12 (22.6)	19 (38.8)	15 (28.3)	12 (22.6)	16 (32.7)
Decreased appetite	12 (22.6)	15 (28.3)	14 (28.6)	11 (20.8)	15 (28.3)	13 (26.5)
Arthralgia	13 (24.5)	13 (24.5)	20 (40.8)	13 (24.5)	13 (24.5)	19 (38.8)
Hemoglobin decreased	13 (24.5)	12 (22.6)	20 (40.8)	9 (17.0)	11 (20.8)	15 (30.6)
Myalgia	7 (13.2)	6 (11.3)	13 (26.5)	7 (13.2)	6 (11.3)	13 (26.5)
Hematocrit decreased	8 (15.1)	8 (15.1)	13 (26.5)	5 (9.4)	7 (13.2)	10 (20.4)
Nasopharyngitis	10 (18.9)	16 (30.2)	5 (10.2)	8 (15.1)	12 (22.6)	3 (6.1)

AE adverse event, *PR* pegylated interferon α-2a and ribavirin, *SAE* serious adverse event, *SMV* simeprevir

[a] Patients discontinued PR treatment regardless of completion or discontinuation of simeprevir

[b] Common AEs were classified as those occurring in >25 % of patients in the simeprevir group in either study

medications. Six serious AEs (SAEs) were reported in five patients in CONCERTO-2 (erythema multiforme, hypoesthesia, anemia, laceration, pyelonephritis acute, and calculus ureteric). Seven SAEs were reported in six patients in CONCERTO-3 (malaise and nausea, pneumonia, cerebral hemorrhage, appendicitis, herpes zoster, and breast cancer female). No individual SAE was reported in more than one patient.

In CONCERTO-2, no AEs resulted in permanent discontinuation of simeprevir. In CONCERTO-3, one patient experienced an AE of abnormal hepatic function, which led to discontinuation of simeprevir. This patient continued to receive treatment with PR and subsequently experienced two SAEs (nausea and malaise), resulting in discontinuation of PR. In CONCERTO-2, three patients permanently discontinued all treatment because of AEs. These included anemia, interstitial lung disease, and erythema multiforme. One patient permanently discontinued PR during the PR-only period due to erythema multiforme. One further patient in CONCERTO-3 discontinued PR during the PR-only period due to a breast cancer-related SAE.

Rash (of any type) was reported in 43.4 % (SMV12/PR) and 49.1 % (SMV24/PR) of patients in CONCERTO-2 and 49.0 % of patients in CONCERTO-3. Erythema was reported in 3.8 %–6.1 % of patients across the two studies. The majority of rashes were of grade 1/2 severity. Only one grade 3 rash was reported for two patients in CONCERTO-2. Grade 3 rash was not reported in any patient in CONCERTO-3, and no grade 4 rash was reported in either study.

In both CONCERTO-2 and CONCERTO-3, median total bilirubin levels increased from baseline during the
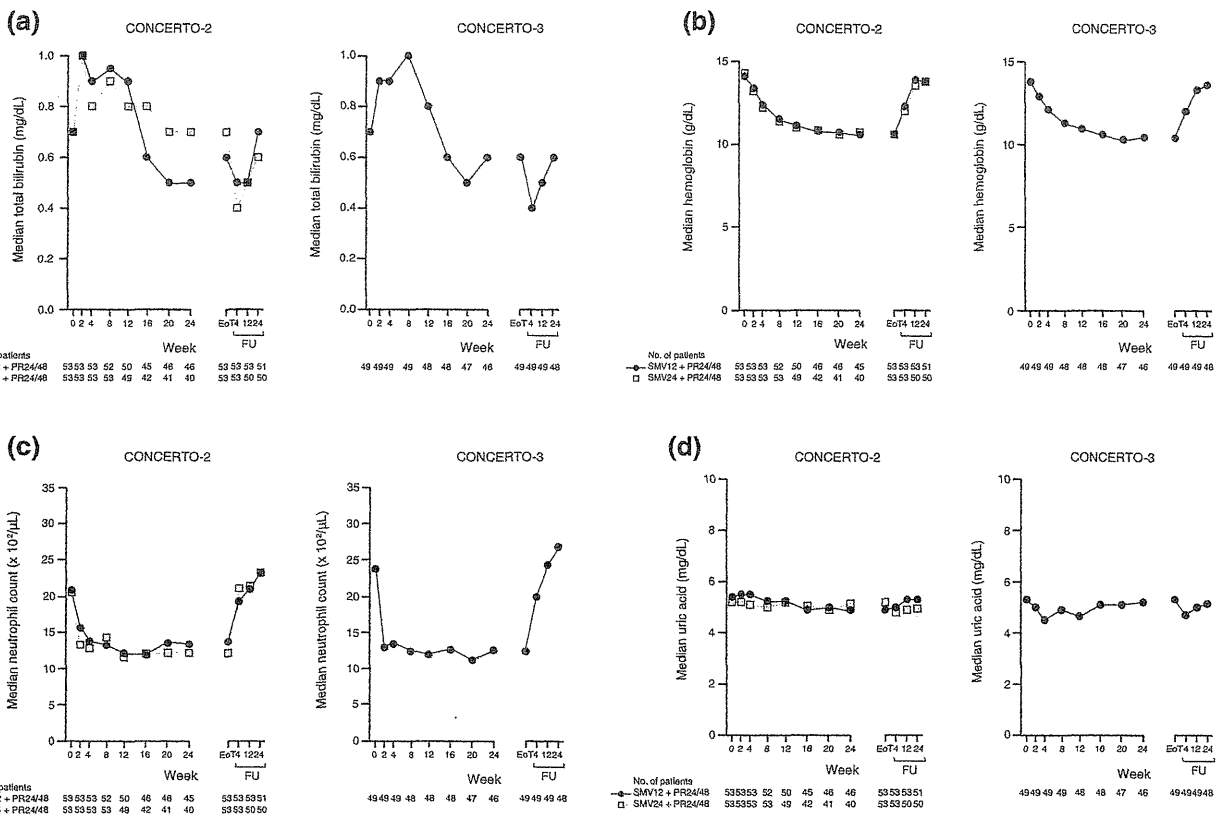
Fig. 2 Median plasma levels of a total bilirubin, b hemoglobin, c neutrophil counts, and d uric acid over the duration of follow-up. In CONCERTO-2 only one patient in each SMV treatment group continued treatment with PR from week 24 onwards, so these data are not shown. *EoT* end of treatment, *FU* follow-up, *PR* peginterferon-α-2a/ribavirin, *SMV* simeprevir

first 2 weeks and then gradually decreased to near baseline levels from week 4 to the end of treatment (Fig. 2). Bilirubin elevations were transient, rapidly returning to baseline or below after the end of simeprevir treatment, and were not associated with increases in ALT or AST.

Overall changes in platelets, neutrophils, and hemoglobin were similar between the two studies, with all of these parameters returning to baseline levels by the end of the post-treatment follow-up period (Fig. 2, Supplementary Figure 4). In CONCERTO-2, one patient discontinued simeprevir and PR due to grade 3 anemia (hemoglobin 7.5 g/dL).

No clinically relevant changes or consistent trends in the other laboratory parameters (including serum creatinine and uric acid) were reported (Fig. 2, Supplementary Figure 4).

Discussion

CONCERTO-2 and CONCERTO-3 evaluated the efficacy and safety of simeprevir as part of a treatment regimen including PR in treatment-experienced patients with genotype 1 and high HCV RNA levels in Japan. The vast majority of study participants were infected with HCV genotype 1b, reflecting the high prevalence of this subtype in Japan [2], and most had received previous treatment with PR. CONCERTO-2 and CONCERTO-3 enrolled two distinct cohorts: patients who had failed to respond to previous IFN-based therapy (CONCERTO-2) and patients who had relapsed after previous IFN-based therapy (CONCERTO-3). This allowed for an independent estimate of virologic response according to prior response to previous IFN-based therapy.

Our results are consistent with previous trials, conducted mainly in Europe and the U.S., showing high virologic response rates with simeprevir in treatment-experienced patients with HCV genotype 1 infection [23, 24]. We reported SVR12 rates of 52.8 % (SMV12/PR) and 35.8 % (SMV24/PR) in prior non-responders and 95.9 % (SMV12/PR) in prior relapsers. These results were statistically significant ($p \le 0.0001$) for each study compared with the null hypothesis on the basis of the published clinical results after re-treatment with PR therapy (prior relapsers, 50 %; prior non-responders, 14 %).

CONCERTO-2 was not powered for statistical comparisons between the SMV12/PR and SMV24/PR

treatment groups. Consequently, the numerically less favorable SVR12 rates and viral relapse rates for patients in the SMV24/PR treatment group (compared to SMV12/PR) should be interpreted with caution. This study was not able to determine whether there is an additional efficacy benefit by prolonging simeprevir therapy beyond 12 weeks.

A limitation of CONCERTO-2 is that prior partial-response versus null-response status to previous IFN-based therapy was not clearly documented; therefore, randomization was not stratified based on these criteria (and may not have been balanced). Any such imbalance could have contributed to the numerical differences in SVR12 rates between the two groups, as it has previously been reported that SVR rates are lower among null responders versus partial responders [25]. In addition, it is possible that the differences in the proportion of patients who discontinued combination therapy with SMV/PR by week 24 contributed to the numerical difference in SVR12 rates between the treatment groups.

Given that patients enrolled in CONCERTO-3 had previously responded to IFN-based therapy before experiencing a relapse (compared to patients in CONCERTO-2, who had failed to respond to IFN-based therapy), the considerably higher SVR12 rate in CONCERTO-3 versus CONCERTO-2 was not unexpected.

SVR12 was the primary efficacy endpoint for both studies. SVR24 is a widely used virologic parameter for evaluating the efficacy of antiviral agents for the treatment of patients with HCV infection. For PR therapy it is well documented in the literature that the majority of viral relapses occur within the first 12 weeks of stopping therapy and that viral relapse rarely occurs between 12 and 24 weeks of follow-up [26]. Furthermore, a strong correlation between SVR12 and SVR24 rates has been demonstrated in completed Phase II/III Japanese [20] and European/U.S. studies of simeprevir [23, 27]. Therefore, the SVR12 rate was considered appropriate as a primary endpoint for both studies.

Both the CONCERTO-2 and CONCERTO-3 studies evaluated an RGT strategy allowing for individualized treatment duration based on HCV RNA levels at week 4 and week 12. According to the RGT criteria, the vast majority of patients in both studies were eligible to shorten their PR treatment duration to just 24 weeks instead of the standard 48 weeks. Among these patients, SVR12 rates of 48.7 % and 60.5 % in prior non-responders and 95.7 % in prior relapsers were observed. These findings suggest that RGT is a valid approach for Japanese patients with HCV infection, providing the opportunity for shortened duration of PR therapy, which may ultimately translate into a shorter duration of AEs [28].

The results of the subgroup analysis by *IL28B* genotype demonstrated that there were no clinically relevant differences in efficacy according to genotype, although the number of patients in each subgroup was small. Consistent with our observation, however, another study has demonstrated that the *IL28B* genotype in IFN-based treatment-experienced patients (both prior relapsers and non-responders) receiving treatment with telaprevir/PR did not predict SVR rates [29].

The number of prior non-responders to IFN-based therapy who experienced viral breakthrough was low (12.3 %), and no prior relapsers experienced viral breakthrough. Rates of viral relapse were 44.9 % and 8.2 %, respectively. The majority of patients with viral breakthrough or relapse had emerging mutations in the HCV NS3 protease domain. These mutations were mostly D168V (alone, or in combination with other mutations) and consistent with earlier reports of mutations associated with reduced susceptibility to simeprevir in vitro [30]. Further investigation of the relevance of HCV NS3 protease domain polymorphisms is required in Japanese patients with HCV, most of whom are infected with HCV genotype 1b. Of note, mutations in the NS3 HCV protease domain at position 168 (mainly D168V) do not confer resistance to the first-generation protease inhibitor telaprevir, but are associated with resistance to second-generation agents such as vaniprevir and faldaprevir [31].

Simeprevir was generally well tolerated in both prior non-responders and prior relapsers. There was no noticeable difference in the incidence or profile of AEs, or discontinuations due to AEs between the two studies. Anemia and rash-related AEs have been documented as common, and sometimes severe, AEs associated with triple therapy regimens that include the first-generation PIs boceprevir and telaprevir [14–19]. Although rash and anemia AEs were relatively common in CONCERTO-2 and CONCERTO-3, the incidences of SAEs or grade 3/4 rash or anemia were low, as were the rates of treatment discontinuations due to these AEs. In both patient populations, mild hyperbilirubinemia was observed before week 4 during the SMV/PR treatment period, but bilirubin levels generally decreased after the first 2 weeks of treatment, returned to near baseline levels after completion of treatment, and were not associated with a concomitant increase in serum transaminases. In vitro data suggest that the observed increase in bilirubin levels during simeprevir therapy may be attributable to simeprevir-mediated inhibition of the transporters OATP1B1 and MRP2, which are involved in bilirubin clearance [32].

In conclusion, in treatment-experienced patients with HCV genotype 1 infection—including patients who failed to respond to, or had prior relapse on, previous IFN-based therapy—re-treatment with 12 weeks of oral simeprevir QD in combination with PR achieves high SVR rates, shortens the overall duration of treatment in

the majority of patients, and is well tolerated. These findings represent an important advance in the management of treatment-experienced patients with HCV genotype 1 infection.

References

1. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect. 2011;17:107–15.
2. Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. Intervirology. 2010;53:39–43.
3. Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol. 2009;44(Suppl 19):102–7.
4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol. 2011;55:245–64.
5. Izumi N. Diagnostic and treatment algorithm of the Japanese society of hepatology: a consensus-based practice guideline. Oncology. 2010;78(Suppl 1):78–86.
6. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347:975–82.

7. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, 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.
8. Iino S, Okita K, Omata M, Kumada H, Hayashi N, Tanigawa H. Clinical efficacy of PEG-interferon alpha-2b and ribavirin combination therapy for 48 weeks in chronic hepatitis C patients with genotype 1 and high viral load: retrospective comparison with interferon alpha-2b and ribavirin combination therapy for 24 weeks. Kantansui. 2004;49:1099–121.
9. Kuboki M, Iino S, Okuno T, Omata M, Kiyosawa K, Kumada H, et al. Peginterferon alpha-2a (40 KD) plus ribavirin for the treatment of chronic hepatitis C in Japanese patients. J Gastroenterol Hepatol. 2007;22:645–52.
10. Jacobson IM, Gonzalez SA, Ahmed F, Lebovics E, Min AD, Bodenheimer HC Jr, et al. A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. Am J Gastroenterol. 2005;100:2453–62.
11. Welsch C, Jesudian A, Zeuzem S, Jacobson I. New direct-acting antiviral agents for the treatment of hepatitis C virus infection and perspectives. Gut. 2012;61(Suppl 1):i36–46.
12. Chayama K, Hayes CN, Ohishi W, Kawakami Y. Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines. J Gastroenterol. 2013;48:1–12.
13. Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H. Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. J Viral Hepat. 2012;19:e134–42.
14. Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. J Hepatol. 2012;56:78–84.
15. Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. N Engl J Med. 2011;365:1014–24.
16. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011;364:2405–16.
17. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med. 2011;364:1207–17.
18. Hezode C. Boceprevir and telaprevir for the treatment of chronic hepatitis C: safety management in clinical practice. Liver Int. 2012;32(Suppl 1):32–8.
19. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med. 2011;364:1195–206.
20. Hayashi N, Seto C, Kato M, Komada Y, Goto S. Once-daily simeprevir (TMC435) with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1-infected patients in Japan: the DRAGON study. J Gastroenterol. 2014;49:138–47.
21. Kaiser S, Lutze B, Hass HG, Werner CR. High sustained virologic response rates in HCV genotype 1 relapser patients retreated with peginterferon alfa-2a (40KD) plus ribavirin for 72 weeks. Hepatology. 2008;48:1140A (abstract).
22. Jensen DM, Marcellin P, Freilich B, Andreone P, Di BA, Brandao-Mello CE, et al. Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. Ann Intern Med. 2009;150:528–40.
23. Zeuzem S, Berg T, Gane E, Ferenci P, Foster GR, Fried MW, et al. Simeprevir increases rate of sustained virologic response among treatment-experienced patients with HCV genotype-1 infection: a phase IIb trial. Gastroenterology. 2014;146:430–41.e6.

24. Manns M, Reesink H, Berg T, Dusheiko G, Flisiak R, Marcellin P, et al. Rapid viral response of once-daily TMC435 plus pegylated interferon/ribavirin in hepatitis C genotype-1 patients: a randomized trial. Antivir Ther. 2011;16:1021–33.

25. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection. N Engl J Med. 2011;364:2417–28.

26. Martinot-Peignoux M, Stern C, Maylin S, Ripault MP, Boyer N, Leclere L, et al. Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. Hepatology. 2010;51:1122–6.

27. Fried MW, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobsen I, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. Hepatology. 2013;58:1918–29.

28. Reddy KR, Lin F, Zoulim F. Response-guided and -unguided treatment of chronic hepatitis C. Liver Int. 2012;32(Suppl 1):64–73.

29. Pol S, Aerssens J, Zeuzem S, et al. Limited impact of IL28B genotype on response rates in telaprevir-treated patients with prior treatment failure. J Hepatol. 2013;58:883–9.

30. Lenz O, Verbinnen T, Lin TI, et al. In vitro resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother. 2010;54:1878–87.

31. Halfon P, Locarnini S. Hepatitis C virus resistance to protease inhibitors. J Hepatol. 2011;55:192–206.

32. Huisman MT, Snoeys J, Monbaliu J, Martens M, Sekar V, Raoof A. In vitro studies investigating the mechanism of interaction between TMC435 and hepatic transporters. Poster 278 presented at the 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, USA, 29 Oct-2 Nov, 2010.

Simeprevir with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1 patients in Japan: CONCERTO-1, a phase III trial

Norio Hayashi[1], Namiki Izumi[2], Hiromitsu Kumada[3], Takeshi Okanoue[4], Hirohito Tsubouchi[5], Hiroshi Yatsuhashi[6], Mai Kato[7], Rito Ki[7], Yuji Komada[7], Chiharu Seto[7], Shoichiro Goto[7,*]

[1]Kansai Rosai Hospital, Hyogo, Japan; [2]Musashino Red-Cross Hospital, Tokyo, Japan; [3]Toranomon Hospital, Tokyo, Japan; [4]Saiseikai Suita Hospital, Osaka, Japan; [5]Kagoshima University Medical and Dental Hospital, Kagoshima, Japan; [6]National Hospital Organization Nagasaki Medical Center, Nagasaki, Japan; [7]Research & Development, Janssen Pharmaceutical K.K., Tokyo, Japan

Background & Aims: In a Japanese Phase II study, the hepatitis C virus NS3/4A protease inhibitor simeprevir demonstrated potent antiviral activity and significantly improved sustained virologic response rates when added to peginterferon α-2a/ribavirin in treatment-naïve patients infected with hepatitis C virus genotype 1.

Methods: CONCERTO-1 was a Phase III, randomized, double-blind, placebo-controlled trial. Treatment-naïve adults (≤70 years) with chronic hepatitis C virus genotype 1 infection (hepatitis C virus RNA ≥5 $\log_{10}$ IU/ml) were randomized (2:1) to simeprevir 100 mg once-daily with peginterferon α-2a/ribavirin for 12 weeks then response-guided therapy with peginterferon α-2a/ribavirin for 12 or 36 weeks, or to placebo with peginterferon α-2a/ribavirin for 12 weeks then peginterferon α-2a/ribavirin for 36 weeks.

Results: Overall, 183 patients were treated. Sustained virologic response 12 weeks after treatment end (primary efficacy endpoint) was achieved in 88.6% of simeprevir- and 61.7% of placebo-treated patients (p <0.0001 for stratum-adjusted between-group difference). Overall, 91.9% of simeprevir-treated patients met response-guided therapy criteria and completed treatment at week 24; sustained virologic response rate at 12 weeks in these patients was 92.0%. One simeprevir- (0.8%) and two placebo-treated patients (3.3%) experienced viral breakthrough; respective viral relapse rates were 7.6% and 30.6%. Overall adverse event profile in simeprevir-treated patients was comparable to that in patients who received peginterferon α-2a/ribavirin alone.

Conclusions: Simeprevir once daily with peginterferon α-2a/ribavirin significantly improved sustained virologic response rate 12 weeks after treatment end in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection, with a shorter 24-week treatment duration in most patients.

Introduction

Japan has one of the highest rates of hepatitis C virus (HCV) infection worldwide, with around 2 million people estimated to be infected [1]. Of these, about 70% are infected with HCV genotype 1b, 20% with genotype 2a, and the remainder with genotype 2b or other genotypes. Hepatocellular carcinoma is a leading cause of cancer mortality in Japan, with approximately 70% of cases related to HCV infection [2].

The goal of chronic HCV infection treatment is virus eradication, to prevent progression to cirrhosis and hepatocellular carcinoma. Until recently, standard of care was combination therapy with peginterferon (PegIFN) and ribavirin (RBV) for 24–48 weeks [3,4], resulting in sustained virologic response (SVR) in approximately 50% of patients [5,6]. However, HCV genotype 1 appears less responsive to PegIFN-based therapy than other genotypes [7].

The development of direct-acting antiviral agents represents a major breakthrough in the treatment of chronic HCV infection. These have been shown to improve SVR rates when combined with PegIFN/RBV in treatment-naïve and treatment-experienced patients [8,9]. Current Japanese guidelines recommend triple therapy with the HCV NS3/4A protease inhibitor (PI) telaprevir plus PegIFN/RBV for chronic genotype 1 HCV infection [10]. However, first-generation HCV PIs, such as telaprevir and boceprevir, are associated with two- or three-times daily dosing, the potential for adverse events (AEs) including anemia, rash, and renal dysfunction; and relatively rapid emergence of resistance in patients who do not achieve SVR [8,9,11–13].

Simeprevir (TMC435) is a potent, oral, once-daily (QD), HCV NS3/4A PI which has recently been approved in Japan [14]. In a Phase I study, simeprevir plasma exposure was found to be higher in healthy Japanese adult male volunteers than in healthy

Research Article

Caucasian volunteers [15]. Based on this finding, simeprevir doses of 50 and 100 mg QD were selected for use in further studies in Japan. A Phase II study in Japanese treatment-naïve patients with HCV genotype 1 infection and high viral load reported simeprevir (50 or 100 mg QD, for 12 or 24 weeks) in combination with PegIFNα-2a/RBV to be generally well tolerated and associated with improved SVR rates and shorter 24-week treatment duration in most patients [16]. The Clinical Optimization of New treatment strategy with TMC435 in Combination with peginterferon plus Ribavirin for Treatment-naïve and treatment-experienced patients infected with HCV genotype 1 (One; CONCERTO) studies were initiated to further explore efficacy and safety of simeprevir combined with PegIFN/RBV in patients with HCV genotype 1 infection in Japan. We present results of the CONCERTO-1 study (ClinicalTrials.gov: NCT01292239) in treatment-naïve patients.

Patients and methods

Patients

Treatment-naïve male and female patients aged 20–70 years with documented chronic genotype 1 HCV infection and plasma HCV RNA $\geqslant 5.0 \log_{10}$ IU/ml at screening were eligible. Key exclusion criteria included liver cirrhosis, hepatic failure, any other liver disease of non-HCV etiology and co-infection with HIV-1, HIV-2, hepatitis B, or non-genotype 1 HCV. Additional exclusion criteria are summarized in the Supplementary data. All patients provided written informed consent before study entry.

Study design

This Phase III, multicenter, randomized, double-blind, placebo-controlled trial assessed efficacy and safety of simeprevir combined with PegIFNα-2a/RBV in treatment-naïve patients with chronic genotype 1 HCV infection. The study was conducted at 37 sites in Japan from January 17, 2011 to October 22, 2012. The study protocol conformed to Good Clinical Practice Guidelines and to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institutions' human research committee. (ClinicalTrials.gov: NCT01292239).

Eligible patients, stratified by age (<65 or $\geqslant 65$ years old) and IL28B genotype (rs8099917; TT, TG, and GG), were randomized centrally 2:1 to simeprevir 100 mg QD plus PegIFNα-2a/RBV for 12 weeks followed by response-guided therapy (RGT) with PegIFNα-2a/RBV alone for 12 or 36 weeks, or placebo with PegIFNα-2a/RBV for 12 weeks followed by PegIFNα-2a/RBV for 36 weeks (see Supplementary data for details of treatment administration). Randomization was balanced by using randomly permuted blocks. Patients, study personnel, and the sponsor were blinded to treatment groups. According to RGT criteria, PegIFNα-2a/RBV therapy could be stopped at week 24 in simeprevir-treated patients who achieved HCV RNA <1.2 $\log_{10}$ IU/ml detectable or undetectable at week 4 and had undetectable HCV RNA at week 12. RGT was not permitted in the placebo group. All patients were followed for 72 weeks after treatment initiation.

Virologic stopping criteria were implemented to ensure that patients with a suboptimal response discontinued treatment in a timely manner in order to limit the risk of drug resistance and reduce unnecessary exposure to PegIFNα-2a/RBV. Patients with HCV RNA >3.0 $\log_{10}$ IU/ml at week 4 discontinued simeprevir or placebo but continued PegIFNα-2a/RBV. Patients with HCV RNA $\geqslant 1.2 \log_{10}$ IU/ml at week 36 stopped PegIFNα-2a/RBV.

The primary efficacy endpoint was the proportion of patients with undetectable plasma HCV RNA at end of treatment and 12 weeks after end of treatment (SVR12). Other efficacy endpoints included: the proportion of patients with undetectable plasma HCV RNA at end of treatment and 24 weeks after end of treatment (SVR24); the proportion of patients with undetectable plasma HCV RNA at week 4 (rapid virologic response) or week 12 (complete early virologic response); incidence of viral breakthrough (>1 $\log_{10}$ IU/ml increase in plasma HCV RNA level from the lowest level reached, or plasma HCV RNA level >2.0 $\log_{10}$ IU/ml in patients whose plasma HCV RNA level had previously been <1.2 $\log_{10}$ IU/ml detectable or undetectable); viral relapse (detectable or quantifiable plasma HCV RNA at last available measurement during post-treatment fol-

low-up in patients who had undetectable plasma HCV RNA at end of treatment), and the proportion of patients who had achieved normalization of ALT levels.

Study assessments

To assess virologic response, plasma HCV RNA was quantified at screening, baseline, day 3, weeks 1, 2, 3, 4, 8, 12, 16, 20, 24, 28, 36, 48, 60, and 72 using the COBAS® TaqMan® HCV Auto Assay System (Roche, USA; lower limit of quantification, 1.2 $\log_{10}$ IU/ml). Plasma HCV RNA levels were also measured at weeks 42 and 52 in patients receiving PegIFNα-2a/RBV until week 48. ALT levels were measured at regular intervals throughout the study. Normal ALT levels, i.e., <40 IU/ml, were defined according to laboratory reference ranges.

Sequence analysis of the HCV NS3 protease domain was performed at baseline and in patients with simeprevir treatment failure (see Supplementary data for further details).

Safety was evaluated throughout the study. AEs were assessed during the treatment period and 28 days after the last dose of study medication. AE severity was graded by investigators using the World Health Organization grading scale. Certain AEs were identified as being of special or clinical interest (see Supplementary data for further details). Vital sign monitoring, electrocardiogram, and clinical laboratory tests were performed at regular intervals during treatment and the post-treatment follow-up period. Severity of laboratory abnormalities was classified according to the World Health Organization grading scale.

Statistical analysis

The sample size of the study (183 subjects) was calculated based on published data [16], and was considered to be sufficient to show superiority of simeprevir vs. placebo for the proportion of subjects achieving SVR12 with 90% power (see Supplementary data for further details). Efficacy analyses were performed on the full analysis set, which comprised all randomized patients who had received $\geqslant 1$ dose of study medication for whom post-baseline efficacy assessment data were available. SVR rates between treatment groups were compared using the Cochran-Mantel-Haenszel test adjusted for the stratification factors (age and IL28B genotype). The 95% confidence interval of the stratum-adjusted difference in the proportions between the treatment groups was calculated.

The safety population comprised all patients who received $\geqslant 1$ dose of study medication. Incidence of AEs and other safety endpoints was summarized for each treatment group.

Statistical analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

Overall, 223 patients were screened for study participation and 188 were randomized, and 183 received $\geqslant 1$ dose of study medication (123 received simeprevir and 60 received placebo) (Fig. 1). Eleven patients (6.0%) discontinued the study during follow-up (3.3% and 11.7% in the simeprevir and placebo groups, respectively). The most common reason for study discontinuation was withdrawal of consent. In the simeprevir group, 92.7% completed the treatment period, as did 75.0% in the placebo group. Reasons for non-completion of all study medication are summarized in the Supplementary data.

Baseline demographic and disease characteristics were generally similar between treatment groups (Table 1). All patients were Japanese, 34.4% were male, and median age was 55 years (range 23–69 years). Most patients had the major alleles TT for IL28B 8099917 and CC for IL28B 12979860 associated with higher likelihood of response to pegIFN/RBV (66.7% and 64.2% in the simeprevir group, respectively, and 70.0% for both in the placebo group). Almost all patients had HCV genotype 1b (98.4%), except for three patients with HCV genotype 1a (two in the simeprevir
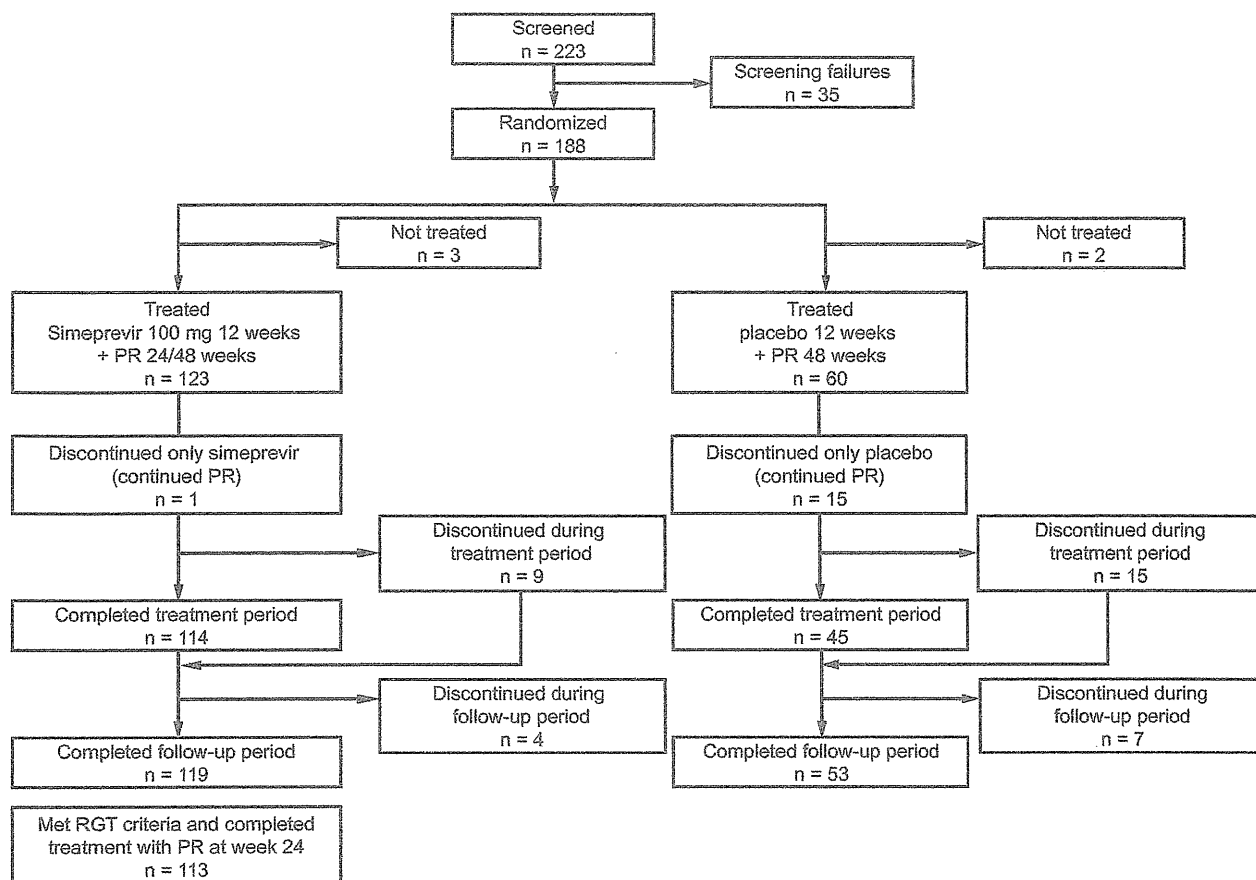
```
                    ┌─────────────┐
                    │  Screened   │
                    │   n = 223   │
                    └─────────────┘
                           │              ┌──────────────────┐
                           │─────────────▶│ Screening failures│
                           │              │     n = 35        │
                           ▼              └──────────────────┘
                    ┌─────────────┐
                    │ Randomized  │
                    │   n = 188   │
                    └─────────────┘
```

Fig. 1. **Subject disposition.** PR, peginterferon/ribavirin; RGT, response-guided therapy.

group and one in the placebo group). Median baseline HCV RNA was 6.3 $\log_{10}$ IU/ml (range 3.3–7.4 $\log_{10}$ IU/ml), with most patients in both groups having baseline HCV RNA levels $\geqslant$6 $\log_{10}$ IU/ml (74.0% for simeprevir and 76.7% for placebo).

Sustained virologic response

SVR12 was achieved in 88.6% of patients receiving simeprevir vs. 61.7% of patients receiving placebo (Table 2). The stratum-adjusted difference in SVR12 rate between the two groups was statistically significant (27.5% [95% confidence interval, 14.38–40.56%]; p <0.0001). Most (113/123; 91.9%) simeprevir-treated patients met RGT criteria and completed treatment at week 24. The SVR12 rate in these patients was 92.0%. Only one patient in the simeprevir group did not meet RGT criteria; this patient discontinued treatment with simeprevir only and completed treatment with PegIFNα-2a/RBV. The remaining nine patients discontinued the study before assessment of RGT criteria at week 24.

The proportion of patients achieving SVR24 was 88.6% in the simeprevir group and 56.7% in the placebo group (Table 2). Efficacy of simeprevir combined with PegIFNα-2a/RBV was statistically significantly superior to placebo combined with PegIFNα-2a/RBV (p <0.0001).

On-treatment virologic response

Onset of antiviral activity was more rapid with simeprevir than with placebo (Fig. 2). In the simeprevir group, 84.4% of patients had undetectable HCV RNA at week 4 vs. 13.3% in the placebo group (Table 2). The proportion of patients with undetectable HCV RNA at week 12 was also higher with simeprevir than with placebo (99.2% and 68.5%, respectively) (Table 2).

A notable difference in the proportion of patients achieving undetectable HCV RNA at weeks 4, 12, and 24 according to *IL28B* genotype at rs8099917 was seen between the simeprevir and the placebo groups (Fig. 3). With simeprevir, undetectable HCV RNA levels at week 4 were achieved by 87.8% of patients with the *IL28B* major allele (TT) and 75.6% of those with *IL28B* minor alleles (TG and GG). The proportion of simeprevir-treated patients with undetectable HCV RNA levels was increased at week 12 (96.3% with TT and 97.6% with TG and GG) and remained high at week 24 (92.7% with TT and 90.2% with TG and GG), irrespective of *IL28B* genotype. Undetectable HCV RNA levels at week 4 were achieved in 19.0% of placebo-treated patients with the *IL28B* major allele. No placebo-treated patients with *IL28B* minor alleles achieved undetectable HCV RNA at this time. Undetectable HCV RNA levels were achieved in 85.7% of placebo-treated patients with the *IL28B* major allele at weeks 12 and 24, and in

Research Article

Table 1. Baseline demographics and clinical characteristics.

Characteristic	Simeprevir group (n = 123)	Placebo group (n = 60)
Male, n (%)	39 (31.7)	24 (40.0)
Age (yr), median (range)	56.0 (23-69)	54.5 (30-69)
Age ≥65 years, n (%)	22 (17.9)	10 (16.7)
BMI (kg/m²), median (range)	22.0 (16.9-32.9)	22.5 (17.3-33.2)
IL28B genotype (rs8099917), n (%)		
TT	82 (66.7)	42 (70.0)
TG/GG	41 (33.3)	18 (30.0)
IL28B genotype (rs12979860), n (%)		
CC	79 (64.2)	42 (70.0)
CT/TT	44 (35.8)	18 (30.0)
HCV genotype, n (%)		
1a	2 (1.6)	1 (1.7)
1b	121 (98.4)	59 (98.3)
Baseline HCV RNA (Log_{10} IU/ml), median (range)	6.3 (4.5-7.2)	6.4 (3.3-7.4)
Baseline HCV RNA category (Log_{10} IU/ml), n (%)		
<6.0	32 (26.0)	14 (23.3)
≥6.0 to <7.0	85 (69.1)	45 (75.0)
≥7.0	6 (4.9)	1 (1.7)
Metavir fibrosis stage,* n/N (%)		
0: No fibrosis	3/41 (7.3)	0
1: Periportal fibrosis expansion	28/41 (68.3)	18/24 (75.0)
2: P-P septae (>1 septum)	8/41 (19.5)	5/24 (20.8)
3: P-C septae	2/41 (4.9)	1/24 (4.2)
4: Cirrhosis	0	0
Platelets (×10⁹/L), n (%)		
<150	30 (24.4)	10 (16.7)
≥150	93 (75.6)	50 (83.3)
ALT (IU/ml)		
<50	74 (60.2)	39 (65.0)
≥50	49 (39.8)	21 (35.0)
Total bilirubin (mg/dl), median (range)	0.7 (0.3-1.7)	0.7 (0.2-1.5)
Hemoglobin (g/dl), median (range)	13.9 (11.1-17.2)	14.1 (12.3-17.0)
Neutrophils (×10²/μl), median (range)	24.2 (11.4-54.7)	22.5 (11.6-45.8)
Platelets (×10⁴/μl), median (range)	18.3 (9.5-29.0)	20.2 (10.6-31.3)

*Available for patients who had a liver biopsy within 2 years before informed consent or during the screening period.
BMI, body mass index; HCV, hepatitis C virus; P-P, portal-portal; P-C, portal-central; ALT, alanine aminotransferase.

11.1% and 44.4% of placebo-treated patients with *IL28B* minor alleles at week 12 and week 24, respectively.

Viral breakthrough, viral relapse, and treatment failure

Viral breakthrough was observed in one patient (0.8%) in the simeprevir group, occurring at week 17 during treatment with Peg-IFNα-2a/RBV alone. Viral breakthrough was reported in two patients in the placebo group (3.3%) at weeks 2 and 21.

Viral relapse rates were lower in the simeprevir group than in the placebo group (7.6% *vs.* 30.6%, respectively). Viral relapse mostly occurred at follow-up week 12 (9/9 patients in the simeprevir group; 12/15 patients in the placebo group).

The proportion of patients meeting virologic stopping criteria was lower in the simeprevir group compared with the placebo group (0.8% *vs.* 40.0%, respectively). All of these patients met virologic stopping criteria at week 4 (plasma HCV RNA levels >3.0 log_{10} IU/ml).

Sustained virologic response according to baseline characteristics

SVR12 rates were higher in the simeprevir group than in the placebo group for all baseline demographic and disease characteristic subgroups. SVR12 rate by baseline demographic and disease characteristics is summarized in Supplementary Table 1.

NS3 sequencing analysis

Most patients (87.4% in the simeprevir group; 84.5% in the placebo group) had no baseline polymorphisms at any NS3 positions associated with reduced antiviral activity to simeprevir *in vitro*. Further information is provided in the Supplementary data section.

Alanine aminotransferase

In most patients with abnormal ALT levels at baseline, ALT levels were normalized by end of treatment (73.5% in the simeprevir group; 76.2% in the placebo group).

Table 2. Virologic response rates.

Endpoint, n/N (%)*	Simeprevir group (n = 123)	Placebo group (n = 60)
SVR12		
All patients	109/123 (88.6)	37/60 (61.7)
Patients meeting RGT criteria	104/113 (92.0)	n.a.
SVR24	109/123 (88.6)	34/60 (56.7)
Undetectable HCV RNA at Wk 4 of treatment*	103/122 (84.4)	8/60 (13.3)
Undetectable HCV RNA at Wk 12 of treatment*	117/118 (99.2)	37/54 (68.5)
Viral breakthrough	1/123 (0.8)	2/60 (3.3)
Viral relapse**	9/118 (7.6)	15/49 (30.6)

SVR12, sustained virologic response 12 weeks after the end of treatment; RGT, response guided therapy; SVR24, sustained virologic response 24 weeks after the end of treatment; n.a., not available.
*Subjects who discontinued all study medication prior to assessment of undetectable HCV RNA at week 4 and week 12 of treatment were not included in the denominator for calculation of these on-treatment response rates.
**Viral relapse was only assessed in patients with undetectable HCV RNA at end of treatment and with at least one follow-up HCV RNA measurement.
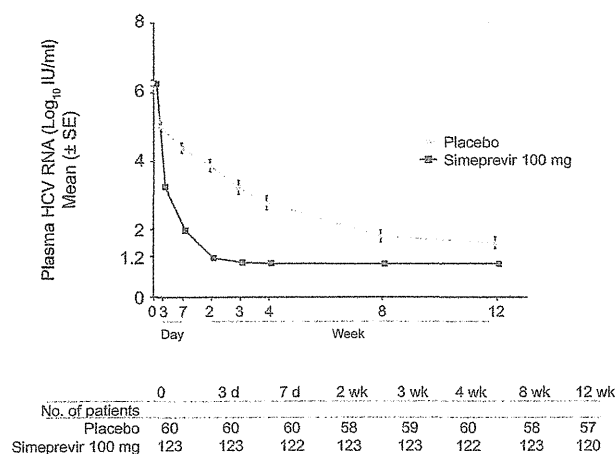

No. of patients	0	3 d	7 d	2 wk	3 wk	4 wk	8 wk	12 wk
Placebo	60	60	60	58	59	60	58	57
Simeprevir 100 mg	123	123	122	123	123	122	123	120

Fig. 2. Mean change from baseline in plasma HCV RNA levels up to week 12. HCV, hepatitis C virus; SE, standard error.

Safety

For the entire treatment period, no clinically significant differences in the type and incidence of AEs were observed between groups (Table 3; Supplementary Table 2). Most AEs were grade 1/2 (72.4% in the simeprevir group; 66.7% in the placebo group). Rates of treatment discontinuation and PegIFNα-2a/RBV dose modification due to AEs were similar between groups, with no AEs leading to permanent discontinuation of simeprevir only. Serious AEs were observed in four patients (3.3%) in the simeprevir group and six patients (10.0%) in the placebo group. There were no deaths reported.

For AEs of special/clinical interest (Table 3), incidence was higher in the simeprevir group than in the placebo group for increased bilirubin-related AEs (22.8% vs. 10.0%) and photosensitivity conditions (1.6% vs. 0%). Incidence was lower in the simeprevir group than in the placebo group for rash (any type) and selected gastrointestinal events. For anemia, neutropenia, and pruritus-related AEs, incidence rates were similar in both groups. In the simeprevir group, all AEs of special/clinical interest were grade 1/2, except for grade 3 anemia-related AEs in one patient
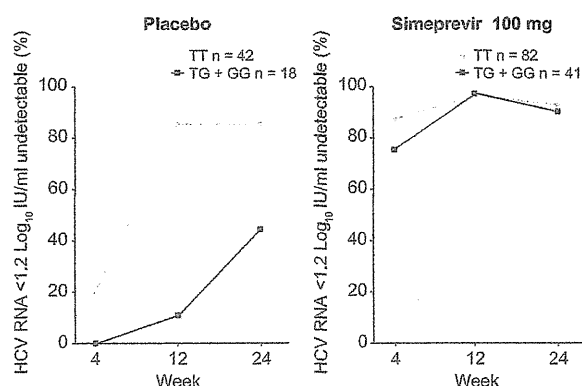

Fig. 3. Proportion of patients with plasma HCV RNA <1.2 log$_{10}$ IU/ml undetectable according to IL28B genotype at rs8099917. HCV, hepatitis C virus.

(0.8%) and grade 3/4 neutropenia-related AEs in 28 patients (22.8%). None of the neutropenia-related AEs were serious and none led to discontinuation of any study medications.

Fig. 4 shows changes in laboratory parameters over the entire duration of treatment and follow-up. Notably, only one patient in the simeprevir group continued treatment with PegIFNα-2a/RBV from week 24 onwards. Except for total bilirubin, no differences between groups were observed in median change from baseline for any of these laboratory parameters. Median total bilirubin levels increased in the simeprevir group from baseline to week 2 and then decreased to near baseline levels from week 4 to week 12. During this period, median levels remained within normal limits. From week 12, median values returned to baseline. Increases in total bilirubin levels were not associated with increased ALT or AST levels.

No consistent clinically significant differences in any other laboratory parameters, electrocardiogram parameters, or vital signs were observed between groups.

Discussion

This study was undertaken to assess efficacy and safety of simeprevir in combination with PegIFNα-2a/RBV in treatment-naïve

Table 3. Summary of AEs reported in the entire study treatment period and during treatment with simeprevir plus PegIFNα-2a and ribavirin (safety population).

No. of patients, n (%)	Entire treatment period		Simeprevir/Placebo + PegIFNα-2a/RBV	
	Simeprevir group (n = 123)	Placebo group (n = 60)	Simeprevir group (n = 123)	Placebo group (n = 60)
Treatment discontinuation due to AE				
Simeprevir/placebo only[†]	0	1 (1.7)	0	1 (1.7)
All study medication[‡]	6 (4.9)	5 (8.3)	4 (3.3)	3 (5.0)
Dose modification due to AE				
PegIFNα-2a	47 (38.2)	24 (40.0)	n.a.	n.a.
RBV	67 (54.5)	27 (45.0)	n.a.	n.a.
SAE	4 (3.3)	6 (10.0)	3 (2.4)	3 (5.0)
Death	0	0	0	0
AEs of special/clinical interest[*]				
Increased bilirubin-related AEs	28 (22.8)	6 (10.0)	28 (22.8)	6 (10.0)
Blood bilirubin increased	20 (16.3)	4 (6.7)	20 (16.3)	4 (6.7)
Hyperbilirubinemia	8 (6.5)	2 (3.3)	8 (6.5)	2 (3.3)
Rash (any type)	71 (57.7)	42 (70.0)	64 (52.0)	27 (45.0)
Rash	57 (46.3)	37 (61.7)	54 (43.9)	24 (40.0)
Erythema	17 (13.8)	4 (6.7)	13 (10.6)	2 (3.3)
Other events	6 (4.9)	3 (5.0)	4 (3.3)	2 (3.3)
Pruritus-related AEs	37 (30.1)	19 (31.7)	29 (23.6)	5 (8.3)
Pruritus	35 (28.5)	18 (30.0)	28 (22.8)	5 (8.3)
Pruritus generalized	2 (1.6)	1 (1.7)	1 (0.8)	0 (0)
Photosensitivity conditions	2 (1.6)	0	1 (0.8)	0 (0)
Photosensitivity reaction	2 (1.6)	0	1 (0.8)	0 (0)
Neutropenia-related AEs	97 (78.9)	49 (81.7)	90 (73.2)	42 (70.0)
White blood cell count decreased	78 (63.4)	41 (68.3)	74 (60.2)	35 (58.3)
Neutrophil count decreased	69 (56.1)	37 (61.7)	59 (48.0)	30 (50.0)
Neutropenia	8 (6.5)	1 (1.7)	8 (6.5)	1 (1.7)
Anemia-related AEs	97 (78.9)	45 (75.0)	74 (60.2)	30 (50.0)
Anemia	70 (56.9)	36 (60.0)	55 (44.7)	24 (40.0)
Hemoglobin decreased	27 (22.0)	9 (15.0)	19 (15.4)	6 (10.0)
Selected gastrointestinal events	53 (43.1)	38 (63.3)	46 (37.4)	28 (46.7)
Decreased appetite	28 (22.8)	20 (33.3)	28 (22.8)	14 (23.3)
Diarrhea	20 (16.3)	17 (28.3)	14 (11.4)	12 (20.0)
Nausea	16 (13.0)	12 (20.0)	15 (12.2)	9 (15.0)
Constipation	9 (7.3)	5 (8.3)	7 (5.7)	2 (3.3)
Vomiting	6 (4.9)	5 (8.3)	4 (3.3)	1 (1.7)

[†]Permanent discontinuation of simeprevir or placebo alone, PegIFNα-2a/RBV continued.
[‡]Discontinuation of simeprevir/placebo and PegIFNα-2a/RBV at the same time, or discontinuation of PegIFNα-2a/RBV after completion or discontinuation of simeprevir/placebo).
[*]See Supplementary data for further details.
AEs, adverse events; PegIFNα-2a, peginterferon α-2a; RBV, ribavirin; SAE, serious adverse event; n.a., not available.

patients with chronic HCV genotype 1 infection and high viral load in Japan. A simeprevir dose of 100 mg with a 12 week duration for triple therapy was selected for use in this study based on results of a previous Phase II trial [16]. Oral once-daily treatment with simeprevir 100 mg for 12 weeks was associated with a significant improvement in SVR12 rate in this patient population. An RGT strategy was employed to allow individualized shortening of PegIFNα-2a/RBV treatment duration to 24 weeks, based on early virologic response. Almost all simeprevir-treated patients (91.9%) met RGT criteria and were eligible to stop PegIFNα-2a/RBV at week 24. The SVR12 rate in these patients was 92.0%, supporting

this approach. A shorter overall treatment duration is highly desirable in patients with chronic HCV infection as it reduces PegIFNα-2a/RBV exposure and the potential for treatment-related AEs [17].

Even in patients with unfavorable demographic and baseline disease characteristics for a response to PegIFNα-2a/RBV (e.g., older age, high baseline HCV RNA, and low baseline platelet counts), treatment with simeprevir was associated with significantly improved SVR rates (Supplementary Table 1). As expected, IL28B single nucleotide polymorphism was predictive of therapeutic effect for PegIFNα-2a/RBV. It is well documented that
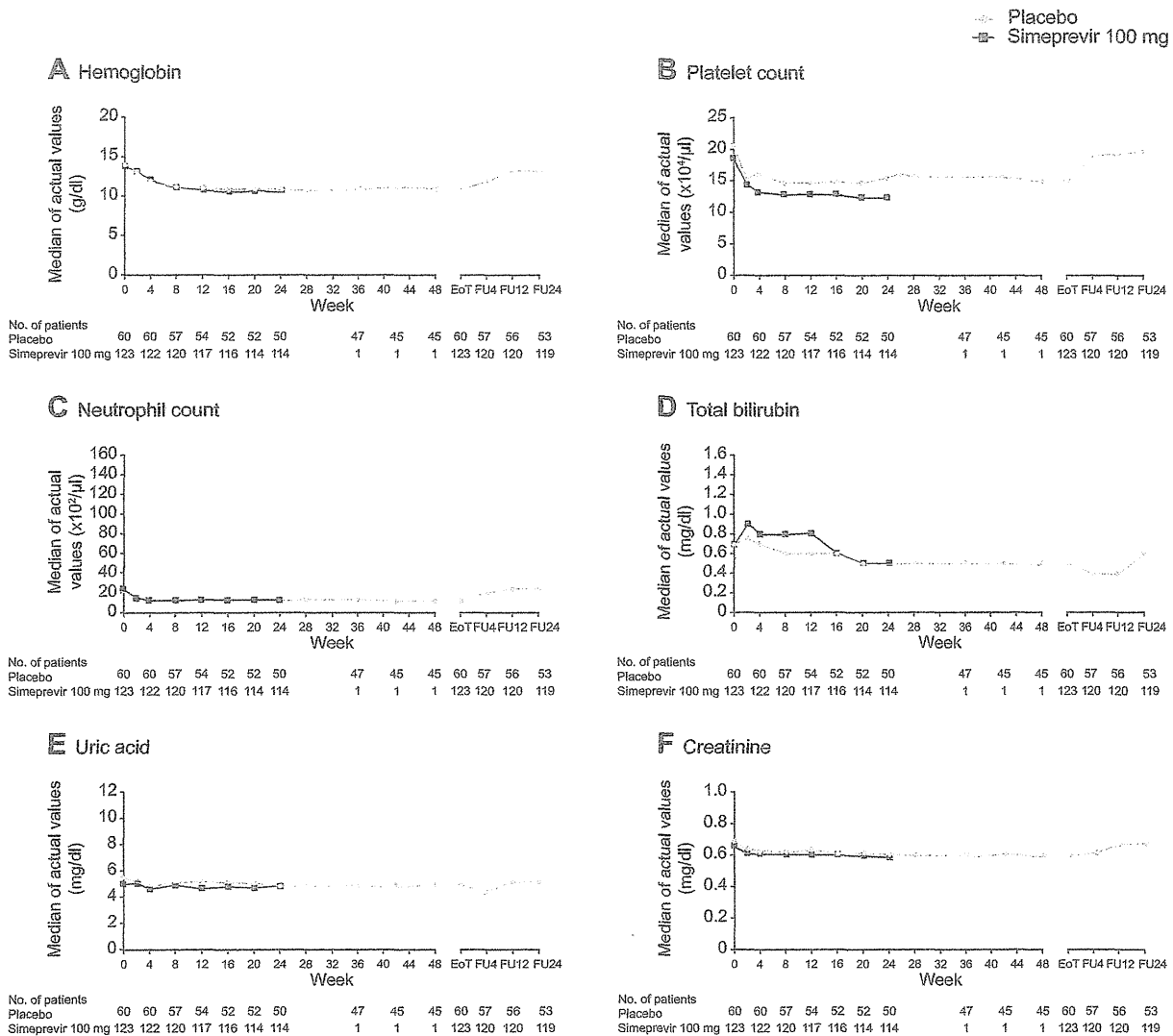
Fig. 4. Median changes in serum levels. (A) hemoglobin, (B) platelet count, (C) neutrophil count, (D) total bilirubin, (E) uric acid, and (F) creatinine over the entire duration of treatment and follow-up.*Only one patient in the simeprevir group continued treatment with peginterferon α-2a/ribavirin from week 24 onwards. EoT, end of treatment; FU, follow-up.

patients carrying *IL28B* minor alleles show greater resistance to PegIFN/RBV than those carrying major alleles [18–21]. Approximately two-thirds of patients in this study had favorable *IL28B* genotypes associated with higher likelihood of response to peg-IFN/RBV therapy. Nevertheless, our results suggest that addition of simeprevir to PegIFNα-2a/RBV reduces the impact of unfavorable *IL28B* minor alleles at rs8099917 and rs12979860. Consistent with the epidemiology of HCV infection in Japan [1], genotype 1b was dominant among patients enrolled in this study (98.4%). It has been suggested that HCV genotype 1a infection has a lower genetic barrier to resistance relative to genotype 1b infection [22].

The SVR rate in patients treated with PegIFNα-2a/RBV for 48 weeks is consistent with the original assumption for sample size estimation and is similar to that reported in a previous Phase

III study of PegIFNα-2a/RBV in a comparable Japanese population [5]. The SVR rate observed in simeprevir-treated patients in this study is within the range reported in a previous dose-ranging Phase II study of simeprevir in treatment-naïve patients with chronic HCV genotype 1 infection in Japan [16].

Viral breakthrough was observed in one patient (0.8%) in the simeprevir group and two patients (3.3%) in the placebo group. Viral relapse occurred infrequently in simeprevir-treated patients compared with the placebo group, being observed in 7.6% of simeprevir-treated patients with undetectable plasma HCV RNA at end of treatment compared with 30.6% of patients who received placebo.

Sequence analysis of the HCV NS3 protease domain focused on detecting previously characterized HCV genotype 1 amino acid substitutions in the NS3 region that have been associated

with reduced susceptibility to simeprevir and other HCV NS3 PIs in vitro (43, 80, 155, 156, and 168) (see Supplementary data) [23,24]. Overall, 86.4% of patients did not have baseline polymorphisms at these positions. Emerging mutations were detected in most simeprevir-treated patients who experienced treatment failure, with D168V the most frequent emerging mutation. Emerging mutations identified at the time of treatment failure were generally not detectable at later time points, suggesting that these mutations do not persist and may resolve over time.

Simeprevir had a clinically favorable safety and tolerability profile in this patient population, with no notable differences in the type and incidence of AEs or discontinuations due to AEs observed between the simeprevir and placebo groups. In contrast to first-generation PIs, such as telaprevir [8,9,12,13], incidence of anemia- and rash-related AEs observed in simeprevir-treated patients was no higher than in placebo-treated patients, and no cases of severe anemia or severe rash were seen in simeprevir-treated patients in this study. Renal dysfunction has also been identified as a potential safety concern with currently marketed first-generation HCV PIs, including telaprevir; no clinically relevant changes in renal function parameters were reported in this study. Cases of sepsis and pneumonia have recently been reported in telaprevir-treated patients during post-marketing surveillance in Japan (Mitsubishi Tanabe, personal communication). However, no severe infectious diseases were reported in this study. Mild reversible transient increases in bilirubin levels were observed in simeprevir-treated patients, as reported previously [14,16]. In vitro data suggest that this results from inhibition of OATP1B1 and MRP2 transporters by simeprevir, as both play a role in bilirubin clearance [25].

In summary, results of the CONCERTO-1 study in treatment-naïve patients with HCV genotype 1 and high viral load show that oral once-daily simeprevir in combination with PegIFNα-2a/RBV significantly improves SVR rates and shortens treatment duration in most patients. Simeprevir has now been approved in Japan for the treatment of chronic HCV infection. Ongoing studies are investigating simeprevir in IFN-free combinations, including all oral regimens [26].

Conflict of interest

Drs Okanoue, Tsubouchi, Kumada, and Hayashi consult and advise Janssen Pharmaceuticals. Drs Ki, Komada, Seto, and Goto are employed by Janssen Pharmaceuticals.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.04.004.

References

[1] Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. Intervirology 2010;53:39–43.
[2] Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol 2009;44:102–107.
[3] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 2011;55:245–264.
[4] Izumi N. Diagnostic and treatment algorithm of the Japanese society of hepatology: a consensus-based practice guideline. Oncology 2010;78:78–86.
[5] Kuboki M, Iino S, Okuno T, Omata M, Kiyosawa K, Kumada H, et al. Peginterferon alpha-2a (40 KD) plus ribavirin for the treatment of chronic hepatitis C in Japanese patients. J Gastroenterol Hepatol 2007;22:645–652.
[6] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
[7] McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580–593.
[8] Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1207–1217.
[9] Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 2011;364:2405–2416.
[10] Chayama K, Hayes CN, Ohishi W, Kawakami Y. Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines. J Gastroenterol 2013;48:1–12.
[11] Buti M, Agarwal K, Horsmans Y, Sievert W, Janczewska E, Zeuzem S, et al. Telaprevir twice daily is noninferior to telaprevir every 8 h for patients with chronic hepatitis C. Gastroenterology 2014;146:744–753.
[12] Hezode C. Boceprevir and telaprevir for the treatment of chronic hepatitis C: safety management in clinical practice. Liver Int 2012;32:32–38.
[13] Thompson AJ, Locarnini SA, Beard MR. Resistance to anti-HCV protease inhibitors. Curr Opin Virol 2011;1:599–606.
[14] Tanwar S, Trembling PM, Dusheiko GM. TMC435 for the treatment of chronic hepatitis C. Expert Opin Investig Drugs 2012;21:1193–1209.
[15] Verloes R, Shishido A. Phase I safety and PK of TMC435 in healthy volunteers and safety, PK and short-term efficacy in chronic hepatitis C infected individuals (abstract O-32). In: Japanese Hepatology Congress, Kobe, Japan, June 4–5, 2009.
[16] Hayashi N, Seto C, Kato M, Komada Y, Goto S. Once-daily simeprevir (TMC435) with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1-infected patients in Japan: the DRAGON study. J Gastroenterol 2014;49:138–147.
[17] Reddy KR, Lin F, Zoulim F. Response-guided and -unguided treatment of chronic hepatitis C. Liver Int 2012;32:64–73.
[18] Estrabaud E, Vidaud M, Marcellin P, Asselah T. Genomics and HCV infection: progression of fibrosis and treatment response. J Hepatol 2012;57:1110–1125.
[19] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
[20] Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, et al. HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. Gut 2011;60:261–267.
[21] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
[22] Cento V, Mirabelli C, Salpini R, Dimonte S, Artese A, Costa G, et al. HCV genotypes are differently prone to the development of resistance to linear and macrocyclic protease inhibitors. PLoS One 2012;7:e39652.

[23] Kieffer TL, Kwong AD, Picchio GR. Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). J Antimicrob Chemother 2010;65:202–212.

[24] Lenz O, Verbinnen T, Lin T-I, Vijgen L, Cummings MD, Lindberg J, et al. In vitro resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 2010;54:1878–1887.

[25] Huisman MT, Snoeys J, Monbaliu J, Martens M, Sekar V, Raoof A. In vitro studies investigating the mechanism of interaction between TMC435 and hepatic transporters. In: Poster 278 presented at the 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, USA, 29 October–2 November, 2010.

[26] Schinazi R, Halfon P, Marcellin P, Asselah T. HCV direct-acting antiviral agents: the best interferon-free combinations. Liver Int 2014;34:69–78.

— 389 —

Short Communication

Potential of a no-touch pincer ablation procedure for small hepatocellular carcinoma that uses a multipolar radiofrequency ablation system: An experimental animal study

Yusuke Kawamura,[1,2] Kenji Ikeda,[1,2] Taito Fukushima,[1,2] Tasuku Hara,[1,2] Tetsuya Hosaka,[1,2] Masahiro Kobayashi,[1,2] Satoshi Saitoh,[1,2] Hitomi Sezaki,[1,2] Norio Akuta,[1,2] Fumitaka Suzuki,[1,2] Yoshiyuki Suzuki,[1,2] Yasuji Arase[1,2] and Hiromitsu Kumada[1,2]

[1]Department of Hepatology, [2]Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan

Aim: Treatment of hepatocellular carcinoma located on the liver surface is frequently difficult because direct puncture of the tumor must be avoided during needle insertion. The aim of this study was to investigate the utility of a no-touch pincer ablation procedure that uses a multipolar radiofrequency ablation (RFA) system for a tumor located on the liver surface.

Methods: The experimental animals were three pigs, and RFA was performed with two internally cooled bipolar electrodes. Three ablative procedures were compared: linear insertion at regular 13-mm intervals (pattern 1; virtual target tumor size, <10 mm); fan-shape insertion, maximum interval 20 mm (pattern 2; virtual target tumor size, <15 mm); and 25 mm (pattern 3; virtual target tumor size, <20 mm). All electrodes were inserted at a 30-mm depth. For patterns 1 and 2, ablation was performed on three other parts of the liver, and for pattern 3, ablation was performed on two other parts.

Results: For the median transverse and longitudinal diameter to the shaft, with the pattern 1 procedure, the ablative areas were 32 mm × 30 mm, and with the pattern 2 procedure, the ablative areas were 27 mm × 30 mm with carbonization of the liver surface. In contrast, with the pattern 3 procedure, the ablative areas were 45 mm × 26 mm; however, the ablative margin did not reach the surface, and carbonization was not apparent.

Conclusion: The no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small nodules that protrude from the liver surface.

Key words: bipolar, hepatocellular carcinoma, multipolar, no-touch ablation, radiofrequency ablation

INTRODUCTION

AMONG THE AVAILABLE treatment options for hepatocellular carcinoma (HCC), surgical resection is generally considered to be a local eradication method that can provide a satisfactory long-term outcome.[1–8]

Recent advances in imaging procedures have led to increased detection of early-stage HCC and to improved survival due to the increased identification of patients in whom hepatic resection is possible.[9,10]

For patients who are not eligible for surgery for various reasons (e.g. lack of sufficient liver function for surgical resection), percutaneous local therapy is a viable therapeutic option. Several local ablation therapies are available, including percutaneous ethanol injection, percutaneous acetic acid injection, cryotherapy, percutaneous microwave coagulation therapy and radiofrequency ablation (RFA). In addition to surgical resection, local ablation therapies, particularly RFA, are considered to be local eradication methods for HCC that can provide good long-term outcomes.[11] Therefore, in recent years, RFA has become a widely used option for the primary treatment of small-size HCC. However, we often

encounter cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver surface. In addition, a relationship between percutaneous local approaches to HCC (including tumor biopsy) and tumor seeding has been reported previously,[12,13] and with regard to the risk of treatment-related tumor seeding, the following risk factors have been reported: tumor size, tumor location (subcapsular portion), α-fetoprotein level, tumor stage and histopathological grade.[14,15] Therefore, a no-touch approach to local therapy may be considered an ideal treatment method for HCC.

Recently, a multipolar ablation system became available. Until now, in Japan, monopolar electrodes have typically been used, and the present cases are usually treated with some technical arrangement. For example, in the case of using a multi-tined expandable electrode, after obliquely inserting the electrode to avoid direct puncture of the target tumor, the multi needles are expanded toward the target tumor via non-tumor tissue, or in the case of using an internally cooled electrode, multiple insertions are made to avoid direct puncture of the target tumor, and RFA is performed after each insertion. However, these methods do not always provide enough of a treatment effect due to the influence of uncertain treatment procedures and natural, direct puncture to a tumor is indispensable. In contrast, a multipolar ablation system that uses an internally cooled bipolar electrode can combine the use of one to three electrodes at the same treatment session. When three electrodes are used, this system can treat large tumors; however, in the case of small tumors, it is not really necessary to use three electrodes to treat the target tumor. In addition, when we used this multipolar ablation system, usually electrodes were inserted into HCC, but in theory, this system can use no-touch ablation. However, to our knowledge, there are no technical reports that describe a non-direct punctual RFA method that uses a bipolar ablation system for HCC located on the liver surface. In this experimental animal study, we assumed that a small (<20 mm) HCC nodule protruded from the liver surface, and examined proper pincer ablation methods using two internally cooled bipolar electrodes.

METHODS

Summary of experimental procedures

WE USED A bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH [Telto,

Germany]) and two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). RFA was applied in the livers of three normal female domestic pigs (each pig's weight was 60 kg) under general anesthesia maintained until killing. The abdomen was opened so that the needle could be inserted under an ultrasonography (US) guide directly into the upper region of the liver where the thickness was larger than 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed two to three times in each liver for evaluation of the "no-touch pincer ablation procedure". After the experiments were completed, the animal was killed, and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Protocol of the no-touch pincer ablation procedure

We used a bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH), and all ablation procedures were performed with two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). Internal liquid circulation of the applicator enables the efficiency of coagulation to be increased. The delivery rate was set to 30 mL/min of saline solution at room temperature. The liquid flow was provided by a triple peristaltic pump, which is part of the system. The electrodes were operated by a power control unit working at 470 kHz and providing a maximum output power of 250 W (OLYMPUS Winter & Ibe GmbH). In this study, output power and total energy in each session were fixed at 60 W and 25 kJ, respectively, according to the dosimetry table for the bipolar RFA system (CelonPOWER System; OLYMPUS Winter & Ibe GmbH).

With regard to the ablation protocol, we performed the following three types of ablation procedure: linear insertion, at regular 13-mm intervals (pattern 1); fan-shape insertion, maximum interval of 20 mm (pattern 2); and 25 mm (pattern 3). All electrodes were inserted at a 30-mm depth from the liver surface under a US guide (Fig. 1). Each ablation procedure was performed for the following number of times: pattern 1, three sessions; pattern 2, three sessions; and pattern 3, two sessions. In this study, we assumed that the size of the virtual target tumor was less than 10 mm in pattern 1,
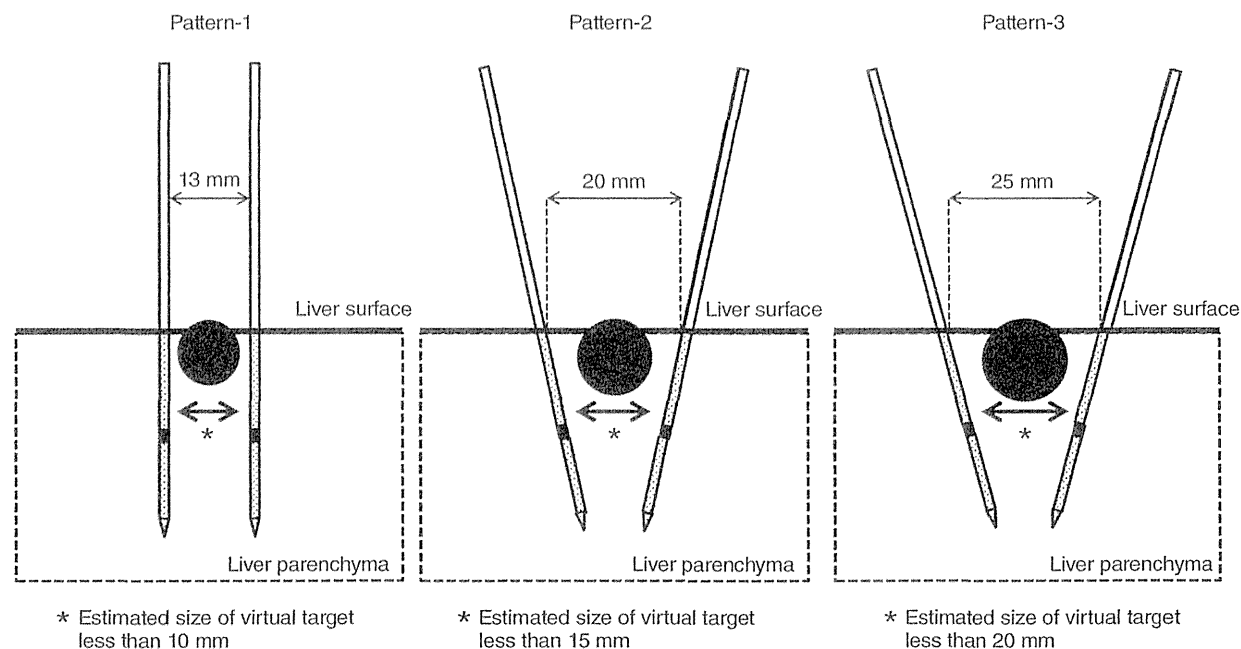
Figure 1 Protocol for a pincer ablation procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface.

less than 15 mm in pattern 2 and less than 20 mm in pattern 3.

Measurement procedure of the ablative margin

After completion of the experiments, the animal was killed and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone).

Statistical analysis

The size of the ablated zone and the duration of ablation were compared among the three groups with the Kruskal–Wallis test. All values are expressed as medians. A *P*-value of less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Features of the no-touch pincer ablation procedure

THE THREE TYPES of pincer ablation procedure applied to the pig liver were performed in the area shown in Figure 2(a).
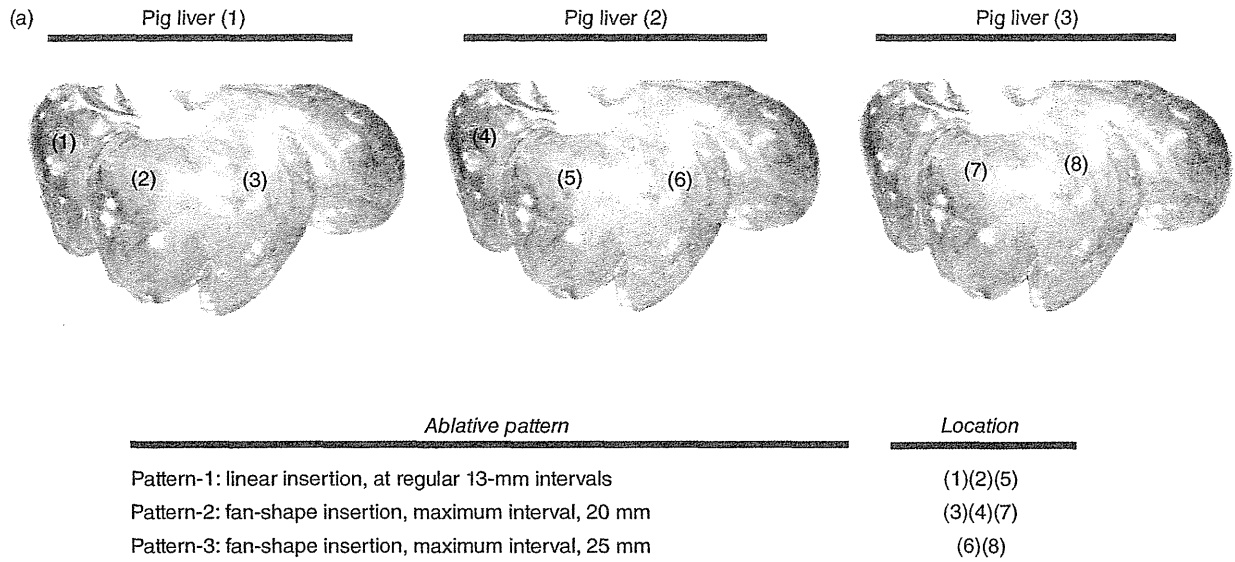
Table 1 summarizes the features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface.

In the median (range) transverse and longitudinal diameter to the shaft, ablative areas were: pattern 1, 32 (27–35) mm × 30 (30–35) mm; pattern 2, 27 (25–35) mm × 30 (30–32) mm; and pattern 3, 45 (40–50) × 26 (25–27) mm. There were no significant differences in the size of each ablative area among the three ablation procedures. However, with the pattern 3 procedure, the transverse diameter to the shaft was larger than with the other procedures, and as a result, the ablative form was flatter. On the other hand, patterns 1 and 2 acquired sufficient ablative areas that covered the liver surface with carbonization of the surface; however, with pattern 3, the ablative areas did not reach the liver surface, and carbonization of the liver surface was not apparent (Fig. 2b–d).

In addition, there were no significant differences among ablation procedures in the duration of ablative time.

DISCUSSION

WE OFTEN ENCOUNTER cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver

(a)

Pig liver (1)	Pig liver (2)	Pig liver (3)

Ablative pattern	Location
Pattern-1: linear insertion, at regular 13-mm intervals	(1)(2)(5)
Pattern-2: fan-shape insertion, maximum interval, 20 mm	(3)(4)(7)
Pattern-3: fan-shape insertion, maximum interval, 25 mm	(6)(8)

(b) Representative ablative images: pattern-1

T30 × 2 60W 25kj (1)

20   30   40   50   60   70   80   90   100   110   120   130

Dotted arrows indicate insertion line of electrode.
Arrowheads indicate ablative area (white zone).

★ Ablative margin (white zone) covered liver surface.
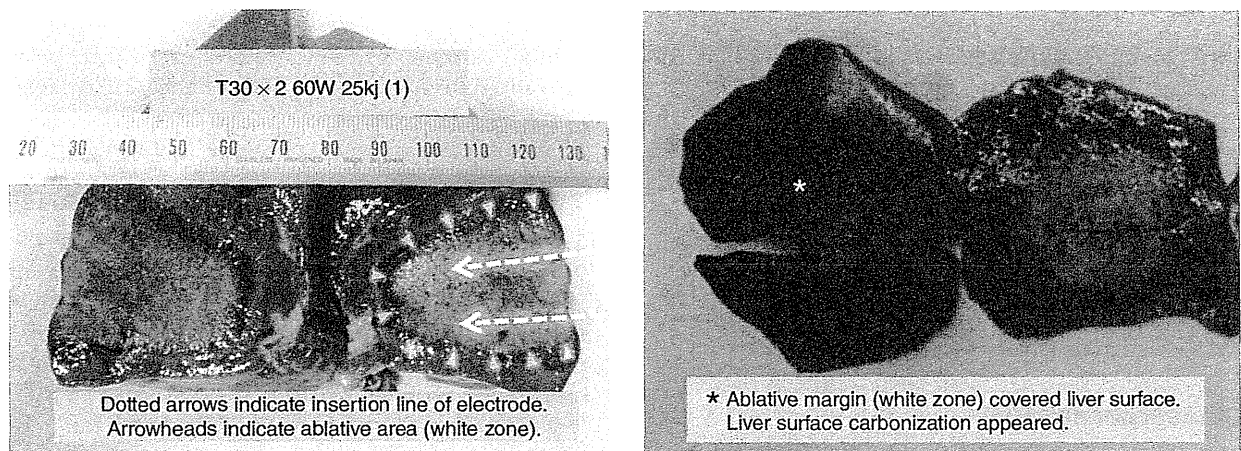Liver surface carbonization appeared.

Figure 2 (a) Schema of the ablative areas of each pincer ablation procedure in the three pig livers. (b) One of the ablative shapes and the margin achieved with the pattern 1 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we inserted the electrodes linearly (maximum interval for each electrode was 13 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (c) One of the ablative shapes and the margin achieved with the pattern 2 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 20 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (d) Ablative shape and margin achieved with the Pattern 3 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 25 mm). The ablative area close to the liver surface was larger than with the other procedures. However, the ablative margin did not cover the liver surface, and carbonization of the liver surface was not apparent.

© 2013 The Japan Society of Hepatology

(c)   Representative ablative images: pattern-2
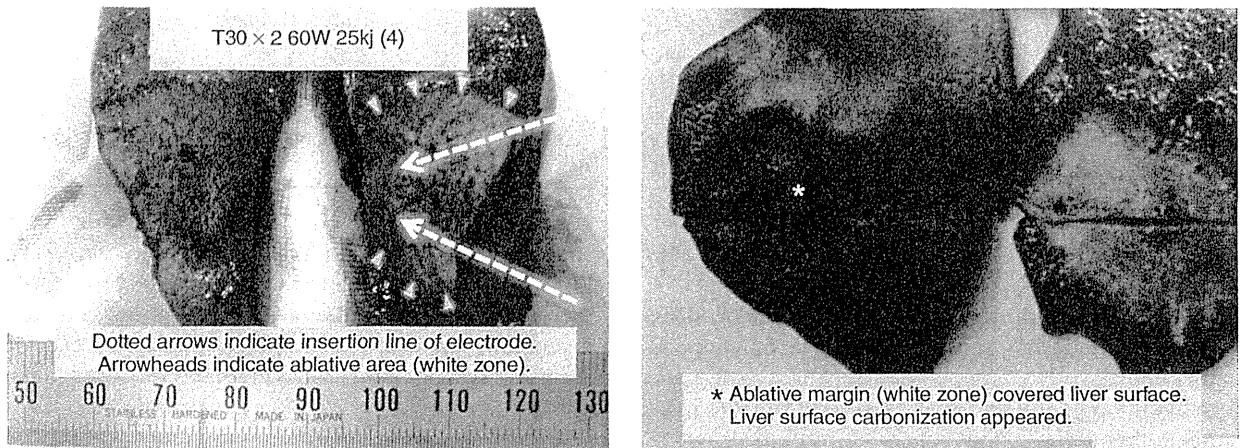
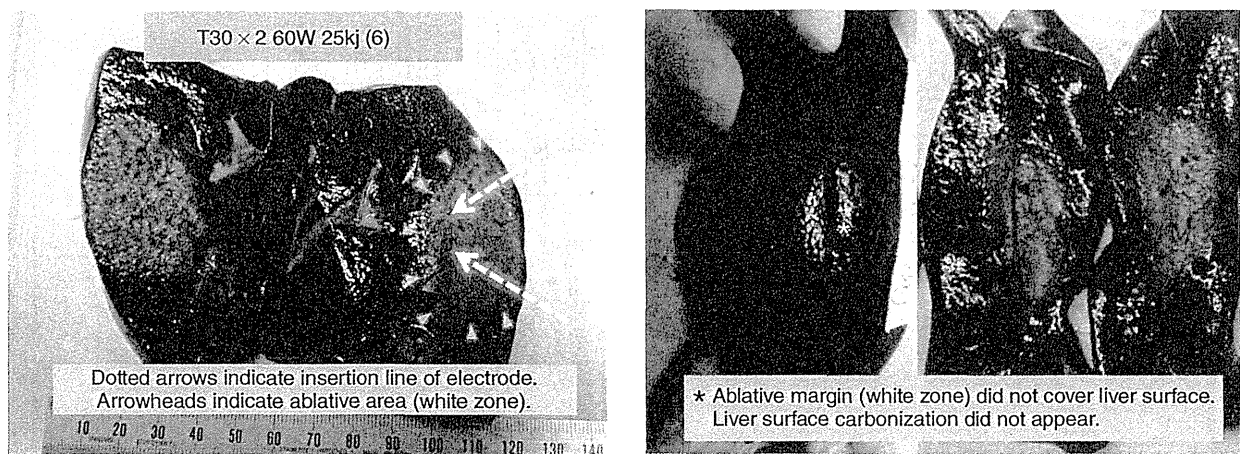(d)   Representative ablative images: pattern-3

Figure 2 *Continued*

surface. In these situations, a multipolar ablation system that uses internally cooled bipolar electrodes may be suitable for treatment. With a multipolar ablation system, we can combine the use of one to three electrodes at the same treatment session, and when three electrodes are used, this system can treat a large tumor. However, in the case of small tumors (<20 mm), it is not really necessary to use three electrodes for treatment of the target tumor. However, in the dosimetry table of this bipolar system in Figure 3, which was made from previously reported early clinical data[16] and basic analy-

sis, when two internally cooled bipolar electrodes are used (30 mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH), the recommended interval of each electrode in this system was 13 mm. With this regulation, we can treat only small tumors (<13 mm) when we perfume no-touch pincer ablation using two electrodes. Therefore, in this study we assumed a virtual target tumor with a tumor diameter less than 20 mm, and investigated the efficacy of a no-touch pincer ablation procedure and the maximum size of the tumor using two internally cooled bipolar electrodes for nodules that