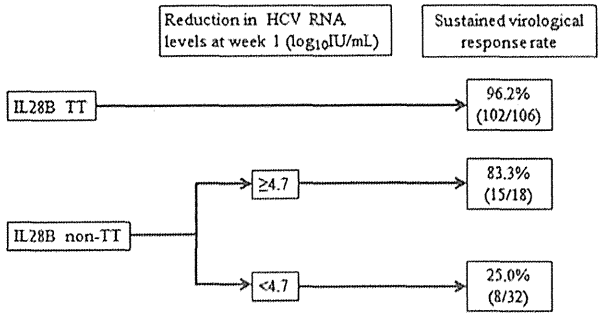

Fig. 4 Prediction of a sustained virological response (SVR) by the *IL28B* (rs8099917) genotype and reduction in HCV RNA level at week 1 after the start of therapy. In patients with the TT genotype, the SVR rate was high (96.2 %), regardless of the reduction in HCV RNA at week 1. In contrast, in patients with the non-TT genotype, the SVR rate was significantly higher in patients with a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA level at week 1 after the start of therapy than in those with a reduction of <4.7 log_{10}IU/mL in the HCV RNA levels at week 1 [15 of 18 patients (83.3 %) vs. 8 of 32 patients (25.0 %), $P = 0.0001$]

of ≥4.7 log_{10}IU/mL in the HCV RNA levels at week 1, the sensitivity, specificity, PPV, NPV, and accuracy for SVR were 62.5, 88.9, 83.3, 75.0, and 78.0 %, respectively. Furthermore, in patients with the non-TT genotype, when both a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA levels at week 1 and RVR were used, the sensitivity, specificity, PPV, NPV, and accuracy for SVR were 60.9, 100, 100, 75.0, and 82.0 %, respectively.

Discussion

Multiple logistic regression analysis revealed that the *IL28B* genotype was the most significant factor predicting SVR to a 24-week regimen of TVR-based triple combination therapy. The impact of the *IL28B* genotype on SVR found for this treatment regimen was in agreement with the findings of previous studies in Japan [7, 11, 14–16, 24]. In addition, a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA levels at week 1 after the start of therapy was identified as a strong independent on-treatment predictor for SVR in a multiple logistic regression analysis.

The reduction in HCV RNA levels at week 1 was particularly relevant in patients with the *IL28B* non-TT genotype. Whereas patients with the *IL28B* TT genotype showed a high SVR rate regardless of the on-treatment response of HCV RNA, a significant difference in SVR rate was observed based on the reduction in HCV RNA levels at week 1 in patients with the unfavorable non-TT genotype. In this patient subpopulation, the reduction in HCV RNA level at week 1 was the factor most strongly associated with SVR, and this finding is of clinical value to identify patients with a low likelihood of achieving SVR as early as possible. Furusyo et al. [11] previously reported that the serum HCV RNA levels at day 3 presented a significant difference between SVR and non-SVR patients. The ability of the very early viral response to predict SVR shown by both Furusyo et al. and our study may be explained by the strong antiviral effect of TVR. However, Furusyo et al. did not enter serum HCV RNA levels at day 3 into a multiple logistic regression analysis to identify significant independent predictors of SVR. Therefore, in that study, it was not clear whether the serum HCV RNA level at day 3 was an independent factor of SVR when including host-related, virus-related, and on-treatment factors. In the present study, the median serum HCV levels at week 1 was significantly lower for SVR patients (1.9 log_{10}IU/mL) than for non-SVR patients (2.2 log_{10}IU/mL) ($P = 0.0136$, data not shown). In the present study, the reduction in HCV RNA levels at week 1 after the start of therapy was an independent predictive factor for SVR. This reduction in HCV RNA level at week 1 may represent early viral kinetics closely correlated with the antiviral effect. The predictive ability of the reduction in HCV RNA level at day 3 and week 1 after the start of therapy should therefore be compared based on the *IL28B* genotype.

This study is the first report to demonstrate that a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA level at week 1 is a useful on-treatment predictive factor associated with SVR to a 24-week TVR-based triple combination therapy in clinical practice, especially in patients with the *IL28B* non-TT genotype. In 'real-world' clinical practice in some cases, it may be impossible to differentiate between previous null and partial responders because of the absence of relevant historical data from medical records. Therefore, for these treatment-experienced patients, *IL28B* genotyping may have clinical utility, as it may serve as a pretreatment marker for interferon responsiveness to guide patients and physicians. In patients with the *IL28B* non-TT genotype, both a reduction of <4.7 log_{10}IU/mL in the HCV RNA levels at week 1 and positivity for HCV RNA at week 4 (non-RVR) indicated a high likelihood of treatment failure. Hence, these patients should not undergo TVR-based triple combination therapy to avoid unnecessary treatment. This study identified that measurement of the HCV RNA level not only at week 4, but also at week 1, provides important information for predicting SVR, particularly in patients with the *IL28B* non-TT genotype.

There were some limitations to this study. First, the number of patients was too low to conclusively identify factors contributing to SVR. In particular, the number of non-responders was very small. Second, TVR-resistant variants were not analyzed. Resistant variants have been reported to occur in 56 % of HCV genotype 1b patients who did not achieve SVR [35]. Therefore, resistance variants should be identified in patients with treatment

failure. Third, this study regimen was limited to T12PR24. Only a 24-week TVR-based triple combination therapy (triple therapy for 12 weeks followed by an additional 12 weeks of PEG-IFN and RBV) is allowed by the Japanese National Insurance System. In the US, Canada, and EU, triple combination therapy is administered for either 12 or 36 additional weeks after PEG-IFN and RBV, according to the response-guided regimen based on the early viral response in each category, i.e., treatment-naïve patients and previous relapsers or partial responders and null responders.

Recently, the second-generation direct-acting antiviral agent simeprevir (SMV), which is once-daily oral NS3/4A protease inhibitor, was approved in September 2013 in Japan. Hayashi et al. [36] reported a Japanese phase II study. In treatment-naïve patients, the SVR rate was 77–92 % by triple combination therapy with SMV, PEG-IFN-α-2a and RBV. During the first 3–7 days of SMV-based therapy, an initial rapid reduction in HCV RNA was evident. Mean reduction in HCV RNA at week 1 in our study in TVR-based therapy was 4.5 $\log_{10}$IU/mL (data not shown). Mean reduction in HCV RNA at week 1 was not shown with the numerical value in this SMV-based therapy, but that seems to be similar to our TVR-based therapy. However, in this study, the *IL28B* genotypes were not investigated. Therefore, in clinical practice, from now on, prospective studies should be necessary to confirm whether the reduction in HCV RNA at week 1 is predictive for SVR in SMV-based therapy based on the *IL28B* genotype as well as in TVR-based therapy.

In conclusion, this prospective, multicenter study of a 24-week TVR-based triple combination therapy for Japanese genotype 1b CHC patients showed that the *IL28B* SNP genotype is the most important baseline factor for predicting SVR, and a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1, i.e., viral kinetics earlier than week 4, could be a useful on-treatment predictor of SVR, especially in patients with the *IL28B* non-TT genotype. Further large-scale prospective studies including SMV-based triple combination therapy are necessary to confirm these findings and develop the individual tailoring and optimization of therapeutics.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ghany MG, Nelson DR, Strader DB, American Association for Study of Liver Diseases, et al. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011;54:1433–44.
2. McHutchison JG, Everson GT, Gordon SC, PROVE1 Study Team, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med. 2009;360:1827–38.
3. Hézode C, Forestier N, Dusheiko G, PROVE2 Study Team, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med. 2009;360:1839–50.
4. Kumada H, Toyota J, Okanoue T, et al. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. J Hepatol. 2012;56:78–84.
5. McHutchison JG, Manns MP, Muir AJ, PROVE3 Study Team, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med. 2010;362:1292–303.
6. Zeuzem S, Andreone P, Pol S, REALIZE Study Team, et al. Telaprevir for retreatment of HCV infection. N Engl J Med. 2011;364:2417–28.
7. Chayama K, Hayes CN, Abe H, et al. IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and telaprevir triple therapy in patients with genotype 1 hepatitis C. J Infect Dis. 2011;204:84–93.
8. Muir AJ, Poordad FF, McHutchison JG, et al. Retreatment with telaprevir combination therapy in hepatitis C patients with well-characterized prior treatment response. Hepatology. 2011;54:1538–46.
9. Akuta N, Suzuki F, Seko Y, et al. Determinants of response to triple therapy of telaprevir, peginterferon, and ribavirin in previous non-responders infected with HCV genotype 1. J Med Virol. 2012;84:1097–105.
10. Hayashi N, Okanoue T, Tsubouchi H, et al. 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.
11. Furusyo N, Ogawa E, Nakamuta M, et al. Telaprevir can be successfully and safely used to treat older patients with genotype 1b chronic hepatitis C. J Hepatol. 2013;59:205–12.
12. Muir AJ. IL28B in the era of direct-acting antivirals for hepatitis C. J Clin Gastroenterol. 2013;47:222–7.
13. 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.
14. Akuta N, Suzuki F, Fukushima T, et al. Prediction of treatment efficacy and telaprevir-resistant variants after triple therapy in patients infected with HCV genotype 1. J Clin Microbiol. 2013;51:2862–8.
15. Tsubota A, Shimada N, Atsukawa M, et al. Impact of IL28B polymorphisms on 24-week telaprevir-based combination therapy for Asian chronic hepatitis C patients with HCV genotype 1b. J Gastroenterol Hepatol. 2013 (Epub ahead of print).
16. Shimada N, Tsubota A, Atsukawa M, et al. α-Fetoprotein is a surrogate marker for predicting treatment failure in telaprevir-based triple combination therapy for genotype 1b chronic hepatitis C Japanese patients with the IL28B minor genotype. J Med Virol. 2013 (Epub ahead of print).
17. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009;41:1105–9.
18. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet. 2009;41:1100–4.
19. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401.
20. Tsubota A, Shimada N, Yoshizawa K, et al. Contribution of ribavirin transporter gene polymorphism to treatment response in

peginterferon plus ribavirin therapy for HCV genotype 1b patients. Liver Int. 2012;32:826–36.
21. Toyoda H, Kumada T, Shimada N, et al. Baseline factors and early viral response (week 4) to antiviral therapy with peginterferon and ribavirin for predicting sustained virologic response in patients infected with hepatitis C virus genotype 1: a multicenter study. J Med Virol. 2013;85:65–70.
22. Toyoda H, Kumada T, Shimada N, et al. Significance of a reduction in HCV RNA levels at 4 and 12 weeks in patients infected with HCV genotype 1b for the prediction of the outcome of combination therapy with peginterferon and ribavirin. BMC Infect Dis. 2012;12:324.
23. Yoshizawa K, Abe H, Aida Y, et al. Serum apolipoprotein B-100 concentration predicts the virological response to pegylated interferon plus ribavirin combination therapy in patients infected with chronic hepatitis C virus genotype 1b. J Med Virol. 2013;85:1180–90.
24. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology. 2010;52:421–9.
25. Chayama K, Hayes CN, Ohishi W, et al. Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines. J Gastroenterol. 2013;48:1–12.
26. Editors of the Drafting Committee for Hepatitis Management Guidelines: The Japan Society of Hepatology. Guidelines for the management of hepatitis C virus infection: first edition, May 2012. The Japan Society of Hepatology. Hepatol Res. 2013;2013(43):1–34.
27. Jacobson IM, McHutchison JG, Dusheiko G, ADVANCE Study Team, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011;364:2405–16.
28. McHutchison JG, Lawitz EJ, Shiffman ML, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS, IDEAL Study Team, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med. 2009;361:580–93.
29. Sherman KE, Flamm SL, Afdhal NH, ILLUMINATE Study Team, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. N Engl J Med. 2011;365:1014–24.
30. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. Hepatology. 1994;20:15–20.
31. Simmonds P, Mellor J, Sakuldamrongpanich T, et al. Evolutionary analysis of variants of hepatitis C virus found in South-East Asia: comparison with classifications based upon sequence similarity. J Gen Virol. 1996;77:3013–24.
32. Akuta N, Suzuki F, Sezaki H, et al. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology. 2005;48:372–80.
33. Akuta N, Suzuki F, Kawamura Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol. 2007;46:403–10.
34. Enomoto N, Sakuma I, Asahina Y, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med. 1996;11(334):77–81.
35. Sullivan JC, De Meyer S, Bartels DJ, et al. Evolution of treatment-emergent resistant variants in telaprevir phase 3 clinical trials. Clin Infect Dis. 2013;57:221–9.
36. Hayashi N, Seto C, Kato M, et al. Once-daily simeprevir (TMC435) with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1-infected patients in Japan: the DRAGON study. J Gastroenterol. 2013 (Epub ahead of print).