

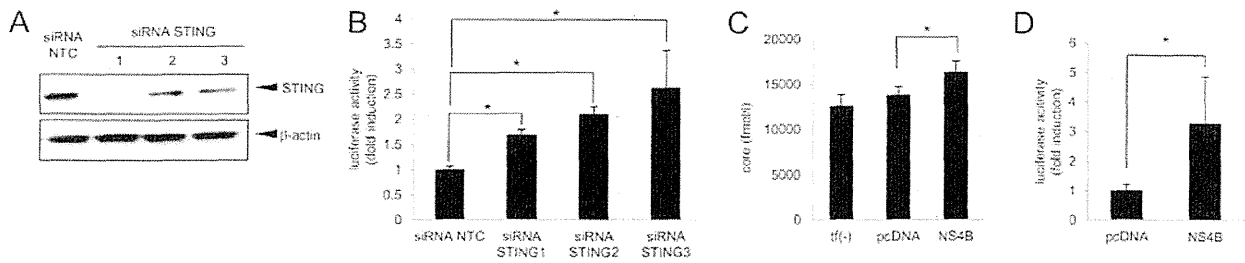
Fig. 5. Binding of NS4B to STING blocks molecular the interaction between Cardif and STING. (A,C) NS4B expression plasmid was cotransfected with STING or Cardif expression plasmid into HEK293T cells (A) or Huh7 cells (C). After 24 hours, cell lysates were subjected to immunoprecipitation using anti-HA or anti-Flag and were immunoblotted with anti-myc. (B,D) Cardif and STING expression plasmids were cotransfected with various amounts of NS4B plasmid in HEK293T cells (B) or Huh7 cells (D). After 24 hours, cells lysates were subjected to immunoprecipitation using anti-Flag and were immunoblotted with anti-HA.

interaction with STING, may hinder the direct molecular interaction between Cardif and STING. To verify this hypothesis, we performed immunoprecipitation assays. First, we transfected plasmids that expressed NS4B and Cardif, or NS4B and STING, in HEK293T cells or Huh7 cells, and performed immunoprecipitation. NS4B strongly bound to STING in both HEK293T cells and Huh7 cells, suggesting specific molecular interactions, whereas NS4B and Cardif did not show any obvious interaction (Fig. 5A,C). Consistent with previous reports, STING and Cardif showed significant interaction (Fig. 5B,D). Interestingly, those interactions were decreased by coexpression of NS4B, depending on its input amount, and finally blocked completely in both HEK293T and Huh7 cells (Fig. 5B,D). Collectively, the results above demonstrate that NS4B disrupts the interaction between Cardif and STING possibly through competitive binding to STING.

**Effects on HCV Infection and Replication Levels by STING Knockdown and NS4B Overexpression.** We next studied the impact of STING-mediated IFN production and its regulation by NS4B on HCV infection and cellular replication. First, we transfected three STING-targeted siRNAs into Huh7/Feo cells (Fig. 6A). As shown in Fig. 6B, STING knockdown cells conferred significantly higher permissibility to HCV replication. We next transfected HCV-JFH1 RNA into Huh7 cells that were transiently transfected with NS4B. As shown

in Fig. 6C, HCV core protein expression was significantly higher in NS4B-overexpressed cells. Furthermore, HCV replication was increased significantly in Huh7/Feo cells overexpressing NS4B (Fig. 6D). Taken together, the results above demonstrate that STING and NS4B may negatively or positively regulate cellular permissiveness to HCV replication.

**The N-terminal Domain of NS4B Is Essential for Suppressing IFN- $\beta$  Promoter Activity Mediated by RIG-I, Cardif, and STING.** It has been reported that the N-terminal domain of several forms of flaviviral NS4B shows structural homology with STING.<sup>24</sup> We therefore investigated whether the STING homology domain in NS4B is responsible for suppression of IFN- $\beta$  production. We constructed two truncated NS4B expression plasmids, which covered the N terminus (NS4Bt1-84, amino acids 1 through 84) containing the STING homology domain and the C terminus (NS4Bt85-261, amino acids 85 through 261), respectively (Fig. 7A). Immunoblotting showed that NS4Bt1-84 and NS4Bt85-261 yielded protein bands of ~9 kDa and ~20 kDa, respectively. Aberrant bands in the truncated NS4B may be due to alternative post-translational processing. HEK293T cells were transfected with  $\Delta$ RIG-I, Cardif, or STING, and NS3/4A or the truncated NS4B, along with IFN- $\beta$ -Fluc plasmid, and a reporter assay was performed. NS4Bt1-84 significantly suppressed RIG-I, Cardif, and STING-

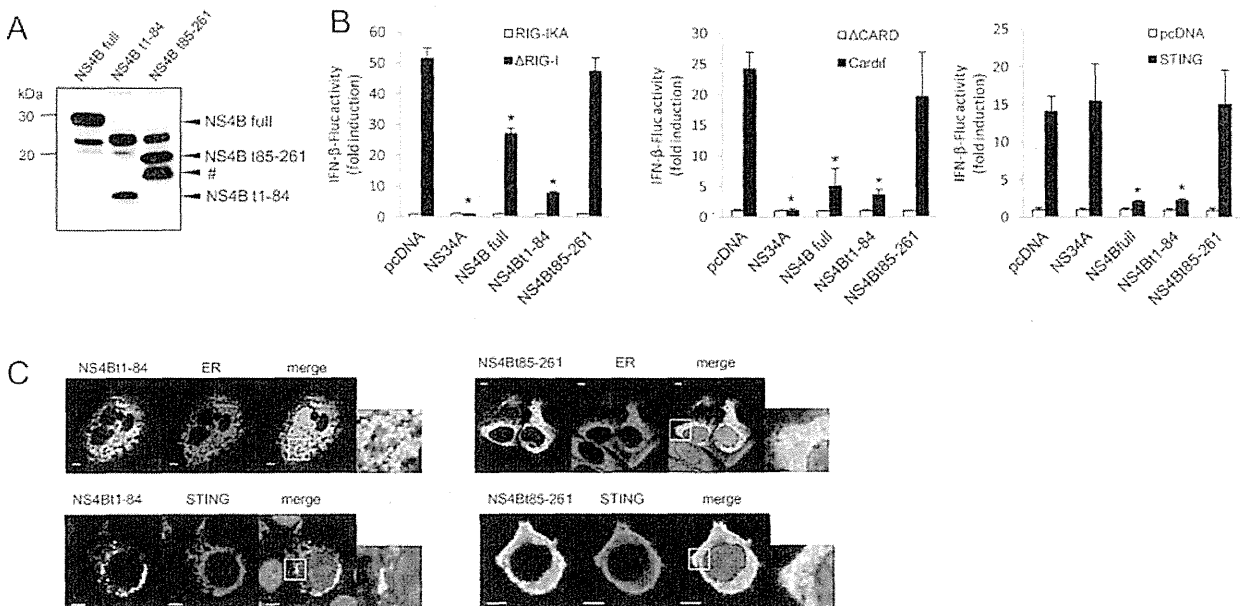


**Fig. 6.** Effects on HCV replication levels by STING knockdown and NS4B overexpression. (A) Effects of siRNA knockdown of STING by siRNA. Huh7 cells were transfected with STING-targeted siRNAs (siRNA STING-1, -2, and -3, respectively) or negative control siRNA (siRNA NTC). Seventy-two hours after transfection, cells were harvested and expression levels of STING protein were detected by immunoblotting. (B) Huh7 cells expressing HCV-Feo subgenomic replicon (Huh7/Feo)<sup>27,28</sup> were transfected with STING-targeted siRNAs or negative control siRNA. Seventy-two hours after transfection, cells were harvested, and internal luciferase activities were measured. The y axis indicates luciferase activity shown as a ratio of transfection-negative control. Assays were performed in triplicate, and error bars indicate the mean  $\pm$  SD. \* $P < 0.05$  compared with corresponding negative controls. (C) Empty plasmid or plasmid expressing NS4B was transfected into Huh7 cells. After 24 hours, HCV-JFH1 RNA was transfected into these cells. Seventy-two hours after virus transfection, HCV core antigen levels in culture medium were measured. Assays were performed in triplicate, and error bars indicate the mean  $\pm$  SD. \* $P < 0.05$  compared with corresponding negative controls. (D) Huh7 cells expressing HCV-Feo replicon (Huh7/Feo)<sup>27,28</sup> were transfected with NS4B expressing plasmid or empty plasmid (pcDNA). Forty-eight hours after transfection, internal luciferase activities were measured. The y axis indicates luciferase activity shown as a ratio of the transfection-negative control. Assays were performed in triplicate, and error bars indicate the mean  $\pm$  SD. \* $P < 0.05$  compared with corresponding negative controls.

induced IFN- $\beta$  promoter activity, whereas NS4Bt85-261 did not (Fig. 7B). These results suggest that the N-terminal domain of NS4B is responsible for association with STING. Fluorescent microscopy indicated

that both NS4Bt1-84 and NS4Bt85-261 colocalized with ER and STING (Fig. 7C).

**NS4B Suppresses IFN Production Signaling Cooperatively with NS3/4A.** It has been reported that



**Fig. 7.** The N-terminal domain of NS4B is essential for suppressing IFN- $\beta$  promoter activity induced by RIG-I, Cardif, or STING. (A) Immunoblotting of NS4B and truncated NS4B, NS4B t1-84, and NS4Bt85-261. HEK293T cells were transfected with NS4B or truncated NS4B. After 24 hours, the cells were lysed and immunoblot assays were performed. The band indicated by the pound sign (#) is a truncated NS4B, probably generated via alternative posttranslational processing. (B) Plasmids expressing  $\Delta$ RIG-I, Cardif, or STING as well as NS3/4A or the indicated truncated form of NS4B were cotransfected with pIFN- $\beta$ -Fluc and pRL-CMV in HEK293T cells. Dual luciferase assays were performed 24 hours after transfection. Plasmids expressing RIG-IKA,  $\Delta$ CARD, or pcDNA were used as negative controls. The y axis indicates IFN- $\beta$ -Fluc activity shown as relative values. Assays were performed in triplicate, and error bars indicate the mean  $\pm$  SD. \* $P < 0.05$  compared with corresponding negative controls. (C) Plasmids expressing NS4Bt1-84-myc or NS4Bt85-261-myc were transfected with or without plasmids expressing HA-STING in HEK293T cells. After 24 hours, the cells were fixed and immunostained. Nuclei were stained with DAPI. Cells were observed by confocal microscopy. Scale bars indicate 5  $\mu$ m.

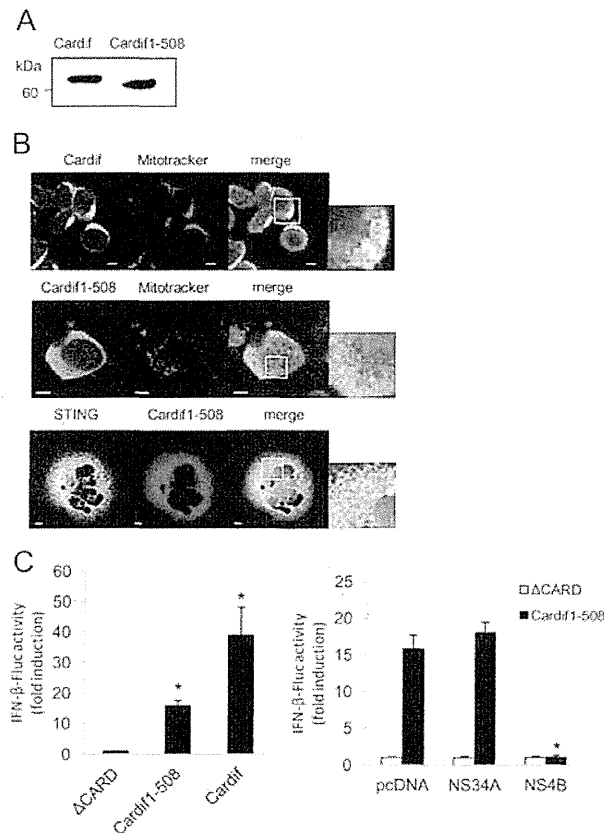


Fig. 8. NS4B suppressed IFN- $\beta$  production pathway independently of and cooperatively with NS3/4A. (A) Immunoblotting of Cardif and truncated Cardif (Cardif1-508). HEK293T cells were transfected with Cardif or truncated Cardif (Cardif1-508). After 24 hours, the cells were lysed and immunoblot assays were performed. (B) Subcellular localization of Cardif and truncated Cardif (Cardif1-508). HEK293T cells were immunostained with anti-Cardif antibody or HEK293T cells were transfected with myc-tagged truncated Cardif (Cardif1-508-myc), and after 24 hours the cells were immunostained with anti-myc. Mitochondria were stained with Mitotracker (red) and nuclei were stained with DAPI (blue). Plasmid expressing myc-tagged truncated Cardif (Cardif1-508) and plasmid expressing HA-tagged STING were transfected into HEK293T cells. The cells were immunostained with anti-myc and anti-HA antibodies and analyzed by confocal laser microscopy. Scale bars = 10  $\mu$ m. (C) Plasmids expressing Cardif or truncated Cardif (Cardif1-508) and pIFN- $\beta$ -Fluc and pRL-CMV were transfected with or without plasmid expressing NS3/4A or NS4B into HEK293T cells as indicated. Dual luciferase assays were performed 24 hours after transfection. Plasmid expressing  $\Delta$ CARD or pcDNA was used as a negative control. The y axis indicates IFN- $\beta$ -Fluc activity shown as relative values. Assays were performed in triplicate, and error bars indicate the mean  $\pm$  SD. \* $P$  < 0.05.

HCV NS3/4A serine protease cleaves Cardif between Cys-508 and His-509, releases Cardif from the mitochondrial membrane, and blocks RIG-I-induced IFN- $\beta$  production. We next assessed whether NS4B suppresses IFN- $\beta$  production in the presence of Cardif cleaved by NS3/4A protease (Cardif1-508, Fig. 8A). The truncation of Cardif-C-terminal residue abolished mitochondrial localization but still colocalized with

STING (Fig. 8B). The reporter assay showed that Cardif1-508 induced weak IFN- $\beta$  activation. Interestingly, NS4B completely blocked the residual function of the Cardif1-508 protein to activate IFN- $\beta$  expression, suggesting an additive effect of NS3/4A and NS4B on the RIG-I-activating pathway (Fig. 8C).

## Discussion

It has been reported that viruses, including HCV, target IFN signaling to establish persistent replication in host cells.<sup>35</sup> We have reported that NS4B blocks the transcriptional activation of ISRE induced by overexpression of RIG-I and Cardif, but not by TBK1 or IKK $\epsilon$ .<sup>19</sup> In the present study, we have shown that NS4B directly and specifically binds STING, an ER-residing scaffolding protein of Cardif and TBK1 and an inducer of IFN- $\beta$  production (Figs. 3 and 5), and blocked the interaction between STING and Cardif (Fig. 5B,D) resulting in strong suppression of RIG-I-mediated phosphorylation of IRF-3 and expressional induction of IFN- $\beta$  (Fig. 1). Furthermore, HCV replication was increased by knock-down of STING or overexpression of NS4B (Fig. 6). Taken together, our results demonstrate that HCV-NS4B strongly blocks virus-induced, RIG-I-mediated activation of IFN- $\beta$  production signaling through targeting STING, which constitutes a novel mechanism of viral evasion from innate immune responses and establishment of persistent viral replication.

Our results also showed that the effects of NS4B on the RIG-I signaling were independent of NS3/4A-mediated cleavage of Cardif. Reporter assays showed that a cleaved form of Cardif (Cardif1-508) partially retained activity for the induction of IFN- $\beta$  promoter activation. The residual IFN- $\beta$  promoter activation was suppressed almost completely by NS4B but not by NS3/4A (Fig. 8C). These findings show that there are at least two mechanisms by which HCV can abrogate RIG-I-mediated IFN production signaling to accomplish abrogation of cellular antiviral responses.

NS4B and STING are ER proteins,<sup>20,21,40</sup> whereas Cardif is localized on the outer mitochondrial membrane.<sup>9</sup> Consistent with those reports, our immunostaining experiments demonstrated that most NS4B protein colocalized with STING (Fig. 2), and their association was localized on MAM (Fig. 2E). In addition to the significant colocalization of STING and NS4B, STING partially colocalized with Cardif at the boundary region of the two proteins (Fig. 2B). Furthermore, immunoprecipitation experiments showed that overexpression of NS4B completely blocked the interaction of STING with Cardif (Fig. 5B). Ishikawa et al.<sup>24</sup> reported

that STING could associate with Cardif by MAM interaction. Castanier et al.<sup>41</sup> reported that Cardif-STING interaction was enhanced in cells with elongated mitochondria. In addition, Horner et al.<sup>42,43</sup> observed NS3/4A targeting of MAM-anchored synapse and cleavage of Cardif at MAM but not in mitochondria. These results led us to speculate that interaction between STING and Cardif was enhanced by altering their subcellular localization during viral infection and that NS4B inhibits Cardif activation by interfering with the association between STING and Cardif on MAM-like NS3/4A behavior against host innate immunity.

HCV-NS4B is an ER-localized 27-kDa protein with several functions in the HCV life cycle. Cellular expression of NS4B induces convolution of the ER membrane and formation of a membranous web that harbors HCV replicase complex.<sup>44,45</sup> NS4B also has RNA-binding capacity.<sup>46</sup> In addition, several point mutations of NS4B were found to alter viral replication activity.<sup>33,46,47</sup> The studies above indicate that NS4B provides an important protein-protein or protein-RNA interaction platform within the HCV replication complex and is essential for viral RNA replication. However, there are few reports on the involvement of NS4B with antiviral immune responses. Consistent with our previous study, Moriyama et al.<sup>48</sup> reported that NS4B partially inhibited dsRNA-induced but not TRIF-induced activation of IFN- $\beta$ . In NS4B-expressing cells, IFN- $\alpha$  induced activation of STAT1 was suppressed.<sup>49</sup> The present study has demonstrated that NS4B functions against the host IFN response, such that NS4B directly interacts with STING and suppresses downstream signaling, resulting in the induction of IFN production.

STING contains a domain homologous to the N terminus of NS4B derived from several flaviviruses, including HCV. In our previous NS4B truncation assay, the NS4B N-terminal domain (amino acids 1-110) was important for suppression of RIG-I-induced IFN- $\beta$  expression.<sup>19</sup> Consistent with these results, N-terminally truncated NS4B (NS4Bt1-84) significantly suppressed STING and Cardif-induced IFN- $\beta$  promoter activation, whereas the C terminus of NS4B (NS4Bt85-261) did not (Fig. 7). These results reinforce our hypothesis that NS4B binds STING at its homology domain and blocks the ability of STING to induce IFN- $\beta$  production.

A small molecule inhibitor of NS4B has been developed and is under preliminary clinical trials.<sup>50</sup> Einav et al.<sup>51</sup> identified clemizole hydrochloride, an H1 histamine receptor antagonist, as an inhibitor of the RNA-binding function of NS4B and HCV RNA replication. A phase 1B clinical trial of clemizole in hepati-

tis C patients has been completed.<sup>52</sup> Other two NS4B inhibitors which are a compound of amiloride analog and anguizole are under preclinical development.<sup>53,54</sup> The possibility remains that such NS4B inhibitors may suppress HCV replication partly through inhibiting the ability of NS4B to suppress IFN- $\beta$  production and restore cellular antiviral responses.

In conclusion, IFN production signaling induced by HCV infection and mediated by RIG-I is suppressed by NS4B through a direct interaction with STING. These virus-host interactions help to elucidate the mechanisms of persistent HCV infection and constitute a potential target to block HCV infection.

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## References

- Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev* 2001; 14:778-809.
- Taniguchi T, Takaoka A. The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr Opin Immunol* 2002;14:111-116.
- Sakamoto N, Watanabe M. New therapeutic approaches to hepatitis C virus. *J Gastroenterol* 2009;44:643-649.
- Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. *J Virol* 2001;75:7059-7066.
- Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004;5:730-737.
- Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, et al. 5'-Triphosphate RNA is the ligand for RIG-I. *Science* 2006;314:994-997.
- Takahashi K, Yoneyama M, Nishihori T, Hirai R, Kumeta H, Narita R, et al. Nonself RNA-sensing mechanism of RIG-I helicase and activation of antiviral immune responses. *Mol Cell* 2008;29:428-440.
- Kawai T. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005;6:981-988.
- Serh RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF- $\kappa$ B and IRF 3. *Cell* 2005;122:669-682.
- Xu LG. VISA is an adapter protein required for virus-triggered IFN- $\beta$  signaling. *Mol Cell* 2005;19:727-740.
- Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 2005;437: 1167-1172.
- Yoneyama M, Sahara W, Fukuhara Y, Fukuda M, Nishida E, Fujita T. Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. *EMBO J* 1998;17:1087-1095.

13. Lin W, Kim SS, Yeung E, Kamegaya Y, Blackard JT, Kim KA, et al. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. *J Virol* 2006;80:9226-9235.
14. Suda G, Sakamoto N, Itsui Y, Nakagawa M, Tasaka-Fujita M, Funaoka Y, et al. IL-6-mediated intersubgenotypic variation of interferon sensitivity in hepatitis C virus genotype 2a/2b chimeric clones. *Virology* 2010;407:80-90.
15. Funaoka Y, Sakamoto N, Suda G, Itsui Y, Nakagawa M, Kakinuma S, et al. Analysis of interferon signaling by infectious hepatitis C virus clones with substitutions of core amino acids 70 and 91. *J Virol* 2011;85:5986-5994.
16. Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM, et al. Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. *Proc Natl Acad Sci U S A* 2006;103:6001-6006.
17. Li X-D, Sun L, Seth RB, Pineda G, Chen ZJ. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci U S A* 2005;102:17717-17722.
18. Baril M, Racine M-E, Penin F, Lamarre D. MAVS Dimer Is a Crucial Signaling Component of Innate Immunity and the Target of Hepatitis C Virus NS3/4A Protease. *J Virol*. 2009;83:1299-1311.
19. Tasaka M, Sakamoto N, Itakura Y, Nakagawa M, Itsui Y, Sekine-Osajima Y, et al. Hepatitis C virus non-structural proteins responsible for suppression of the RIG-I/Cardif-induced interferon response. *J Gen Virol* 2007;88:3323-3333.
20. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 2008;455:674-678.
21. Sun W, Li Y, Chen L, Chen H, You F, Zhou X, et al. ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization. *Proc Natl Acad Sci U S A* 2009;106:8653-8658.
22. Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, et al. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 2008;29:538-550.
23. Jin L. MPYS, a novel membrane tetraspanner, is associated with major histocompatibility complex class II and mediates transduction of apoptotic signals. *Mol Cell Biol* 2008;28:5014-5026.
24. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 2009;461:788-792.
25. Yanagi M, Purcell RH, Emerson SU, Bukh J. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. *Proc Natl Acad Sci U S A* 1997;94:8738-8743.
26. Lin R, Iacoste J, Nakhaei P, Sun Q, Yang L, Paz S, et al. Dissociation of a MAVS/IPS-1/VISA/Cardif-IKKepsilon molecular complex from the mitochondrial outer membrane by hepatitis C virus NS3-4A proteolytic cleavage. *J Virol* 2006;80:6072-6083.
27. Yokota T, Sakamoto N, Enomoto N, Tanabe Y, Miyagishi M, Maekawa S, et al. Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. *EMBO Rep* 2003;4:602-608.
28. Tanabe Y, Sakamoto N, Enomoto N, Kurosaki M, Ueda E, Maekawa S, et al. Synergistic inhibition of intracellular hepatitis C virus replication by combination of ribavirin and interferon- alpha. *J Infect Dis* 2004;189:1129-1139.
29. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005;11:791-796.
30. Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, et al. Complete replication of hepatitis C virus in cell culture. *Science* 2005;309:623-626.
31. Nakagawa M, Sakamoto N, Enomoto N, Tanabe Y, Kanazawa N, Koyama T, et al. Specific inhibition of hepatitis C virus replication by cyclosporin A. *Biochem Biophys Res Commun* 2004;313:42-47.
32. Yamashiro T, Sakamoto N, Kurosaki M, Kanazawa N, Tanabe Y, Nakagawa M, et al. Negative regulation of intracellular hepatitis C virus replication by interferon regulatory factor 3. *J Gastroenterol* 2006;41:750-757.
33. Lindstrom H, Lundin M, Haggstrom S, Persson MA. Mutations of the hepatitis C virus protein NS4B on either side of the ER membrane affect the efficiency of subgenomic replicons. *Virus Res* 2006;121:169-178.
34. Hayashi T, Rizzuto R, Hajnoczky G, Su TP. MAM: more than just a housekeeper. *Trends Cell Biol* 2009;19:81-88.
35. Lewin TM, Van Horn CG, Krisans SK, Coleman RA. Rat liver acyl-CoA synthetase 4 is a peripheral-membrane protein located in two distinct subcellular organelles, peroxisomes, and mitochondrial-associated membrane. *Arch Biochem Biophys* 2002;404:263-270.
36. Simmen T, Aslan JE, Blagoveschenskaya AD, Thomas L, Wan L, Xiang Y, et al. PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. *EMBO J* 2005;24:717-729.
37. Kerppola TK. Design and implementation of bimolecular fluorescence complementation (BiFC) assays for the visualization of protein interactions in living cells. *Nat Protoc* 2006;1:1278-1286.
38. Kerppola TK. Bimolecular fluorescence complementation (BiFC) analysis as a probe of protein interactions in living cells. *Annu Rev Biophys* 2008;37:465-487.
39. Kato H. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 2006;441:101-105.
40. Saichou T, Fujita N, Hayashi T, Takahara K, Satoh T, Lee H, et al. Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. *Proc Natl Acad Sci U S A* 2009;106:20842-20846.
41. Castanier C, Garcin D, Vazquez A, Arnoult D. Mitochondrial dynamics regulate the RIG-I-like receptor antiviral pathway. *EMBO Rep* 2009;11:133-138.
42. Horner SM, Liu HM, Park HS, Briley J, Gale M. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. *Proc Natl Acad Sci U S A* 2011;108:14590-14595.
43. Horner SM, Park HS, Gale M Jr. Control of innate immune signaling and membrane targeting by the hepatitis C virus NS3/4A protease are governed by the NS3 helix  $\alpha 0$ . *J Virol* 2012;86:3112-3120.
44. Egger D, Wolk B, Gosert R, Bianchi L, Blum HE, Moradpour D, et al. Expression of Hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex. *J Virol* 2002;76:5974-5984.
45. Gretton SN, Taylor AI, McLauchlan J. Mobility of the hepatitis C virus NS4B protein on the endoplasmic reticulum membrane and membrane-associated foci. *J Gen Virol* 2005;86:1415-1421.
46. Einav S, Elazar M, Danieli T, Glenn JS. A nucleotide binding motif in hepatitis C virus (HCV) NS4B mediates HCV RNA replication. *J Virol* 2004;78:11288-11295.
47. Elazar M, Liu P, Rice CM, Glenn JS. An N-terminal amphipathic helix in hepatitis C virus (HCV) NS4B mediates membrane association, correct localization of replication complex proteins, and HCV RNA replication. *J Virol* 2004;78:11393-11400.
48. Moriyama M, Kato N, Otsuka M, Shao RX, Taniguchi H, Kawabe T, et al. Interferon-beta is activated by hepatitis C virus NS5B and inhibited by NS4A, NS4B, and NS5A. *Hepato Int* 2007;1:302-310.
49. Xu J, Liu S, Xu Y, Tien P, Gao G. Identification of the nonstructural protein 4B of hepatitis C virus as a factor that inhibits the antiviral activity of interferon-alpha. *Virus Res* 2009;141:55-62.
50. Hofmann WP, Zeuzem S. A new standard of care for the treatment of chronic HCV infection. *Nat Rev Gastroenterol Hepatol* 2011;8:257-264.
51. Einav S, Gerber D, Bryson PD, Sklan EH, Elazar M, Maerkl SJ, et al. Discovery of a hepatitis C target and its pharmacological inhibitors by microfluidic affinity analysis. *Nat Biotech* 2008;26:1019-1027.
52. Rai R, Deval J. New opportunities in anti-hepatitis C virus drug discovery: targeting NS4B. *Antiviral Res* 2011;90:93-101.
53. Cho NJ, Dvory-Sobol H, Lee C, Cho SJ, Bryson P, Masek M, et al. Identification of a class of HCV inhibitors directed against the non-structural protein NS4B. *Sci Transl Med* 2010;2:15ra16.
54. Bryson PD, Cho NJ, Einav S, Lee C, Tai V, Bechtel J, et al. A small molecule inhibits HCV replication and alters NS4B's subcellular distribution. *Antiviral Res* 2010;87:1-8.

## Special Report

# Guidelines for the Management of Hepatitis C Virus Infection

First edition, May 2012, The Japan Society of Hepatology

Editors of the Drafting Committee for Hepatitis Management Guidelines: The Japan Society of Hepatology<sup>\*,\*\*</sup>

## 1. INTRODUCTION

THE JAPAN SOCIETY of Hepatology (JSH) has, until now, produced “A Management Guide for Chronic Hepatitis and Liver Cirrhosis”, “A Management Guide for NASH and NAFLD”, and “A Treatment Manual for Hepatocellular Carcinoma”. The only official guidelines produced by the Society have been the “Clinical Practice Guidelines for Hepatocellular Carcinoma Based on Scientific Evidence”, however, and we had not yet developed guidelines for hepatitis.

As a scientific body that promotes hepatology research, we considered it necessary to publish our official position on the diagnosis and treatment of hepatitis. The regular JSH board meeting on 19 October 2011

approved the establishment of the Drafting Committee for Hepatitis Management Guidelines.

The Committee decided that our first priority was the production of guidelines for the management of hepatitis C, most urgently needed by Society members, so we began with the production of these “Guidelines for the Management of Hepatitis C Virus Infection (First Edition)”. We hope and anticipate that these guidelines will be used throughout Japan in the management of hepatitis C.

This is a field that changes rapidly with the accumulation of new evidence, accompanied by changes in the level of evidence, so we have elected not to show evidence levels. We plan to revise these guidelines at appropriate intervals, as new evidence comes to hand.

Reproduction of these guidelines is forbidden without authorization.

May 2012

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## 2. GENERAL STRATEGY AGAINST HEPATITIS C VIRUS INFECTION

FOLLOWING THE IDENTIFICATION of the hepatitis C virus (HCV) by Choo *et al.* in the USA in 1989,<sup>1</sup> it became clear that over 90% of patients previously diagnosed with non-A non-B hepatitis, and over 50% of those diagnosed with alcoholic hepatitis, in fact suffered from liver disease caused by HCV. Currently, there are an estimated 170 million carriers worldwide, and 1.5–2 million in Japan. Even in healthy adults, once an HCV infection occurs, only approximately 30% resolve completely in the acute phase. HCV

infection is prolonged in approximately 70% of cases, causing chronic hepatitis. Once an HCV infection has become chronic, spontaneous elimination of the virus is rare (0.2% annual rate), and persistent inflammation can induce fibrosis, progressing to cirrhosis or hepatocellular carcinoma (HCC).<sup>2</sup> Interferon (IFN) therapy commenced in 1986, when Hoofnagle *et al.* administered human recombinant IFN- $\alpha$  to patients with non-A non-B hepatitis, confirming normalization of transaminase levels.<sup>3</sup> IFN therapy has been used in the general clinical setting in Western countries since 1991, and in Japan since 1992. Since that time, with the development of the polymerase chain reaction (PCR) method, a revolutionary new technology for viral detection, quiescence of hepatitis has been confirmed in patients in whom HCV RNA was eradicated by IFN therapy;<sup>4</sup> furthermore, inhibition of progression of liver disease and hepatocellular carcinogenesis has been demonstrated in these patients.<sup>5-8</sup>

The aim of treatment of chronic hepatitis C is to improve the long-term prognosis of chronic liver disease (CLD) associated with persistent HCV infection; in other words, to prevent mortality associated with HCC and CLD. Sustained virological response (SVR) rates have improved with the standard therapy combining pegylated interferon (Peg-IFN) and ribavirin. SVR rates are no better than 40-50% in patients with genotype 1 infection who have high viral loads, however, so HCV cannot be eliminated in around half of these patients. In recent years, a number of new antiviral agents have been developed with the aims of increased therapeutic efficacy and decreased adverse reactions. In November 2011, the first generation protease inhibitor telaprevir became available for clinical use in patients with HCV genotype 1 infection and high viral loads. Triple therapy with telaprevir, Peg-IFN- $\alpha$ -2b and ribavirin has shown an increased antiviral effect, improving initial SVR rates to around 70% in treatment-naïve cases, but adverse reactions are also increased, including severe anemia and serious skin rashes.<sup>9-13</sup> In Japan, trials are underway with triple therapy comprising a second generation protease inhibitor (TMC435,<sup>14</sup> MK7009<sup>15</sup> or BI-201335), Peg-IFN and ribavirin, as well as IFN-free oral antiviral therapy comprising a protease inhibitor and an NS5A inhibitor.<sup>16</sup> Much is anticipated from the next generation direct antiviral agents (DAA), reported to have considerably fewer adverse reactions, and even greater antiviral effects, with SVR rates exceeding 80% in treatment-naïve cases.

Therapeutic guidelines for chronic hepatitis C should be formulated with the above-mentioned background

in mind, with careful consideration of the appropriateness of the presently available antiviral therapies for each individual patient.

### Indications for antiviral therapy for HCV infection

In general, in patients with chronic hepatitis C, liver disease progresses gradually in association with elevation of alanine aminotransferase (ALT) levels, and the risk of developing cancer increases with the progression of fibrosis.<sup>8</sup> Conversely, cancers are rarely seen arising from a normal liver with no inflammation or fibrosis. Accordingly, in general, antiviral therapy is indicated in all chronic hepatitis C patients with elevated ALT levels (ALT >30 IU/L), indicating hepatic inflammation, or a decreased platelet count (platelet count <150 000/ $\mu$ L), reflecting the degree of liver fibrosis. The indication for antiviral therapy should be individualized for patients with ALT  $\leq$ 30 IU/L and a platelet count  $\geq$ 150 000/ $\mu$ L, considering the risk of developing HCC is low.

Early viral eradication is required in the group at high risk of developing cancer. In patients with HCV infection, three factors have been identified as independent risk factors for hepatocellular carcinogenesis: (i) advanced age; (ii) advanced fibrosis; and (iii) male sex.<sup>3-7</sup> Accordingly, the risk of developing cancer is particularly high in patients with multiple risk factors, and early introduction of antiviral therapy should be considered in this group.

### Basic guidelines for treatment of chronic hepatitis C

In developing these guidelines, we formulated separate treatment plans according to the risk of developing cancer in different subgroups of patients with chronic hepatitis C, for elderly and non-elderly patients, and those with advanced fibrosis and mild fibrosis. Analyses of hepatocellular carcinogenesis in older patients with chronic hepatitis C show that the risk of cancer increases with increasing age, although the definition of "older age" varies, considered by some to be greater than 55, 60 or 65 years. In these guidelines, we have defined "elderly" as  $\geq$ 66 years old, based on Japanese clinical trials of telaprevir conducted with subjects aged  $\leq$ 65 years,<sup>11</sup> and the increased risk of HCC over the age of 65 years.<sup>17</sup> Furthermore, although we have defined "advanced fibrosis" as a METAVIR score  $\geq$ F2, or platelet count of <150 000/ $\mu$ L, it should be kept in mind that the risk of cancer is particularly high in the

patient group with a METAVIR score  $\geq$ F3, or platelet count of  $<$ 120 000/ $\mu$ L.

For the group at high risk of developing HCC (elderly and advanced fibrosis), antiviral therapy should be commenced as soon as possible with due consideration to tolerability. Early commencement of antiviral therapy is also desirable in the medium-risk group (elderly or advanced fibrosis). However, some in the particularly high-risk group, elderly and/or with advanced fibrosis, are non-responders, so in order to avoid adverse reactions and the development of drug-resistant mutations, the treatment discontinuation criteria should be kept in mind during antiviral therapy. On the other hand, in the low-risk group comprising non-elderly patients without advanced fibrosis, early introduction of antiviral therapy is not always necessary. In some patients, it may be possible to await the introduction of the new generation antiviral agents, so the present indication for antiviral therapy should be decided after consideration of anticipated therapeutic effect, adverse reactions and the risk of HCC.

In any patient group, in case it is difficult with any presently available antiviral regimens to ensure viral eradication, and ALT levels are elevated ( $\geq$ 30 IU/L), patients should be administered long-term low-dose Peg-IFN or supportive therapy, for example, stronger neo-minophagen C (SNMC), ursodeoxycholic acid (UDCA). If an adequate therapeutic effect is not achieved, and iron overload is suspected, then the addition of, or changeover to, therapeutic phlebotomy should be considered. The aim of these therapies is to keep the ALT level  $\leq$ 30 IU/L, maintaining it as low as possible. Strict control of the ALT level is particularly necessary in the group at high risk of developing HCC. Low-dose Peg-IFN therapy should be discontinued if no improvement is seen within 6 months in the ALT level (to  $\leq$ 40 IU/L) or the  $\alpha$ -fetoprotein (AFP) level (to  $\leq$ 10 ng/mL).<sup>18,19</sup>

#### Recommendations:

- 1 In general, antiviral therapy is indicated in all chronic hepatitis C patients with elevated ALT levels ( $>$ 30 IU/L) or a decreased platelet count ( $<$ 150 000/ $\mu$ L).
- 2 The indication for antiviral therapy should be individualized for patients with ALT levels  $\leq$ 30 IU/L and a platelet count  $\geq$ 150 000/ $\mu$ L, considering the risk of developing HCC is low.
- 3 For the group at high risk of developing HCC (elderly and advanced fibrosis), antiviral therapy should be commenced as soon as possible with due consideration to tolerability.

4 Following commencement of antiviral therapy in patients either elderly or with advanced fibrosis, in order to avoid adverse reactions and the development of drug-resistant mutations, the treatment discontinuation criteria, used for the early detection of non-responders, should be kept in mind during antiviral therapy.

5 In the low-risk group (non-elderly, non-advanced fibrosis), the present indication for antiviral therapy should be decided after consideration of anticipated therapeutic effect, adverse reactions and the risk of HCC.

6 If viral eradication is not achieved, long-term low-dose Peg-IFN or supportive therapy (SNMC or UDCA) should be administered with the aim of preventing progression of liver disease and preventing hepatocellular carcinogenesis. If an adequate therapeutic effect is not achieved, and iron overload is suspected, then the addition of, or changeover to, therapeutic phlebotomy should be considered.

7 Low-dose Peg-IFN therapy should be discontinued if no improvement is seen within 6 months in the ALT level (to  $\leq$ 40 IU/L) or the AFP level (to  $\leq$ 10 ng/mL).

### 3. INTERFERON THERAPY

#### 3.1 Interferon

THE  $\alpha$ - AND  $\beta$ -types of IFN have been approved for use in the treatment of chronic hepatitis C. IFN- $\alpha$  preparations come in non-pegylated and pegylated forms, depending on whether polyethylene glycol (PEG) has been attached. The former comes in the form of natural human IFN- $\alpha$  and recombinant IFN- $\alpha$ -2b, and the latter as Peg-IFN- $\alpha$ -2a and Peg-IFN- $\alpha$ -2b. IFN- $\beta$  preparations comprise natural non-pegylated-IFN- $\beta$ .

#### IFN- $\alpha$

Standard non-pegylated-IFN- $\alpha$  is unstable, with a plasma half-life of 3–8 h, and becomes undetectable after 24 h.<sup>20</sup> Administration at least three times per week is therefore required when treating chronic hepatitis C. Adverse reactions, including fever, chills and headache, are common with non-pegylated-IFN due to repeated rises and falls in the plasma levels. Of the non-pegylated IFNs, natural human IFN- $\alpha$  is approved for self-injection, and patients only need to attend hospital once every 2 weeks. Furthermore, patients can self-inject at night before retiring, better taking advantage of diurnal variations in plasma cortisol levels, and minimizing fever and other adverse reactions.<sup>21–23</sup>



### Peg-IFN- $\alpha$

PEG is a water-soluble neutral molecule with no toxicity of itself. The number of ethylene oxide subunits determines the molecular weight. The aims of pegylating IFN are twofold: (i) to alter its *in vivo* pharmacodynamic properties; and (ii) protect the IFN molecule from recognition and elimination by the host immune defenses. Peg-IFN- $\alpha$  used in the treatment of chronic hepatitis C comes in the form of Peg-IFN- $\alpha$ -2a, with a 40-kD PEG branch chain covalently attached to IFN- $\alpha$ -2a, and Peg-IFN- $\alpha$ -2b, with a 12-kD PEG branch chain attached via a urethane bond to IFN- $\alpha$ -2b. They reach a maximum concentration (Cmax) at 72–96 and 15–44 h after administration, respectively, and after a single dose maintain plasma levels within the therapeutic range for approximately 168 and 80 h, respectively.<sup>24</sup> As the molecular weight of PEG attached to IFN in this way increases, the intracorporeal retention time also increases, although the pharmacological effect decreases in inverse proportion. The IFN activity of Peg-IFN- $\alpha$ -2a is 7% that of non-pegylated-IFN- $\alpha$ -2a, whereas the IFN activity of Peg-IFN- $\alpha$ -2b is 28% that of non-pegylated-IFN- $\alpha$ -2b, with the latter more active. Accordingly, the actual antiviral effect is determined in a complex fashion by the balance between intracorporeal retention time and IFN activity, as well as the patient's body type and weight. Peg-IFN- $\alpha$ -2a is approved as monotherapy and in combination with ribavirin for national medical insurance coverage, whereas Peg-IFN- $\alpha$ -2b is approved in combination with ribavirin with or without telaprevir.

The two forms of Peg-IFN- $\alpha$  have different standard doses. The standard dosage regimen for Peg-IFN- $\alpha$ -2a is fixed at 180  $\mu$ g/week, and the dose of Peg-IFN- $\alpha$ -2b varies according to the patient's weight, the standard dosage regimen being 1.5  $\mu$ g/kg per week.

### IFN- $\beta$

Interferon- $\beta$  is a natural IFN that can be used in a non-pegylated form, and is approved as monotherapy and in combination with ribavirin for medical insurance coverage. It is administered at least three times per week as an i.v. injection or i.v. infusion. Although IFN- $\beta$  binds to the same type I IFN receptor as IFN- $\alpha$ , and has a similar antiviral effect to IFN- $\alpha$ , their adverse reaction profiles differ. A retrospective study of natural human IFN- $\beta$  + ribavirin combination therapy in the treatment of 40 cases with genotype 1b HCV infections reported fewer discontinuations due to adverse reactions, and only mild decreases in platelet counts.<sup>25</sup> Even patients with a history of discontinuing IFN- $\alpha$  therapy due

to depression tolerated IFN- $\beta$  + ribavirin combination therapy well in terms of depressive symptoms and other adverse reactions.<sup>26–28</sup> IFN therapy with natural human IFN- $\beta$  is therefore recommended in patients in whom IFN- $\alpha$  therapy is not tolerated, for example, those with a history of depression.

Anti-IFN- $\alpha$  neutralizing antibodies were detected in 15% of non-responders to Peg-IFN- $\alpha$  + ribavirin therapy in one study.<sup>29</sup> Anti-IFN- $\alpha$  neutralizing antibodies do not block IFN- $\beta$  activity, so a changeover to natural human IFN- $\beta$  should be considered in cases of non-response to Peg-IFN- $\alpha$  + ribavirin due to these neutralizing antibodies.

Natural human IFN- $\beta$  can be administered twice daily in divided doses, providing a more potent antiviral effect than once daily dosing as measured by the HCV dynamics.<sup>30</sup> Divided dosing IFN- $\beta$  induction prior to Peg-IFN- $\alpha$  + ribavirin therapy has been trialed.<sup>31</sup>

### Antiviral effects of IFN<sup>32–34</sup>

IFN acts through binding to type I IFN receptors on the target cell membrane. Type I IFN receptors are common to IFN- $\alpha$  and IFN- $\beta$ , and binding of either IFN type to the receptor causes activation of the tyrosine-protein kinase, Janus kinase 1 (JAK1). This induces phosphorylation of tyrosine residues in the intracellular domain of the receptor, resulting in phosphorylation and formation of dimer complexes of signal transducer and activator of transcription 1 (STAT1), which transmit signal to the cell nucleus. This in turn induces and upregulates expression of IFN-stimulated genes (ISG). The family of ISG includes a wide variety of antiviral and immunoregulatory genes, and the antiviral effects of IFN are thought to derive from proteins induced by ISG.

### Adverse reactions

Adverse reactions to IFN therapy are experienced by almost all patients. The most common are influenza-like symptoms, such as general malaise, fever, headache and aching joints, and are reported by 60–95% of patients. Most influenza-like syndrome can be controlled with anti-inflammatory analgesic medication. Blood tests show leukopenia, with white blood cell counts <1000/mm<sup>3</sup> seen in approximately 60% of patients. Serious infections associated with neutropenia are, however, considered rare.<sup>35</sup> White blood cell, neutrophil and platelet counts tend to decrease for the first 4 weeks of IFN therapy, then often remain stable without further decline. Neuropsychiatric symptoms such as depression and insomnia occur in 5–10% of patients, and are more

common in those with pre-existent neuropsychiatric symptoms or a history of depression.<sup>36</sup> Neuropsychiatric symptoms are classified into depression-specific symptoms and depression-related autonomic nervous symptoms, with selective serotonin re-uptake inhibitors (SSRI) reported to be useful in treating the former.<sup>37–39</sup> IFN can also trigger or aggravate autoimmune diseases such as chronic thyroiditis, so the utmost caution is required when administering IFN to patients with autoimmune diseases. Interstitial pneumonia, another reported adverse reaction to IFN therapy, can be serious and even life-threatening. It usually occurs after 2 months of therapy, or in the later stages of treatment. A rapid and appropriate management is required following the onset of respiratory symptoms such as a dry cough or dyspnea, including an immediate chest CT scan. Determination of serum KL-6 levels is also useful in the diagnosis of interstitial pneumonia. Other reported adverse reactions to IFN therapy include cardiomyopathy and fundal hemorrhage.

The adverse reaction profile of Peg-IFN differs somewhat to that of non-pegylated-IFN. In a Japanese clinical trial of Peg-IFN- $\alpha$ -2a monotherapy, the adverse reactions with a higher reported frequency than non-pegylated-IFN- $\alpha$ -2a were skin reactions such as erythema at the injection site and hematological reactions such as decreases in the white blood cell counts or platelet counts. On the other hand, mild to moderate adverse reactions such as influenza-like syndrome, including fever and joint pains, or malaise and loss of appetite, were milder than with standard non-pegylated-IFN- $\alpha$ -2a.<sup>40</sup>

#### Recommendations:

- 1 Reported adverse reactions to IFN therapy include influenza-like syndrome, decrease of blood cell counts, neuropsychiatric symptoms, autoimmune phenomena, interstitial pneumonia, cardiomyopathy and fundal hemorrhage.
- 2 Pegylation stabilizes serum IFN levels, ameliorating influenza-like syndrome such as fever and joint pains.
- 3 Patients self-injecting of natural human IFN- $\alpha$  at night minimizes influenza-like syndrome.
- 4 IFN- $\beta$  should be considered in patients unable to tolerate IFN- $\alpha$  due to depression or other causes.

#### Is there any difference between Peg-IFN- $\alpha$ -2a and Peg-IFN- $\alpha$ -2b therapeutic efficacy and adverse reactions?

In Japan at present, two Peg-IFN formulations are available for use in Peg-IFN + ribavirin therapy, Peg-IFN-

$\alpha$ -2a and Peg-IFN- $\alpha$ -2b. McHutchison *et al.* conducted a large multi-center study comparing the efficacy of these two agents. In this randomized controlled trial (RCT) conducted at 118 institutions, with 3070 patients with IFN-naive genotype 1 HCV infection, the SVR rate in the Peg-IFN- $\alpha$ -2a 180- $\mu$ g group was 40.9% and that in the Peg-IFN- $\alpha$ -2b group 39.8%, with no difference seen between groups, and no significant difference was seen between groups in terms of tolerability.<sup>41</sup> On the other hand, two Italian single-center studies with 441 and 320 patients with IFN-naive genotype 1–4 HCV infection, respectively, found no significant difference between groups in the incidence of adverse events, but reported significantly higher SVR rates in the Peg-IFN- $\alpha$ -2a group than in the Peg-IFN- $\alpha$ -2b group.<sup>42,43</sup> A recent systematic review examining 12 RCT of the efficacy and safety of these two agents found no difference between them in terms of adverse events causing discontinuation. The overall SVR rates based on 8 RCT were 47% for the Peg-IFN- $\alpha$ -2a group and 41% for the Peg-IFN- $\alpha$ -2b group, significantly higher in the former (risk ratio, 1.11; 95% confidence interval [CI], 1.04–1.19;  $P = 0.004$ ).<sup>44</sup> However, a conclusion has not been reached to recommend either agent, due to heterogeneity of the patient populations in HCV genotype, race and Peg-IFN- $\alpha$ -2b dosage in the different RCT, as well as problems with the quality of the RCT in terms of subject numbers and withdrawals, and only limited data concerning adverse events. In Japan, studies have been conducted comparing both agents, but the final results have yet to be published.

Accordingly, at present, Peg-IFN- $\alpha$ -2a and Peg-IFN- $\alpha$ -2b are considered similar from the viewpoints of efficacy and adverse reactions, and there is no definitive evidence supporting a recommendation of either formulation in clinical practice. To improve therapeutic efficacy further, more important considerations will be optimization of the dosage and duration of treatment with other agents, such as ribavirin, for each individual patient, as well as formulation of a treatment plan with consideration of factors that influence therapeutic efficacy for each patient, and control of adverse reactions.

#### Inhibition of HCC by IFN monotherapy

Many reports have emerged from Japan regarding inhibition of hepatocellular carcinogenesis by IFN therapy. Ikeda *et al.*<sup>6</sup> performed a retrospective analysis of cumulative hepatocellular carcinogenesis rates in patients with chronic hepatitis C who underwent IFN monotherapy as initial treatment, stratified for therapeutic

efficacy. The 10-year hepatocellular carcinogenesis rate was 12.0% in the untreated group ( $n = 452$ ), 15.0% in the IFN nonresponsive group, with no SVR and abnormal ALT levels ( $n = 1076$ ) and 1.5% in the SVR group ( $n = 676$ ), significantly lower in the latter. Even in the incomplete response group, with no SVR but normalization of ALT levels ( $n = 298$ ), the 10-year hepatocellular carcinogenesis rate was 2.0%, indicating suppression of HCC.<sup>6</sup> Imai *et al.*<sup>45</sup> and Kasahara *et al.*<sup>7</sup> have reported similar results, with IFN therapy inhibiting hepatocellular carcinogenesis in the normalized ALT group. Furthermore, Yoshida *et al.*<sup>8</sup> conducted a large-scale retrospective study with 2890 patients, reporting that IFN therapy and resultant SVR reduce the risk of developing HCC, including patients in whom ALT levels improved to within two times the upper limit of normal. They further reported that the calculated rate of progression of hepatic fibrosis was  $-0.28$ /year in IFN responders, indicating amelioration of hepatic fibrosis associated with viral clearance, and even in patients who failed to respond to IFN, the rate of progression of  $-0.2$ /year indicated inhibition of progression of hepatic fibrosis.<sup>8</sup> Okanoue *et al.* also reported inhibitory effect on development of HCC dependent on the degree of progression of hepatic fibrosis, and amelioration of fibrosis with IFN therapy.<sup>46</sup> Nishiguchi *et al.* conducted a prospective study with patients with HCV-associated cirrhosis, finding HCV eradication or prolonged normalization of ALT levels by IFN therapy significantly reduced the risk of HCC and liver failure.<sup>47</sup>

Overseas, Di Bisceglie *et al.* conducted the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial, a prospective randomized controlled study of whether low-dose Peg-IFN- $\alpha$  maintenance therapy can reduce the rate of liver disease-associated events, including HCC, in non-responders to Peg-IFN- $\alpha$  + ribavirin therapy. They recruited a cohort of 1050 HCV-infected patients with bridging fibrosis or cirrhosis who had not had an SVR to previous therapy with Peg-IFN- $\alpha$  + ribavirin therapy, and randomly allocated them to a group administered Peg-IFN- $\alpha$ -2a 90  $\mu$ g/week for 3.5 years or an untreated control group. They compared incidences during the observation period of outcome variables including the following: death, development of HCC, development of hepatic decompensation and exacerbation of histological fibrosis. After 3.8 years of observation in both groups, 157 patients reached one of the end-points, 34.1% of the treatment group and 33.8% of the control group, with no significant difference seen between groups (hazard ratio [HR], 1.01; 95% CI, 0.81–1.27).<sup>48</sup> They also exam-

ined the risk of hepatocellular carcinogenesis in this cohort, with 48 patients (4.8%) developing HCC during the observation period. The 5-year cumulative HCC rate was 5.4% in the treatment group and 5.0% in the control group, with no significant difference seen between groups ( $P = 0.78$ ).<sup>49</sup> The conclusion was that, at this stage, low-dose Peg-IFN- $\alpha$ -2a maintenance therapy does not reduce the rate of liver disease-associated events, including HCC, in non-responders to Peg-IFN- $\alpha$  + ribavirin therapy. Similar results were achieved in a study using Peg IFN- $\alpha$ -2b.<sup>50</sup>

However, Lok *et al.* recently published the results of an extended analysis of the HALT-C cohort. Extending the observation period beyond the previous analysis to a median 6.1 years (greatest, 8.7 years), they reported HCC in 88 patients (8.4%). Including both patients with and without cirrhosis, the 7-year cumulative HCC rate was 7.2% in the treatment group and 9.6% in the control group, with no significant difference seen between groups (HR, 0.77; 95% CI, 0.51–1.18;  $P = 0.24$ ), showing no clear inhibition of hepatocellular carcinogenesis by IFN therapy. If we limit the analysis to patients with cirrhosis, however, the 7-year cumulative HCC rate was 7.8% in the treatment group and 24.2% in the control group, showing a significant reduction in the risk of HCC with low-dose Peg-IFN- $\alpha$ -2a maintenance therapy (HR, 0.45; 95% CI, 0.24–0.83;  $P = 0.01$ ). However, this effect was not significant in patients without cirrhosis, as the 7-year cumulative HCC rate was 8.3% in the treatment group and 6.8% in the control group, actually tending to be higher in the group administered Peg-IFN- $\alpha$  (HR, 1.44; 95% CI, 0.77–2.69;  $P = 0.26$ ).<sup>51</sup>

Inhibition of hepatocellular carcinogenesis by low-dose Peg-IFN- $\alpha$ -2a monotherapy was also examined in Japan in a multicenter collaborative trial. The subjects were 59 patients administered Peg-IFN- $\alpha$ -2a monotherapy and a control group comprising 59 patients matched for age, sex, degree of fibrosis, platelet counts and serum bilirubin levels. They reported a significantly lower cumulative HCC rate in the Peg-IFN- $\alpha$ -2a monotherapy group ( $P = 0.0187$ ) with a relative risk (RR) of 0.167. The reduction in risk of HCC was particularly marked in patients with advanced fibrosis (F3–4) (RR, 0.0847;  $P = 0.0036$ ). Even in patients who failed to eradicate HCV RNA, the HCC rate was significantly lower in those who achieved either an ALT level  $<40$  IU/L or AFP  $<10$  ng/mL at treatment week 24.<sup>49</sup> Improvement in ALT and AFP levels with Peg-IFN- $\alpha$ -2a monotherapy has been reported in other Japanese studies.<sup>52,53</sup>

We can understand that with extension of the observation period, the results of the HALT-C trial confirm that low-dose Peg-IFN- $\alpha$  maintenance therapy reduces the risk of HCC in patients with cirrhosis. The effect was unclear on analysis of the entire cohort in patients without cirrhosis, however, and the results suggest that reduction in the risk of HCC did not emerge until after at least 4 years of low-dose Peg-IFN- $\alpha$  maintenance therapy. On the other hand, the above-mentioned Japanese studies confirmed that IFN therapy significantly reduced the incidence of HCC in patients with sustained normalization of ALT levels, even if SVR was not achieved. In the above-mentioned multicenter collaborative trial of Peg-IFN- $\alpha$ -2a monotherapy, a significant reduction in the HCC was seen, even when patients without cirrhosis were included, and with shorter observation periods. In this way, the results of the HALT-C trial do not agree with Japanese findings. Possible reasons for this discrepancy may include the fact that the average age of the HALT-C cohort was 52 years, younger than the average age of Japanese patients with chronic hepatitis C, and the low overall incidence of HCC. Asahina *et al.* reported that in Japanese patients with chronic hepatitis C, even with the same degree of fibrosis the risk of HCC is considerably higher in older patients, whereas in patients with cirrhosis, there is no significant difference in the risk of HCC associated with aging.<sup>17</sup> We cannot therefore exclude the possibility that differences between Japan and the USA in the ages of patients with chronic hepatitis C and the risk of HCC influenced the results of the HALT-C trial for patients without cirrhosis. Furthermore, there were a considerable number of deaths and liver transplantation events in the HALT-C cohort, the incidences of which were significantly different between patients on low-dose Peg-IFN maintenance therapy and control groups among patients without cirrhosis.<sup>54</sup> These deaths and liver transplantation events give rise to bias in analyses of the risk of HCC. From the above, a certain degree of caution is needed in interpreting the results of the HALT-C trial.

#### Inhibition of HCC by IFN monotherapy in the elderly

As mentioned above, Japanese patients with chronic hepatitis C are older than their Western counterparts, and the risk of developing HCC is higher in elderly patients, even after correction for other risk factors. Although the risk of HCC is significantly reduced with SVR, even in elderly patients, in comparison with younger patients they are more likely to fail to achieve

SVR, and to discontinue treatment due to adverse reactions.<sup>17</sup> With these considerations of therapeutic efficacy and adverse reactions in mind, in Japan, long-term IFN monotherapy is widely used in elderly patients, the aim of treatment being inhibition of hepatocellular carcinogenesis by reducing inflammation rather than viral eradication.

Arase *et al.*<sup>18</sup> examined the reduction in hepatocellular carcinogenesis by IFN therapy in a study with 120 subjects aged  $\geq 60$  years with either chronic hepatitis C or liver cirrhosis. They were treated with natural IFN- $\alpha$  3 MU three times weekly for an average of 2.47 years, and compared with an age- and sex-matched control group not administered IFN comprising 240 subjects. As a result, the 10-year HCC rate was 17.3% in the IFN group and 32.8% in the control group, with an RR of 0.3. AFP levels decreased significantly in the IFN group than in the control group, and the incidence of HCC was particularly low in patients with AFP levels  $< 10$  ng/mL.<sup>18</sup> Nomura *et al.* also conducted a study with 44 patients with genotype 1 HCV infection aged  $\geq 60$  years. They were treated with natural IFN 3 MU three times weekly for 3 years, and compared with a control group not administered IFN, matched for age, sex and hepatic histological findings, comprising 44 subjects. They reported a significantly lower cumulative rate of HCC in the IFN group.<sup>55</sup>

#### Recommendations:

- 1 Eradication of HCV by IFN therapy lowers the risk of HCC.
- 2 Even if HCV cannot be eradicated, we can expect the risk of developing HCC to be reduced through lowering ALT or AFP levels by long-term natural IFN- $\alpha$  therapy or long-term Peg-IFN- $\alpha$ -2a monotherapy.

#### Inhibition of HCC recurrence by IFN therapy

Not only is IFN administered with the aim of inhibiting hepatocellular carcinogenesis in patients with chronic hepatitis C and cirrhosis who have not yet developed HCC, it is also given to patients who have undergone complete ablation or resection of HCC nodules with the aims of preventing recurrence and improving survival rates. Shiratori *et al.*<sup>56</sup> randomly allocated patients who had undergone complete ablation of HCC nodules, using ethanol injection, to a group administered IFN for 48 weeks and an untreated control group, comparing recurrence rates and survival rates. They reported no significant difference between groups in the rate of first recurrence; however, the rates of second and subsequent recurrences were significantly lower in the IFN group, as

were survival rates. These results indicated the usefulness of IFN therapy following complete tumor ablation.<sup>56</sup> Sakaguchi *et al.*<sup>57</sup> and Kudo *et al.*<sup>58</sup> administered low-dose long-term IFN- $\alpha$ -2b or IFN- $\alpha$ -2a therapy to 127 patients with HCC who had undergone complete ablation. Comparison with an untreated control group matched for sex, age and platelet count showed a significant reduction in recurrences after the first recurrence, and a significant improvement in survival rates, the RR for survival being 0.21.<sup>57,58</sup> Another study of IFN + ribavirin therapy following hepatic arterial embolization or radiofrequency ablation reported viral eradication in half of patients, with lower tumor recurrence rates and prolonged survival.<sup>59</sup>

**Recommendation:**

*IFN therapy following complete ablation of HCC can be expected to reduce tumor recurrence rates and improve survival rates.*

**Necessity of follow up of patients who achieved an SVR**

SVR is defined as undetectable levels of serum HCV RNA 24 weeks after the completion of IFN treatment. HCV RNA clearance is usually sustained in cases of SVR, with HCV RNA remaining undetectable in 99–100% over the average 5.6-year observation period (range, 1–8.3 years) in patients with SVR to IFN + ribavirin therapy.<sup>60,61</sup> In studies conducted prior to 2000, however, a somewhat lower proportion at 96–98% of patients remained serum HCV RNA negative.<sup>62–66</sup> Possible causes for this discrepancy are that IFN monotherapy was the mainstay in the earlier studies, and that the sensitivity of testing for HCV RNA was lower at the time, suggesting the possibility of false-positive assessments of SVR.

As described above, achievement of SVR gives sustained clearance of HCV RNA, and significantly reduces the risk of HCC in patients with chronic hepatitis C.<sup>6–8,45,46</sup> However, HCC has been reported to develop during follow up even in patients who have achieved SVR. A number of Japanese studies have addressed hepatocellular carcinogenesis following SVR, with reported HCC rates of 0.9–4.2% over mean observation periods of 3.3–8.0 years. Risk factors for HCC include advanced age, male sex, advanced fibrosis, alcohol consumption, hepatic steatosis and insulin resistance. The interval between achieving SVR and detection of HCC is most often reported as being within 10 years, although some studies reported an interval of greater than 10 years.<sup>8,17,46,67–71</sup> There is a lack of consensus regarding how long patients should be screened for

HCC following SVR, but depending on the risk factors for hepatocellular carcinogenesis in each individual patient, screening for HCC should continue for at least 5–10 years after achieving SVR.

**Recommendation:**

*Risk factors for developing HCC in virological responders include advanced age, male sex, advanced fibrosis, alcohol consumption, hepatic steatosis and insulin resistance. Even following SVR, screening for HCC should continue with consideration of the known risk factors for each individual.*

**3.2 Ribavirin**

Ribavirin, a purine nucleotide analog with a chemical structure resembling guanosine, shows antiviral activity against a wide range of RNA and DNA viruses.<sup>72</sup> Proposed mechanisms for the actions of ribavirin include T-helper cell 1 dominant immune induction, induction of viral mutagenesis, inhibition of RNA polymerase and depletion of intracellular guanosine triphosphate pools.<sup>73</sup> Ribavirin monotherapy for chronic hepatitis C improves ALT levels, but does not decrease HCV RNA or improve liver histology.<sup>74–76</sup> However, IFN- $\alpha$ -2b + ribavirin combination therapy is superior to IFN- $\alpha$ -2b monotherapy in terms of viral clearance and improved ALT levels.<sup>77</sup>

Ribavirin is generally used in combination with one of the Peg-IFN preparations, Peg-IFN- $\alpha$ -2a or Peg-IFN- $\alpha$ -2b. In comparison with Peg-IFN monotherapy, higher end-of-treatment HCV RNA clearance rates are achieved with Peg-IFN + ribavirin combination therapy, but most importantly, the addition of ribavirin markedly decreases the risk of relapse following completion of treatment.<sup>78,79</sup> Presently in Japan, apart from the Peg-IFN preparations, ribavirin can also be used in combination with standard non-pegylated-IFN- $\alpha$ -2a or IFN- $\beta$ . When the pretreatment hemoglobin (Hb) level is  $\geq 14$  g/dL, the daily dose of ribavirin is 600 mg for patients weighing  $\leq 60$  kg, 800 mg for 61–80 kg and 1000 mg for  $>80$  kg.<sup>80,81</sup>

**Therapeutic results**

The efficacy of Peg-IFN + ribavirin combination therapy was confirmed in two Japanese phase III clinical trials.<sup>82,83</sup> In Japanese clinical studies with patients with genotype 1b chronic hepatitis C with high viral loads ( $>100$  kIU/mL), the SVR rate with 48 weeks of Peg-IFN- $\alpha$ -2b + ribavirin therapy was 48% (121/254), and that for 48 weeks of Peg-IFN- $\alpha$ -2a + ribavirin therapy was

59% (57/96).<sup>83,84</sup> In another study, other than with genotype 1b and high viral loads, a high SVR rate of 89% (40/45) was achieved with 24 weeks of Peg-IFN- $\alpha$ -2b + ribavirin therapy.<sup>85</sup>

### Adverse reactions

Ribavirin is administered p.o. twice daily, after breakfast and dinner. The peak plasma concentration is reached 1–2 h after ingestion, and with repeated administration plasma levels reach equilibrium after 4–8 weeks of treatment. Ribavirin accumulates in the body, remaining in the liver, muscle and within erythrocytes for long periods. Ribavirin is mostly eliminated by the renal route, and caution is required when prescribing to patients with renal disease or impaired renal function. It is contraindicated in patients with a creatinine clearance  $\leq 50$  mL/min. Ribavirin is not eliminated by dialysis, so it is generally contraindicated in patients with renal failure on dialysis.

The main adverse reaction to ribavirin is hemolytic anemia, so caution is required when considering ribavirin therapy in patients with anemia or heart conditions (e.g. ischemic heart disease, heart failure, arrhythmia). In a Japanese clinical trial of Peg-IFN- $\alpha$ -2b + ribavirin combination therapy, treatment was discontinued in 8–11% of patients, and the ribavirin dose reduced in 20%, due to anemia. Dose reduction was more common in patients with a pretreatment Hb  $< 14$  g/dL, neutrophil count  $< 2000/\mu\text{L}$  or platelet count  $< 120\,000/\mu\text{L}$ , and in females. In particular, a reduction in the dose of Peg-IFN or ribavirin was required in 80% of patients aged  $\geq 65$  years with a pretreatment Hb  $\leq 13$  g/dL. The rate of discontinuation of treatment was high in patients with a decline in Hb  $\geq 2$  g/dL at 2 weeks from the start of treatment, so the authors suggest reducing the ribavirin dose by 200 mg/day at this point.<sup>86</sup> The criteria for ribavirin dose reduction or discontinuation when a decline in Hb occurs during treatment (in patients without heart conditions) are: reduce the daily dose by 200 mg (400 mg if started at 1000 mg) for Hb  $< 10$  g/dL, and discontinue if Hb is  $< 8.5$  g/dL.<sup>80,81</sup> In one of the above-mentioned Japanese clinical studies, the SVR rate was 62.5% when no reduction in the IFN or ribavirin dose was needed, 45.7–53.3% when a dose reduction or temporary withdrawal was needed, falling to 19.2% when treatment was discontinued.<sup>83</sup> Accordingly, to achieve SVR it is important to control any decline in Hb appropriately, complete the treatment without discontinuation and as much as possible avoid any dose reductions or temporary withdrawals.

It has become evident that two functional variants in the inosine triphosphatase (*ITPA*) gene on chromosome 20 (rs7270101 and rs1127354) are associated with severe anemia during Peg-IFN + ribavirin therapy.<sup>87,88</sup> Of the *ITPA* polymorphism (rs1127354), the CC genotype (major-homo) was strongly associated with treatment-induced anemia in comparison with the CA + AA genotypes, and the CC genotype was an independent risk factor for ribavirin dose reduction.<sup>89</sup> Accordingly, patients with the CC genotype and low Hb need to be monitored for further decline in Hb during treatment.

Other adverse reactions associated with ribavirin include lymphopenia, hyperuricaemia, pruritus, rashes, cough and nasal congestion. Teratogenicity has been reported in animal studies with ribavirin, so it is contraindicated in pregnant women, women who may be pregnant and breastfeeding women. Transfer into the seminal fluid cannot be ruled out, so when ribavirin is administered to women who might become pregnant, or men whose partner might become pregnant, they should be advised to use contraception during treatment and for 6 months after its completion.

#### Recommendations:

- 1 In comparison with Peg-IFN monotherapy, HCV RNA is more likely to be undetectable at the end of treatment with Peg-IFN + ribavirin combination therapy, and the risk of relapse following completion of treatment is markedly decreased.
- 2 The main adverse reaction to ribavirin is hemolytic anemia, so caution is required when considering ribavirin therapy in patients with anemia or heart conditions.
- 3 To achieve SVR it is important to control any decline in Hb appropriately, complete the treatment without discontinuation, and as much as possible avoid any dose reductions or temporary withdrawals.
- 4 SNPs (rs7270101 and rs1127354) in the *ITPA* gene are associated with severe anemia during Peg-IFN + ribavirin therapy.
- 5 Due to concerns regarding teratogenicity, ribavirin is contraindicated in pregnant and breastfeeding women. Women who might become pregnant, and men whose partner might become pregnant, should be advised to use contraception.

### 3.3 Telaprevir

Telaprevir, discovered through optimization of  $\alpha$ -ketoamide scaffolds, is an antiviral agent that can be administered p.o.<sup>90</sup> A protease inhibitor, telaprevir

directly inhibits NS3-4A serine protease, a HCV gene non-structural protein that plays an important role in HCV replication, thereby strongly inhibiting viral replication.<sup>91</sup> Telaprevir inhibits replication of the HCV genotype 1 particularly strongly, and was approved in September 2011 for use in Japan in combination with Peg-IFN and ribavirin in the treatment of genotype 1 chronic hepatitis C with a high viral load ( $\geq 5.0$  log IU/mL).

### Therapeutic results

**Treatment-naïve patients.** The duration of telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy is 24 weeks, with all three agents for the first 12 weeks, then Peg-IFN- $\alpha$ -2b + ribavirin dual therapy for the remaining 12 weeks. In a Japanese phase III clinical trial of 24 weeks of triple therapy for IFN-naïve patients (aged  $\leq 65$  years), the SVR was 73% (92/126), significantly higher than that of 49% (31/63) for the control group, given Peg-IFN- $\alpha$ -2b + ribavirin dual therapy for 48 weeks (Table 1). The relapse rate was 17% (21/126), the breakthrough rate 3% (4/126) and the non-response rate 1% (1/126). No correlation was seen between sex or viral load at commencement and SVR, whereas the SVR rate was higher in patients aged  $< 50$  years than in those aged  $\geq 50$  years (85% vs 67%,  $P = 0.034$ ).<sup>11</sup>

Analysis of therapeutic efficacy according to adherence showed that the SVR rate in patients who discontinued none of the three agents was 84% (66/79), 60% (12/20) in those who discontinued telaprevir alone and 52% (14/27) in those who discontinued all three agents. The SVR rate was high at 79% (85/108) in patients with  $\geq 60\%$  adherence to telaprevir and 39% (7/18) in those with  $< 60\%$  adherence. Similarly, the SVR rate was high at 84% (68/81) in patients with  $\geq 80\%$

adherence to Peg-IFN- $\alpha$ -2b, and was less than 60% in those with  $< 80\%$  adherence. The SVR rate was high at 93% (13/14) in patients with  $\geq 80\%$  adherence to ribavirin, and although the SVR rate decreased as adherence declined, it was still 53% (8/15) in those with  $< 20\%$  adherence to ribavirin.<sup>11</sup>

In terms of virological kinetics, the SVR rate was 75% (81/108) in patients achieving a rapid virological response (RVR) (Table 2) and 61% (11/18) in those failing to achieve an RVR. The SVR rate was 80% (70/88) in patients achieving an extended RVR (eRVR) and 58% (22/38) in those failing to achieve an eRVR (Table 3).<sup>11</sup>

**Relapsers and non-responders to previous treatment.** A Japanese trial of 24 weeks' triple therapy for relapsers and non-responders to previous treatment yielded SVR rates of 88% (96/109) and 34% (11/32), respectively (Table 4). No correlation was seen between sex, age or viral load at commencement and SVR. Analysis of therapeutic efficacy according to adherence showed that the SVR rate was 91% (93/102) in relapsers with  $\geq 40\%$  adherence to telaprevir and 43% (3/7) in those with  $< 40\%$  adherence. In non-responders to previous treatment, the SVR rate was 40% (10/25) with  $\geq 80\%$  adherence to telaprevir and 17% (1/6) in those with 60–80% adherence. The SVR rate was  $\geq 80\%$  in relapsers to previous treatment with  $\geq 40\%$  adherence to Peg-IFN- $\alpha$ -2b and 48% (11/23) in non-responders to previous treatment only with  $\geq 80\%$  adherence. The SVR rate was high at  $\geq 85\%$  in relapsers with  $\geq 20\%$  adherence to ribavirin and 33–38% in non-responders even with 40–80% adherence to ribavirin.<sup>9</sup>

In terms of virological kinetics, among relapsers the SVR rate was 92% (90/98) in those achieving an RVR and 55% (6/11) in those failing to achieve an RVR. For non-responders to previous treatment, the SVR rate was 39% (9/23) in those achieving an RVR and 22% (2/9) in those failing to achieve an RVR. The SVR rate was 96% (84/88) in relapsers achieving an eRVR and 57% (12/21) in those failing to achieve an eRVR; whereas for non-responders to previous treatment the SVR rate was 47% (9/19) in those achieving an eRVR and 15% (2/13) in those failing to achieve an eRVR (Table 3).

### Recommendations:

- 1 The SVR rate was 73% in IFN-naïve patients administered telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy for 24 weeks, significantly higher than that of 49% in the control group administered Peg-IFN- $\alpha$ -2b + ribavirin dual therapy for 48 weeks.

Table 1 Therapeutic results for telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy in treatment-naïve patients (SVR rate, %) (reproduced from <sup>11</sup>)

	Telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy	Peg-IFN- $\alpha$ -2b + ribavirin dual therapy	<i>P</i>
SVR	73.0	49.2	0.002
Relapse	16.7	22.2	
Breakthrough	3.2	1.6	
Non-response	0.8	20.6	$< 0.0001$

Peg-IFN, pegylated interferon; SVR, sustained viral response.

2 *Telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy achieved SVR rates in relapsers and non-responders to previous treatment of 88% and 34%, respectively.*

Table 2 Virological response definitions

Virological response	Definition
Rapid virological response (RVR)	Serum hepatitis C virus (HCV) RNA undetectable at treatment week 4
Extended RVR (eRVR)	Serum HCV RNA undetectable at both treatment week 4 and 12
Early virological response (EVR)	cEVR or pEVR
Complete EVR (cEVR)	Serum HCV RNA undetectable at treatment week 12
Partial EVR (pEVR)	Serum HCV RNA detectable at treatment week 12 but decrease $\geq 2 \log_{10}$ IU/mL
End of treatment response (ETR)	Serum HCV RNA undetectable at the end of treatment
Sustained virological response (SVR)	Serum HCV RNA undetectable 24 weeks after the completion of treatment
Breakthrough	Reappearance of HCV RNA at any time during treatment having once been undetectable
Relapse	Reappearance of HCV RNA following treatment having been undetectable during treatment
Non-responder	Serum HCV RNA never undetectable during treatment
Null responder	Serum HCV RNA decrease $< 2 \log_{10}$ IU/mL at treatment week 12
Partial responder	Greater than $2 \log_{10}$ IU/mL decrease in serum HCV RNA level from baseline at treatment week 12, but serum HCV RNA detectable at treatment week 24

N.B.: The 2009 American Association for the Study of Liver Diseases (AASLD) "Diagnosis, management, and treatment of hepatitis C: an update" define non-responder, null responder and partial responder as "failure to clear HCV RNA from serum after 24 weeks of therapy", "failure to decrease HCV RNA by  $< 2 \log$ s after 24 week of therapy" and "2 log decrease in HCV RNA but still HCV RNA positive at week 24", respectively.<sup>116</sup> However, the 2011 version, updated to include telaprevir and boceprevir, dropped the non-responder category, and redefined null responder and partial responder as "failure to decrease HCV RNA level by at least 2 log IU/mL at treatment week 12" and "decrease in HCV RNA level by at least 2 log IU/mL at treatment week 12 but HCV RNA still detected at treatment week 24", respectively.<sup>101</sup> In these guidelines, we define null and partial responder as per the 2011 AASLD guidelines, and further define non-responder as encompassing both null and partial responders.

## Adverse reactions

The incidence of adverse reactions is greater for telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy than for Peg-IFN + ribavirin dual therapy. The most important adverse reactions are skin disorders and anemia.

In one study, skin disorders were reported in 85% (226/267) of patients, of greater severity than with dual therapy. The timing of onset was within 7 days after commencement of treatment in 56% (150/267) and within 28 days in 77% (205/267). More than 50% of the body surface was affected in 5% (19/355) of patients. Constitutional symptoms such as fever and lymphadenopathy were reported in 7% of patients, and serious skin disorders, including Stevens–Johnson syndrome, drug-induced hypersensitivity syndrome and erythema multiforme with mucosal involvement, in 1.5% (4/267).<sup>92</sup> Accordingly, strict attention should be given to any skin changes. A dermatologist should be consulted in the management of any treatment-induced skin disorders, and appropriate treatment promptly commenced in accordance with the symptom severity, topical corticosteroids and oral anti-allergic drugs for milder cases, and systemic corticosteroids for more severe cases. Most cases can be managed with topical corticosteroids and oral anti-allergic drugs. However, when skin disorders occur the hepatologist should not treat them his/herself, but should always consult a dermatologist colleague, even for mild symptoms, and follow their instructions regarding the possibility of exacerbation, and the use of topical and systemic medication to control symptoms. Subsequent collaboration is also important. The decision whether telaprevir therapy can be continued should also be made in consultation with the dermatologist, with due consideration of therapeutic efficacy and adverse reactions.

Anemia is an important adverse reaction to Peg-IFN- $\alpha$ -2b + ribavirin dual therapy, with a strong correlation between the SNP (rs1127354) of the ITPA gene and a decline in Hb during treatment.<sup>87,88,93</sup> The addition of telaprevir to dual therapy causes even more severe anemia. In the above-mentioned Japanese clinical trial for treatment-naïve patients, the incidence of grade 1 anemia (Hb 9.5–11.0 g/dL) was 39.7% in the group administered telaprevir + Peg-IFN + ribavirin triple therapy and 50.8% in the group on Peg-IFN + ribavirin dual therapy, whereas the incidence of grade 2 anemia (Hb 8.0–9.5 g/dL) was 27.0% and 17.5%, respectively, and grade 3 anemia (Hb  $< 8.0$  g/dL) occurred only in the triple therapy group.<sup>11</sup> The rate of treatment discontinuation due to anemia is also high with triple therapy.



Table 3 Therapeutic results for telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy stratified by RVR and eRVR (SVR rate, %) (reproduced from <sup>9,11</sup>)

	RVR		eRVR	
	Achieved	Not achieved	Achieved	Not achieved
Initial treatment	75% (81/108)	61% (11/18)	80% (70/88)	58% (22/38)
Relapse	92% (90/98)	55% (6/11)	96% (84/88)	57% (12/21)
Non-response	39% (9/23)	22% (2/9)	47% (9/19)	15% (2/13)

eRVR, extended rapid viral response; RVR, rapid viral response; SVR, sustained viral response.

Similarly to dual therapy, with triple therapy including telaprevir, significantly greater decreases in Hb early in the treatment period are seen with the CC genotype of the ITPA gene than with the CA/AA genotypes. Rapid decreases in Hb are seen up to treatment week 4 in patients with the CC genotype.<sup>94</sup> Risk factors for a Hb level <11.0 g/dL at treatment week 4 were female sex, body mass index <23, CC genotype of the ITPA gene and age  $\geq$ 50 years. Risk factors for a Hb level <8.5 g/dL, one of the discontinuation criteria, were patients weighing <60 kg and aged  $\geq$ 61 years. Patients with any of these risk factors should be carefully monitored for changes in Hb levels.

The response to a decline in Hb should be regular Hb measurements and an early reduction in the ribavirin dose. As mentioned above, in Japanese clinical trials of initial therapy and retreatment, reductions in the ribavirin dose had relatively little effect on therapeutic efficacy.<sup>9,11</sup> In particular, SVR rates  $\geq$ 85% were achieved in relapsers as long as at least 20% of the intended ribavirin dose was administered.<sup>9</sup>

Some other noteworthy adverse reactions seen in the early treatment period that have come to light through post-marketing surveillance are raised serum creatinine (renal dysfunction) and hyperuricaemia. As these generally appear within the first week of treatment, patients should be monitored for rises in serum creatinine and

uric acid soon after treatment commences. Japanese clinical trials of triple therapy including telaprevir did not include patients with cirrhosis, so its safety in these patients has not been established. Clinicians should be aware that triple therapy is not approved for patients with cirrhosis under the national medical insurance scheme.

#### Recommendations:

- 1 *Serious skin disorders can occur with telaprevir + Peg-IFN + ribavirin triple therapy. When skin disorders occur, the hepatologist should not treat them his/herself, but should always consult a dermatologist colleague, even for mild symptoms, and follow their instructions regarding the possibility of exacerbation, and the use of topical and systemic medication to control symptoms. The decision whether telaprevir therapy can be continued should also be made in consultation with the dermatologist, with due consideration of therapeutic efficacy and adverse reactions.*
- 2 *A decline in Hb should be managed with regular Hb measurements and an early reduction in the ribavirin dose.*
- 3 *Serum creatinine and uric acid levels may rise early in the treatment period.*
- 4 *In patients with liver cirrhosis, the safety of triple therapy including telaprevir has not been established, and thus triple therapy is not approved for cirrhosis by national medical insurance in Japan.*

Table 4 Therapeutic results for telaprevir + Peg-IFN- $\alpha$ -2b + ribavirin triple therapy in relapsers and non-responders to previous treatment (SVR rate, %) (reproduced from <sup>9</sup>)

	Relapsers	Non-responders to previous treatment
SVR	88.1	34.4
Relapse	7.3	40.6
Breakthrough	0.9	18.8
Non-response	0.9	6.3

SVR, sustained viral response.

#### Drug interactions

Telaprevir strongly inhibits the CYP3A4/5 drug metabolizing enzyme, and may increase plasma levels of co-administered drugs that are also substrates of CYP3A4/5. Telaprevir is also metabolized by CYP3A4, so co-administration with inducers of CYP3A4 may lower plasma telaprevir levels. As a result, co-administration of a number of agents with telaprevir is contraindicated (Table 5) and caution is advised with

Table 5 Drugs contraindicated for co-administration with telaprevir with trade names (reproduced from <sup>95</sup>)

Contraindicated drug (generic name)	Trade names
Quinidine sulfate hydrate	Quinidine sulfate
Bepiridil hydrochloride hydrate	Vascor, BepriCor
Flecainide acetate	Tambocor
Propafenone hydrochloride	Rythmol, Pronon
Amiodarone hydrochloride	Cordarone, Ancaron
Pimozide	Orap
Ergotamine tartrate	Cafergot, Ergomar
Dihydroergotamine mesilate	Migranal, Dihydergot
Ergometrine maleate	Oxytocin
Methylergometrine maleate	Methergine, Utergin
Triazolam	Halcion, Hypam, Trilam
Lovastatin/simvastatin	Crestor/Zocor
Atorvastatin calcium hydrate	Lipitor, Caduet
Alfuzosin	Uroxatral
Vardenafil hydrochloride hydrate	Levitra
Sildenafil citrate (for treatment of pulmonary hypertension)	Viagra, Revatio
Tadalafil (for treatment of pulmonary hypertension)	Cialis, Adcirca
Blonanserin	Lonasen
Colchicine (when administered to patients with liver or kidney disease)	Colgout, Lengout
Rifampicin	Aptecin, Rifadin, Rimactane

many others.<sup>95</sup> The package insert should be referred to before administrating telaprevir.

#### Recommendation:

*Telaprevir strongly inhibits the CYP3A4/5 drug metabolizing enzyme and is also a substrate, so co-administration of a number of agents is contraindicated or requires caution. The package insert should be referred to before administrating telaprevir.*

#### Drug resistance

Telaprevir-resistant mutations (V36, T54, R155, A156, V170) have been reported in cases of viral breakthrough with telaprevir monotherapy,<sup>96–98</sup> as well as in cases on virological non-response and relapse with triple therapy.<sup>99,100</sup> The reported incidences of telaprevir resistance are 12% with initial therapy and 22% with retreatment. One study found telaprevir-resistant viruses in 80–90% of cases of viral breakthrough, virological non-response and relapse.<sup>101</sup> Resistance is more common in

genotype 1a than genotype 1b HCV. In most cases, these telaprevir-resistant viruses become undetectable, reverting to wild type, over time.<sup>97,98</sup>

#### 3.4 Initial treatment—Genotype 1 with high viral load

A number of new agents are under development for the treatment of HCV genotype 1 with a high viral load ( $\geq 5.0 \log_{10}$  IU/mL using real-time PCR, HCV core antigen  $\geq 300$  fmol/L), which is often refractory to treatment. These include HCV-selective antiviral agents in the form of enzyme inhibitors (protease inhibitors, polymerase inhibitors, NS5A inhibitors), new IFN formulations, ribavirin prodrugs and immunopotential agents. At present, however, the only therapies available for clinical use in Japan are antiviral combinations based on an IFN formulation, in other words Peg IFN (IFN)  $\pm$  ribavirin  $\pm$  telaprevir. Peg-IFN + ribavirin therapy was approved available for use in Japan in 2004, improving therapeutic efficacy but with the addition of adverse reactions such as anemia. Subsequent detailed studies in a large number of IFN-treated subjects have identified correlations between viral, host and drug factors on the one hand and therapeutic effect and adverse reactions on the other hand. At present, we are moving away from uniform therapies in accordance with HCV genotype and viral load towards optimizing therapy for each individual patient, with the emphasis on response-guided therapy that adjusts the duration of treatment according to the response. In 2009, IFN- $\beta$  + ribavirin therapy, which has a relatively good safety profile with fewer adverse reactions such as depression, gained approval under the national medical insurance scheme in Japan.

In 2011, telaprevir + Peg-IFN + ribavirin triple therapy became available for use in Japan. The addition of telaprevir to Peg-IFN + ribavirin improves the therapeutic efficacy, also shortening the treatment duration from 48 (or 72) weeks to 24 weeks, but with the addition of adverse reactions such as severe anemia and serious skin disorders. Japanese clinical trials of this triple therapy were conducted with patients aged  $\leq 65$  years with platelet counts  $\geq 100\ 000/\mu\text{L}$ , so we need to gather scientific evidence regarding therapeutic efficacy and adverse reactions in patients at high risk of developing HCC, including elderly patients and those with advanced hepatic fibrosis.

Recently interleukin (IL)-28B SNP and substitutions of amino acids in the HCV core and NS5A regions are widely recognized as important pretreatment predictors of therapeutic efficacy. Accordingly, although not

approved by national medical insurance, if possible, IL-28B SNP and substitutions of amino acids in the HCV core and NS5A regions should be tested pre-treatment, and antiviral therapy selected based on the results. These tests can be referred to outside laboratories.

#### **Peg-IFN + ribavirin combination therapy**

*Prediction of therapeutic efficacy using pretreatment factors.* Factors that predict therapeutic efficacy prior to commencement of Peg-IFN + ribavirin combination therapy can be divided into host and viral factors. Of the host factors, testing for IL-28B SNP is useful. Patient groups with a minor allele G at rs8099917 (TG/GG) show greater resistance to Peg-IFN + ribavirin therapy than those with a major allele (TT).<sup>102–104</sup> Other predictors of antiviral efficacy are age and degree of fibrosis.<sup>105</sup> SVR rates are relatively high in non-elderly and non-advanced fibrosis patients, and generally low in elderly patients and those with advanced fibrosis. Even among the elderly, SVR rates are known to be particularly low in elderly female patients.<sup>106,107</sup>

Of the viral factors, amino acid 70 and 91 substitutions in the HCV core region,<sup>108,109</sup> and amino acid substitutions in the HCV NS5A region (interferon sensitivity-determining region [ISDR]),<sup>110–112</sup> are independent predictive factors for the therapeutic efficacy of Peg-IFN + ribavirin therapy. Therapeutic efficacy is high in patients with wild-type at amino acid 70 in the HCV core region and increases with the number of amino acid substitutions in the HCV NS5A region. Amino acid substitutions outside the HCV NS5A ISDR (IFN/ribavirin-resistance determining region; IRRDR) are also known to affect the therapeutic efficacy of this regimen.<sup>113</sup>

#### *Recommendations:*

- 1 *Prior to commencement of Peg-IFN + ribavirin combination therapy, host factors that predict antiviral efficacy are IL-28B SNP, age and degree of fibrosis. Of the viral factors, amino acid 70 and 91 substitutions in the HCV core region and amino acid substitutions in the HCV NS5A region are independent predictive factors for the therapeutic efficacy of Peg-IFN + ribavirin therapy.*
- 2 *If possible, IL-28B SNP and amino acid substitutions in the HCV core and NS5A regions should be tested to enable accurate prediction of therapeutic efficacy. These tests can be referred to outside laboratories, but are not approved by national medical insurance.*

*Prediction of therapeutic efficacy using response to treatment: Response-guided therapy and treatment discontinuation criteria.* The response to treatment by each individual patient after commencing Peg-IFN + ribavirin combination therapy, in other words HCV RNA dynamics, is a good marker for predicting SVR.<sup>114</sup> The risk of resistant viral mutations is low with Peg-IFN + ribavirin therapy, so it is possible, as well as useful, to commence treatment and then formulate a treatment plan based on the early response to therapy (response-guided therapy). As the physical and financial burdens on patients of Peg-IFN + ribavirin therapy are great, when the decrease in the HCV RNA load is very poor and SVR cannot be anticipated, in line with the treatment discontinuation criteria, it is recommended that antiviral therapy with the aim of achieving SVR should be discontinued.

In two Japanese clinical trials of 24 weeks' Peg-IFN + ribavirin combination therapy, HCV RNA was measured using the Amplicor PCR method (Roche Molecular Systems, Pleasanton, CA, USA). The SVR rate was  $\geq 70\%$  when HCV RNA became undetectable before treatment week 12, lower when HCV RNA became undetectable after treatment week 12 and SVR did not occur when HCV RNA was still detectable at treatment week 24.<sup>82,83</sup> An overseas study also reported that, in particular, the early viral response (EVR) at treatment week 12 (Table 2), either complete EVR (undetectable HCV RNA) or partial EVR ( $\geq 2$  log IU/ml decrease in HCV RNA), was significantly associated with SVR.<sup>115</sup> Based on this result, the American Association for the Study of Liver Diseases (AASLD) guidelines recommend that when Peg-IFN + ribavirin combination therapy (48 weeks) fails to reduce HCV RNA  $\geq 2$  log IU/ml at treatment week 12 in patients with genotype 1, or if HCV RNA remains positive at week 24, treatment should be discontinued.<sup>116</sup>

For patients in whom HCV RNA becomes undetectable between treatment weeks 13 and 24, it has become evident that increasing the treatment period to 72 weeks improves SVR rates.<sup>117–120</sup> Real-time PCR testing has also shown that SVR can be achieved in the group in whom HCV RNA becomes undetectable between treatment weeks 25 and 36; therefore, in Japan, extension of the treatment period to 72 weeks is recommended in patients in whom HCV RNA becomes undetectable between treatment weeks 13 and 36. Extended treatment periods have been reported to be particularly useful in patient groups with amino acid 70 and 91 substitutions in the HCV core region and wild-type at ISDR in the HCV NS5A region.<sup>121</sup> In patients with advanced hepatic fibrosis or elderly patients in whom

HCV RNA becomes undetectable between treatment weeks 9 and 12, relapse rates are high if treatment is stopped at treatment week 48. Accordingly, extending the treatment period to 72 weeks is an option worth considering, although it is not approved by national medical insurance.<sup>122</sup>

In recent years, real-time PCR testing has enabled measurement of HCV RNA over a wide range of viral loads. This suggests the possibility of assessing each individual patient's response to treatment through the rate of decrease in HCV RNA earlier than treatment week 12. In other words, analysis of SVR rates in patients administered response-guided Peg-IFN + ribavirin combination therapy stratified according to the rate of decrease in HCV RNA at treatment week 4 yielded SVR rates of 4%, 18%, 55%, 66% and 89%, respectively, for HCV RNA decreases <1 log, 1–2 log, 2–3 log, 3–4 log and  $\geq 4$  log (but still detectable), and 100% for undetectable HCV RNA. A strong correlation was seen between the rate of HCV decrease at treatment week 4 and SVR ( $P < 0.001$ ) (Table 6).<sup>111</sup> On the other hand, HCV RNA did not become undetectable in patients on the same therapy with an HCV decrease <1 log at treatment week 8, or <2 log at treatment week 12, so at least discontinuation of treatment aimed at achieving SVR is recommended for these patient groups at week 8 or 12.

In patients at high risk of hepatocellular carcinogenesis, such as the elderly and those with advanced fibrosis, consideration should be given to continuing Peg-IFN + ribavirin combination therapy, aiming for biochemical improvement rather than SVR. In a Japanese trial, in relapsers and non-responders in whom viral clearance could not be achieved, the ALT normalization rates 6 months after completion of treatment were 56% (5/9) and 62% (8/13), respectively. Among patients with normalized ALT levels 6 months after treatment, long-term biochemical response persisting until 2 years post-treatment was achieved in all but one patient with normalized ALT levels.<sup>123</sup> Accordingly, in patients at high risk of HCC, if normalization of AST/ALT levels has been achieved at treatment week 36 with combination therapy, even if HCV RNA remains

detectable, it is worthwhile continuing treatment to 48 weeks in total.<sup>122</sup>

#### Recommendations:

- 1 After commencement of Peg-IFN + ribavirin combination therapy, the timing of HCV RNA clearance and the rate of decrease in HCV RNA over time are useful markers of therapeutic efficacy.
- 2 The rate of decrease in HCV RNA at treatment week 4 is a good marker of SVR.
- 3 Extension of the treatment period to 72 weeks is recommended in patients in whom HCV RNA becomes undetectable between treatment weeks 13 and 36. In patients with advanced hepatic fibrosis or elderly patients in whom HCV RNA becomes undetectable between treatment weeks 9 and 12, relapse rates are high if treatment is stopped at treatment week 48. Accordingly, extending the treatment period to 72 weeks is an option worth considering, although it is not approved by national medical insurance.
- 4 Discontinuation of treatment should be considered in patients with a HCV decrease <1 log at treatment week 8 or <2 log at treatment week 12, and treatment should be discontinued if HCV clearance is not achieved before treatment week 36 even with an HCV RNA decrease  $\geq 2$  log at treatment week 12.
- 5 In patients at high risk of HCC, if normalization of AST/ALT levels has been achieved at treatment week 36 with combination therapy, even if the discontinuation criteria are met, continuing treatment to 48 weeks in total should be considered.

*Adherence and therapeutic efficacy.* Overseas, clinical trials have confirmed the influence of adherence to Peg-IFN + ribavirin combination therapy on therapeutic effect.<sup>115,124–127</sup> In another study, SVR rates were significantly higher in patients who took  $\geq 80\%$  of the intended dose of both Peg-IFN and ribavirin than in other patients (51% vs 34%). The influence of dose reductions on therapeutic effect was most marked in patients with dose reductions before treatment week 12.<sup>24</sup> In one Japanese study, a significant correlation was found between the EVR rate and the Peg-IFN dose at treatment week 12, but not the ribavirin dose. With Peg-IFN- $\alpha$ -2b doses

Table 6 The decline of serum HCV-RNA at treatment week 4 and SVR rates with response-guided therapy in patients with Genotype 1/high viral load. HCV RNA decline vs. SVR,  $P < 0.001$

HCV RNA decrease (log)	<1 log	1–2 log	2–3 log	3–4 log	$\geq 4$ log	Undetectable HCV
SVR rate	4% (2/53)	18% (10/55)	55% (48/87)	66% (58/88)	89% (70/79)	100% (29/29)

HCV, hepatitis C virus; SVR, sustained viral response.