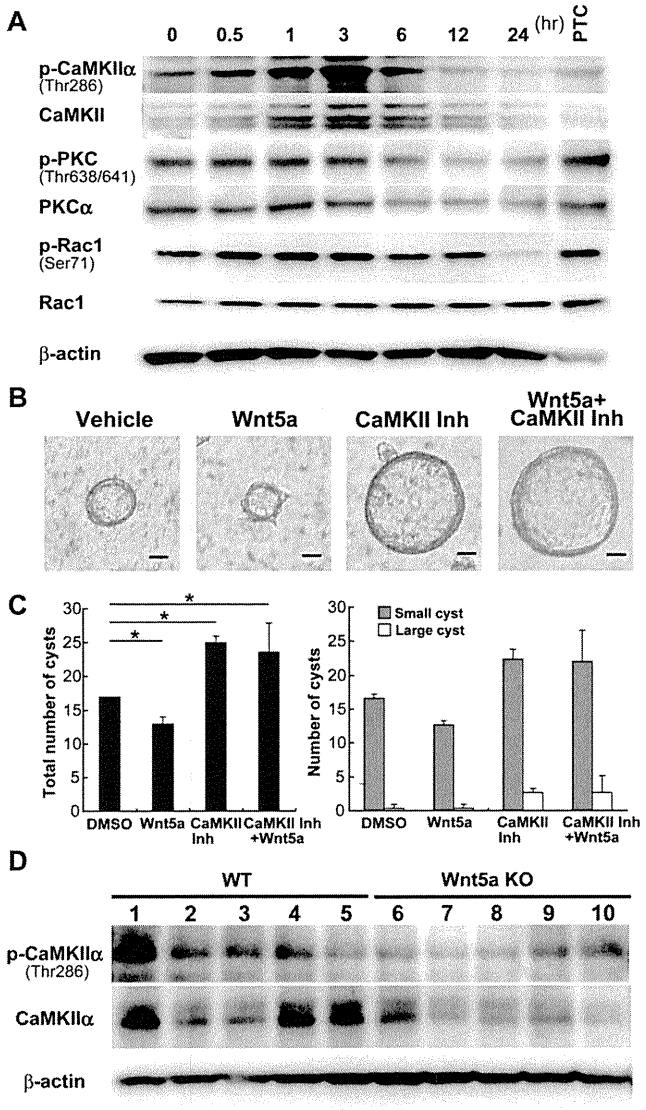

Fig. 6. Phosphorylation of CaMKII is regulated by Wnt5a stimulation in fetal liver. (A) Immunoblot analysis of p-CaMKII, p-PKC, and p-Rac1 in HPPL at pretreatment (0), and then 0.5, 1, 3, 6, 12, and 24 hours after stimulation by Wnt5a. Homogenate of whole E14.5 embryo served as a positive control (PTC). Wnt5a treatment increased the levels of both total CaMKII and p-CaMKII in HPPL, but did not change the levels of p-PKC and p-Rac1. (B) Representative phase-contrast images of cysts derived from HPPL supplemented either with vehicle (DMSO), 100 ng/mL Wnt5a, CaMKII inhibitor (KN62), or 100 ng/mL Wnt5a plus CaMKII inhibitor. Scale bars: 100 μm. (C) Numbers of bile duct-like cysts derived from HPPL in five random fields per well in cultures supplemented with vehicle (DMSO), Wnt5a, CaMKII inhibitor (KN62), or Wnt5a plus CaMKII inhibitor. The effect of Wnt5a on HPPL cysts was cancelled by KN62 treatment. $*P < 0.05$. (D) Immunoblot analysis of p-CaMKII in E16.5 WT and Wnt5a KO livers demonstrating a decrease in p-CaMKII level in Wnt5a KO livers. Mice 1-5 and mice 6-10 are E16.5 WT and Wnt5a KOs, respectively. Results are represented as mean ± SD of three individual experiments.

inhibitors of these candidate molecules in HPPL-derived cysts, where Wnt5a is expressed (Supporting Fig. 7A). Relative to controls, inhibitors specific to CaMKII (KN93 and KN62) resulted in a significant increase in numbers of both small and large bile duct–like cysts derived from HPPL (Figs. 5A and B). In contrast, other inhibitors, including Y-27632 (Rho-kinase inhibitor), NSC23766 (Rac1 inhibitor), cyclosporin A (calcineurin inhibitor), and Go6976 (PKC inhibitor), had no effect on the number or size of HPPL-derived cysts (Fig. 5C). We examined the expression of biliary markers in HPPL-derived cysts treated with CaMKII inhibitor (KN62). Expression of MRP3, a key primary active transporter in biliary cells, in HPPL-derived cysts increased significantly with supplemental CaMKII inhibitor (Fig. 5D). There were no significant differences in mRNA levels of ALB, HNF4α, and β-catenin–related molecules between HPPL-derived cysts treated with CaMKII inhibitor and those treated with vehicle (Supporting Fig. 8). The protein level of AFP in HPPL-derived cysts treated with CaMKII inhibitor was lower than that in vehicle-supplemented controls, whereas the levels of CK19 and PCNA did not change (Fig. 5E). These data indicate that CaMKII activity suppresses the formation of HPPL-derived cysts, whereas activities of other Wnt5a-mediated candidates did not influence the efficacy of cyst formation.

Phosphorylation of CaMKII in Primary Hepatoblasts. To investigate the activation state of CaMKII in fetal and neonatal WT livers, we used immunoblots of liver homogenates derived from E14.5, E16.5, and E18.5 and postnatal day (P) 1, P7, and P14 mice to measure CaMKII phosphorylation levels.

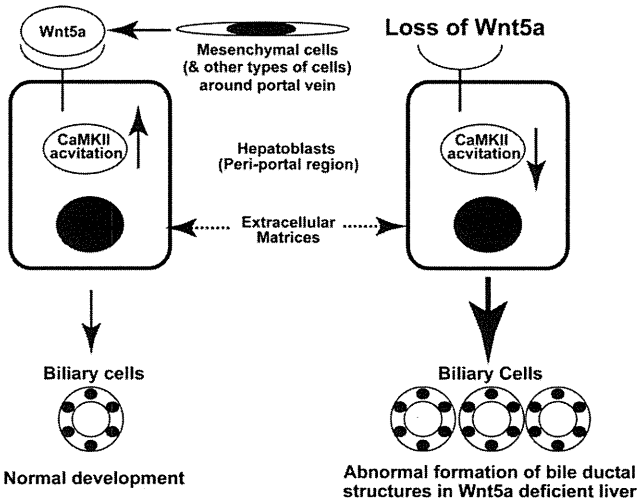

Fig. 7. Schema for the biliary differentiation of hepatoblasts in Wnt5a KO liver. Wnt5a is expressed in mesenchymal cells and other types of cells in midgestational fetal liver, and increases the level of CaMKII activation in hepatoblasts. The microenvironment around the portal vein, which consists of mesenchymal cells, other types of cells, and extracellular matrices, regulates appropriate differentiation of hepatoblasts into biliary cells, whereas loss of Wnt5a in such microenvironment leads to down-regulation of CaMKII activation in hepatoblasts and abnormally increased formation of bile ducts.

Phosphorylation at threonine-286, specifically, has been reported to maintain CaMKII in an active state.[25] Phosphorylation of PKC, a kinase that did not affect cyst formation in HPPL cells, was also examined. Whereas we detected both phosphorylated CaMKII (p-CaMKII) and PKC (p-PKC) in each fetal and neonatal liver homogenate, levels of phosphorylated CaMKII increased gradually over time (Supporting Fig. 9A, top panel), similar to the pattern of Wnt5a expression during liver development (Fig. 1A). In contrast, developmental changes in the steady-state levels and phosphorylation of PKC in these samples (Supporting Fig. 9A, lower panels) did not correspond to Wnt5a expression patterns.

Using immunostaining of FACS-purified primary hepatoblasts with anti p-CaMKII Ab, we detected p-CaMKII in >90% of FACS-purified primary hepatoblasts (Supporting Fig. 9B, upper panels); p-PKC was also detected with anti p-PKC antibodies in these cells (Supporting Fig. 9B, lower panels). These data demonstrate that both CaMKII and PKC are in an active state in primary hepatoblasts.

Wnt5a Regulates the Phosphorylation of CaMKII in Fetal Liver. To verify whether CaMKII activation is controlled by Wnt5a, levels of p-CaMKII in HPPL grown in the absence or presence of Wnt5a were examined. Immunoblot analysis revealed that Wnt5a stimulation increased the level of phosphorylated CaMKII, with p-CaMKII levels peaking 3 hours after Wnt5a supplementation and then decreasing to baseline levels after 12 hours (Fig. 6A and Supporting Fig. 10A). Similar to a previous report,[15] total CaMKII protein levels in HPPL also increased after CaMKII activation. Ratios of p-CaMKII/CaMKII also increased, peaking 3 hours after Wnt5a supplementation (Supporting Fig. 10B). In contrast, Wnt5a had no effect on p-PKC and p-Rac1 levels in HPPL (Supporting Figs. 10C and D) nor on nuclear translocation of NFAT (representative downstream molecule of calcineurin; data not shown).

We also tested the combined effect of Wnt5a plus a CaMKII inhibitor (KN62) on cyst formation in HPPL-derived cells. The number and size of cysts in HPPL-derived cells decreased with Wnt5a alone, and increased with CaMKII inhibitor alone. When used in combination (HPPL treated with both CaMKII inhibitor plus Wnt5a), the number and size of cysts was similar to CaMKII inhibitor alone, and significantly higher than cells treated with Wnt5a alone (Figs. 6B and C).

We also used immunoblots to compare p-CaMKII levels in WT and Wnt5a KO fetal liver homogenates. Levels of p-CaMKII were significantly lower in Wnt5a KO relative to WT fetal livers (Fig. 6D); quantification using densitometry revealed that p-CaMKII levels in Wnt5a KO livers were also significantly lower than those in littermate WT livers (Supporting Fig. 10E), indicating that Wnt5a mediates an increase in CaMKII phosphorylation in fetal liver.

Discussion

This study provides the first evidence of a physiological role for Wnt5a in liver development, in that Wnt5a was observed to suppress the formation of bile ducts derived from hepatoblasts. Our data showed increased expression of Sox9, Notch1, Notch2, and Jagged1 in Wnt5a KO livers (Fig. 2B and Supporting Fig. 3A), as well as abnormally increased formation of primitive ductal structures (Figs. 2E and F). In Wnt5a KO livers, the numbers of $HNF1\beta^{+}HNF4\alpha^{-}$ biliary precursor cells and primitive ductal structures were increased around the portal vein only (zone 1), whereas such cells were not observed in zone 2 or 3 (Figs. 2D-F). At E14.5, $HNF1\beta^{+}HNF4\alpha^{-}$ biliary precursor cells were not detected in Wnt5a KO livers similar to WT livers (Supporting Fig. 11A). These results suggested that lineage commitment of hepatoblasts into biliary cells is determined by the microenvironment around the portal vein, depending on the presence or absence of Wnt5a protein. The lungs and intestine of systemic Wnt5a KO mice were abnormal, while tissue structures of the pancreas and kidneys were almost normal (Supporting Fig. 12). Immunostaining analysis showed that $p75NTR^{+}$ cells were detected in E18.5 Wnt5a KO livers, similar to WT livers (Supporting Fig. 11B). These results implied that development of mesenchymal cells in E18.5 Wnt5a KO livers is not impaired compared with that in littermate WT livers. Wnt5a expression was significantly higher in mesenchymal cells than in hepatoblasts or other types of cells in midgestational WT fetal liver (Fig. 1B). Thus, the microenvironment around the portal vein, which consists of mesenchymal cells, other types of cells, and extracellular matrices, regulates appropriate cell fate decision of hepatoblasts, whereas loss of Wnt5a in such developmental niche leads to abnormally increased formation of primitive ductal structures (Fig. 7). Further investigation of this hypothesis will require conditional deletion of Wnt5a-downstream molecules in hepatoblasts at late gestational fetal stages.

Maturation of hepatoblasts to a hepatocyte lineage is regulated by several factors, including oncostatin M, HGF, and extracellular matrices.[24] Our data showed that hepatic maturation of primary hepatic stem/progenitor cells was promoted in cultures supplemented with Wnt5a (Figs. 4A and B). On the other hand, no

significant changes in hepatocyte marker expression were detected in Wnt5a KO relative to WT livers. It may be that there is functional redundancy among different Wnt family ligands *in vivo*, since several noncanonical-signaling Wnt ligands (Wnt4, Wnt5a, and Wnt11) are expressed in normal fetal liver.[26] In support of the hypothesis that other noncanonical Wnt ligands may compensate for Wnt5a, Supporting Fig. 13A shows that Wnt4 expression levels in liver increase significantly in Wnt5a KO versus WT littermates. These data strongly support our hypothesis that the effect of Wnt5a on hepatic maturation is compensated by other noncanonical Wnt ligands, such as Wnt4.

CaMKII, a serine/threonine protein kinase present in essentially every tissue, regulates important functions including modulation of ion channel activity, cellular transport, and cell morphology in neural tissues.[27] A Wnt5a-CaMKII pathway has been reported to induce osteoblastogenesis by attenuating adipogenesis in mesenchymal bone marrow stem cells.[15] Our results show that in liver, inhibition of CaMKII activity promoted bile duct–like cyst formation (Figs. 5A and B), and that phosphorylation of CaMKII is dependent on Wnt5a stimulation (Fig. 6). Although these results provide strong support for our hypothesis that Wnt5a stimulates CaMKII in hepatoblasts, we have not identified which molecules function downstream of CaMKII.

CaMKII has been reported to activate the transforming growth factor β–activated kinase 1 (TAK1)-Nemo-like kinase (NLK) pathway, and that resulting phosphorylation of T cell factor inhibits β-catenin–dependent transcription.[28] On the other hand, CaMKII-TAK1-NLK signaling induces bone marrow mesenchymal stem cells to undergo osteoblastogenesis depending on specific downstream signaling cascades.[15] Our expression analysis showed that expression levels of *Cyclin D1* and *c-Myc* (the direct target molecules of β-catenin activation) did not change in Wnt5a KO mice *in vivo* (Supporting Fig. 4) nor in HPPL-derived cysts treated with CaMKII inhibitor *in vitro* (Supporting Fig. 8), compared with the respective control samples. Preliminary data (not shown) demonstrated that the levels of TAK1 mRNA and protein during development did not correlate with those of Wnt5a and p-CaMKII in whole liver lysates. Moreover, Wnt5a stimulation did not increase the level of activated β-catenin in HPPL (Supporting Figs. 13B and C). These results suggest that the Wnt5a-CaMKII pathway does not activate β-catenin in hepatoblasts. On the other hand, Wnt5a stimulation increased the level of stabilized p53 (phosphorylated at Ser15) in HPPL (Supporting Figs. 13B and D), suggesting that stabilization of p53 is associated with Wnt5a-CaMKII signaling. Further study will be needed to clarify this issue.

Recent studies have shown pathological roles for Wnt5a in various organs; addition of recombinant Wnt5a significantly reduced the migratory capacity of colorectal cancer cell line.[29] Whereas increased Wnt5a expression correlates with advanced stages of gastric cancer with poor prognosis,[30] there is no definitive data about Wnt5a in the progression of hepatocellular carcinomas. In this study, we reveal one function of Wnt5a in fetal liver in the suppression the biliary differentiation of hepatic stem/progenitor cells. To clarify the pathological role of Wnt5a in liver disease, inducible systemic Wnt5a KO mice or liver-specific CaMKII KO mice would be needed in future studies. Any future evidence demonstrating a role for Wnt5a in adult hepatic stem/progenitor cells and cancer stem cells may lead to studies of Wnt5a signaling as a therapeutic target against abnormal bile ductal formation in the liver or cholangiocellular carcinoma.

Acknowledgment: We thank Prof. A. Miyajima (the University of Tokyo) for the gift of anti-CK19 antibody. We thank K. Itoh, K. Okada, and A. Yanagida (Institute of Medical Science, the University of Tokyo) for excellent technical assistance.

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Hepatology Research

Hepatology Research 2014; 44 (Suppl. 1): 59–70 doi: 10.1111/hepr.12272

Special Report

JSH Guidelines for the Management of Hepatitis C Virus Infection: A 2014 Update for Genotype 1

Drafting Committee for Hepatitis Management Guidelines, the Japan Society of Hepatology*,**

1. INTRODUCTION

RECENTLY, THE MANAGEMENT of chronic hepatitis C virus (HCV) has been greatly advanced with introduction of direct-acting antiviral agents (DAAs) in clinical setting. In Japan, the first DAA, telaprevir (TVR), was approved for patients with chronic hepatitis C in 2011. Along with this, the Japan Society of Hepatology (JSH) produced the first clinical practice guideline for the management of HCV infection, "Guidelines for the Management of Hepatitis C Virus Infection" in May 2012 (English version, 2013[1]). It is our great pleasure that these Guidelines were welcomed and utilized by physicians and other health care providers in daily clinical practices in Japan.

Meanwhile, in September 2013, a second-generation DAA, simeprevir (SMV), was approved for use in Japan. According to Phase III trials in Japan and overseas, SMV has a robust therapeutic effect with better safety profiles compared to TVR. As a result, we have decided to update the clinical guidelines for HCV with launch of this new DAA. SMV has now been approved for use in patients with chronic hepatitis C with genotype 1 and high viral load, and therefore these current Guidelines are updated for patients in this group.

As stated in the previous Guidelines, this is a field that changes rapidly with the accumulation of new evidence, and evidence levels are not shown in the recommendations. At present, several other therapeutic agents are expected to be approved for daily use and we plan to revise these guidelines at appropriate intervals, as new evidence comes to hand.

2. SIMEPREVIR (SMV)

INHIBITORS OF HEPATITIS C virus (HCV) NS3-4A protease are classified into 2 groups on the basis of their molecular structures, linear inhibitors with no branches and macrocyclic inhibitors containing macrocycles. Macrocyclic small molecule compounds show superior affinity and selectivity for therapeutic target proteins.[2] Whereas TVR is a first-generation protease inhibitor with linear structure, SMV is a second-generation protease inhibitor with macrocyclic structure discovered during the optimization process for early protease inhibitors.[3] In vitro resistance testing has yielded different drug resistance profiles, due to their different structures, with cross resistance to SMV seen in TVR resistant mutations at amino acids 155 and 156, whereas mutations at amino acids 36, 54 and 170 were sensitive to SMV, and mutations at amino acids 80 and

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168 resistant to SMV alone.[4] Pharmacokinetic studies have shown that once daily administration of SMV provides effective plasma levels 24 h post-dose.[5] SMV shows inhibitory activity against HCV genotypes 1, 2, 4, 5 and 6, with particularly strong anti-proliferative action against genotypes 1a and 1b. In September 2013, the use of SMV in clinical setting was approved in combination with Peg-IFN + RBV in patients with chronic hepatitis C with genotype 1 and a high viral load (≥5.0 log IU/mL).

2.1 Therapeutic results

Phase II trials of SMV + Peg-IFN + RBV combination therapy for genotype 1 chronic hepatitis C include the Japanese DRAGON study (treatment-naïve patients),[6] and the overseas PILLAR study (treatment-naïve patients)[7] and the ASPIRE trial (relapsers following previous treatment and non-responders to previous treatment).[8] Based on the results of these studies, the SMV dosage was set at 100 mg once daily for clinical phase III studies in Japan, and 150 mg once daily for overseas studies. Published Japanese clinical phase III studies comprise the CONCERTO-1 (treatment-naïve patients),[9] CONCERTO-2 (non-responders to previous treatment),[10] CONCERTO-3 (relapsers following previous treatment),[10] and CONCERTO-4 (treatment-naïve patients, non-responders, and relapsers) trials.[11] Published overseas clinical phase III studies comprise the QUEST-1 (treatment-naïve patients),[12] QUEST-2 (treatment-naïve patients),[13] and PROMISE (relapsers) studies.[14] The subjects for the Japanese clinical trials were patients with chronic hepatitis C (excluding cirrhosis) with genotype 1 and a high viral load (≥5.0 log IU/mL), aged 20–70 years (Table 1).

2.1.1 Treatment-naïve patients

The protocol for the Japanese CONCERTO-1 trial,[9] conducted with IFN-naïve subjects, administered SMV 100 mg once daily + Peg-IFNα-2a + RBV triple therapy for the first 12 weeks, then Peg-IFNα-2a + RBV dual therapy for 12 or 36 weeks according to the response-guided therapy (RGT). Using this RGT, subjects with HCV RNA < 1.2 log IU/mL or undetectable after 4 weeks' treatment, and undetectable after 12 weeks, were administered Peg-IFNα-2a + RBV for 12 weeks (total treatment duration 24 weeks), and all other subjects for 36 weeks (total treatment duration 48 weeks). As a result, 99% of subjects met the response-guided criteria, and underwent 24 weeks of treatment. The SVR24 rate was 89% (109/123) for the triple therapy group, significantly higher than that of 57% (34/60) in the control group (Fig. 1).

Peg-IFNα-2b was used in the CONCERTO-4 trial,[11] conducted with IFN-naïve subjects, the same response-

Table 1A Characteristics of patients enrolled in CONCERTO-1/2/3

	Treatment-naïve		*Non-responders*		*Relapsers*
	SMV 12W	PBO	SMV 12W	SMV 24W	SMV 12W
	(n = 123)	(n = 60)	(n = 53)	(n = 53)	(n = 49)
male, %	31.7	40.0	50.9	49.1	40.8
age *	56 (23–69)	54.5 (30–69)	60 (30–70)	60 (24–70)	61 (22–70)
≥65, %	17.9	16.7	26.4	22.6	24.5
BMI, kg/m² *	22.0 (16.9–32.9)	22.5 (17.3–33.2)	22.3 (16.8–29.5)	21.9 (19.2–33.4)	22.3 (17.9–32.2)
IL28B SNP (rs8099917), %					
TT	61.7	70	15.1	11.3	71.4
TG	31.7	28.3	83	86.8	28.6
GG	1.6	1.7	1.9	1.9	0
HCV genotype 1b, %	98.4	98.3	100	94.3	98
HCV RNA at baseline, LogIU/mL *	6.3 (4.5–7.2)	6.4 (3.3–7.4)	6.4 (4.6–7.3)	6.4 (5.1–7.0)	6.5 (5.0–7.0)
previous IFN Tx					
IFN mono			7.5	3.8	4.1
IFN+RBV			7.5	7.5	8.2
Peg-IFN mono			0	1.9	4.1
Peg-IFN+RBV			84.9	86.8	83.7

* expressed as median (range).

Table 1B Characteristics of patients enrolled in CONCERTO-4

	Treatment-naïve	*Non-responders*	*Relapsers*
	SMV 12W	SMV 12W	SMV 12W, PR 48W
	(n = 24)	(n = 29)	(n = 26)
male, %	33.3	55.2	50
age *	60 (37–68)	60 (38–70)	53 (45–69)
≥65, %	20.8	31	15.4
BMI, kg/m^2 *	23.0 (18.1–30.2)	22.5 (18.1–31.9)	22.4 (16.9–34.3)
IL28B SNP (rs8099917), %			
TT	66.7	89.7	7.7
TG	33.3	10.3	80.8
GG	0	0	11.5
HCV genotype 1b, %	100	100	96.2
HCV RNA at baseline, LogIU/mL *	6.6 (5.4–7.0)	6.6 (4.9–7.4)	6.5 (5.1–7.4)
previous IFN Tx			
IFN mono		3.4	0
IFN+RBV		0	11.5
Peg-IFN mono		0	0
Peg-IFN+RBV		96.6	88.5

* expressed as median (range).

guided criteria were set, all subjects met the criteria and underwent 24 weeks of treatment, yielding an SVR24 rate of 92% (22/24) (Fig. 2).

In the overseas QUEST-1 study,[12] subjects were administered SMV 150 mg once daily + Peg-IFNα-2a + RBV triple therapy for the first 12 weeks, then response-guided criteria were set as for the CONCERTO-1 trial, with 85% of subjects meeting the criteria and undergoing 24 weeks of treatment. The overall SVR12 rate was 80%; 71% (105/147) in genotype 1a and 90% (105/117) in genotype 1b. The QUEST-2 study[13] set two groups, with either Peg-IFNα-2a or Peg-IFNα-2b, otherwise following the same

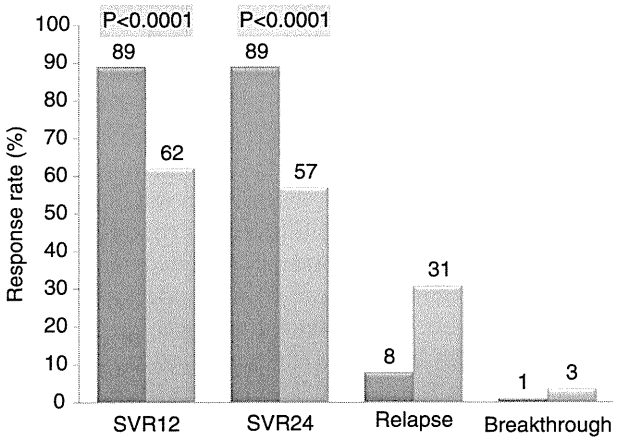

Figure 1 Therapeutic results for SMV + Peg-IFNα-2a + RBV triple therapy for treatment-naïve patients (from CONCERTO-1 trial[9]). ■, SMV + Peg-IFNα-2a + RBV; ■, Peg-IFNα-2a + RBV.

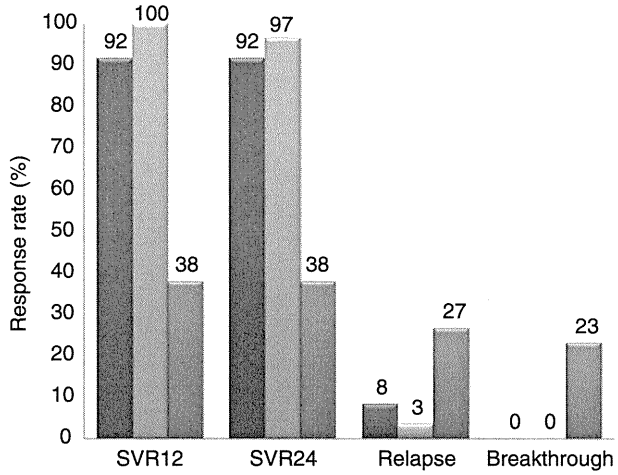

Figure 2 Therapeutic results for SMV + Peg-IFNα-2b + RBV triple therapy for treatment-naïve patients, non-responders, and relapsers (from CONCERTO-4 trial[11]). ■, treatment-naïve cases; ■, relapsers; ■, non-responders. Total treatment duration was 24W for treatment-naive and relapsers, and 48W for non-responders.

protocol as the QUEST-1 study for treatment durations. As a result, 91% of subjects met the criteria and underwent 24 weeks of treatment. The overall SVR12 rate was 81%; 80% (86/107) and 82% (123/150) in genotype 1a and 1b, respectively. The SVR12 rate for Peg-IFNα-2a and Peg-IFNα-2b was 88% and 78%, respectively. In both these studies, triple therapy including SMV yielded significantly higher SVR rates than for 48 weeks of Peg-IFN + RBV dual therapy.

In this way, clinical trials of SMV-based triple therapy regimens were conducted using a response-guided protocol that set a treatment duration of 24 or 48 weeks, with almost all subjects meeting the criteria for the shorter duration. The SVR rate for IFN-naïve subjects in the Japanese studies was 89–92%, and in the overseas studies it was 82–90% for genotype 1b, significantly higher than the SVR rate in the control groups administered 48 weeks of Peg-IFN + RBV dual therapy.

2.1.2 Relapsers following previous treatment

The Japanese CONCERTO-3 trial,[10] conducted with subjects who relapsed following previous IFN therapy, was conducted using a similar protocol to the CONCERTO-1 trial.[9] All subjects met the response-guided criteria and underwent 24 weeks of treatment, yielding an SVR24 rate of 90% (44/49) (Fig. 3). Similarly, the CONCERTO-4 trial,[11] conducted with relapsers, followed a similar therapeutic protocol to the CONCERTO-3 trial,[10] using Peg-IFNα-2b. All subjects met the response-guided criteria and underwent 24 weeks of treatment, yielding an SVR24 rate of 97% (28/29) (Fig. 2).

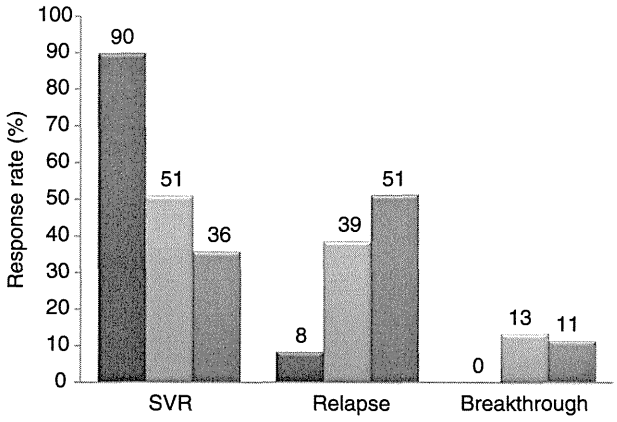

Figure 3 Therapeutic results for SMV + Peg-IFNα-2a + RBV triple therapy for non-responders and relapsers (from CONCERTO-2 and CONCERTO-3 trials[10]). ■, relapsers; ■, non-responders (SMV for 12 wks); ■, non-responders (SMV for 24 wks).

The overseas PROMISE study,[14] conducted with relapsers, was performed using a similar protocol to the QUEST-1 study. As a result, 93% of subjects met the response-guided criteria and underwent 24 weeks of treatment. The overall SVR12 rate was 79%; 70% (78/111) in genotype 1a and 86% (128/149) in genotype 1b.

In this way, in clinical trials of SMV-based triple therapy regimens with relapsers following previous IFN therapy, majority of subjects met the response-guided criteria and underwent 24 weeks of treatment. The SVR rate for the Japanese studies was 90–97%, and in the overseas studies it was 86% for genotype 1b, significantly higher than the SVR rate in the control groups administered 48 weeks of Peg-IFN + RBV dual therapy.

2.1.3 Non-responders to previous treatment

In the Japanese CONCERTO-2 trial,[10] non-responders to previous IFN therapy were administered SMV + Peg-IFNα-2a + RBV triple therapy for 12 weeks (SMV 12W group) or 24 weeks (SMV 24W group). The total treatment duration for both groups was set using response-guided criteria similar to those for the CONCERTO-1 trial,[9] with 96% and 98% of subjects, who completed 24 weeks of treatment respectively, meeting the criteria and finishing the treatment at 24 weeks. The SVR24 rate was 51% (27/53) for the SMV 12W group, and 36% (19/53) for the SMV 24W group (Fig. 3). In the CONCERTO-4 trial,[11] non-responders were administered SMV + Peg-IFNα-2b + RBV triple therapy for 12 weeks, followed by Peg-IFNα-2b + RBV dual therapy for 36 weeks, for a total treatment duration of 48 weeks. The SVR24 rate was 38% (10/26) (Fig. 2).

Although the Japanese CONCERTO-2[10] and CONCERTO-4[11] trials were conducted with non-responders, they did not conduct any further analyses subdividing non-responders into partial responders, with a decrease in the HCV RNA level by ≥2 log IU/mL at week 12 of the previous treatment, and null responders, with a decrease < 2 log IU/mL. On the other hand, the overseas phase II ASPIRE trial,[8] conducted with relapsers and non-responders, reported therapeutic results separately for partial responders and null responders. This trial assigned subjects to one of 3 groups, all with a total treatment period of 48 weeks. They were administered SMV + Peg-IFNα-2a + RBV triple therapy for 12 weeks or 24 weeks, followed by Peg-IFNα-2a + RBV dual therapy for the remaining time, or triple therapy for the entire 48 weeks. SMV was administered in a daily dosage of either 100 mg or 150 mg. The SVR rate for the SMV 12, 24 and 48 week

Table 2 Drugs contraindicated for co-administration with SMV (reproduced from[16])

Generic name	Trade name
Efavirenz	Stocrin
Rifampicin	Rifadin
Rifabutin	Mycobutin

groups was 70%, 66% and 61%, respectively, at the 100 mg dosage, and 67%, 72% and 80% at the 150 mg dosage, with no difference seen between groups due to treatment duration. The SVR rate in relapsers was 85% for both the 100 mg and 150 mg dosages. On the other hand, the SVR rate for partial responders and null responders was 57% and 46%, respectively, at the 100 mg dosage of SMV, and 75% and 51% at the 150 mg dosage. This indicates that within the non-responders, a higher SVR rate is achieved in partial responders than in null responders. In particular, if we confine the analysis to genotype 1b, common in Japanese patients, the SVR rate for partial responders and null responders was 68% and 56%, respectively, at the 100 mg dosage of SMV, and 88% and 58% at the 150 mg dosage. In genotype 1a, the SVR rate for partial/null responders was 56%/33% at 100 mg and 42%/33% at 150 mg.[8]

Recommendations

- ***The SVR rate in IFN-naïve subjects was significantly higher for SMV + Peg-IFN + RBV triple therapy than for Peg-IFN + RBV dual therapy for 48 weeks.***
- ***A high SVR rate of 90–97% was achieved with SMV + Peg-IFN + RBV triple therapy in relapsers following previous IFN therapy.***
- ***An SVR rate of 36–51% was achieved with SMV + Peg-IFN + RBV triple therapy in non-responders to previous IFN therapy.***
- ***In an overseas trial, subanalysis of non-responders to previous IFN therapy showed a higher SVR rate in partial responders than in null responders, although there is no data available regarding Japanese subjects.***

2.2 Adverse reactions

In the CONCERT-1 trial,[9] the treatment completion rate was 92.7%. Only 4.9% of subjects in the triple therapy group discontinued treatment due to adverse events, as against 8.3% of subjects in the Peg-IFNα-2a + RBV dual therapy group, with no significant difference between groups.

Elevated bilirubin levels were seen in 40.7% of subjects administered SMV, but these were mild, transient increases not associated with elevated AST or ALT levels. Bilirubin levels in grade 1 (1.1–1.5 mg/dL) were seen in 25.2%, grade 2 (1.6–2.5 mg/dL) in 14.6%, and grade 3 (2.6–5.0 mg/dL) in 0.8%, with no cases of grade 4 (> 5.0 mg/dL). Elevated bilirubin levels are reported to be caused by inhibition of hepatic transporter activity by SMV.[15]

The type and incidence of adverse reactions, including anemia, skin conditions, renal dysfunction, hyperuricemia, malaise, and gastrointestinal symptoms, were similar for SMV + Peg-IFN + RBV triple therapy and for Peg-IFN + RBV dual therapy. The incidence and degree of anemia was similar for both treatment groups; for the SMV-based triple therapy group, the lowest hemoglobin level was ≥10.6 g/dL in 29.3% of subjects, grade 1 anemia (Hb 9.5–10.5 g/dL) in 41.5%, grade 2 anemia (8.0–9.4 g/dL) in 29.3%, and no cases of grade 3 anemia (<8.0 g/dL).

Skin conditions were reported in 57.7% of subjects, all grade 1 or 2, with similar incidences, degrees of severity, and discontinuation rates in the two treatment groups. No serious cutaneous reactions, such as Stevens-Johnson syndrome (SJS) or drug-induced hypersensitivity syndrome (DIHS), were reported.

Recommendations

- ***A transient, mild elevation in bilirubin levels may be seen in patients undergoing SMV + Peg-IFN + RBV triple therapy, caused by inhibition of hepatic transporter activity.***
- ***The type and incidence of other adverse reactions are similar to those seen with Peg-IFN + RBV dual therapy, yielding high completion rates.***

2.3 Drug interactions

Since SMV is mainly metabolized by CYP3A, co-administration with inhibitors or inducers of CYP3A may affect plasma levels of SMV. In particular, co-administration with strong inducers of CYP3A may enhance the metabolism and markedly lower plasma SMV levels, resulting in attenuating the therapeutic effects. As a result, co-administration of drugs listed in Table 1 is contraindicated.[16]

In addition, since SMV inhibits OATP1B1 and P-glycoprotein, co-administration with drugs transported through these channels may reduce plasma levels of those drugs. The package insert should be referred to before administrating SMV.

Recommendations

- ***Since SMV is mainly metabolized by CYP3A and inhibits OATP1A1 and P-glycoprotein, co-administration of***

some drugs is contraindicated. The package insert should be referred to before administrating SMV.

2.4 Drug resistance

The CONCERTO-2 and CONCERTO-3 trials,[10] conducted with non-responders and relapsers, investigated gene mutations in the NS3 protease region in cases of treatment failure, including breakthrough, meeting the discontinuation criteria due to insufficient antiviral effect, HCV RNA positive at completion of treatment, and relapse following completion. Testing for genetic mutations was possible in 59 out of 61 cases of treatment failure, in 54 (92%) of whom mutations conferring SMV resistance were detected. Almost all of these were amino acid 168 substitutions (52/54), with 42 cases of substitution including D168V (35 single D168V substitutions, 7 mixed or multiple substitutions), and 10 single or mixed D168A/H/T/E/X substitutions. For the two cases with no D168 substitutions detected, a single Q80L substitution was seen in one, and mixed Q80K and R155K substitutions in the other. Genotype 1b was present in 97% of the subjects of these studies, and the overseas ASPIRE study also reported that D168V substitutions are responsible for almost all SMV resistance in genotype 1b, whereas R155K substitutions are mainly responsible for SMV resistance in genotype 1a.[17]

Overseas clinical trials have reported that the presence of Q80K polymorphism pretreatment in patients with genotype 1a may reduce the SVR rate.[8,12,13] As Q80K polymorphism is detected in 23–41% of patients with genotype 1a, this may be a predictive factor for therapeutic efficacy. Q80K polymorphism is rare in patients with genotype 1b.[8]

Recommendations

- ***Resistant mutations are found in a high proportion of patients in whom SMV + Peg-IFN + RBV triple therapy is ineffective. Almost all of these mutations were D168V substitutions in genotype 1b.***
- ***SVR rates may be reduced in patients with genotype 1a and Q80K polymorphism pretreatment. Q80K polymorphism is rare in patients with genotype 1b.***

3. TREATMENT-NAÏVE PATIENTS

A NUMBER OF new agents are under development for the treatment of HCV genotype 1 and high viral load (≥5.0 log IU/mL using real-time PCR, HCV core antigen ≥300 fmol/L) infections. These include HCV selective antiviral agents (protease inhibitors, polymerase inhibitors, NS5A inhibitors), new IFN preparations, RBV prodrugs, and agents with immunostimulant effects. At present, however, what we have available for general clinical use are antiviral therapies based on IFN preparations, in other words Peg-IFN (IFN) ± RBV ± protease inhibitors (SMV, TVR). In 2011 TVR + Peg-IFN + RBV triple therapy became available for use in Japan. Use of this combination reduced the duration of treatment for 48 or 72 weeks to 24 weeks, and provided a marked improvement in therapeutic efficacy, albeit some problems with adverse reactions. In December 2013, national medical insurance coverage approved the use of SMV,[9–11] a second generation protease inhibitor, for the treatment of genotype 1 high viral load infections. The duration of treatment for SMV + Peg-IFN + RBV triple therapy is 24 weeks, the same as for TVR-based triple therapy. However, once daily dosing for the former, as well as high SVR rates of 80–90% in Japanese clinical trials with treatment naïve subjects (DRAGON,[6] CONCERTO-1,[9] and CONCERTO-4[11]), and similar rates of adverse reactions to the control Peg-IFN + RBV dual therapy group, make SMV + Peg-IFN + RBV triple therapy the present treatment of first choice.

There are no clear discontinuation criteria for SMV-based triple therapy, and very few patients in whom this regimen is contraindicated, so in general the discontinuation criteria for TVR-based triple therapy should be followed.

In some patients, however, in whom adverse reactions are a concern, and the risk of carcinogenesis is considered low, it may be possible to await the introduction of the new agents with more favorable safety profiles.

3.1 Predictors of therapeutic efficacy of SMV-based combination therapy

3.1.1 IL28B

In the Japanese CONCERTO −1 trials using SMV-based combination therapy, subanalysis according to IL28B alleles (rs8099917 SNP) yielded an SVR24 rate of 94% (77/82) for the TT allele, and 78% (32/41) for the TG/GG alleles.[9] This represents a relatively high SVR rate for the TG or GG minor alleles achieved with SMV-based combination therapy, unlike Peg-IFN + RBV dual therapy, whose therapeutic efficacy is strongly affected by IL28B polymorphism (Fig. 4). A similar trend was seen in the CONCERTO-4 trial, with an SVR24 rate of 100% (16/16) for the TT allele, and 75% (6/8) for the TG/GG alleles, although subject numbers were small.[11]

In the overseas QUEST-1 and QUEST-2 trials using SMV-based combination therapy, SVR12 rates stratified

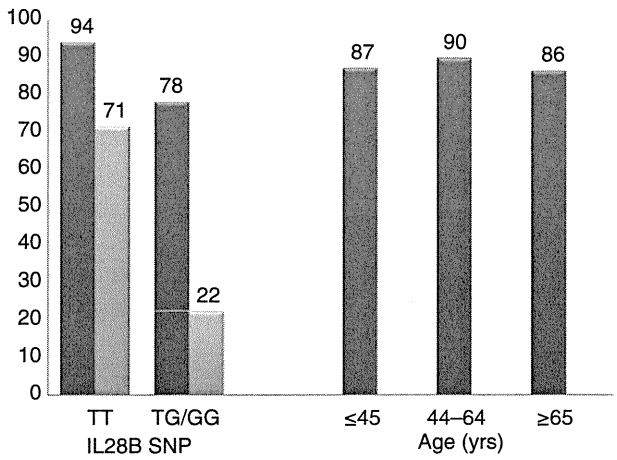

Figure 4 Results in treatment-naïve patients using the SMV + Peg-IFNα-2a + RBV triple therapy regimen; influence of IL28B polymorphism and age (CONCERTO-1 trial[9]). ■, SMV + Peg-IFNα-2a + RBV; ■, Peg-IFNα-2a + RBV.

for IL28B alleles (rs12979860 SNP) were 97% (72/77) and 96% (72/77) respectively for the CC allele, 76% (114/150) and 80% (114/142) for the CT allele, and 65% (24/37) and 58% (23/40) for the TT allele, showing a similar trend to the Japanese studies (Table 3).

3.1.2 Age and fibrosis

SVR24 rates stratified for age in the CONCERTO-1 trial were 87% (20/23) for subjects ≤ 45, 90% (70/78) for those aged 44–64, and 86% (19/22) for those ≥65. No clear differences were seen in SVR rates according to age for those ≤70 years old (Fig. 4). As for fibrosis, QUEST-1 and QUEST-2 examined the relationship between hepatic fibrosis and SVR12 rates, finding SVR12 rates of 83% and 85% respectively for F0-2, 78% and 67% for F3, and 58% and 65% for F4 (Table 3). These results suggest a correlation between the degree of hepatic fibrosis and the efficacy of SMV-based combination therapy. However, the classification F4 is not included in Japanese clinical trials, and there have been no reports of therapeutic results stratified for the degree of hepatic fibrosis.

Taken together, the results of Japanese and overseas clinical trials showed no clear age-related differences in therapeutic effect of SMV + Peg-IFN + RBV triple therapy. Although IL28B SNPs and the degree of fibrosis may influence therapeutic efficacy, SVR rates of 60–80% were still achieved in patients with IL28B minor alleles and advanced fibrosis ≥ F3. Accordingly, at present we cannot say that age, IL28B SNPs or the degree of fibrosis exerts any great influence on the therapeutic efficacy of this treatment regimen.

Recommendations

- ***SMV + Peg-IFN + RBV triple therapy is at present the treatment of first choice in IFN-naïve patients.***
- ***IL28B polymorphism has little influence on the SVR rate in IFN-naïve patients undergoing SMV + Peg-IFN + RBV triple therapy, with relatively high SVR rates achieved even in patients with the TG/GG minor alleles.***
- ***In Japanese clinical trials conducted with subjects aged ≤ 70, no clear correlation could be identified between age and SVR rates.***
- ***Although Japanese data is lacking, the results of overseas clinical trials indicate that advanced hepatic fibrosis may influence SVR rates.***
- ***From the above, in general, if treatment is likely to be tolerated, SMV-based triple therapy is indicated in all patients who meet the criteria for antiviral therapy (ALT > 30 U/L or platelet count <150 000/μL), irrespective of IL28B SNP status.***
- ***In some patients, however, in whom adverse reactions are a concern, and the risk of carcinogenesis is***

Table 3 Overseas results with SMV + Peg-IFN + RBV triple therapy; influence of IL28B polymorphism and age (SVR12, %) (QUEST-1,[12] QUEST-2[13] and PROMISE trials[14])

		IL28B SNP			Fibrosis (METAVIR)		
		CC	CT	TT	F0-2	F3	F4
QUEST-1	SMV+Peg-IFN+Rib	97	76	65	83	78	58
	Peg-IFN+Rib	78	42	24			
QUEST-2	SMV+Peg-IFN+Rib	96	80	58	85	67	65
	Peg-IFN+Rib	81	41	19			
PROMISE	SMV+Peg-IFN+Rib	89	78	65	82	73	74
	Peg-IFN+Rib	53	34	18			

considered low, it may be possible to await the introduction of the new agents with more favorable safety profiles.

3.2 Selection of antiviral therapy in treatment-naïve patients (Fig. 5)

3.2.1 Elderly patients

In this patient group at high risk of hepatocellular carcinogenesis, the best possible antiviral therapy should be promptly commenced. However, the possibility of adverse reactions, and the possibility that viral eradication may not be achieved, should be thoroughly explained to the patient in advance. Although the introduction of TVR + Peg-IFN + RBV triple therapy improved SVR rates in comparison to Peg-IFN + RBV dual therapy,[1] postmarketing surveys revealed serious adverse reactions in approximately 40% of elderly patients. Accordingly, it is recommended that TVR therapy should be commenced at a reduced dosage of 1500 mg/day,[18] although great caution is still required in its use in this age group. On the other hand, clinical trials of SMV + Peg-IFN + RBV triple therapy for treatment-naïve patients have reported an SVR rate of 86% (19/22) in elderly patients aged ≥ 65 (and ≤70), indicating a therapeutic efficacy similar to that seen in non-elderly patients (Fig. 4). Furthermore, very little difference is seen between SMV-based triple therapy and Peg-IFN + RBV dual therapy in terms of safety. Accordingly, SMV + Peg-IFN + RBV triple therapy should be commenced as soon as possible if treatment is likely to be tolerated.

If antiviral therapy is not introduced due to concerns about tolerability, and ALT levels are abnormal, protective therapy (stronger neo-minophagen C; SNMC and/or ursodeoxycholic acid; UDCA) should be commenced.[1] Long-term low dose Peg-IFN (IFN) therapy is another option.[1]

Recommendations

- ***Elderly patients are at high risk of hepatocellular carcinogenesis, and should commence antiviral therapy promptly.***
- ***SMV + Peg-IFN + RBV triple therapy is the antiviral treatment of first choice in treatment-naïve elderly patients.***
- ***If antiviral therapy is not introduced and ALT levels are abnormal, protective therapy (SNMC, UDCA) should be commenced. Long-term low dose Peg-IFN (IFN) therapy is another option.***

3.2.2 Non-elderly patients

Although the risk of hepatocellular carcinogenesis is relatively low in non-elderly patients, the introduction of antiviral therapy is inevitably necessary in cases of advanced hepatic fibrosis, as in elderly patients. In general, SMV + Peg-IFN + RBV triple therapy should be administered to patients with advanced fibrosis. Also consider IFNβ + RBV combination therapy in patients with depressive symptoms.[1] The risk of carcinogenesis is considered lower in patients with mild fibrosis, so it may be reasonable to await the advent of newer agents with fewer adverse reactions. Determination of IL28B SNP status may be of benefit when the decision whether to commence treatment is a difficult one. However, as mentioned above, clinical trials of SMV + Peg-IFN + RBV triple therapy in treatment-naïve subjects reported SVR rates of approximately 80% in patients

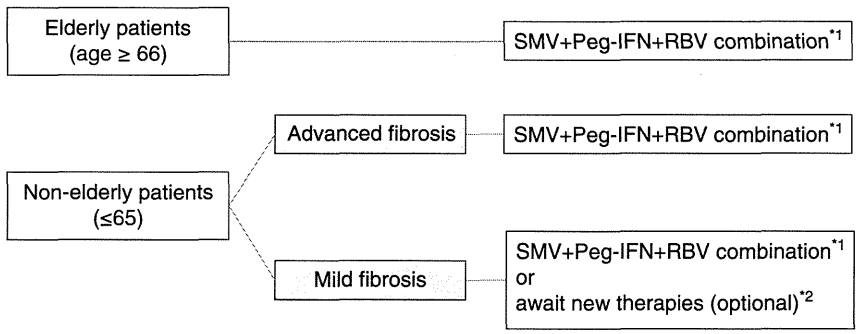

Figure 5 Treatment flow chart for treatment-naïve patients. Use IL28B testing as a reference if available. Follow therapy protocol for treatment-naïve patients if previous therapy was Peg-IFN (IFN) monotherapy or details of previous therapy with Peg-IFN (IFN) and RBV are unknown. Consider IFNβ + RBV combination if depressive symptoms present. *1 TVR + Peg-IFN + RBV triple therapy is another option (TVR should be commenced at a reduced dosage of 1500 mg/day in the elderly). *2 Protective therapy or low dose Peg-IFN(IFN) therapy if abnormal ALT levels.

with IL28B minor alleles (Fig. 4). SMV-based triple therapy should therefore be considered in all patients who meet the criteria for antiviral therapy (ALT > 30 U/L or platelet count < 150 000/μL)[1] if treatment is likely to be tolerated, irrespective of IL28B SNP status. If antiviral therapy is not introduced, and ALT levels are abnormal, protective therapy should be commenced.[1]

Recommendations

- ***Although the risk of hepatocellular carcinogenesis is relatively low in non-elderly patients, the introduction of antiviral therapy is inevitably necessary in cases of advanced hepatic fibrosis, as in elderly patients. Waiting for advent of newer agents with fewer adverse reactions is an option in patients with mild fibrosis.***
- ***In general, SMV + Peg-IFN + RBV triple therapy should be administered to treatment-naïve non-elderly patients with advanced fibrosis.***
- ***Although treatment may be delayed in non-elderly patients with mild fibrosis, SMV-based triple therapy should be considered in all patients who meet the criteria for antiviral therapy (ALT > 30 U/L or platelet count < 150 000/μL) if treatment is likely to be tolerated. If antiviral therapy is not introduced, and ALT levels are abnormal, protective therapy should be commenced.***

4. PREVIOUSLY-TREATED CASES (RETREATMENT)

4.1 Predictors of therapeutic efficacy of SMV-based combination therapy

SEVERAL LINES OF clinical studies indicate that, in retreatment using SMV + Peg-IFN + RBV combination therapy, response to the previous treatment is the best indicator of the efficacy of retreatment when IFN/Peg-IFN + RBV combination therapy is ineffective.[10,11,17] In the overseas phase II trial (ASPIRE trial), administering SMV + Peg-IFN + RBV triple therapy to previously treated subjects, Peg-IFN + RBV combination therapy was administered for 48 weeks, in combination with SMV 100 mg or 150 mg/day for the first 12 or 24 weeks, or the entire 48 weeks. As described above, SVR rates for the different SMV dosages (100/150 mg/day) were 85%/85% in relapsers, 57%/75% in partial responders, and 46%/51% in null responders. No differences were seen in SVR rates according to dosage, whereas the response to previous therapy did influence SVR rates, with a greater therapeutic effect seen in partial responders than in null responders.[17] Similarly, in Japanese phase III trials (CONCERTO-2/3[10]) administering SMV + Peg-IFN + RBV triple therapy to previously treated subjects, SVR rates in relapsers and non-responders were 90% (44/49) and 51% (27/53), respectively (Fig. 3). In the CONCERTO-4[11] using Peg-IFNα-2b, the SVR rate was 97% (28/29) in relapsers, and 38% (10/26) in non-responders, a similar result to the CONCERTO-2/3[10] trials using Peg-IFNα-2a (Fig. 2).

Examination of the therapeutic efficacy of SMV-based combination therapy in relapsers, stratified for IL28B SNP status, revealed SVR24 rates of 91% (32/35) for the TT allele, and 86% (12/14) for the TG/GG alleles in the CONCERTO-3 trial (Fig. 6), and 96% (25/26) for the TT allele, and 100% (3/3) for the TG/GG alleles in the CONCERTO-4 trial. High SVR rates were achieved in relapsers in both studies, irrespective of IL28B SNP status. On the other hand, in the CONCERTO-2 trial,[10] conducted with non-responders, SVR24 rates stratified for IL28B SNP status were 50% (7/14) for the TT allele, and 42% (39/92) for the TG/GG alleles (Fig. 6), again showing no difference in SVR rates associated with IL28B polymorphism.

In the overseas PROMISE trial,[14] conducted with relapsers, SVR12 rates stratified for IL28B alleles (rs12979860 SNP) were 89% (55/62) for the CC allele, 78% (131/167) for the CT allele, and 65% (20/31) for the TT allele. Examination of the relationship between hepatic fibrosis and SVR12 rates yielded SVR12 rates of 82% for F0-2, 73% for F3, and 74% for F4 (Table 3). These results demonstrated that, unlike treatment-naïve cases, high SVR rates can be achieved irrespective of the

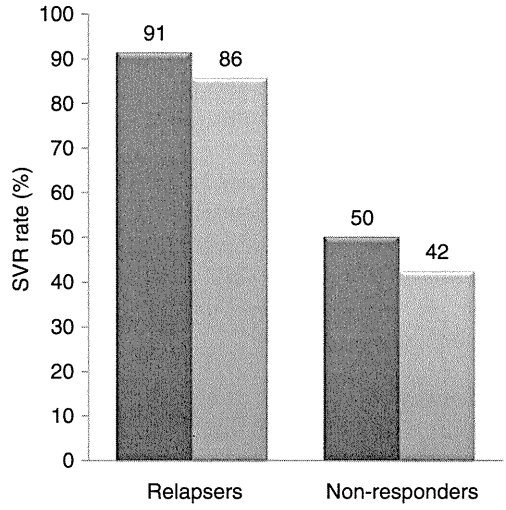

Figure 6 Results of treatment using SMV + Peg-IFNα-2a + RBV triple therapy in relapsers and non-responders depending on IL28B status (CONCERTO-2/3 trial[10]). ■, TT; ■, TG/GG.

degree of hepatic fibrosis in relapsers. However, the classification F4 is not included in Japanese clinical trials, and there have been no reports of therapeutic results stratified for the degree of hepatic fibrosis.

In this way, the response to previous therapy is at present the most important predictive factor for SVR rates achieved by SMV + Peg-IFN + RBV triple therapy.

There is presently no evidence available concerning the therapeutic efficacy of SMV + Peg-IFN + RBV triple therapy in non-responders to previous TVR + Peg-IFN + RBV triple therapy. However, retreatment with SMV-based therapy, with particular caution regarding adverse reactions, is an option in patients previously administered TVR-based therapy who were unable to tolerate adequate dosages of one or more agents due to adverse reactions.

When previously treated patients undergo retreatment with a combination including RBV, if RBV was not included in the previous IFN or Peg-IFN monotherapy regimen, the response to the earlier therapy is not a strong predictive factor for the efficacy of further treatment, so in general follow the treatment protocol for treatment-naïve patients. If the HCV RNA decrease at week 12 of the previous treatment is unknown, but it is clear that HCV RNA did not become negative, follow the retreatment protocol for null responders.

Recommendations

- *The response to previous therapy is the best indicator for the response to retreatment in patients who were non-responders to previous IFN/Peg-IFN + RBV combination therapy. The relationship between IL28B SNPs and therapeutic efficacy is unclear at present.*
- *Retreatment with RBV combination therapy in patients previously administered IFN or Peg-IFN monotherapy should in general follow the treatment protocol for treatment-naïve cases. If the HCV RNA decrease at week 12 of the previous treatment is unknown, but it is clear that HCV RNA did not become negative, follow the null response retreatment protocol.*
- *There is presently no evidence available concerning the therapeutic efficacy of SMV + Peg-IFN + RBV triple therapy in non-responders to previous TVR + Peg-IFN + RBV triple therapy.*

4.2 Selection of antiviral therapy in previously-treated patients (retreatment) (Fig. 7A, 7B)

4.2.1 Elderly patients

SMV + Peg-IFN + RBV triple therapy should be commenced promptly if treatment is likely to be tolerated. In particular, relapsers and partial responders are favorable indications. As for null responders, in the overseas clinical trial (ASPIRE), SVR rates of approximately 50% were achieved when SMV + Peg-IFN + RBV combination therapy administered to null responders to previous treatment. Introduction of this regimen is therefore recommended to null responders, although it may be an option to await the advent of newer agents with fewer adverse reactions if problems with tolerability are anticipated. TVR + Peg-IFN + RBV triple therapy is another option, although it is recommended that TVR therapy should be commenced at a reduced dosage of 1500 mg/day as in treatment-naïve cases, and great caution is still required in its use.

The risk of hepatocellular carcinogenesis is high in elderly patients, and when viral eradication cannot be achieved protective therapies (SNMC, UDCA) should be administered with the aims of biochemical improvement and inhibiting hepatocellular carcinogenesis.[1] Long-term low dose Peg-IFN (IFN) therapy is another option.[1]

Recommendations

- *In retreatment of elderly patients, if treatment is likely to be tolerated, SMV + Peg-IFN + RBV triple therapy should be administered to relapsers and partial responders.*
- *SVR rates of approximately 50% are achieved with SMV + Peg-IFN + RBV combination therapy in null responders to previous treatment, and introduction of this regimen is therefore recommended to null responders as well. If problems with tolerability are anticipated, it may be an option to await the advent of newer agents with fewer adverse reactions.*
- *When viral therapy is not administered, protective therapies should be administered to patients with abnormal ALT levels. Long-term low dose Peg-IFN (IFN) therapy is another option.*

4.2.2 Non-elderly patients

As with elderly patients, as a general rule non-elderly patients with advanced fibrosis and associated high risk of hepatocellular carcinogenesis should be administered SMV + Peg-IFN + RBV combination therapy. Even in patients with mild fibrosis and a lower risk of carcinogenesis, a high SVR rate of approximately 90% is achieved with SMV + Peg-IFN + RBV combination therapy in relapsers and partial responders. Therefore, if treatment is likely to be tolerated, SMV-based triple therapy should be administered to this patient group.

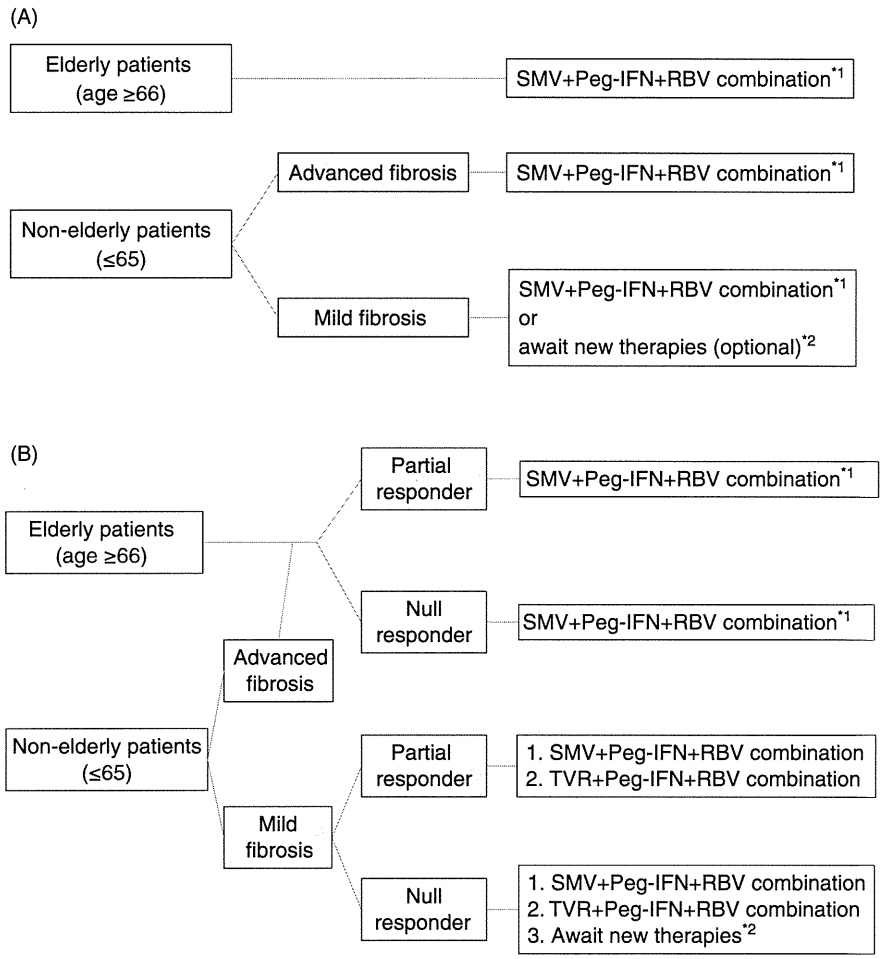

Figure 7 Treatment flow chart for previously-treated patients (retreatment). A. Relapsers. B. Non-responders. "Previously-treated" refers to previous treatment with Peg-IFN(IFN)/RBV combination therapy. Consider IFNβ + RBV combination if depressive symptoms present. Follow the null response retreatment protocol in non-responders if the quantitative decrease in HCV RNA at week 12 of the previous treatment is unknown. *1 TVR + Peg-IFN + RBV triple therapy is another option (TVR should be commenced at a reduced dosage of 1500 mg/day in the elderly). *2 Protective therapy or low dose Peg-IFN/IFN therapy if abnormal ALT levels.

On the other hand, for non-elderly null responders with mild fibrosis, if adverse reactions are a concern, it may be reasonable to await the advent of newer agents with fewer adverse reactions. When there are no problems with tolerability, SMV + Peg-IFN + RBV combination therapy can be commenced in patients who meet the therapeutic indications for antiviral therapy (ALT > 30 U/L or platelet count < 150 000/μL).

TVR + Peg-IFN + RBV triple therapy is an alternative option in cases with mild fibrosis, where safety is relatively guaranteed.

Recommendations

- *In general, SMV + Peg-IFN + RBV triple therapy should be administered for retreatment of non-elderly patients with advanced fibrosis, as for elderly patients.*
- *Even in patients with mild fibrosis, a high SVR rate of approximately 90% is achieved with SMV + Peg-IFN + RBV combination therapy in relapsers and partial responders. If treatment is likely to be tolerated, SMV-based triple therapy should be therefore administered to this patient group.*
- *On the other hand, for non-elderly null responders with mild fibrosis, if adverse reactions are a concern, it may be reasonable to await the advent of newer agents with fewer adverse reactions. When there are no problems with tolerability, SMV + Peg-IFN + RBV combination therapy can be commenced in patients who meet the therapeutic indications for antiviral*

therapy (ALT > 30 U/L or platelet count < 150 000/μL).

- ***In non-responders (partial and null responders), TVR + Peg-IFN + RBV triple therapy is an alternative option in cases with mild fibrosis, if treatment is likely to be tolerated.***

CONFLICTS OF INTEREST

THE MEMBERS OF Drafting Committee for Hepatitis Management Guidelines have received royalty from SRL, lecture fees from Ajinomoto Pharmaceuticals, MSD, Daiichi-Sankyo, Dainippon-Sumitomo Pharma, Mitsubishi Tanabe Pharma, Chugai Pharmaceutical, Bristol-Myers-Squibb, Janssen Pharmaceutical Companies, and research support from Eisai, MSD, Kan Research Institute, Chugai Pharmaceutical, Mitsubishi Tanabe Pharma, Dainippon-Sumitomo Pharma, Toray, Minophagen Pharmaceutical.

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Hepatology Research

Hepatology Research 2014; 44 (Suppl. 1): 1–58 doi: 10.1111/hepr.12269

Special Report

JSH Guidelines for the Management of Hepatitis B Virus Infection

Drafting Committee for Hepatitis Management Guidelines and the Japan Society of Hepatology*,**

PREFACE

THE JAPAN SOCIETY of Hepatology established the Drafting Committee for Hepatitis Management Guidelines in November 2011, and published the Guidelines for the Management of Hepatitis C in May 2012 (English version, Jan 2013). Thence the Committee decided our next task of high priority is to produce the practical guidelines for hepatitis B, also a significant burden to the health care system. Here the Committee has launched the Guidelines for the Management of Hepatitis B Virus Infection. As with hepatitis C virus, 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 update these guidelines at appropriate intervals, as new evidence comes to hand.

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1. INTRODUCTION

1.1 Hepatitis B virus

IT IS ESTIMATED that there are 400 million patients of persistent hepatitis B virus (HBV) infection in the world.[1] In Japan, the HBV infection rate is around 1%. HBV infection at birth or during infancy leads to persistent infection in over 90% of cases. Approximately 90% of these undergo seroconversion from HBe antigen (HBeAg) positive at the initial stage to anti-HBe antibody positive and become inactive carriers, and in virtually all cases the condition effectively stabilizes. But in the remaining 10% the virus remains active, leading to chronic hepatitis, and in around 2% of cases annually, there is further progression to liver cirrhosis, with potential for hepatocellular carcinoma (HCC) and liver failure.[2–4]

Clinical research on HBV dates back to the discovery of the Australia antigen (later renamed HBs antigen; HBsAg) by Blumberg *et al.* in 1964. Prince *et al.* and Okouchi *et al.* subsequently reported a link between the Australia antigen and hepatitis. And there have been various other discoveries demonstrating that the existence of an asymptomatic carrier, who does not develop hepatitis following HBV infection and indicating HBV as a cause of chronic liver diseases. The base form of HBV, known as the Dane particle, was discovered in 1970, followed by the identification of HBeAg in 1972. In 1979, the whole HBV genome was successfully cloned from virus particles, enabling measurement of the virus gene (HBV DNA) for the first time.

In Japan, screening for the HBsAg was introduced at blood centers in 1972. 1986 was the year of the introduction of an anti-HBV vaccine and immunoglobulin for newborns designed to prevent vertical (mother-to-child) infection. This was highly effective in arresting the development of new HBV carriers through vertical infection, causing a marked decline in HBsAg positive rates among juveniles. The incidence of acute hepatitis caused by HBV infection, however, has not declined, mainly as a result of horizontal transmission associated with sexual activity. In recent years, there has been an increase in infection rates for the HBV genotype A, which frequently causes persistent infection.[5]

1.2 Natural history of patients with persistent HBV infection

HBV in itself is considered to have little or no cytotoxicity. Hepatocellular damages are generally caused by cellular immunity associated with cytotoxic T cells, which represent the host's immune response attacking HBV infected cells. Other immunity-associated cells such as antigen-specific helper T cells, macrophages, natural killer cells and natural killer T cells also contribute to inflammation and illness. Patients suffering from persistent HBV infection generally are categorized into four phases defined by the host immune response and the replication of HBV DNA, as shown in Figure 1.

(1) Immune tolerance phase

In infants, when the host immune response is immature, HBV infection inevitably leads to persistent infection. This is followed by a state of immune tolerance, with high levels of HBeAg and HBV DNA replication activity. The host in this phase is termed as an asymptomatic carrier, with ALT levels within the normal range and negligible activity of hepatitis. Infectivity is high. In most cases, infection during infancy is followed by a prolonged immune tolerance period lasting from a few to more than 20 years.

(2) Immune clearance phase

By adulthood, the immune response to HBV becomes an active one, which develops active hepatitis in the immune clearance phase. During the process of HBeAg seroconversion, with disappearance of HBeAg and appearance of anti-HBe antibody, the replication of

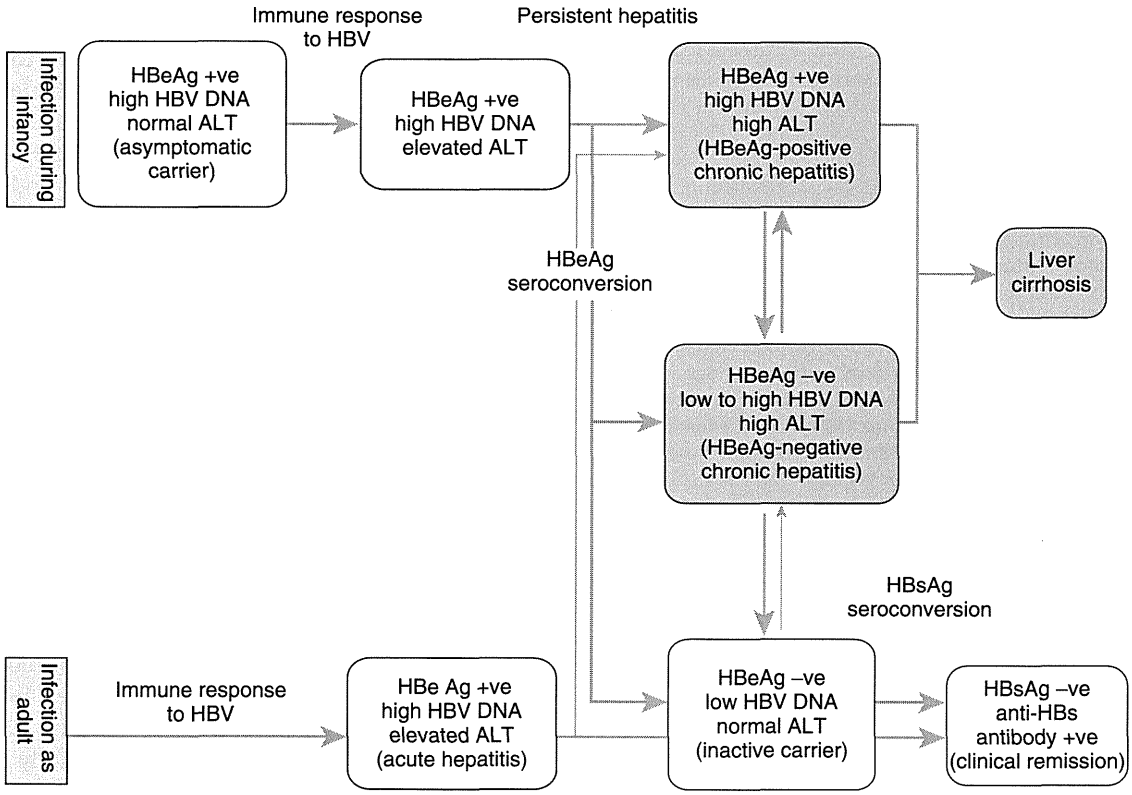

Figure 1 Natural course of persistent HBV infection.

HBV DNA is inhibited, thereby encouraging quiescence of hepatitis. However liver disease can progress in cases of persistent hepatitis that remain HBeAg positive for extended periods (HBeAg-positive hepatitis).

(3) Low replicative phase (inactive phase)

HBeAg seroconversion usually results in quiescence of hepatitis, with HBV DNA levels dropping below 4 log copies/mL (inactive carrier). In 10–20% of cases, however, HBeAg seroconversion is followed by increased HBV replication in the HBeAg negative state, causing the exacerbation of hepatitis (HBeAg-negative hepatitis). In a further 4–20% of cases, the HBeAg actually reappears and anti-HBe antibody disappears, a phenomenon known as reverse seroconversion.

(4) Remission phase

In some cases, HBeAg seroconversion causes appearance of anti-HBs antibody and disappearance of HBsAg. In the remission phase, improvement is seen in both blood tests and liver biopsy findings. The natural rate of disappearance of HBsAg in patients with persistent HBV infection is thought to be around 1%.

The natural course of persistent HBV infection can be therefore a progression from HBeAg-positive asymptomatic carrier, through HBeAg-positive (or negative) chronic hepatitis, to cirrhosis. HCC occurs at an annual rate of 5–8% in patients with cirrhosis. At the same time, however, in inactive carriers, in whom HBV DNA declines and serum ALT values are persistently normal following HBeAg seroconversion without any therapeutic intervention, there is a lower risk of progression and hepatocarcinogenesis with a good long-term prognosis. Thus it is important that treatment of patients with persistent HBV infection should be based on a thorough understanding of the natural course as described above.

Where infection occurs after the patient has reached adulthood, an immune reaction will normally develop against HBV during the early stages of infection. After a period of acute hepatitis, the virus is eliminated and quiescence occurs. With the rising incidence of HBV genotype A in recent years, however, we have seen an increasing number of adult infection cases progressing to chronic hepatitis.[5]

1.3 Treatment goals – what should we aim for?

The treatment goal of antiviral therapy for persistent HBV infection is to improve the life expectancy and quality of life (QOL) of the patient with HBV infection.

HBV infection is directly associated with the life expectancy in three ways, due to acute liver failure, chronic liver failure, and HCC. Of these three, acute liver failure usually presents the most difficult challenge in terms of prediction and prevention. Management usually centers on preventing HBV reactivation associated with immunosuppressant agents. Meanwhile, the risk factors for chronic liver failure and HCC associated with persistent HBV infection are known, and can be successfully eliminated via antiviral therapy in order to reduce the risk of disease. In other words, we can say that the treatment goal of antiviral therapy in patients with persistent HBV infection should be to inhibit activity of hepatitis and progression of hepatic fibrosis in order to prevent chronic liver failure and reduce the risk of HCC, thereby improving the life expectancy and QOL of the patient with HBV infection. HBsAg is considered the most effective surrogate marker for achieving this ultimate goal, and HBsAg elimination should be defined as the long-term goal of antiviral therapy in patients with persistent HBV infection (Table 1).

Antiviral therapy has three short term goals leading to the elimination of HBsAg: persistent normalization of ALT (≤30 U/L), HBeAg negative and anti-HBe antibody positive (HBeAg seroconversion in HBeAg-positive cases and maintain HBeAg negative status in HBeAg-negative cases), and suppression of HBV DNA replication.

Target serum HBV DNA levels differ between chronic hepatitis and cirrhosis, and also depending on the therapeutic agents. Nucleos(t)ide analogue (NA) therapy is highly effective at producing negative HBV DNA, and at maintaining a negative status through treatment. Thus the on-treatment goal should be to attain an HBV DNA negative status, as determined using high-sensitivity real-time PCR, for both chronic hepatitis and cirrhosis alike. For interferon (IFN) therapy, since HBeAg seroconversion and HBsAg reduction or elimination are expected outcomes following completion of therapy, there is no need for an on-treatment goal of reduced HBV DNA. It should be recommended to complete the full course of therapy over 24 to 48 weeks.

The off-treatment goal (i.e., after IFN therapy has concluded and NAs are no longer administered) is the absence of active hepatitis with no risk of further progression on no medication. Accordingly, the target at 24 to 48 weeks after the end of treatment is set as <4.0 log copies/mL for chronic hepatitis, and negative HBV DNA for cirrhosis.

Recommendations

- ***The treatment goal for antiviral therapy in patients with persistent HBV infection is to prevent liver failure and inhibit HCC by suppressing activity of hepatitis***

Table 1. Treatment goals for antiviral therapy

	Chronic hepatitis	Liver cirrhosis
Long-term goal	HBsAg elimination	HBsAg elimination
Short-term goals		
ALT	Persistent normal*1	Persistent normal*1
HBeAg	Negative*2	Negative*2
HBV DNA*3		
On-treatment (Ongoing NA therapy)	Negative	Negative
Off-treatment (IFN completed/NA therapy ceased*4)	< 4 log copies/ml	Negative*5

Notes
*1. Normal range of ALT is defined as ≤30 U/L.
*2. Conversion to HBeAg-negative in HBeAg-positive cases, and maintain HBeAg-negative in HBeAg-negative cases.
*3. As measured using high-sensitivity PCR (real-time PCR).
*4. At 24–48 weeks following completion of antiviral therapy.
*5. NA therapy should not to be ceased in patients with cirrhosis.

and progression of liver fibrosis, thereby improving the patient's life expectancy and overall QOL.

- ***HBsAg is considered the most effective surrogate marker for attaining this treatment goal. The long-term goal of antiviral therapy is to eliminate HBsAg.***
- ***The three short-term goals of antiviral treatment prior to elimination of HBsAg are persistent normalization of ALT, HBeAg negative and positive anti-HBe antibody, and suppression of HBV DNA replication.***
- ***The on-treatment goal is negative HBV DNA; this applies to both chronic hepatitis and cirrhosis.***
- ***Since HBeAg seroconversion and reduction (or elimination) of HBsAg are expected outcomes following completion of therapy, on-treatment HBV DNA target levels are not applied, and it should be recommended to complete a full course of treatment of 24 to 48 weeks.***
- ***The off-treatment goals (following IFN therapy and cessation of NAs) are <4.0 log copies/mL HBV DNA (chronic hepatitis), and negative HBV DNA (cirrhosis).***

1.4 Pharmacotherapy – which agents should we use?

Currently IFN and NAs are employed in antiviral therapy for persistent HBV infection. Table 2 lists the approval process of main antiviral therapy agents used in Japan by national medical insurance.

IFN therapy is intended to achieve lasting benefits from a limited treatment period. IFN therapy was first introduced to Japan in 1987. Initially, it was limited to a 28-day course of treatment, although this was extended to 6 months in 2002. In 2011, Peg-IFN (pegylated interferon) was approved for treatment of chronic hepatitis B in clinical settings. In addition to inhibiting the replication of HBV DNA, IFN has both antiviral and immunomodulatory effects. Therapeutic effects of IFN further improved with the advent of Peg-IFN.

IFN therapy offers some key advantages. Treatment is for a fixed period, and if an adequate therapeutic response is achieved, no further treatment is required. IFN therapy can therefore produce lasting therapeutic benefits in the drug-free state. Furthermore, overseas studies have reported that IFN therapy is also highly effective at eliminating HBsAg over the long term. However, disadvantages include the fact that only 20–30% of HBeAg positive cases and 20–40% of HBeAg negative cases respond well to Peg-IFN treatment; patients are required to attend hospital weekly; there are several possible adverse reactions associated with treatment; and finally, Peg-IFN treatment for cirrhosis is not currently approved by Japanese national medical insurance.

Meanwhile, NAs are a form of antiviral agent originally developed as a pharmacological therapy for

Table 2 Approval process of antiviral therapy in Japan

1987	Conventional interferon (28-day course, HBeAg positive only)
2002	Conventional interferon (six-month course, HBeAg positive only)
2000	Lamivudine
2004	Adefovir
2006	Entecavir
2011	Peg-IFN