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REVIEW

Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines

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Abstract Hepatitis C virus (HCV) infection is a serious health problem leading to cirrhosis, liver failure and hepatocellular carcinoma. The recent introduction of telaprevir, which was approved in November 2011, in combination with peg-interferon and ribavirin is expected to markedly improve the eradication rate of the virus. However, side effects of triple therapy may be severe. In a phase three III clinical trial, 2250 mg of telaprevir, which is the same dosage used in clinical trials in Western countries, was given to Japanese patients. As this dosage is considered to be relatively high for Japanese patients, who typically have lower weight than patients in Western countries, reduction of telaprevir is recommended in the 2012 revision of the guidelines established by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labour and Welfare of Japan. Other protease inhibitors with fewer side effects are now in clinical trials in Japan. Alternatively, treatment of patients with combination of direct acting antivirals without interferon has been reported. In this review we summarize current treatment options in Japan and discuss how we treat patients with chronic HCV infection.

Keywords Telaprevir · Triple therapy · Antiviral resistance · Anemia · Dose reduction

Abbreviations

HCV	Hepatitis C virus
DAAs	Direct acting anti-virals
SVR	Sustained virological response
RVR	Rapid virological response

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Introduction

At least 1.5 million people in Japan and more than 200 million people worldwide are chronically infected with the hepatitis C virus [1, 2]. Due to an aging patient population, the health burden of chronic HCV infection in Japan is expected to increase over the next several decades [3]. Chronic infection develops in 60–80 % of symptomatic patients, leading to higher risk of cirrhosis, hepatocellular carcinoma, and end-stage liver disease. Chronic HCV infection is also one of the primary indications for liver transplantation [3], and ultimately 5–7 % of patients die from complications related to HCV infection [4–7].

The goal of HCV therapy is successful eradication of the virus and resolution of liver disease. Success is defined as

the absence of detectable virus 24 weeks following the end of treatment. In some patients, the virus becomes undetectable by the end of treatment (end of treatment response) but then rebounds in the absence of therapy (relapse or transient response). Viral breakthrough occurs when the virus rebounds during the course of therapy. In non-responders, the virus remains detectable throughout therapy.

Therapy for chronic HCV infection

Hepatitis C virus genotypes vary by region and susceptibility to interferon treatment [8]. Genotype 1 is the most common genotype worldwide and in Japan [8]. Weekly injections of pegylated interferon (peg-interferon) and daily oral administration of ribavirin constitute the standard therapy for genotype 1 chronic HCV [9]. However, combination therapy is costly and poorly tolerated, requires long-term treatment (48 weeks), and is successful in only 42–52 % of patients [10–12].

The success rate of HCV therapy in Japan is expected to improve greatly following the November 2011 approval of telaprevir (VX-950/MP-424; Incivek; Vertex Pharmaceuticals, Inc., Cambridge, MA, USA), the first in a class of new direct acting antiviral (DAA) drugs. Teleprevir and a related drug, boceprevir (Victrelis), were also recently approved for treatment of genotype 1 in the US, Canada, and the European Union. While boceprevir is not approved for use in Japan, a meta-analysis found no difference in outcomes between the two drugs, except for slightly higher efficacy among prior relapsers using telaprevir [13].

Telaprevir and direct acting antiviral drugs

DAAs act by specifically inhibiting essential viral targets. Telaprevir is an NS3/4 serine protease inhibitor that mimics the carboxy-terminal region of the NS3 protease and binds slowly and tightly to the protease [14]. The NS3-4A protein is also an attractive target due to its additional role in degrading immune signaling molecules [15]. Consequently, targeting NS3-4A may not only disrupt viral replication but may also help to restore innate antiviral responses [16, 17]. However, treatment with telaprevir alone often results in a rapid decline in viral load followed by viral breakthrough due to rapid selection for resistance mutations [18, 19]. Triple therapy with peg-interferon, ribavirin, and telaprevir appears to be required to suppress viral breakthrough and achieve SVR [20].

Telaprevir clinical trials outside of Japan

Phase II studies

Several phase II and III clinical trials have established the safety and efficacy of telaprevir in the treatment of HCV genotype 1 (Table 1). The PROVE I [20] and PROVE II [21] phase II studies showed SVR rates significantly higher for triple therapy compared to the standard of care (61 vs. 41 %, 69 vs. 46 %, respectively) after 12 weeks of triple therapy followed by another 12 weeks of peg-interferon plus ribavirin combination therapy. Both studies found that reducing the length of peg-interferon and ribavirin to 12 weeks erased the advantage of triple therapy over standard therapy, and PROVE II revealed that ribavirin is required to suppress viral breakthrough [20, 21]. PROVE III examined the efficacy of triple therapy in patients who failed to achieve SVR during prior interferon therapy and reported improved SVR rates among patients with prior nonresponse (39 %), relapse (69 %), or viral breakthrough (57 %) [22].

Phase III studies

The phase III ADVANCE study compared duration of telaprevir therapy in treatment-naive patients using three treatment arms, a control peg-interferon plus ribavirin group and 8 and 12 week telaprevir triple therapy groups followed by response-guided peg-interferon plus ribavirin combination therapy [23] (Table 1). SVR rates were 69 % for the 8 week telaprevir treatment and 75 % for the 12 week telaprevir treatment, compared to 44 % for standard peg-interferon plus ribavirin combination therapy. The phase III REALISE study assessed response to triple therapy in patients with prior treatment failure [24]. Prior relapsers, partial responders, and null responders were randomized to a 48 week peg-interferon plus ribavirin control group or to 48 week triple therapy groups with 12 weeks of telaprevir with or without a 4 week peg-interferon plus ribavirin lead-in phase. SVR rates in the triple therapy group were 66 % with the lead-in phase and 64 % without it, compared to only 17 % in the control group. When analyzed by response to prior treatment, prior relapsers showed the strongest improvement in SVR rates, but triple therapy also appears to benefit prior null and partial responders as well [24–26]. Based on these studies, the U.S. Food and Drug Administration (FDA) approved response-guided therapy (RGT) for prior relapsers who achieved extended rapid virological response (eRVR) [27]. This allows prior relapsers to discontinue all treatment after 24 weeks if HCV RNA is undetectable at weeks 4 and 12. In Japan, duration of triple therapy is 24 weeks without regard for response to prior treatment.

Table 1 Summary of telaprevir clinical trials

Study	Design	Results
PROVE I	Phase II; $N = 233$	SVR
McHutchison et al. [20]	T12PR24: 12 week TVR + 24 week PR	PR: 41 %
	T12P48: 12 week TVR + 24 week PR	T12PR24: 61 %
	PR48: 12 week placebo + 48 week PR	T12P48: 67 %
PROVE II	Phase II; $N = 334$	SVR
Hezode et al. [21]	T12PR24: 12 week TVR + 24 week PR	PR48: 46 %
	PR48: 12 week placebo + 48 week PR	T12PR24: 69 %
PROVE III	Phase II; $N = 465$	SVR
McHutchison et al. [22]	Patients with prior PR treatment failure	T12PR24: 51 %
	T12PR24: 12 week TVR + 24 week PR	T24PR48: 53 %
	T24PR48: 12 week TVR + 24 week PR	T24P24: 24 %
	T24P24: 12 week TVR + 24 week PR	PR48: 14 %
	PR48: 12 week placebo + 48 week PR	
ADVANCE	Phase III double-blind; $N = 1088$	SVR
Jacobson et al. [23]	Treatment-naïve patients	T8PR: 69 %
	T8PR: 8 week TVR + 24 or 48 week PR RGT	T12PR: 75 %
	T12PR: 12 week TVR + 24 or 48 week PR RGT	PR: 44 %
	PR: 12 week placebo + 48 week PR	
ILLUMINATE	Phase III open-label; $N = 540$	SVR
Sherman et al. [55]	Treatment-naïve patients	T12PR24: 92 %
	T12PR24: 12 week TVR + 24 or 48 week PR RTG	T12PR48: 88 %
	T12PR48: 12 week TVR + 48 week PR	
REALIZE	Phase III; $N = 662$	SVR by treatment
Zeuzem et al. [24]	Patients with prior PR treatment failure	T12P48: 64 %
	T12PR48: 8 week TVR + 48 week PR	Lead-in T12P48: 66 %
	Lead-in T12PR48: 4 week PR + 8 week TVR + 48 week PR	PR48: 17 %
	PR48: 12 week placebo + 48 week PR	SVR by prior history
		Relapsers: 83–88 %
		Partial responders: 54–58 %
		Non-responders: 29–33 %
Yamada et al. [32]	Phase Ib; $N = 10$	ETR: 10 %
	Treatment-naive Japanese patients	
	TVR monotherapy: 12 week	
Ozeki et al. [19]	Phase IIa; $N = 4$; single-arm, open label	SVR (off-study): 100 %
	Older female Japanese patients with prior PR treatment failure	
	TVR monotherapy: 24 week + off-study PR	
Toyota et al. [33]	Phase II; $N = 15$; single-arm, open-label	SVR: 7 %
	Treatment-naive Japanese patients	
	TVR monotherapy: 24 week	
Kumada et al. [28]	Phase III; $N = 189$	SVR
	Treatment-naïve Japanese patients	TR12P24: 73 %
	TR12P24: 12 week TVR + 12 week PR	P48: 49 %
	P48: 48 week PR	

Table 1 continued

Study	Design	Results
Hayashi et al. [31]	Phase III; $N = 141$ Patients with prior PR treatment failure TR12P24: 12 week TVR + 12 week PR P48: 48 week PR	SVR Relapsers: 88 % PR48: 34 %

TVR telaprevir, *PR* peg-interferon plus ribavirin combination therapy, *RGT* response-guided therapy—24 week PR if undetectable HCV RNA at weeks 4 and 12 (eRVR); otherwise 48 week PR, *ETR* end-of-treatment response

Clinical trials of telaprevir in Japan

Triple therapy in treatment-naive patients

Although Asians are under-represented in the above studies (1–2 %), several phase II and III clinical trials have also been performed in Japan (Table 1). In Kumada et al. [28], 126 patients were randomly assigned to 12 weeks of telaprevir triple therapy followed by 12 weeks of combination therapy, and 63 patients were assigned to 48 weeks of combination therapy. Early viral dynamics varied greatly between the two groups, with more rapid and extensive loss of HCV RNA and a significantly higher rate of SVR in the triple therapy group (73.0 vs. 49.2 %). Rates of viral breakthrough and relapse did not differ between the treatment groups. However, patients who underwent triple therapy experienced a significantly higher incidence of side effects during the telaprevir phase of the treatment. Because HCV patients in Japan tend to be more than 10 years older than patients in Western countries and include a higher proportion of women, ribavirin-induced anemia is of particular concern [29]. Moderate or severe anemia developed in 38.1 % of patients in the triple therapy group compared to 17.5 % in the combination therapy group [30]. The ribavirin dose was adjusted accordingly, resulting in a lower total ribavirin dose in the triple therapy group. However, ribavirin dose reduction did not significantly impact treatment efficacy. Skin disorders were about twice as common in triple therapy patients (46.8 vs. 23.8 %), and severe skin lesions were only observed in this group. Due to the higher SVR rate and shorter duration of triple therapy, the study authors recommend triple therapy over combination therapy for treatment of HCV genotype 1 in Japan but stress the need for careful monitoring of hemoglobin levels and close coordination with a dermatologist.

Triple therapy in patients with prior treatment failure

In a second phase III clinical trial in Japan, Hayashi et al. [31] examined the safety and efficacy of triple therapy for difficult-to-treat patients who either relapsed (109) or failed to respond to prior interferon therapy (32). As in the previous studies, patients were treated to 12 weeks of triple therapy followed by 12 weeks of combination therapy. SVR rates were 88.1 % for prior relapsers and 34.4 % for prior non-responders. Adverse events were common but moderate. 82 % of patients experienced rash or other skin disorders, mainly during the telaprevir phase, and nearly all (98.6 %) patients required ribavirin dose reduction for anemia, although ribavirin dose reduction had no effect on SVR rate down to about 20 % of the planned dose. Telaprevir was discontinued in 21.3 % of patients, and all drugs were discontinued in 16.3 % of patients. SVR rates in prior relapsers were significantly higher among men than women (93.9 vs. 79.1 %), but there was no difference among prior non-responders. Rates of viral breakthrough (18.8 %) and relapse (40.6 %) were significantly higher among prior non-responders and were more common after completion of the telaprevir phase, suggesting that extension of telaprevir therapy past 12 weeks or continuation of combination therapy past 24 weeks may improve response for prior non-responders. The study authors recommend weekly hemoglobin monitoring and note that even sharp reductions in ribavirin dose my allow therapy to continue without adversely affecting outcome.

Side effects of telaprevir in clinical trials in Japan

An early phase Ib study was conducted in Japan to examine the safety, tolerability, and antiviral profile of telaprevir monotherapy over 12 weeks in 10 treatment-naive patients with high viral loads of genotype 1b [32]. Telaprevir was well tolerated and no serious adverse events occurred, but 80 % of patients developed a rash and 70 % experienced anemia. Telaprevir monotherapy demonstrated potent antiviral activity, with HCV RNA levels decreasing by 2.3 $\log_{10}$ by 16 h and by 5.2 $\log_{10}$ after 2 weeks. HCV RNA dropped to the limit of detection or became undetectable in all patients during the course of therapy, but only one patient achieved an end-of-treatment response. Viral breakthrough occurred in 8 patients, mainly due to Ala156 mutation. However, resistance mutants reverted to wild type during the 24 week follow-up period.

Another study examined safety and efficacy of telaprevir monotherapy over a longer duration of 24 weeks with a larger number of patients and a greater range of viral loads [33]. The only patient who achieved SVR also had the lowest baseline viral load (3.55 $\log_{10}$ IU/ml), but three other patients were able to achieve an end-of-treatment response. HCV RNA levels decreased rapidly (average −5 $\log_{10}$ IU/ml), and HCV RNA became undetectable in 5 patients within 8 weeks. 10 out of 15 patients (66 %) discontinued the drug due to viral breakthrough, adverse events, or other causes. Incidence of adverse events was high (14/15 patients) and 7 out of 15 patients (47 %) developed anemia, but most incidences were mild to moderate, and anemia did not lead to discontinuation of therapy. T54A and A156V variants were the most common and were not detectable at earlier time points. Secondary substitutions at V158I and I132L were also observed.

SVR rates tend to be lower among women than men over 50 in Japan (53 vs. 22 %), and dose reductions and discontinuation of treatment in standard therapy are high in this group [34]. Ozeki et al. [19] examined 24 weeks of telaprevir monotherapy in a group of four older female patients predicted to be difficult to treat due to age, sex, and Core70 and ISDR substitutions. All patients required telaprevir dose reduction due to anemia but did not require discontinuation. Resistance variants were detected in three patients, and two patients experienced viral breakthrough. Additional substitutions and variants emerged as therapy progressed. However, at the end of the telaprevir administration, all four patients were given at least 48 weeks of standard therapy, and all patients were able to achieve SVR. Although this approach results in longer duration of therapy, it avoids the need for simultaneous administration of the three drugs and takes advantage of the fact that resistance mutants selected during telaprevir therapy often have reduced fitness compared to the wild type and are more susceptible to standard therapy.

Telaprevir antiviral resistance

Pre-existence of resistance mutations and selection for resistance may be an inevitable consequence of DAA therapy [35]. The high replication rate of HCV high (10^{12} viruses per day) coupled with the low fidelity of HCV polymerase results in a high mutation rate (10^{-3}–10^{-5} per day) and the presence of viral quasispecies. Single and double substitutions from the consensus sequence are expected to exist at low frequency prior to therapy. The relative proportion of these variants increases rapidly in the viral population as the wild-type virus is eradicated. De novo mutations appear to play only a minor role in the emergence of resistance mutations, suggesting that a genetic barrier of three to four mutations might be sufficient to reduce selection based on pre-existing mutants. At the same time, mutations conferring resistance often have reduced fitness and may require compensatory mutations in order to compete with wild-type viruses. Nonetheless, HCV sub-genotypes vary substantially in sequence, and some are likely to have a reduced genetic barrier against certain DAAs. For example, viral genotypes 1a and 1b already have different genetic barriers to telaprevir resistance; amino acid substitution of amino acid 155 requires only one nucleotide change in genotype 1a, whereas genotype 1b requires two nucleotide substitutions [36, 37]. Resistance substitutions at six major sites within the NS3 HCV protease have been reported, including at amino acids 36, 54, 155, 156, 168, and 170, and some substitutions are known to act synergistically [35]. At least 50 direct-acting antiviral drugs are at some stage of development, but these belong to a small number of distinct drug classes, increasing the risk of cross-resistance. Although wild-type strains are typically restored following removal of the drug due to viral breakthrough, prior treatment experience with DAAs, especially in high-risk subpopulations such as injection drug users, may increase the risk of transferring partially resistant strains during new infections.

Patient selection and predictive factors for triple therapy

Telaprevir triple therapy is an extension of peg-interferon plus ribavirin combination therapy. Therefore, factors that predict the outcome of combination therapy might also help to predict outcome of triple therapy. Age, fibrosis, obesity, hepatic steatosis [38], LDL cholesterol, gamma-GTP [39], insulin resistance [40], baseline viral titer [38, 41], and IL28B SNP genotype [42–44] are known to affect response to combination therapy. HCV genotype [41] and genetic variants within the viral genome, including amino acid substitutions at positions 70 (Core70) and 91 (Core91) of the HCV core protein and substitutions within the NS5A interferon sensitivity determining region (ISDR) [45, 46], are also thought to influence response to combination therapy. Akuta et al. [47] reported that Core70 substitution and partial response to prior therapy were significant predictors of SVR for triple therapy, and partial response and alpha-fetoprotein levels were significant predictors of end-of-treatment response. Chayama et al. [26] reported that IL28B SNP genotype, rapid virological response (RVR), and response to prior therapy were predictive of outcome of triple therapy. Prior relapsers achieved high levels of SVR (93 %), whereas patients who failed to respond to combination therapy were also less likely to respond to triple therapy. ITPA SNP genotype did

not influence outcome of therapy, but patients with the anemia-susceptible ITPA SNP rs1127354 genotype typically required ribavirin dose reduction earlier than patients with other genotypes. Predictive factors for SVR identified during the ADVANCE phase III clinical trial include race, viral load, IL28B, RVR, and stage of fibrosis [48]. IL28B and on-treatment factors such as RVR appear to remain important predictors for response to triple therapy and may aid in patient selection and determination of treatment duration [48].

2012 guidelines for treatment of patients with chronic hepatitis C

Two guidelines for treatment of chronic HCV are available in Japan, both providing recommendations for patient selection for telaprevir triple therapy. Triple therapy in Japan consists of 12 weeks of telaprevir (Telavic) in combination with 24 weeks of dual peg-interferon α 2b (Peg-Intron) and 24 weeks of ribavirin (Rebetol).

Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis: 2012 Guideline on Therapy for Chronic Hepatitis C

The following are the most recent guidelines from the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labour and Welfare of Japan (Tables 2, 3, 4, 5, 6). The recommended course of treatment differs depending on HCV genotype, viral titer, and prior history of interferon treatment. Patients with high viral load (>5.0 log IU/ml) of genotype 1 are considered difficult to treat and are recommended for triple therapy in both interferon treatment-naive and treatment-experienced patients (Tables 2, 3). In this group of patients, IL28B SNP genotype, HCV Core70 and ISDR substitutions are strong predictors of treatment outcome and may be used to determine the starting therapy. Patients with rs8099917 TT genotype are recommended for triple therapy. If telaprevir is contraindicated due to age, gender, or hemoglobin levels, peg-interferon plus ribavirin may be used instead (Table 4). However, combination therapy alone without telaprevir is not recommended for patients with rs8099917 TG/GG genotype, Core70 mutant, and wild type ISDR (0–1 substitutions) due to poor response to combination therapy in these patients (Table 4). For treatment-naive patients with low viral loads of either genotype 1 or genotype 2, the recommended treatment is 24–48 weeks of peg-interferon α 2a (Pegasys) (Table 1). Recommended treatment for patients with high viral load of genotype 2 is 24 weeks of dual therapy with ribavirin and either peg-interferon α 2b or interferon β (Feron). In the case of adverse drug reactions, such as depression, or in the case of increased risk of adverse drug reactions due to age, interferon β plus ribavirin should be

Table 2 Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis: 2012 guidelines for chronic hepatitis C therapy for treatment-naive patients

	Genotype 1	Genotype 2
High viral load	Peg-IFN α 2b: Peg-Intron (24 weeks)	Peg-IFN α 2b: Peg-Intron
≥5.0 log IU/mL	+Ribavirin: Rebetol (24 weeks)	+Ribavirin: Rebetol (24 weeks)
≥300 fmol/L	+Telaprevir: Telavic (12 weeks)	IFN β: Feron
≥1 Meq/mL		+Ribavirin: Rebetol (24 weeks)
Low viral load	IFN (24 weeks)	IFN (8–24 weeks)
<5.0 log IU/mL	Peg-IFN α 2a: Pegasys (24–48 weeks)	Peg-IFN α 2a: Pegasys (24–48 weeks)
<300 fmol/L		
<1 Meq/mL		

Table 3 Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis: 2012 guidelines for chronic hepatitis C therapy for previously treated patients

	Genotype 1	Genotype 2
High viral load		
≥5.0 Log IU/mL		
≥300 fmol/L	Peg-IFN α 2b + Ribavirin (24 weeks)	Peg-IFN α 2b + Ribavirin (36 weeks)
		OR
≥1 Meq/mL	+Telaprevir (12 weeks) combined therapy	Peg-IFN α 2a + Ribavirin (36 weeks)
		OR
Low viral load		IFN β + Ribavirin (36 weeks)
<5.0 log IU/mL		
<300 fmol/L		
<1 Meq/mL		

considered for patients, regardless of genotype 1 or 2. Previously treated patients with genotype 1 should be treated with triple therapy, consisting of 12 weeks of telaprevir and 24 weeks of peg-interferon α 2b and ribavirin regardless of viral load (Table 3). Patients with genotype 2 should be given 36 weeks of dual therapy with ribavirin and either peg-interferon α 2a/b or interferon β (Table 3).

Telaprevir triple therapy is associated with an increased risk of anemia, skin lesions, and other side effects compared to peg-interferon plus ribavirin dual therapy, especially among females and older patients [20, 26]. Initial dosages should be determined based on the patient's age, weight, and expected tolerability. However, for female patients with baseline hemoglobin levels between 13 and 14 g/dl or male patients with baseline hemoglobin levels between 12 and 13 g/dl, ribavirin dosage should be reduced by 200 mg and telaprevir dosage should be reduced to 1500 mg (Table 5). Triple therapy is unsafe in patients with baseline hemoglobin levels <12 g/dl. Hemoglobin levels should be closely monitored, and in the case of anemia ribavirin, dosage should be reduced based on both the absolute value of the hemoglobin levels as well as the amount of the reduction (Table 6). Triple therapy should be conducted in cooperation with a dermatologist to manage the high risk of potentially serious skin problems, including Stevens–Johnson syndrome and drug-induced hypersensitivity syndrome. Use of all three drugs should immediately cease in the event of serious skin problems. In the event of cutaneous symptoms, adequate treatment should begin early in consultation with a dermatologist. Benefits and risks of administration of oral steroids or other drugs should be

Table 4 Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis: pretreatment indicators for triple therapy

Indications for therapy involving a host factor (IL28B) and two viral factors (ISDR and Core70) at the start of triple combined therapy including telaprevir in the initial therapy for the treatment-naive patients with high viral load of genotype 1

1. Telaprevir triple therapy is recommended in patients homozygous for the favorable IL28B SNP allele (e.g., rs8099917 T/T genotype) because the anticipated effect of the therapy is high. If telaprevir therapy is likely to be difficult in consideration of the patient's age, gender, hemoglobin level, or other factor, then peg-interferon α or interferon β plus ribavirin combination therapy should be chosen instead
2. Telaprevir triple therapy may be preferred over interferon plus ribavirin combination therapy in patients with an unfavorable IL28B SNP genotype (rs8099917 T/G or G/G), wild-type ISDR (0–1 substitutions), and a Core70 mutation, because the effect of interferon plus ribavirin combination therapy is low in these patients

Table 5 Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis: guidelines for ribavirin and telaprevir dose reduction based on baseline hemoglobin levels

Baseline hemoglobin (g/dl)	Ribavirin	Telaprevir
≥14.0	Conventional dose	Conventional dose (2250 mg)
13.0–14.0	Decrease by 200 mg (females only)	Decrease to 1500 mg (females only)
12.0–13.0	Decrease by 200 mg	Decrease to 1500 mg
<12.0	Triple therapy unsafe	

Initial ribavirin and telaprevir dosages relative to hemoglobin levels are estimated based on the results of clinical trials. Initial dosages should be determined by a specialist based on the patient's age, weight, etc

Table 6 Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis: precautions for triple therapy with peg-interferon α 2b, ribavirin, and telaprevir in case of high viral load of genotype 1

1. Severe anemia occurs more frequently in peg-interferon α 2b plus ribavirin plus telaprevir triple therapy compared to interferon plus ribavirin combination therapy. Care should be taken to monitor hemoglobin levels, and in case of anemia, ribavirin dosage should be adjusted based on consideration of both the absolute value of hemoglobin as well as the amount of hemoglobin reduction. Because the risk of anemia increases with age, peg-interferon α or interferon β plus ribavirin combination therapy is the preferred initial therapy for older female patients or patients with low hemoglobin levels and high viral loads of genotype 1
2. Peg-interferon α 2b plus ribavirin plus telaprevir triple therapy should be conducted in coordination with a dermatologist because serious skin problems such as Stevens–Johnson syndrome and drug-induced hypersensitivity syndrome are likely to occur. In the event of severe skin problems, use of all three drugs should be immediately ceased. If cutaneous symptoms are expressed, adequate treatment should begin at an early date. Course of treatment should be decided in cooperation with a dermatologist in view of the respective risks and benefits, and administration of oral steroids should be considered if necessary
3. Some patients experience an increase in uric acid and creatinine levels rise during the first week of peg-interferon α 2b plus ribavirin plus telaprevir triple therapy. If uric acid levels become aberrant, early administration of a therapeutic agent for hyperuricemia is required

considered, if necessary. Some patients may also experience a rapid increase in uric acid levels at the start of therapy (1–7 days), in which case a therapeutic agent should be administered early to reduce hyperuricemia.

Japan Society of Hepatology: 2012 guidelines for treatment of chronic HCV

The 2012 guidelines supported by the Japan Society of Hepatology (http://www.jsh.or.jp/english/index.html) provide more specific recommendations for patients with high viral load of HCV genotype 1 based on factors including patient age, IL28B SNP genotype, Core70 and ISDR substitutions, prior treatment history, and stage of fibrosis. The English version of this guideline will be published soon in Hepatology Research (2012). Treatment-naive patients with rs8099917 TT genotype should be given triple therapy, if possible, but combination therapy may be substituted if telaprevir is contraindicated (Fig. 1a). Interferon β plus ribavirin may also be substituted in case of depression. Therapy should also be postponed in patients with both the unfavorable IL28B SNP genotype (TG/GG) and Core70 mutation due to the poor expected outcome of therapy. When IL28B and Core70 data are not available, patients should be treated with triple therapy or combination therapy, depending on tolerability and fibrosis stage (Fig. 1b). Therapy may be postponed in nonelderly patients (≤65) with mild fibrosis.

Triple therapy provides a retreatment opportunity for patients who were unable to eradicate the virus during prior therapy. However, not all patients show an improved response, and a patient's response to the prior therapy should be used as a guide for treatment selection, if available. Patients who experienced relapse or partial response are expected to respond well to therapy and should be administered triple therapy or combination therapy depending on age and stage of fibrosis (Fig. 2a). On the other hand, patients who experienced null response during prior therapy should be administered triple therapy, if possible; otherwise, treatment should be postponed, as combination therapy alone is unlikely to be successful. When treatment history is unknown but IL28B SNP and Core70 data are available, guidelines for treatment-naive patients should be followed (Fig. 2a). In the absence of

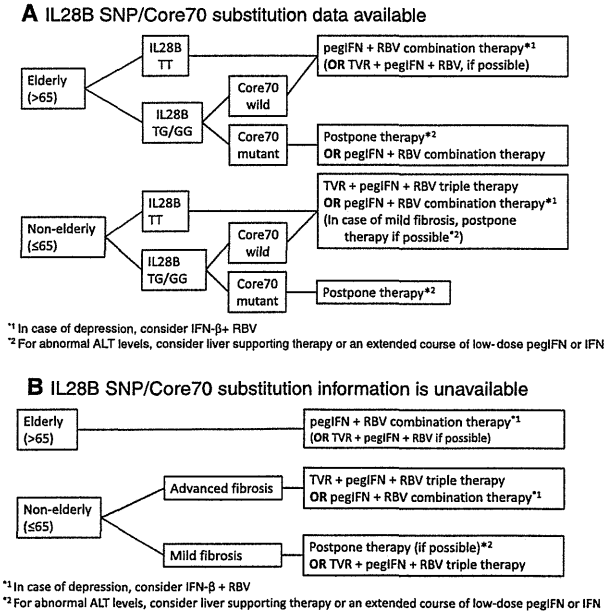

Fig. 1 Japan Society of Hepatology: 2012 treatment guidelines for treatment-naive chronic HCV patients with high viral load of genotype 1. **a** Patients with the favorable IL28B SNP genotype (rs8099917 TT) and/or wild type viral core protein amino acid 70 (Core70) should be treated with triple or combination therapy, if possible, depending on age and fibrosis stage. Patients with both the unfavorable IL28B SNP genotype (TG/GG) and Core70 substitution should postpone therapy due to poor expected outcome. **b** When IL28B SNP genotype and Core70 substitutions are unavailable, treatment is determined based on patient age and stage of fibrosis

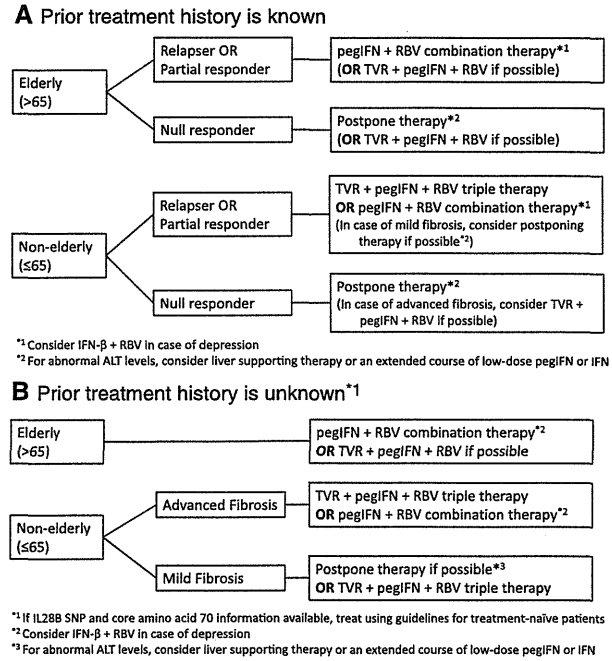

Fig. 2 Japan Society of Hepatology: 2012 treatment guidelines for re-treatment of previously treated chronic HCV patients with high viral load of genotype 1. **a** Patients who experienced relapse or partial response during prior interferon therapy should be treated with triple therapy or combination therapy, if possible, depending on age. Triple therapy is recommended for patients who experienced null response to prior therapy, but if triple therapy is not possible, therapy should be postponed due to poor expected response to combination therapy in these patients. **b** When prior treatment history is unavailable but IL28B SNP and core amino acid 70 (Core70) information is available, guidelines for treatment-naive patients should be followed (Fig. 1a). When both prior treatment history and IL28B SNP and Core70 information are unavailable, triple therapy is recommended for older patients as well as for younger patients with advanced fibrosis. If fibrosis is mild, triple therapy for younger patients should be postponed

both treatment history and IL28B/Core70 data, patients should be treated with triple therapy or combination therapy, depending on tolerability and fibrosis stage (Fig. 2b).

Future therapies

The development, clinical testing, and approval of telaprevir triple therapy is the culmination of a decades-long process [49]. At the same time, however, the introduction of telaprevir and boceprevir represents the first success in a much broader direct antiviral strategy targeting multiple facets of the viral life cycle. Future clinical trials involving triple therapy are likely to lead to further improvements in SVR rate, shorter duration of therapy, and improved management of side effects, especially among specific patient subgroups. Future research will also identify new predictive factors associated with response to DAA therapy, including risk of viral breakthrough and adverse events.

A major goal of future clinical research, however, is to move beyond interferon-based therapy in favor of interferon-free DAA combination therapies. A number of novel DAAs are currently undergoing clinical testing (Table 7), and DAAs are being evaluated in combination with interferon as well as other DAAs (Table 8). Many other drugs and vaccines are currently in some stage of clinical testing (http://www.hcvadvocate.org/hepatitis/hepC/HCVDrugs_2012.pdf). Telaprevir and other DAAs under development are not intended for use in monotherapy due to the low genetic barrier to resistance. However, combinations of DAAs with different viral targets and mechanisms of action should have a higher genetic barrier. For example, in a chimeric mouse model a protease inhibitor (telaprevir) in combination with an RNA polymerase inhibitor (MK-0608) resulted in rapid clearance of HCV RNA without emergence of resistance mutants [50].

Several DAA combination therapies have entered phase II clinical trials in humans. Safety and efficacy of dual therapy with daclatasvir (NS5A inhibitor) and asunaprevir (NS3 protease inhibitor) was examined in two phase II clinical trials in the US and Japan for difficult-to-treat genotype 1 patients with null response to prior interferon therapy [51–53]. The studies differed notably with respect to sub-genotype; 81 % of patients in the US study had genotype 1a, whereas all patients in the Japanese study had genotype 1b. In the Japanese study, 77 % of patients achieved SVR (90 % in the sentinel cohort) [52, 53], whereas in the dual DAA therapy arm of the US study (group A), only 36 % of

Table 7 Direct-acting antiviral (DAA) drugs in clinical testing

	Phase I	Phase II	Phase III	Phase IV
Protease inhibitor	ACH-2684	ABT-450	BI201335	Telaprevir
		ACH-1625	TMC435	
		BMS-650032		
		BMS-791325		
		GS-9256		
		MK-5172		
		MK-7009		
		RG7227		
Polymerase inhibitor	ALS-2158	ANA598	GS-7977	
	ALS-2200	BI207127		
	ABT-072	Filibuvir		
	ABT-333	GS-9190		
	MK-3281	IDX184		
	TMC649128	INX-189		
		GS-938		
		RG7128		
		VX-222		
		VX-759		
NS5A inhibitor	ACH-2928		BMS-790052	
	AZD-7295			
	IDX719			
	PPI-461			
	PPI-688			
NS4B inhibitor	Clemizole			
Entry inhibitor	ITX-5061			

Table 8 Direct-acting antiviral (DAA) combination therapies in clinical testing

Usage	Phase II	Phase III	Phase IV
DAA combinations	ABT-450 + ABT-072	BMS-790052 + BMS-650032[a]	
	ABT-450/r + ABT-267[a]		
	ABT-450 + ABT-333		
	BI201335 + BI207127		
	BMS-790052 + GS-7977		
	BMS-790052 + TMC435		
	Boceprevir + mericitabine		
	GS-9256 + GS-9190		
	GS-7977 + TMC435		
	RG7128 + RG7227		
	Telaprevir + VX-222		
DAA + IFN		Peg + RBV + BI201335	Peg + RBV + telaprevir[b]
		Peg + RBV + BMS-790052	
		Peg + RBV + GS-7977	
		Peg + RBV + TMC435[b]	
		Peg + RBV + MK-7009[b]	
		IFN λ + RBV + BMS-790052[a]	
		IFN λ + RBV + BMS-650032[a]	

DAA combinations, interferon-free combination therapies involving two or more DAAs; DAA + IFN, therapies based on interferon plus ribavirin combination with one or more DAAs; Peg, pegylated interferon, RBV, ribavirin; IFN, interferon; IFN λ, interferon-lambda (type III interferon)

[a] Currently in clinical trials in Japan

[b] Completed clinical trials in Japan

patients achieved SVR, while the other patients either relapsed or had viral breakthrough [51]. In the latter study, the two patients with genotype 1b both achieved SVR. All patients in group B, in which all patients received peg-interferon plus ribavirin in addition to daclatasvir and asunaprevir, achieved SVR at 12 weeks after treatment. These discrepancies may reflect differences between genotypes 1a and 1b in the genetic barrier for resistance to this drug combination [51] and suggest that such treatments may be more amenable in Japan where genotype 1b is common.

In another phase II dual DAA therapy study, treatment-naive genotype 1 patients were administered GS-9256, an NS3 serine protease inhibitor, and tegobuvir (GS-9190), a non-nucleoside NS5B polymerase inhibitor, with or without peg-interferon and ribavirin, followed by standard therapy with peg-interferon plus ribavirin [54]. Only 7 % of patients receiving dual DAA therapy alone achieved RVR, whereas RVR rates increased to between 67 and 100 % among patients who also received peg-interferon and/or ribavirin. Although promising, these studies suggest that interferon and ribavirin will continue to be used in future DAA combination therapies to control viral breakthrough.

Future perspective and conclusion

Although SVR rates still fall far short of 100 %, the recent introduction of telaprevir to standard peg-interferon plus ribavirin therapy greatly increases the chance that a patient with chronic HCV infection will be able to successfully clear the virus, and it offers a promising retreatment opportunity for patients who were unable to clear the virus in previous therapy attempts. Despite the higher SVR rate, however, triple therapy also further limits patient eligibility and increases the burden on patients. This issue is of particular concern in Japan where patients tend to be older than in Western countries and at greater risk for HCC, as well as more likely to face complications or treatment discontinuation due to adverse events.

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Review Article

Impact of interleukin-28B genotype on *in vitro* and *in vivo* systems of hepatitis C virus replication

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Identification of the relationship between the interleukin (IL)-28B genotype and the effect of peginterferon plus ribavirin treatment has had a great impact on the study of antiviral therapy for patients with chronic hepatitis C virus (HCV) infection. Differential expression levels of interferon-stimulated genes (ISG) in the liver and white blood cells based on the *IL-28B* genotype, which may in turn lead to differences in outcome of therapy, indicate that previous studies should be re-evaluated taking the effect of the *IL-28B* single nucleotide polymorphism (SNP) into consideration, although the exact mechanism of how variation in *IL-28B* SNPs affect HCV eradication remains unknown. These results suggest that the genotypes of multiple cell types, including liver and immune cells, contribute to the efficacy of therapy. Studies using human hepatocyte chimeric mice, in which effector cells of the human adaptive immune response are absent, showed that viral load, ISG expression levels and reduction of HCV RNA by interferon are affected by the *IL-28B* genotype. Genetic differences among hepatocytes may, therefore, contribute to differences in baseline viral loads and response to interferon therapy. Further studies should be done to clarify the mechanism of action of *IL-28B* SNP on viral load and effect of interferon treatment. Advances in cell culture systems and human hepatocyte chimeric mice, as well as upcoming *in vitro* and *in vivo* experimental systems, provide an effective platform to examine the effects of host and viral genetic variation on infection and response to interferon.

Key words: cell culture, chimeric mouse, interferon-stimulated genes, λ-interferon, single nucleotide polymorphism

INTRODUCTION

IN 2002, INTERFERON (IFN)-λ1, -λ2 and -λ3, also known as interleukin (IL)-29, IL-28A and *IL-28B*, respectively, were identified as members of a new family of IFN (type III) with antiviral activity.[1–7] In 2009, an association between single nucleotide polymorphism (SNP) genotypes within the *IL-28B* locus and the efficacy of peginterferon plus ribavirin combination therapy was established in a series of landmark genome-wide association studies.[8–12] Ge *et al.* published the first report of an association between the rs12979860 polymorphism and sustained virological response (SVR) following 48 weeks of combination therapy in a large cohort of patients of European or African-American ancestry with genotype 1.[8] This report was followed by studies based on rs8099917 by Tanaka *et al.* and Suppiah *et al.* in 314 Japanese and 848 Australian patients, respectively.[9,10] While the association was initially identified in patients with genotype 1,[8–11] these findings have since been replicated in other hepatitis C virus (HCV) genotypes, although the effect of the SNP appears to be weaker in genotypes 2 and 3.[13–19] Although most studies have focused on combination therapy, Ochi *et al.* showed that the *IL-28B* SNP is also associated with outcome of IFN monotherapy.[12] Although only 20–30% of patients are typically able to resolve acute HCV infection without treatment, Thomas *et al.* showed a strong association between rs12979860 genotype and spontaneous resolution of acute HCV

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infection in 1008 individuals of European and African ancestry.[20] Tillmann *et al.* also observed a higher frequency of spontaneous clearance in patients with the rs12979860 CC genotype in a cohort of 190 German women.[21] These results suggest that the *IL-28B* SNP is robustly associated with resolution of HCV infection and response to IFN therapy across a range of viral genotypes.

IN VITRO REPLICATION OF HCV USING CELL LINES

DEVELOPMENT OF EFFECTIVE therapies for HCV ultimately requires establishing a host cell able to support infection, as well as a virus capable of replicating in this environment.[22] However, HCV propagates poorly in cultured cells, and each step towards development of an infection system has been hampered by challenges. A major step forward involved transfection of the human hepatoma cell line Huh-7 using a viral clone.[23] This system was subsequently improved using permissive cell lines based on cell-culture adaptive mutations, such as Huh-7.5, which contains a point mutation in the retinoic acid-inducible gene (RIG-1).[24,25] The need for cell culture adaptive mutations was overcome using JFH-1, an HCV viral genome isolated from a patient with fulminant hepatitis.[26] High infection and replication rates were later achieved using the combination of JFH-1 and the highly permissive Huh-7.5.1 cell line.[24]

Although HCV can be propagated efficiently in hepatoma cells, these cells have a number of abnormalities[27] limiting their suitability and accuracy as a model of infection and host responses. However, other options are now available, such as micropatterned co-cultures (MPCC), in which primary human hepatocytes can be maintained in a multiwall format.[28] This system makes it possible to support the entire HCV life cycle and provides a high-throughput method for assessing efficacy and toxicity of therapeutic drugs.[28] Another recent advancement was the addition of miR-122 and a HCV receptor to hepatocellular carcinoma-derived HepG2 cells, resulting in efficient viral entry and replication.[29] Hepatic stems cells may offer another approach to examining the relationship between *IL-28B* on HCV infection in cell culture.

EVALUATION OF EFFECT OF IFN-λ IN CELL CULTURE

AS SHOWN IN Table 1, the effect of IFN-λ had been evaluated using a number of human and animal cell models even before the identification of the association between *IL-28B* SNP and outcome of combination therapy. IFN-λ has been investigated in over 100 cell lines in 50 different tissue types representing several different species, including humans, mice, Chinese hamsters and African green monkeys. Following the identification of the role of *IL-28B* in response to therapy, particular attention has been paid to the effect of IFN-λ in human and mouse hepatocytes.

The high odds ratios of SVR in patients with eradication-favorable *IL-28B* genotypes suggest that cells obtained from donors with different *IL-28B* genotypes might respond differently to IFN. To prevent potential confounding and improve comparability among studies, the *IL-28B* genotype of cell culture systems should be evaluated. A recent letter by Bensadoun *et al.* noted that Huh7-derived cell lines may differ in the *IL-28B* genotype even though they originated from a common ancestor.[44] They analyzed *IL-28B* genotype frequencies among Huh7 cell lines using ultra-deep pyrosequencing and showed that one Huh7 cell line was fixed for the eradication-unfavorable rs12979860 TT genotype, whereas descendants in the HCV-permissive replicon Huh7.5.1 line were fixed for the favorable CC genotype, perhaps due to the polyploidal nature of hepatoma cells and selection of specific clones from ancestral polyclonal populations. Therefore, it may be helpful to characterize the genetics of hepatoma cell lines used in HCV research.[44] Nonetheless, hepatoma cell lines have many abnormalities that limit extrapolation of results, and the role of the *IL-28B* SNP may have more or less relevance in a particular cell line.

IN VIVO REPLICATION OF HCV USING HUMAN HEPATOCYTE CHIMERIC MOUSE

HEPATITIS C VIRUS is only able to infect and effectively proliferate in human and chimpanzee hepatocytes. A breakthrough in HCV research occurred when the first small animal model of HCV infection was reported by Mercer *et al.*[45] They transplanted human liver cells into urokinase-type plasminogen activator severe combined immunodeficiency mice to create chimeric mice with human hepatocytes. As it is still difficult to culture human hepatocytes, the chimeric mouse model is ideal to study the nature of liver cells. Liver cells implanted into an individual mouse are usually transplanted from a single donor, and chromosomal alterations seen in cancer cell lines are expected to be rare or absent in this non-tumor liver cell proliferation system. Tateno *et al.* improved the repopulation rate of human liver cells in the mouse liver,[46] which was

Table 1 Human and animal cell models

Author	Cell lines	Species	Tissue	Description
Kotenko *et al.*[30]	COS-1	Monkey, African green	Kidney	SV40 transformed African green monkey kidney
	HT29	Human	Colon	Adenocarcinoma
	16-9	Hamster–human hybrid		Hamster–human somatic cell hybrid line
	CHO-K1	Chinese hamster	Ovary	Subclone of CHO cells
	CV-1	African green monkey	Kidney	Kidney, highly susceptible to SV40 infection
	HeLa S3	Human	Uterine cervix	Cervical epithelioid carcinoma
	A549	Human	Lung	Adenocarcinoma
	HaCaT	Human	Keratinocyte	
	HuH7	Human	Liver	Hepatoma, differentiated
	Raji	Human	Lymphocyte	Lymphoma, Burkitt's
	MOLT-4	Human	Lymphocyte	Leukemia, acute T lymphoblastic
	HL60	Human	Lymphocyte	Leukemia, acute promyelocytic, differentiation-inducible
	K562	Human	Lymphocyte	Leukemia, chronic myelogenous, differentiation-inducible
	SW480	Human	Colon	Adenocarcinoma
	G-361	Human	Melanoma	Malignant melanoma, skin
Sheppard *et al.*[7]	sf9	*Spodoptera frugiperda*	*Spodoptera frugiperda*	Ovary cancer
	Blood mononuclear cells	Human	Peripheral blood mononuclear cells	
	COS-7	Monkey, African green	Kidney	Transformant of CV-1 cells by origin-defective SV-40, SV-40 large T-antigen-expressing
	293 HEK	Human	Kidney	Transformed embryonic kidney by adenovirus (type 5)
	HepG2	Human	Liver	Hepatoma
	HL60	Human	Lymphocyte	Leukemia, acute promyelocytic, differentiation-inducible
	HeLa S3	Human	Uterine cervix	Cervical epithelioid carcinoma
	K562	Human	Lymphocyte	Leukemia, chronic myelogenous, differentiation-inducible
	MOLT-4	Human	Lymphocyte	Leukemia, acute T lymphoblastic
	Raji	Human	Lymphocyte	Lymphoma, Burkitt's
	SW480	Human	Colon adenocarcinoma cell	
	A549	Human	Lung (cancer)	Adenocarcinoma
	G-361	Human	Melanoma	Malignant melanoma, skin

Table 1 *Continued*

Author	Cell lines	Species	Tissue	Description
Donnelly *et al.*[31]	A-431	Human	Epidermoid carcinoma	Epidermoid carcinoma, high expression of epidermal growth factor receptor
	COLO-205	Human	Colon	Adenocarcinoma
	Primary human hepatocytes	Human	Primary human hepatocytes	
	HT-29	Human	Colon	Adenocarcinoma
	COS-7	Monkey, African green	Kidney	Transformant of CV-1 cells by origin-defective SV-40, SV-40 large T-antigen-expressing
Dumoutier *et al.*[32]	BW5147	Mouse	Hemolymphocytic	Lymphoma, T-cell lymphoma (AKR/J mouse)
	HEK293-EBNA	Human	Kidney	Transformed embryonic kidney by adenovirus (type5)
	HEK293	Human	Kidney	Transformed embryonic kidney by adenovirus (type 5)
	P815	Mouse	Hemolymphocytic	Mastocytoma (DBA/2 mouse)
	BWLICR2	Mouse	Thymus	Thymoma
Brand *et al.*[3]	Caco-2	Human	Colon	Colorectal cancer-derived cell
	DLD-1	Human	Colon	Colorectal cancer-derived cell
	SW480	Human	Colon	Colorectal cancer-derived cell
	HCT116	Human	Colon	Colorectal cancer-derived cell
	HT-29	Human	Colon	Colorectal cancer-derived cell
	CCL-6	Human	Colon	Normal colonic tissue and the untransformed cell
	LNCaP	Human	Prostate adenocarcinoma cell	
	Int-407	Human	Colon	Fetal colon
Brand *et al.*[33]	HepG2	Human	Liver	Hepatoma
	Hep3B	Human	Liver	Hepatoma
	HuH-7	Human	Liver	Hepatoma

Meager *et al.*[6]	U-87MG	Human	Glia	Glioblastoma
	U-138MG	Human	Glia	Glioblastoma
	U-373MG	Human	Glia	Glioblastoma
	MO-G-UVW	Human	Glia	Glioblastoma
	CCF-STTG1	Human	Glia	Glioblastoma
	MO-G-CCM	Human	Glia	Glioblastoma
	1321NI	Human	Glia	Glioblastoma
	LN229	Human	Glia	Glioblastoma
	LN319	Human	Glia	Glioblastoma
	LN443	Human	Glia	Glioblastoma
	2D9	Human	Glia	Glioblastoma
	SW480	Human	Bladder	Bladder carcinoma
	T24/83	Human	Bladder	Bladder carcinoma
	PANC-1	Human	Pancreas	Pancreatic carcinoma
	MIA-PA-CA-2	Human	Pancreas	Pancreatic carcinoma
	MG63	Human	Bone	Osteosarcoma cell
	TE671	Human	Cerebellum	Medulloblastoma
	HT1080	Human	Fibrocyte	Fibrosarcoma
	WISH	Human	Amniotic cell	
	RT4	Human	Bladder	Bladder carcinoma
	HepG2	Human	Bladder	Bladder carcinoma
	U1C	Human	Fibrocyte	Fibrosarcoma
	A549	Human	Lung	Adenocarcinoma
	HEK 293	Human	Kidney	Transformed embryonic kidney by adenovirus (type 5)
	Daudi	Human	Lymphocyte	Lymphoma, Burkitt's
	MRC-5	Human	Fibroblast	Normal diploid fibroblast
	HFF	Human	Fibroblast	Normal diploid fibroblast cell
	Hep2C	Human	Cervix	Laryngeal carcinoma
	KD4	Human	Muscle	Rhabdomyosarcoma
	L-929	Mouse	Adipose tissue	Fibrosarcoma
	L-M	Mouse	Adipose tissue	Fibrosarcoma
	MEG-01 s	Human	Myeloid cell	Chronic myelogenous leukemia cell
	TF-1	Human	Erythrocyte	Erythroleukemia
	MEG-01	Human	Lymphocyte	Lymphocytic leukemia
	93D7	Human	Lung	Adenocarcinoma
	A549	Human	lung	Adenocarcinoma
Siren *et al.*[34]	CRL-2407	Human	Lymphocyte	Activated natural killer cell
	NK and T cells	Human	Lymphocytes	

Table 1 *Continued*

Author	Cell lines	Species	Tissue	Description
Doyle *et al.*[4]	HepG2-WT10	Human	Liver	Hepatoma
	AVA5	Human	Liver	HCV replicon derived from Huh7
	HuH7	Human	Liver	Hepatoma
	SK-Hep-1	Human	Liver	The non-hepatocyte liver-derived cells
	HepSMCV	Human	Liver	Hepatic vein smooth muscle cells
	HepSMCA	Human	Liver	Hepatic artery smooth muscle cells
	HepFIB	Human	Liver	Hepatic fibroblasts
	HuHep	Human	Liver	Hepatoma
	U266	Human	B-cell	Myeloma
Mennechet *et al.*[35]	T cells	Human	Peripheral blood mononuclear cells	
Ank *et al.*[1]	Bruce4	Mouse	Embryonic stem cells	
	Hematopoietic stem cell	Mouse	Bone marrow	Hematopoietic stem cell
	tissue cells	Mouse	Skin	(Fibroblasts, keratinocytes, epithelial cells)
Maher *et al.*[36]	HaCaT	Human	Skin	Keratinocyte cell
	2fTGH	Human	Skin	Keratinocyte cell
	B16	Mouse	Skin	Melanoma
	HuH-7.5	Human	Liver	Hepatoma, differentiated
Sommereyns *et al.*[37]	Muscle	Mouse	Muscle	
	Spleen	Mouse	Spleen	
	Spinal cord	Mouse	Spinal cord	
	Liver	Mouse	Liver	
	Kidney	Mouse	Kidney	
	Brain	Mouse	Brain	
	Heart	Mouse	Heart	
	Intestine	Mouse	Intestine	
	Stomach	Mouse	Stomach	
	Lung	Mouse	Lung	
	Epithelial	Mouse	Epithelial	
	Endothelial	Mouse	Endothelial	
Zitzmann *et al.*[38]	BON1	Human	Pancreatic neuroendocrine tumor cells	

Lasfar *et al.*[39]	16-9	Hamster-human	Hamster-human somatic cell hybrid line	
	HT29	Human	Colon	Colorectal cancer-derived cell
	COS-1	African green monkey	Kidney	SV40 transformed African green monkey kidney
	CV-1	African green monkey	Kidney	Kidney, highly susceptible to SV40 infection
	L929	Mouse	Connective tissue	Fibroblast like
	NIH 3T3	Mouse	Embryo	Fibroblast, contact inhibited
	B16	Mouse	Skin	Melanoma
Numasaki *et al.*[40]	MCA205	Mouse	Lymphocyte	Fibrosarcoma cell
	B16	Mouse	Skin	Melanoma
	Yac-1	Mouse	Lymphocyte	A lymphoma cell
Sato *et al.*[41]	B16/F0	Mouse	Skin	Melanoma
	B16/F10	Mouse	Skin	Melanoma
	NIH3T3	Mouse	Embryo	Fibroblast, contact inhibited
	L929	Mouse	Connective tissue	Fibroblast
	COS7	African green monkey	Kidney	Transformant of CV-1 cells by origin-defective SV-40
Wongthida *et al.*[42]	B16(LIF)	Mouse	Skin	Melanoma
	B16ova	Mouse	Ovary	Melanoma cell
	BHK-21	Syrian hamster	Kidney	Subclone of BHK-21
Yoshimoto *et al.*[43]	SCCVII	Mouse	Skin	A murine squamous cell carcinoma cell
	C2C12	Mouse	Muscle	A myoblastoid cell
	B16	Mouse	Melanoma	Melanoma, skin, melanin pigment production (but large portion of cells is amelanotic) (C57BL/6 mouse)
	Bone marrow cells	Mouse	Bone marrow cells (C3H/He mice by flushing femurs with HANKS buffer)	