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

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 ≥1 Meq/mL	Peg-IFN α 2b + Ribavirin (24 weeks) +Telaprevir (12 weeks) combined therapy	Peg-IFN α 2b + Ribavirin (36 weeks) OR Peg-IFN α 2a + Ribavirin (36 weeks) OR IFN β + Ribavirin (36 weeks)
Low viral load <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

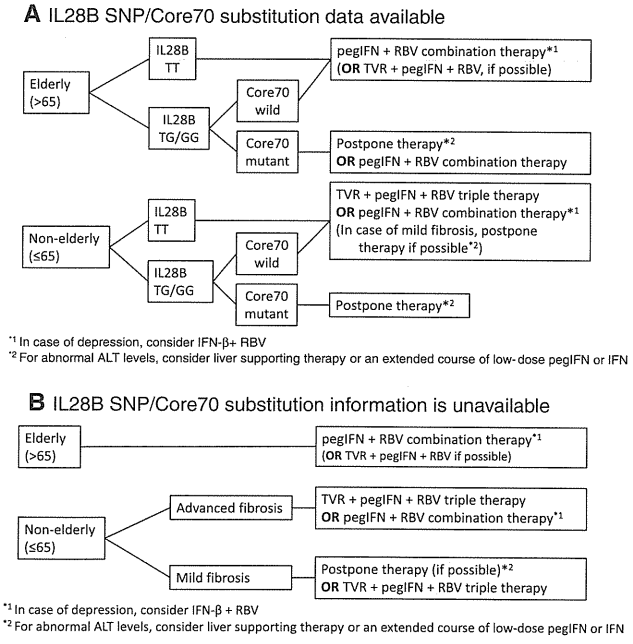

Fig. 1 Japan Society of Hepatology: 2012 treatment guidelines for treatment-naive chronic HCV patients with high viral load of genotype 1. **a** Patients with the favorable IL28B SNP genotype (rs8099917 TT) and/or wild type viral core protein amino acid 70 (Core70) should be treated with triple or combination 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

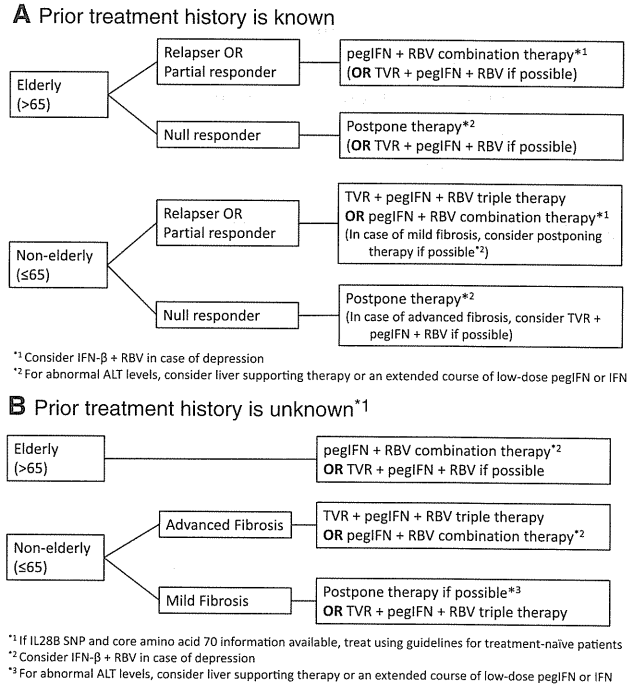

Fig. 2 Japan Society of Hepatology: 2012 treatment guidelines for re-treatment of previously treated chronic HCV patients with high viral load of genotype 1. **a** Patients who experienced relapse or partial response during prior interferon therapy should be treated with triple therapy or combination 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)	

recently reported to be important for HCV infection by Vanwolleghem *et al.*,[47] who showed that 100% HCV infectivity was reached with high quality inocula in mice with human albumin levels of more than 1 mg/mL.

Spontaneous eradication of HCV has been reported to be higher in individuals with the rs12979860CC genotype.[20] In such individuals, components of both the innate and adaptive immune systems likely play a role in eliminating the invading virus in the absence of exogenous IFN. As no human or mouse adaptive immune effector cells are present in human hepatocyte chimeric mice, it is possible to isolate the effects of innate immunity in liver cells using this model. When liver cells are selected from donors with different *IL-28B* genotypes, the model provides an opportunity to study differences in innate immune responses associated with the *IL-28B* genotype.

Clonal infection with HCV genotypes 1a, 1b, 2a and 2b is now possible,[48–50] and reverse genetic approaches combining infectious HCV clones with the human hepatocyte chimeric mouse facilitate studying the effects of viral mutations and host factors, such as the *IL-28B* genotype, on infectivity of the virus. Several factors may influence infectivity of the virus, including serum lipid profile,[51] presence of neutralizing antibodies and presence of antibody-resistant viral quasispecies.[52,53] However, we found no difference in incidence of establishment of infection with genotype 1b clones among mice transplanted with different *IL-28B* genotype liver cells.[54] This result may derive from the specific infection route (intrahepatic injection of RNA to mouse liver) and the large RNA titers used in the study.

EXPRESSION OF INTERFERON-STIMULATED GENE (ISG) AND VIRAL LOAD IN HUMANS AND HUMAN HEPATOCYTE CHIMERIC MICE

ALTHOUGH THE MECHANISM underlying the *IL-28B* polymorphism remains unclear, a number of studies have reported that intrahepatic ISG expression levels are lower[55–57] and viral titers are higher[8,12] in patients with the eradication-favorable *IL-28B* genotype. Honda *et al.* showed that expression of a number of ISGs was higher in patients with the unfavorable rs8099917 TG or GG genotypes compared to those with the TT genotype and that non-responders were significantly over-represented among patients with high ISG expression levels.[57] Using paired liver biopsy samples, Sarasin-Filipowicz demonstrated that ISG expression levels in non-responders were initially higher prior to IFN treatment, but administration of IFN failed to induce ISG expression levels above this baseline level, whereas IFN induced a strong upregulation of ISG expression in patients who achieved a rapid virological response.[58] Shebl *et al.* measured ISG expression levels in uninfected cells and found no evidence of an association with the *IL-28B* genotype, suggesting that the association observed in HCV-infected cells does not reflect normal expression levels in healthy cells and reflects the response to HCV infection.[59]

The chimeric mouse provides a suitable model to test hypotheses concerning the role of the *IL-28B* genotype on innate immune responses and ISG expression. Using this model, we found that ISG expression levels were also lower and viral titers were higher in human hepatocyte chimeric mice with the favorable *IL-28B* genotype.[54] As illustrated in Figure 1, the virus appears to replicate more efficiently in mice with the favorable genotype, perhaps because whenever the viral load is low hepatocytes with the favorable genotype can efficiently clear the virus so that chronic infection only results under higher viral loads.[20,60] At the same time, ISG expression is lower in cells with the favorable allele, which may be advantageous by preventing saturation of the IFN signaling pathway through continual stimulation.[58] However, earlier reports demonstrated that induction of a strong response to IFN-α is dependent on a weak constitutive IFN signal to maintain IFN-dependent expression of insulin-related factor-7, an essential transcription factor involved in IFN signal transduction that has a short half-life.[61] The unfavorable *IL-28B* genotype might alter the balance of this feedback loop in the presence of HCV. Although we observed that ISG expression levels were lower in mice with the favorable genotype prior to IFN treatment (unpubl. data, K. Chayama, Hiroshima University, Japan), ISG expression increased sharply following IFN administration in mice with the favorable genotype and were significantly higher than in mice with an unfavorable genotype.[54]

ISG EXPRESSION LEVELS AND EFFECT OF IFN THERAPY IN HUMANS AND CHIMERIC MICE

PRETREATMENT ISG EXPRESSION levels may be a stronger predictor of outcome of combination therapy than the *IL-28B* genotype,[62] suggesting that the mechanism of the SNP is related to its role in ISG regulation. Lower ISG expression levels are associated with the eradication-favorable *IL-28B* genotype in chronic hepatitis C patients,[55–57] and high baseline expression levels of ISG such as *RIG-I*, *ISG-15* and *USP-18* correlate with poor response to therapy.[63] Using the human

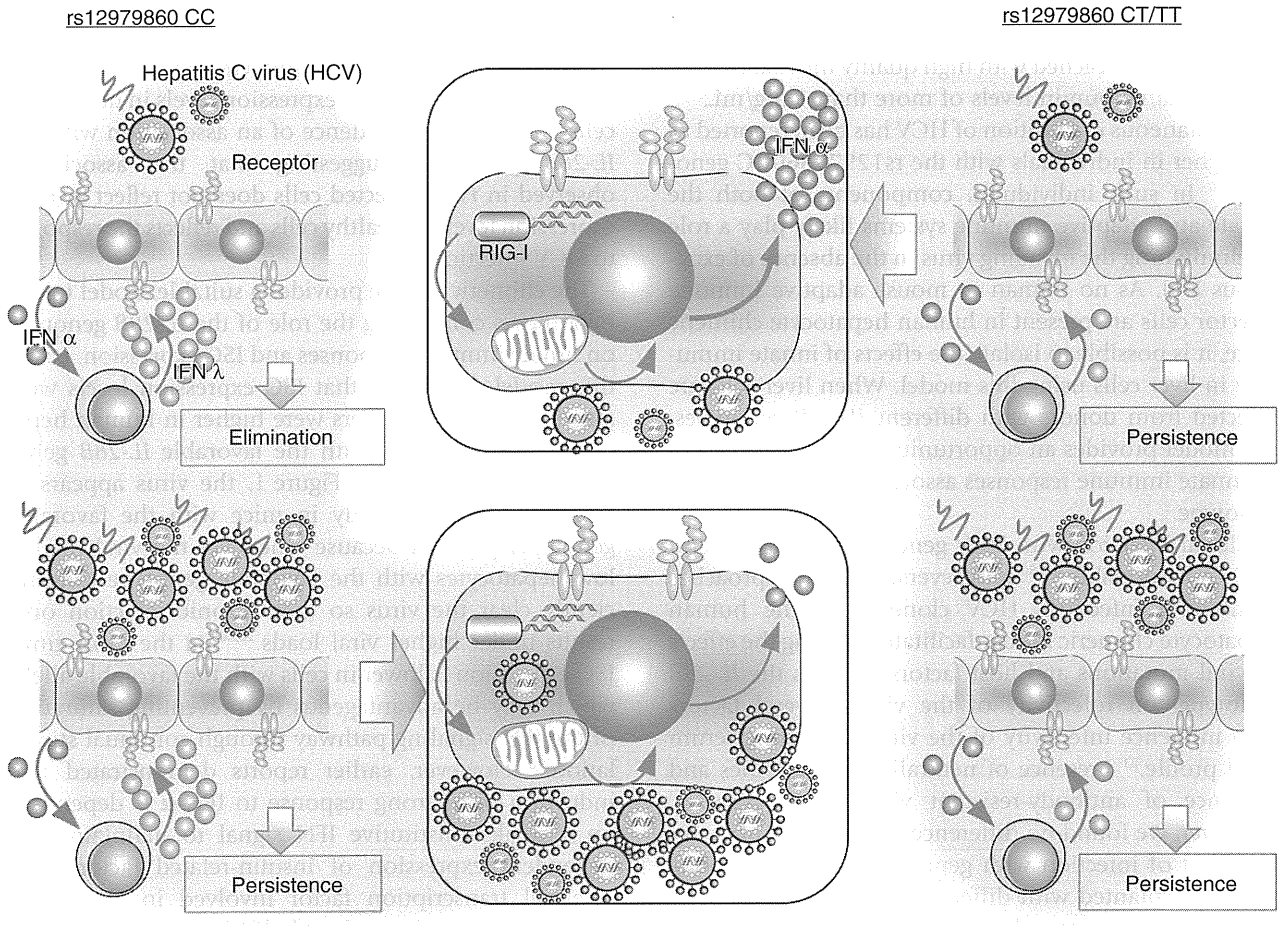

Figure 1 Hypothetical model of virus replication in hepatocytes with eradication-favorable (interleukin [IL]-28B rs12979860 CC or rs8099917 TT) or unfavorable (rs12979860 CT/TT or rs8099917 GT/GG) genotypes. HCV may be eliminated from individuals with the favorable genotype at low titer but may persistently infect those with high titers or the unfavorable genotype (upper right and left panels). Viral sensors continuously detect viral RNA during chronic infection (central panel), resulting in continuous activation of interferon-stimulated genes (ISG), including interferon inhibitory molecules such as *PIAS* and SOCS3. In hepatocytes with the unfavorable genotype, ISG expression may be refractory to further interferon stimulation, resulting in a poor response to therapy, but in hepatocytes with the favorable genotype, low baseline ISG expression may prevent overexpression of interferon signal inhibitors, resulting in stronger ISG induction and a better response to therapy.

hepatocyte chimeric mouse model, we showed that intrahepatic expression levels were significantly higher and HCV RNA reduction was significantly greater in mice with rs12979860CC hepatocytes following IFN administration.[54] Several genes that suppress IFN-dependent antiviral activity, such as *PIAS* and genes in the SOCS family, are themselves ISGs, and may serve as a negative feedback loop to regulate IFN signaling. Consequently, continuous low-level ISG expression in hepatocytes with the unfavorable genotype may dampen IFN sensitivity and prevent effective ISG induction when IFN is administrated during treatment.

RELATIONSHIP BETWEEN *IL-28B* GENOTYPE AND HCV CORE AMINO ACID SUBSTITUTIONS

REVERSE GENETICS USING the chimeric mouse model could facilitate detailed studies of the effect of host and viral factors on IFN sensitivity and ISG expression. HCV core protein substitutions are predictive of outcome of peginterferon plus ribavirin combination therapy,[64,65] and patients with the unfavorable *IL-28B* genotype are more likely to host viruses having core substitutions.[66] To examine these interactions *in*

vivo, we created core amino acid 70 and 91 double wild and double mutant clones using the infectious genotype clone KT-9 and performed infection and IFN treatment experiments. While we observed higher ISG expression levels and a more prominent viral decline in mice transplanted with favorable rs12979860 CC hepatocytes than those with rs12979860 TT, we found no difference between mice infected with core double wild and double mutant HCV.[54]

The core double mutant is associated with steatosis of the liver,[67] so one explanation is that gradual metabolic alterations during long-term infection may be necessary to induce detectable changes in response to IFN treatment. In this case, the short lifespan and sensitivity of chimeric mice may make them unsuitable for examining conditions involving long-term hepatic change. Similarly, spontaneous clearance does not necessarily imply rapid clearance, and so it may not be possible to detect subtle differences in spontaneous clearance due to the *IL-28B* genotype in chimeric mice over a sufficiently long period of time. However, the recent development of permissive mouse hepatocyte lines may improve viral infection rates in mice and avoid some of the limitations of chimeric mice.[68]

POSSIBLE INTERACTION BETWEEN LIVER CELLS AND IMMUNE CELLS

TWO OF THE initial *IL-28B* reports showed that production of IL-28 was higher in individual leukocytes homozygous for the eradication-favorable allele (rs12979860 CC or rs8099917 TT),[8,10] suggesting that stronger IFN-λ expression was responsible for the better response to therapy, although Urban *et al.* found no association between *IL-28B* genotype and *IL-28B* or *IL-28A* expression.[56] Honda *et al.* also found no association between *IL-28B* genotype and *IL-28B* expression, although they point out that it may be difficult to detect differences in expression levels due to the high sequence similarity between *IL-28A* and *IL-28B* and the relatively low expression levels of these genes regardless of genotype.[57] Although IFN-λ inhibits replication of HCV genotypes 1 and 2[69] and induces essentially the same set of ISGs as IFN-α,[62] IFN-λ may act synergistically by enhancing the antiviral efficacy of sub-saturating levels of IFN-α.[69] IFN-α and IFN-λ also have different kinetics. Marcello *et al.* showed that IFN-α induces an early peak followed by a rapid decline, whereas IFN-λ induces slower but more sustained ISG expression.[69] Therefore, coordination of autocrine and paracrine IFN expression is likely complex and may involve interactions between hepatocytes and immune cells such as dendritic cells, cytotoxic T lymphocytes, natural killer cells and natural killer T cells. The use of *in vitro* and *in vivo* experimental systems may help to clarify and isolate the roles of individual components of the cytokine network to elucidate the underlying mechanism of *IL-28B* polymorphisms on outcome of IFN therapy.

FUTURE REMARKS

IDENTIFICATION OF SNPs in the *IL-28B* locus has had a dramatic impact on the study and clinical assessment of HCV infection, but much about its role remains paradoxical and speculative, and more sophisticated *in vitro* and *in vivo* experiments are necessary to uncover the mechanism and use this knowledge to develop more effective IFN therapies. Recommendations for future research include genetic characterization of cell lines, culture of immune cells and hepatocytes using a cell separator, and transplantation of immune cells to human hepatocyte chimeric mice. In addition to improving treatment efficacy for chronic HCV infection, molecular and immunological insights gained from *IL-28B* research may impact a broad area of medicine, including infectious disease and cancer research.

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