scription factors, particularly STAT. Activation by IL-10, IL-22, IL-26 or IFN-λ could be inhibited using neutralizing antibodies to the IL-10R2 chain.[30]

The association of IL-10R2 polymorphism with susceptibility to systemic sclerosis (SSc) was found in rs2834167 (OR = 2.67).[31] An SNP in the 5′-flanking region of IL-10R2, rs999788, harboring linkage disequilibrium also showed the association with SSc.

The antiproliferative activity of IFN-λ1 was completely abolished when both $Tyr^{343}$ and $Tyr^{517}$ of IL-28R1 were mutated to phenylalanine.[32] The influence of tyrosine mutations on the antiviral activity of IFN-λ was determined by BW5147 cells and the Mengo virus system. The antiviral activity was also lost when both $Tyr^{343}$ and $Tyr^{517}$ were mutated into phenylalanine. These two tyrosine residues of IL-28RA are redundantly involved in STAT2 activation.[32] IL-29 activated the IRF-7 promoter, but this effect was abrogated by mutation of either $Tyr^{343}$ or $Tyr^{517}$ separately. These results showed some similarities with tyrosines from type I IFN receptors involved in STAT2 activation. Both residues are required for IL-29 response under suboptimal conditions. By contrast, STAT4 phosphorylation was independent from IL-28R1/IL-10R2 tyrosine residues.

The type I IFN receptor system mediates positive feedback on IFN-λ expression, whereas IL-28R1 signaling does not provide feedback on either type I or type III IFN expression *in vivo*.[14]

## Antiviral effect of IFN-λ

### HIV

The activation of Toll-like receptor (TLR)-3 also exhibited antiviral activity against pseudo-typed HIV-1 infection of the neuronal cells. Human neuronal cells also expressed functional IFN-λ receptor complex, IL-28R1 and IL-10R2, as evidenced by the observations that exogenous IL-29/28A treatment inhibited pseudo-typed HIV-1 infection of the neuronal cells and induced the expression of Apobec3g/3f (Table 3).[24] IFN-λ has the ability to inhibit HIV-1 infection of blood monocyte-derived macrophages, and upregulated intracellular expression of type I IFN and Apobec3g/3f.[33]

However, different data was reported from an independent group even though using a distinct assay system and immune cells. In treatment of IL-28A, the accumulation of CD4, CXCR4 and CCR5 transcripts was increased, particularly in peripheral blood mononuclear cells (PBMC). Pretreatment of PBMC and C8166 cells with type III and I IFN causes increased HIV binding and replication.[34] These effects are likely due to increased

**Table 3** Summary of experimental systems and its antiviral activity

| Virus | Cell type | Type of IFN-λ | Viral replication | In vivo | Type of IFN-λ | Viral replication | Ref. |
|---|---|---|---|---|---|---|---|
| HIV | Macrophage, primary neuronal | IL-29/28A | Inhibited | – | – | – | 24,33 |
| HIV | PBMC and C8166 | IL-28A | Enhanced | – | – | – | 34 |
| EMCV | HepG2, HuH7, HEK293, Raji, A549, HeLa, U937, HT29, SW480 | IL-29/28A/28B | Inhibited | C57BL/6 | IL-28A | No effect | 18,19,27,35–37 |
| HTNV | A549 | IL-29/28A | Inhibited | – | – | – | 38 |
| IAV | mDC, ATII, AM | IL-29 | Inhibited | C57BL/6 | IL-28A/28B | Inhibited | 28,39,40 |
| HBV | HuH7, primary hepatocytes | IL-29/28A | Inhibited | – | – | – | 41–43 |
| HCV | HuH7, HepG2, primary hepatocytes | IL-29/28A | Inhibited | – | – | – | 42–46 |
| HSV-2 | Raji, A549, HeLa, U937 | IL-29/28A/28B | Inhibited | C57BL/6 | IL-28A | Inhibited | 35 |

EMCV, encephalomyocarditis virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV-2, herpes simplex virus 2; HTNV, Hanta virus; IAV, ; IL, interleukin; CI, confidence interval; NVR, null virological response; OR, odds ratio; PBMC, peripheral blood mononuclear cells; SNP, single nucleotide polymorphisms; VR, virological response.

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expression of HIV receptors and co-receptors on the plasma membrane.

### EMCV

λ-Interferons have appreciable antiviral activity against EMCV but limited activity against herpes simplex virus type 2 (HSV-2), whereas IFN-α potently restricted both viruses.[18,19,27,35–37] IL-28A and IL-29, like IFN-α2a, were able to protect HepG2 cells from viral-induced cytopathogenic effect when applied 24 h before adding EMCV. Half-maximal protection ($EC_{50}$) was achieved with less than 2 ng/mL of IL-29 and 30 ng/mL of IL-28A compared to 0.5 ng/mL of IFN-α2a. These results showed that IL-28A and IL-29, like type I IFN, have intrinsic cellular antiviral activity and are able to fully protect HepG2 cells challenged with EMCV (Table 3).[19]

Treatment with IFN-λ *in vivo* did not affect viral load after infection with EMCV, but reduced the viral titer of HSV-2 in the liver, while IFN-α reduced the viral load after infection with EMCV. The discrepancy between the observed antiviral activity *in vitro* and *in vivo* may suggest that IFN-λ exerts a significant portion of its antiviral activity *in vivo* through stimulation of the immune system rather than through induction of the antiviral state.[35]

### Hantavirus

Hanta virus (HTNV), causing hemorrhagic fever with renal syndrome (HFRS) and hanta virus cardiopulmonary syndrome (HCPS), are known to be sensitive to nitric oxide (NO) and to pretreatment with type I and II IFN. HTNV can interfere with the activation of antiviral innate immune responses in patients and inhibit the antiviral effects of all IFN. In HFRS patients, the levels of serum IFN-α and IFN-β are not elevated and the level of serum IFN-λ is decreased.[38] Pretreatment of A549 cells with IFN-λ show antiviral effect against HTNV replication (Table 3). However, an established HTNV infection is insensitive to treatment with IFN-α, -β, -γ and -λ. The levels of STAT1 phosphorylation after IFN treatment is reduced in HTNV-infected cells.

### Influenza A virus

Intranasal application of IFN-λ protected the mice from lethal challenge with influenza A virus, whereas systemic application of IFN-λ failed to mediate protection from disease induced by hepatotropic virus, Rift Valley fever virus and thogotovirus.[28] Protection against influenza virus correlated with the presence of the IFN-induced Mx1 protein in the lung tissue, suggesting that lung epithelial cells carry functional IFN-λ receptors (Table 3). By contrast, no Mx1 protein was found in liver tissue of mice treated with IFN-λ. The liver tissue also failed to respond to IFN-λ synthesized in the virus-infected liver. The author concluded that IFN-λ contributes to inborn resistance against viral pathogens infecting the lung but not the liver.

Alveolar type II epithelial cells (ATII), and mDC are one of the primary targets for influenza A pneumonia. Influenza A virus infection to ATII increased mRNA expression of IFN-β, IFN-λ1 and IFN-λ2.[39,40] On the other hand, IFN-λ1 treatment of ATII induced mRNA expression of ISG, MX1, 2′5′-OAS and ISG56, but not IFN-β, suggesting that IFN-λ1 protect ATII independent of IFN-β.

### Hepatitis B virus

The antiviral activity of IFN-λ against severe hepatitis B virus (HBV) in human hepaotcyte-derived cells has been determined to reveal whether human IFN-λ can affect replication of the hepatitis virus or not. Replication of HBV in a human hepatoma cell line was reduced by approximately 30% following treatment with a high concentration of IL-29. These results suggest that the antiviral activity of IFN-λ1 against HBV may be limited in human cells (Table 3).[41,42]

Robek *et al.* reported the result using IL-28A in concordance with Hong *et al.* Treatment of differentiated HBV-Met cells treated with murine IL-28A inhibited HBV replication by more than 90% at 24 h after the IFN treatment similar to the inhibition observed after treating the cells with 200 U/mL of murine IFN-α. IFN-λ2 induces an antiviral response in hepatocytes that inhibits HBV replication.[43]

### Hepatitis C virus

In HCV assay, IFN-α and IL-29/28A reduced the level of HCV plus strand RNA 3–5 days after IFN addition by *in vitro* assay (Table 3).[43–46] The reduction in HCV replicon RNA was approximately 90% in the subgenomic and more than 99% in the full-length genomic replicon containing cells by day 5 post-treatment. However, this reduction was less than that achieved with 500 U (~5 ng) of IFN-α/mL. IFN-λ also induced the expression of the representative IFN-stimulated gene Mx1 to levels similar to those induced by IFN-α in replicon-containing HuH7 cells. Thus, like HBV replication, HCV replication is sensitive to the antiviral effects of IFN-λ.

In addition, IL-29 may have therapeutic value against chronic viral hepatitis in human patients.[42]

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## CONCLUSIONS

A SERIES OF studies on response to HCV treatment revealed one of clinical significance on IL-28B. The discovered SNP related to PEG-IFN plus RBV therapy provided the opportunity to investigate the difference between IFNs-λ although these three genes were considered to have a similar function. Considering the function of the IL-28B gene, our findings would suggest that IL-28B behaves as a potentiator of ISG under PEG-IFN plus RBV combination therapy and/or inhibits viral replication by itself. On the other hand, these SNP are useful for the prediction of treatment response because of high OR. This is a first step in the tailor-made therapy for HCV infection. Further studies on IFN-λ and the SNP should be investigated including viral factors such as the mutations in the core and NS5A regions as well as HCVRNA levels to improve positive predictive value for applying practical tailor-made therapy.

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***Original Article***

# High sensitivity assay using serum sample for *IL28B* genotyping to predict treatment response in chronic hepatitis C patients

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*Aim:* Recent human genome-wide association studies (GWAS) revealed a strong association between *IL28B* gene variation and the pegylated interferon-α with ribavirin (PEG-IFN-α/RBV) treatment response in chronic hepatitis C patients. Two single nucleotide polymorphisms (SNP), rs8103142 and rs11881222 located in the *IL28B* gene, were found in significant association with the viral clearance. The present study employed these SNPs to develop a new accessible screening method allowing identification of potential non-responders before starting the therapy.

*Methods:* Primer sets were designed to amplify rs8103142 and rs11881222 fragments from genomic DNA extracted from serum samples. This method was validated using microarray typing (GWAS) and applied for genotyping of 68 hepatitis C virus-infected patients with PEG-IFN-α/RBV treatment at baseline.

*Results:* In comparison with GWAS, the screening method showed 100% and 95.6% accuracy in typing of rs8103142 and rs11881222, respectively, indicating incomplete specificity but 100% of sensitivity in both. Genotyping by both SNP showed that 53 (77.9%), 14 (20.6%) and one (1.5%) of the patients were of major homozygous, heterozygous and minor homozygous type, respectively. The majority (85%) of homozygous patients exhibited response to therapy in contrast to heterozygous patients (29%). Among all genotyped only one case was found with the minor homozygous genotype which had late virological response to therapy before relapsing.

*Conclusion:* This study described a highly sensitive assay that can be useful in determining SNP genotypes as well as in predicting the response to IFN-based treatment.

**Key words:** hepatitis C virus, *IL28B*, pegylated interferon-α plus ribavirin, single nucleotide polymorphism typing.

## INTRODUCTION

AN ESTIMATED 3% of the global population has been infected with hepatitis C virus (HCV). More than 170 million chronic carriers are at risk of liver cirrhosis and hepatocellular carcinoma.[1] Combination of pegylated interferon-α with ribavirin (PEG-IFN-α/RBV) is presently the most effective therapy for chronic HCV infection and is widely practiced as a standard treatment. However, not all patients are able to achieve viral clearance. A number of factors were associated with efficiency of the therapy; genotype of the infecting virus is one of them. HCV genotypes 1 and 4 respond poorly to PEG-IFN-α/RBV and often require therapy extended beyond 48 weeks to increase chances of sustained virological response (SVR).[2,3] More than half of the patients infected with genotype 1 could not elicit SVR and suffered from adverse side-effects such as influenza-like symptoms, depression, fever and anemia.[3,4]

Beside viral factors (genotype and viral load), host factors also influence the therapeutic outcome. Age, sex, body mass index and histological grade are considered to determine the individual's treatment regimen and

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outcome.[3,5,6] Recent studies have demonstrated a strong association between genetic variation of the *IL28B* gene on chromosome 19 with response to PEG-IFN-α/RBV therapy[7–9] and natural clearance of HCV.[10] *IL28B* is encoded as a cytokine known as interferon-λ3 (IFN-λ3) which is adjacent to *IL28A* (IFN-λ2) and near *IL29* (IFN-λ1) on chromosome 19. Genome-wide association studies (GWAS) exerted single nucleotide polymorphisms (SNP) in the *IL28B* region to be associated with response to IFN-based treatment among HCV-infected patients of Asian, African and European ancestry.[7–11] The two outstanding SNPs, rs12979860 and rs8099917 (located ~3 kb and 8 kb upstream of *IL28B*, respectively), have been found in strong association with the treatment response and several other SNPs within the *IL28B* gene were found in strong linkage disequilibrium with them (including rs8103142 and rs11881222).[7,9] The minor allele frequency of rs8099917 was significantly higher in the null virological response group (NVR) than the virological response (VR) group. By taking advantage of *IL28B* typing, it may be possible to predict NVR as well as SVR in order to tailor the most suitable treatment regimens.

The featured rs8103142 and rs11881222 SNP are located in the third exon and the third intron of the *IL28B* gene and have significant association with treatment response.[9,12] In this study, we took advantage of the two SNP to develop an accessible screening method. To allow determination of the polymorphism, the *IL28B* gene was performed using conventional polymerase chain reaction (PCR) on serum samples. The newly developed method was validated using microarray typing and enrolled clinical cohort of HCV-1b infected patients receiving PEG-IFN-α/RBV treatment.

## METHODS

### Patients

SERUM SAMPLES WERE collected from Japanese patients infected with HCV genotype 1b, after providing informed consent. All of the subjects had undergone a standard course of PEG-IFN-α/RBV therapy and had their virological response status established before this study.

Null virological response (NVR), transient virological response (TVR) and sustained virological response (SVR) were defined as previously described.[9] This study classified the response outcome into two categories: responders (including those with SVR and TVR) and non-responders (including patients with NVR). Sera were collected from 68 Japanese patients (age range 23–72 years) infected with HCV genotype 1b, who had received the IFN-based treatment. Additionally, serum samples of 23 patients who had genotyping profiles from a GWAS study[9] were included in this study to validate the PCR-based genotyping method outlined in this study.

### DNA extraction and PCR amplification

Human genomic DNA was extracted from 100 μL of serum samples using a QIAamp blood kit according to the manufacturer's instruction (QIAGEN, Tokyo, Japan). To determine SNP genotype of rs8103142 and rs11881222 as well as rs8099917, specific sets of primers were designed within the *IL28B* gene and approximately 8-kb upstream of the non-coding region of the gene, respectively. Nested PCR amplified a short fragment containing rs8103142, rs11881222 or rs8099917 using specific primer pairs (Table 1). The PCR mixture comprised 1 μL DNA, 10 pmol of each primer, 160 μM of deoxyribonucleotide triphosphate, $Mg^{2+}$ and 1.25 U of AmpliTaqGold DNA polymerase (Applied Biosystems, Foster City, CA, USA). The amplification conditions for the three SNP spanning regions was carried out under the following conditions: preincubation for 5 min at 94°C, followed by 40 cycles of three step holds (94°C for 30 s, 65°C for 30 s, and 72°C for 45 s) before final extension at 72°C for 7 min. The amplification products were subjected to electrophoresis in 2.5% agarose gel. Amplified fragments (~200 bp) were sequenced directly in both forward and reverse directions with Prism Big Dye (Fig. 2a) (Applied Biosystems) on an ABI 3100 DNA automated sequencer.

### Nucleotide sequencing and data analysis

Genotyping was based on the chromatograms of nucleotide bases in positions 502 and 685 of rs8103142 and rs11881222, respectively (Figs 1,2b) (nucleotide numbering started at the ATG start codon of the *IL28B* gene). SNP typing of rs8099917 was analyzed in the same criteria. The superimposed chromatogram signals at those three SNP positions were interpreted as heterozygous genotype (Fig. 2b and Table 1). Specificity of this method was assessed by comparison with the SNP genotyping of rs8099917 carried out by GWAS.[9]

## RESULTS

### Validation of *IL28B* typing

ACCORDING TO THE GWAS, rs8099917, located upstream of the *IL28B* gene, is strongly associated with response to therapy of HCV-infected patients.[9] The present study employed PCR and sequencing-based

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**Table 1** Three single nucleotide polymorphisms (SNP) of *IL28B* used in this study. Allele genotype of each SNP was shown. Validation of SNP typing based on polymerase chain reaction and sequencing compared with genome-wide association studies showed the high specificity of this method

| SNP | Location | Allele | | Specificity | Primer name | Sequence | Direction | Size (bp) |
|---|---|---|---|---|---|---|---|---|
| | | Major | Minor | | | | | |
| rs8103142 | Exon 3 | A | G | 100% (23/23) | IL28B/ORF/392F | ttgtcagccatcctccaatcccatcagag | Forward | 224 |
| | | | | | IL28B/ORF/616R | gggccactacagagccaggtgagca | Reverse | |
| rs11881222 | Intron 3 | T | C | 95.6% (22/23) | IL28B/ORF/579F | gcccaccctgccctcccagccctg | Forward | 219 |
| | | | | | IL28B/ORF/787R | ggtgaggggggaggtgaggggaacaggttg | Reverse | |
| rs8099917 | Upstream | T | G | 95.6% (22/23) | rs8099917/F | aagtaacacttgttccttgtaaaagattcc | Forward | 250 |
| | | | | | rs8099917/R | cgctataattaaagatgtgggagaatgcaa | Reverse | |

Primer pairs and predicted product size of each amplified fragment were indicated.

assay to analyze rs8099917 in DNA extracted from serum samples. Of the 23 samples that have been genotyped in GWAS,[9] only one sample showed discrepancy in results obtained here by PCR and sequencing method (Table 1, Fig. 2c). Although rs8099917 has been associated with NVR and has high potential to use as the predictive factor for IFN-based treatment response, it is located far from the *IL28B* coding region. Two additional SNP located in the *IL28B* gene, rs8103142 and rs11881222, which were also significantly associated with the response to HCV therapy, may directly affect the anti-viral response of the IFN-λ3 function. Therefore, these SNP were proposed as a new useful tool to predict NVR or SVR.[9]

Due to the high homology of *IL28A* and *IL28B*, with 96% of amino acid identity,[12] two sets of primers were designed based on the variable regions (Fig. 1). The primers included unique nucleotide sequences of *IL28B* to specifically amplify fragments spanning rs8103142 and rs11881222. To validate the new method, serum of the 23 patients with known genotype identified by GWAS were used to test the specificity of the primer sets of this study. The single clear band of the respective target fragments amplified from serum DNA demonstrated that this method was highly sensitive and specific for the individual SNP (Fig. 2a). Genotyping by our new method showed results identical with the GWAS in 100% and 95.6% of rs8103142 and rs11881222, respectively. In total, of the 23 samples with GWAS data, 11 (47.8%), 10 (43.5%) and two (8.7%) samples were major homozygous, heterozygous and minor homozygous genotypes, respectively. Of the patients with major homozygous genotypes, 72.7% had achieved SVR to IFN-based therapy. However, most of heterozygous and minor homozygous patients, 70% and 100%, respectively, were non-responders. These results in correlation with GWAS, indicated that the method was highly specific and sensitive to amplify the respective SNP in the *IL28B* gene from serum samples.

## *IL28B* typing in PEG-IFN-α/RBV treated patients

Hepatitis C virus genotype 1b infected patients treated with PEG-IFN-α/RBV were examined using this method. The *IL28B* genotype was determined based on both rs8103142 and rs11881222. With results identical for both SNP, most of the patients were of major homozygous genotype (77.9%) (Table 2). Patients with major homozygous allele exhibited higher prevalence

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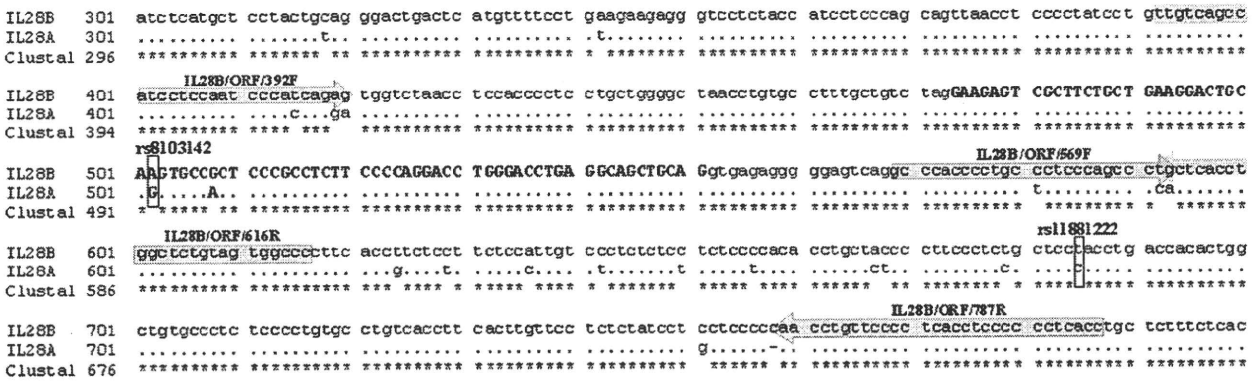


**Figure 1** Nucleotide sequence alignment of *IL28B* and *IL28A* coding region. The gray arrows show the primer-specific site and direction. The numbering of the primers was according to the first AUG start codon. Upper case and lower case represent sequence of exon and intron, respectively. The boxes show the positions of rs8103142 and rs11881222.

of virological response (84.9%) compared to those with minor heterozygous or homozygous allele (5/15, 33.3%) (Table 2). Interestingly, none of the latter patients achieved SVR by PEG-IFN-α/RBV therapy for 48 weeks.

## DISCUSSIONS

TREATMENT WITH PEG-IFN-α/RBV for 48 weeks is currently recommended for patients infected with HCV genotype 1, which is the most common genotype worldwide. However, less than 50% of HCV genotype 1 infected patients achieved SVR to IFN-based therapy.[13] It would be worthwhile to identify those patients who would not benefit from this regimen. In the present study, an effective tool to predict treatment response was developed and evaluated. Genetic variation in *IL28B*, rs8099917, was significantly associated with treatment response.[9] The rs8103142 and rs11881222 were found in linkage disequilibrium with the rs8099917. The rs8103142 is located in the exon 3 and an amino acid change of this location may have an effect on the function of the protein.[9] To achieve higher confidence of the genotyping, this method targets the two SNP (rs8103142 and rs11881222) located in relative proximity to each other within the *IL28B* gene.

Due to relatively low yield of intact human genomic DNA in serum samples, the method was designed for amplification of short DNA. Nested PCR was performed to amplify both alleles of chromosome 19 and increase the detection sensitivity (Fig. 2). Comparison of the results obtained by this screening method with GWAS data revealed its reliability and accuracy (100% accuracy with rs8103142 and 95.5% accuracy with rs11881222). It would be useful to detect the SNP using serum samples as the material is easier to collect, prepare and store in most clinical and research laboratories, however, it might be difficult to exclude possible contamination by nested PCR. Although a real-time detection PCR by TaqMan probe (rs8099917) might be more reliable (100% of the specificity), the sensitivity was lower than that of nested PCR (78.3% and 100%, respectively).

This method applied the conventional PCR with high sensitivity detection even using stored serum samples. The technique was easy and convenient to perform, as neither a high level of experience and training nor complicated analysis were required. Additionally, due to the fact that rs8103142 and rs11881222 are located close to each other, a single PCR (~395 bp using IL28B/ORF/392F and 787R) could be performed to detect genomic DNA from peripheral blood mononuclear cells (PBMC). Trial of the *IL28B* genotype with the single PCR using human DNA from PBMC was successful for 600 HCV-infected Japanese patients (data not shown), indicating that the results by the single PCR using PBMC might be more reliable than those by the nested PCR using serum samples. If patients need information on the *IL28B* SNP genotype, the results using PBMC should be presented.

Most of the major homozygous type patients (72.7%) achieved SVR while none of the heterozygous or minor homozygous patients had SVR to PEG-IFN-α/RBV for 48 weeks. Similar rates were observed when PEG-IFN-α/RBV-treated patients with no GWAS data were examined (Table 2). Although the minor allele was associated with NVR, one of the minor homozygous patients in this study exhibited late virological response and relapsed

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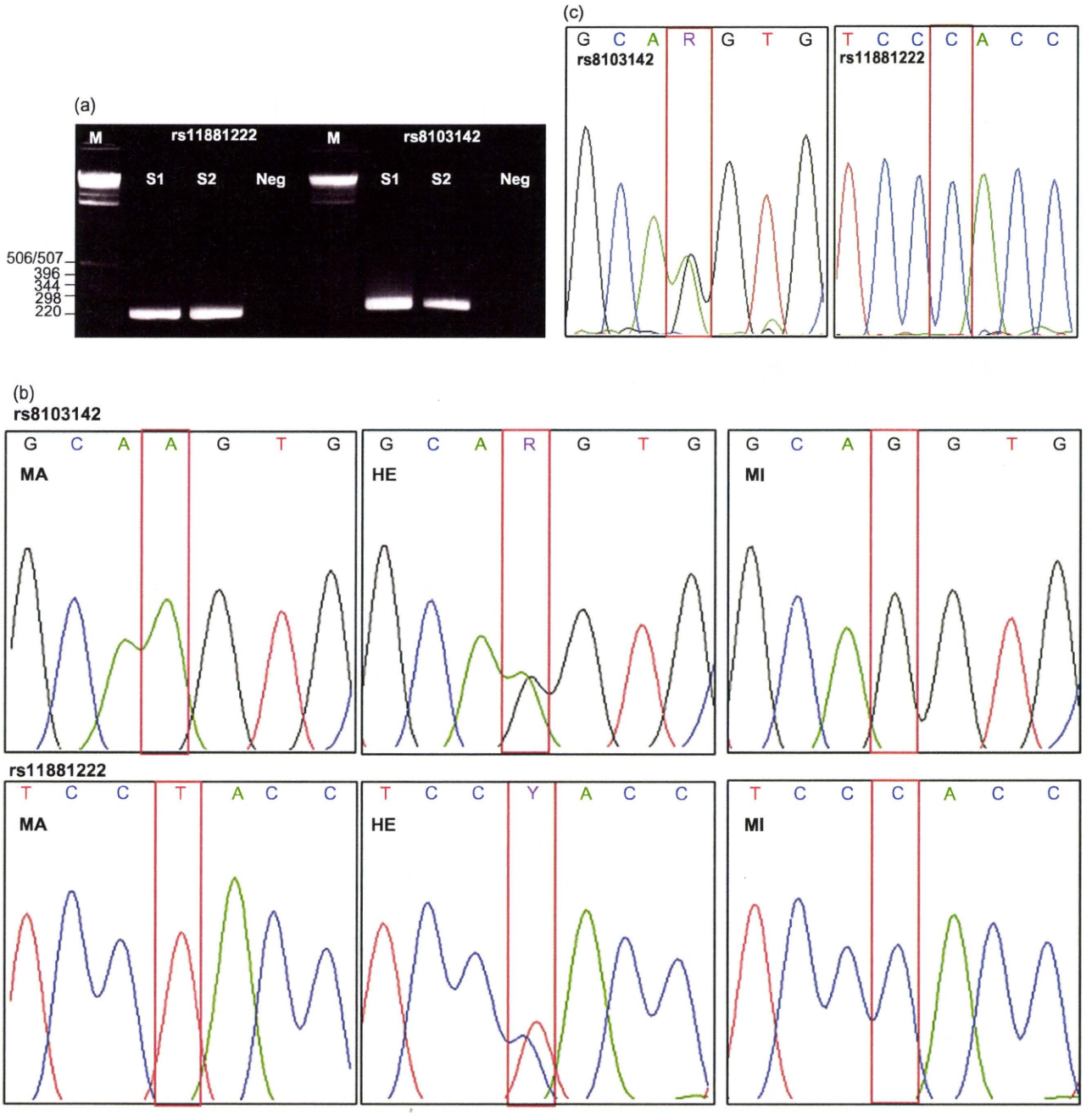


**Figure 2** (a) High sensitivity and specificity of rs11881222 and rs8103142 typing. DNA marker (M) shows approximate product size of samples (S1 and S2) amplified from serum DNA. (b) Nucleotide sequence chromatogram of major homozygous (MA), heterozygous (HE) and minor homozygous (MI) of rs11881222 (lower panel) and rs8103142 (upper panel). (c) A discrepant data between rs8103142 (HE) and rs11881222 (MI).

after the end of 56 weeks of treatment. This may be due to other factors related to viral response.

Individuals with the minor allele genotype displayed significant association with low levels of *IL28A* and *IL28B* expression, which may be associated with the small proportion of SVR patients in heterozygous and minor homozygous groups.[8,9] IFN-λ, a new member of cytokines, has a distinct receptor complex and functions

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**Table 2** Correlation between clinical treatment outcome and SNP typing based on rs8103142 and rs11881222 in hepatitis C virus-infected patients

| | MA ($n = 53$) (77.9%) | HE ($n = 14$) (20.6%) | MI ($n = 1$) (1.5%) | Total ($n = 68$) |
|---|---|---|---|---|
| Mean age, years (SD) | 53.0 (10.8) | 55.6 (10.9) | 61 (0) | 53.6 (10.6) |
| Sex (%) | | | | |
| Female | 23 (43.4) | 6 (42.9) | 0 (0) | 29 (42.7) |
| Male | 30 (56.6) | 8 (57.1) | 1 (100) | 39 (57.4) |
| Response (%) | 45 (84.9) | 4 (28.6) | 1 (100) | 51 (75) |
| Non-response (%) | 8 (15.1) | 10 (71.4) | 0 (0) | 17 (25) |

Single nucleotide polymorphism (SNP) typing based on the two SNP, clinical data as well as pegylated interferon-α plus ribavirin treatment response.
HE, heterozygous; MA, major homozygous; MI, minor homozygous genotype.

similar to type 1 IFN by inducing the JAK–STAT pathway.[12,14] The previous study reported that IFN-α induces IFN-λ expression,[15] suggesting that IFN-α and IFN-λ work synergistically. Consequently, lack of these cytokines or low levels of expression may lead to inadequate transduction signals and become resistant to therapy. Introduction of IFN-λ as an optional new antiviral agent may facilitate better response in the deficient group.

Among viral factors currently considered to be important in prognosis treatment outcome, viral load and genotype are important ones. Variations in the core and NS5A region of HCV genotype 1b and substitutions at amino acids 70 and 91 in the core region were associated with NVR,[16] in addition to host genetic factors may play a role in determining the susceptibility to HCV infection and viral clearance.[17] Most genetic variations presented in the innate immune system such as the interleukin family, tumor necrosis factor and the new member of IFN family (IFN-λ), *IL28A*, *IL28B* and *IL29*, were suggested to have an effect on spontaneous clearance and response to therapy.[12,18,19] Except for *IL28B*, none of them were proved to be significantly associated with response to antiviral therapy.[9,20,21] Hence, this study describes the new convenient approach to predict response to antiviral treatment using DNA from serum samples.

The high-sensitivity technique based on DNA genotyping in serum can be useful in determining SNP genotypes as well as in predicting the response to IFN-based treatment. Although it might be convenient for research purposes to determine *IL28B* SNP in serum, we have to be careful of showing the results in clinics. The method may be useful in IFN-based therapy not only for HCV patients, but potentially for patients infected with other viruses and receiving IFN-based therapy.

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# Hepatitis C Pharmacogenetics: State of the Art in 2010

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**In 2009, a correlated set of polymorphisms in the region of the interleukin-28B (*IL28B*) gene were associated with clearance of genotype 1 hepatitis C virus (HCV) in patients treated with pegylated interferon-alfa and ribavirin. The same polymorphisms were subsequently associated with spontaneous clearance of HCV in untreated patients. The link between *IL28B* genotype and HCV clearance may impact decisions regarding initiation of current therapy, the design and interpretation of clinical studies, the economics of treatment, and the process of regulatory approval for new anti-HCV therapeutic agents.** (HEPATOLOGY 2011;53:336-345)

The current standard of care for chronic infection with hepatitis C virus (HCV) is 24 or 48 weeks of therapy with pegylated interferon-alfa (PEG-IFN) and ribavirin (RBV). Response to therapy is variable, and viral and host characteristics can influence whether patients achieve a sustained virological response (SVR), defined as having undetectable serum HCV RNA at 24 weeks after cessation of treatment. Viral genotype is a predictor of response: patients infected with genotype 1 virus who are treated for 48 weeks with PEG-IFN and RBV have a 40%-50% likelihood of having an SVR, whereas patients with genotype 2 or 3 virus have an SVR rate of 70%-80% after only 24 weeks of PEG-IFN and RBV therapy. Patient genetic ancestry is also a factor in treatment outcome. African American patients with chronic HCV have an almost 50% reduction in SVR rates with PEG-IFN and RBV compared with non-Hispanic patients of European ancestry, and the difference is not explained by socio-demographic characteristics or compliance to treatment.[1,2] Other factors predictive of response to PEG-IFN and RBV include hepatitis C viral load as well as patient age, sex, weight, liver fibrosis stage, and adherence to therapy. Because PEG-IFN and RBV can cause burdensome adverse effects and treatment is prolonged, clinicians often weigh the various viral and host characteristics for each patient before initiating treatment.

In 2009, reports from three genome-wide association studies described several highly correlated common single nucleotide polymorphisms (SNPs) in the vicinity of three IFN-$\lambda$ genes as being highly predictive of response to PEG-IFN and RBV therapy in patients with genotype 1 HCV.[3-5] The three genes encode IFN-$\lambda$1 (*IL29*), IFN-$\lambda$2 (*IL28A*), and IFN-$\lambda$3 (*IL28B*). The same set of SNPs was subsequently associated with natural clearance of HCV.[6,7]

To discuss the implications of the novel pharmacogenetic data on hepatitis C treatment, a meeting of representatives from leading academic medical centers, government agencies, and the pharmaceutical and biotechnology industries took place in Alexandria, VA, on June 4 and 5, 2010. The focus of the meeting was to critically appraise current evidence on the association between genetic markers and response to PEG-IFN and RBV therapy and to provide guidance for incorporating

*Abbreviations: CI, confidence interval; HCV, hepatitis C virus; IL28B, interleukin-28B; ISG, interferon-stimulated gene; ITPA, inosine triphosphatase; OR, odds ratio; PEG-IFN, pegylated interferon-alfa; RBV, ribavirin; RVR, rapid virological response; SNP, single nucleotide polymorphism; SVR, sustained virological response.*

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*A list of Pharmacogenetics and Hepatitis C Meeting participants is provided in the article Appendix.*

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**Table 1. *IL28B* SNPs and Predictive Rates in Various Populations**

| | Percentage Achieving SVR | | | | | |
|---|---|---|---|---|---|---|
| | Total Population | American Caucasian | African American | Hispanic | Japanese | Australian Caucasian |
| rs12979860 | | | | | | |
| Ge et al.[3] | | | | | | |
| No. of patients | 1,137 | 871 | 191 | 75 | | |
| C/C | 79* | 82† | 53‡ | 77§ | | |
| T/C | 38 | 42 | 19 | 43 | | |
| T/T | 26 | 33 | 17 | 21 | | |
| Thompson et al.[8] | | | | | | |
| No. of patients | | 1,171 | 300 | 116 | | |
| C/C | | 69‖ | 48¶ | 56# | | |
| T/C | | 33 | 15 | 38 | | |
| T/T | | 27 | 13 | 27 | | |
| rs8099917 | | | | | | |
| Tanaka et al.[4] | | | | | | |
| No. of patients | | | | | 314 | |
| T/T | | | | | 64** | |
| T/G | | | | | 13 | |
| G/G | | | | | 0 | |
| Suppiah et al.[5] | | | | | | |
| No. of patients | | | | | | 848 |
| T/T | | | | | | 56†† |
| T/G | | | | | | 36 |
| G/G | | | | | | 31 |

*$P = 1.37 \times 10^{-28}$ for T/T versus C/C.
†$P = 1.01 \times 10^{-25}$ for T/T versus C/C.
‡$P = 2.06 \times 10^{-3}$ for T/T versus C/C.
§$P = 4.39 \times 10^{-3}$ for T/T versus C/C.
‖$P < 0.0001$ for T/T versus C/C.
¶$P < 0.0001$ for T/T versus C/C.
#$P = 0.249$ for T/T versus C/C.
**$P = 1.18 \times 10^{-18}$ for G/G or G/T versus T/T.
††$P = 7.75 \times 10^{-4}$ for G/G versus T/T.

genetic data into clinical decision-making and drug development. We report here the current data on *IL28B* in HCV and the panel's recommendations for establishing priorities for *IL28B* research. In addition, recommendations for incorporating genetic data into clinical care and development of therapeutics are outlined.

## Predictive Endpoints of *IL28B* SNPs

The initial published analyses describing genome-wide associations of *IL28B* SNPs and response to PEG-IFN and RBV were derived from several global populations recruited in different clinical trials (Table 1). All three studies reached similar conclusions that underscored the strong predictive effect of *IL28B* genotype on response in treatment-naïve patients.

***United States.*** The first published report came from Ge et al.,[3] who analyzed 1,131 genotype 1 HCV patients for predictors of response to 48 weeks of treatment with PEG-IFN and RBV. Adherence to therapy was a criterion for inclusion: all patients achieving SVR were included, and nonresponders had to be >80% adherent to PEG-IFN and RBV. For the analysis, genetic ancestry was determined explicitly by genetic inference, not self-reporting. Polymorphism rs12979860, which is upstream of the *IL28B* gene on chromosome 19, was strongly associated with SVR, both among patients of European ancestry ($P = 1.06 \times 10^{-25}$) and African American patients ($P = 2.06 \times 10^{-3}$). *IL28B* encodes IFN-$\lambda$3, a cytokine distantly related to type 1 ($\alpha$ and $\beta$) IFNs and the IL-10 family. A set of other variants was also reported as being associated with response, and in patients of European ancestry they were not statistically distinguishable from rs12979860. The C allele at rs12979860 was positively associated with SVR. In patients of European ancestry, ≈80% of patients with the C/C genotype cleared the virus, whereas only ≈30% with the T/T genotype did so. The C/C genotype was also more common in European Americans (39%) than African Americans (16%). The difference in allele frequency between these population groups explains approximately half of the difference in response rates between patients of African American versus European ancestry.

The association between the rs12979860 SNP and SVR appears to be clinically relevant. Thompson et al.[8] reanalyzed the patient population from Ge et al.[3] on an

intent-to-treat basis, meaning that patients were included regardless of adherence. Ethnicity was determined by patient self-reporting. Although including all subjects regardless of adherence does not result in the most powered study design for discovering gene variants influencing therapeutic efficacy, it provides a more accurate picture of the relevance of genotypic information in the clinic, where adherence is variable. Among patients of European ancestry (n = 1,171), SVR was attained by 27% with the T/T genotype, 33% with the C/T genotype, and 69% with the C/C genotype. Among African American patients (n = 300), SVR was attained by 13% with the T/T genotype, 15% with the C/T genotype, and 48% with the C/C genotype. The presence of only one C allele conferred little benefit in treatment response, as was true in the analyses performed by Ge et al.[3] and in the studies of spontaneous clearance reported by Thomas et al.[6] African American patients with the C/C genotype had a significantly higher rate of SVR than European Americans who were non-C/C, indicating that genetic background is more important than ethnicity. However, response rates were lower for African Americans in each genotype category. In a logistic regression analysis of pretreatmeant (baseline) factors, *IL28B* status (C/C versus non-C/C) was the strongest predictor of SVR (odds ratio [OR] 5.2; 95% confidence interval [CI] 4.1-6.7). When on-treatment parameters were considered, rapid virological response (RVR, HCV RNA negativity at week 4) was the strongest predictor of SVR, but only a minority of patients (14% of Caucasians) had rapid response. In patients that did have RVR, *IL28B* remained strongly predictive of SVR, even at 4 weeks after treatment initiation.

***Asia.*** To identify host genes associated with response to PEG-IFN and RBV, Tanaka et al.[4] conducted a genome-wide association study in treatment-adherent Japanese patients with HCV genotype 1 infection. Among the 154 patients, 82 had virological nonresponse (defined as <2 $\log_{10}$ IU/mL reduction in serum HCV RNA at week 12 of treatment), and 72 had a virological response. Two SNPs, rs12980275 and rs8099917, both of which are near *IL28B*, were strongly associated with both virological nonresponse ($P = 1.93 \times 10^{-13}$ [OR 20.3] and $P = 3.11 \times 10^{-15}$ [OR 30.0], respectively) and SVR. Interestingly, the predictive value of these variants in the Japanese study appears to be stronger than what was observed in the studies of African American and European American patients.

The associations were replicated in an independent cohort, and in further fine mapping of the region, seven SNPs near *IL28B* (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917, and rs7248668) showed the most significance. Analysis of linkage disequilibrium (statistical association) among these SNPs showed that all were highly correlated, and there were few grounds for distinguishing them, although rs8099917 was the most significant. It should be noted that rs12979860 was not tested in this study, but it is within the group of associated SNPs (Fig. 1).

Real-time quantitative polymerase chain reaction assays in peripheral blood mononuclear cells showed modestly lower *IL28B* expression levels in individuals carrying the minor alleles of rs8099917 ($P = 0.015$), suggesting that variable *IL28B* expression is associated with a response to PEG-IFN and RBV treatment, although this conclusion remains controversial (see "Mechanisms of Action of Lambda IFNs and Role of *IL28B*" below).

***Australia.*** Suppiah et al.[5] conducted a genome-wide association study of SVR to PEG-IFN and RBV combination therapy in 293 Australian individuals with genotype 1 chronic hepatitis C. The most significantly associated SNPs were then tested in a larger independent cohort of Europeans from the United Kingdom, Germany, Italy, and Australia (N = 555). SVR was associated with the SNP rs8099917 (combined $P = 9.25 \times 10^{-9}$ [OR 1.98; 95% CI 1.57-2.52]).

## *IL28B* for Other HCV Genotypes and in the Context of Coinfection

***HCV Genotypes 2 and 3.*** Although the original studies of *IL28B* polymorphisms were performed in patients with genotype 1 HCV, more recently the association of rs12979860 on response to treatment with PEG-IFN and RBV has been characterized in a cohort of genotype 2 or 3 patients.[9] The patient population consisted of 268 Caucasian patients (genotype 2, n = 213; genotype 3, n = 55) who participated in a multicenter randomized controlled trial from 13 clinical sites in Italy. Patients were randomly assigned to groups that received therapy of either variable or standard (24 weeks) duration. Patients in the variable group who had an RVR (HCV RNA negativity at week 4) were treated for 12 weeks; those without an RVR were treated for 24 weeks. Of patients with the C/C genotype, 82% had an SVR, compared with 75% for genotype C/T and 58% for genotype T/T ($P = 0.0046$ for trend). In contrast to previous observations in North American patients with genotype 1 HCV,[3,8] the SVR rate for genotype C/T patients was intermediate between genotype C/C and T/T patients, suggesting

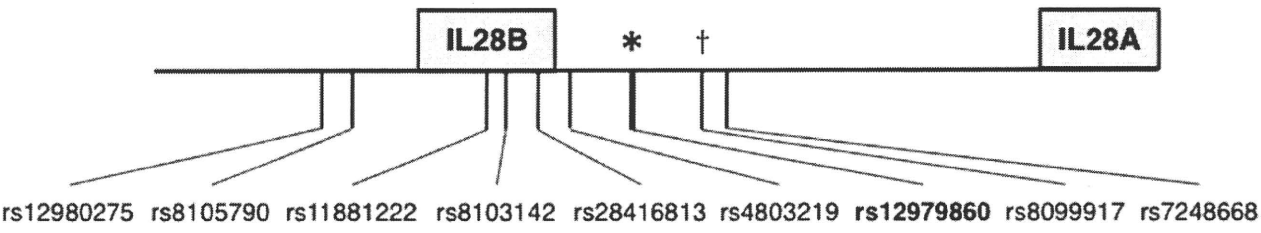


Fig. 1. Variants identified from multiple studies associated with chronic hepatitis C treatment response. All variants shown are associated with treatment response and represent the same genetic signal. The variant rs12979860 (*) was the initial variant discovered by Ge at al.[3] The remaining variants represent the findings by Tanaka et al.,[4] with the primary associated variant, rs8099917 (†), identified by Tanaka et al. and Suppiah et al.[5] Compared with the other variants, the rs12979860 variant is a much stronger predictor of response in individuals of African ancestry; therefore, this single variant would be the best diagnostic in global populations.

the possibility of a more additive effect of the C allele than in the previous setting. Differences between *IL28B* genotypes were greatest among patients who did not have an RVR. Among the 61% of patients who had RVR, SVR was >70% in all *IL28B* genotype groups, and the *IL28B* genotype was not associated with SVR. In contrast, for patients who did not attain RVR, there was a significant difference in SVR on the basis of *IL28B* genotype.

***Human Immunodeficiency Virus Coinfection.*** In a study of patients from two clinical trials at eight major hospitals in Switzerland, the rs8099917 minor allele was associated with progression to chronic HCV infection (OR 2.31; 95% CI 1.74-3.06; $P = 6.07 \times 10^{-9}$).[7] The association was observed in HCV monoinfected patients (OR 2.49; 95% CI 1.64-3.79; $P = 1.96 \times 10^{-5}$) and patients coinfected with HCV and human immunodeficiency virus (OR 2.16; 95% CI 1.47-3.18; $P = 8.24 \times 10^{-5}$). Among all patients, the risk allele was identified in 24% of those with spontaneous HCV clearance, 32% who responded to therapy, and 58% who did not respond ($P = 3.2 \times 10^{-10}$). The strongest association in failure to respond was in patients with HCV genotypes 1 or 4.

***Choosing a Variant for Diagnosis.*** Multiple polymorphisms around the *IL28B* gene are strongly associated with response to standard of care for chronic hepatitis C (Fig. 1), thus raising the issue of which variant or variants to use diagnostically. For patients of European ancestry[3,5] or Japanese ancestry,[4] multiple polymorphisms are statistically indistinguishable from the initially reported variant rs12979860. However, in patients of African ancestry, rs12979860 is clearly a stronger predictor than any other reported variant.[3] In particular, using the data set of Ge et al.,[3] rs8099917 does not associate with SVR in African Americans (OR 0.95; $P = 0.7$), whereas rs12979860 is significantly associated ($P = 0.002$). Therefore, given the current knowledge, the best single choice of variant for diagnostic purposes in global populations or in the clinical trial setting is rs12979860. We note that the causal variants underlying the association between *IL28B* and HCV clearance remains unknown. If one or more causal variants in the region are securely identified in the future, it may be appropriate to consider other or additional diagnostic variants.

## Spontaneous Clearance of HCV

To determine the potential effect of rs12979860 variation on natural resolution of HCV infection, Thomas et al.[6] genotyped this variant in HCV cohorts comprising individuals who spontaneously cleared the virus (n = 388) or had persistent infection (n = 620). The C/C genotype strongly enhanced resolution of HCV infection, with similar clearance rates among individuals of both European and African ancestry. Clearance rates for genotype C/C were approximately double those for T/T and implicate *IL28B* as having a primary role in resolving HCV infection. The rs8099917 genotype T/T has also been strongly associated with spontaneous resolution of HCV infection in Swiss cohorts.[7]

## *IL28B* Genotype and Viral Kinetics

Variation in *IL28B* appears to influence the kinetics of viral response to therapy.

The first 24 to 48 hours after initiation of IFN-alpha therapy is characterized by a rapid dose-dependent decline in viral load, known as the first phase decline, which represents direct inhibition of viral replication.[10] In patients who respond to therapy, after ≈24-48 hours, the viral decline enters a second phase of relatively slow exponential decay, which represents elimination of infected cells. Patients who are not responsive to therapy have a plateau or even a rebound in viral load during this second phase.

After initiation of PEG-IFN and RBV therapy, patients with the C/C genotype at rs12979860 have a greater HCV RNA decline from days 0-28 than patients with the C/T or T/T genotype.[8] Further studies show that the difference can be detected in the first 48 hours of treatment (Fig. 2).[11,12] Among patients

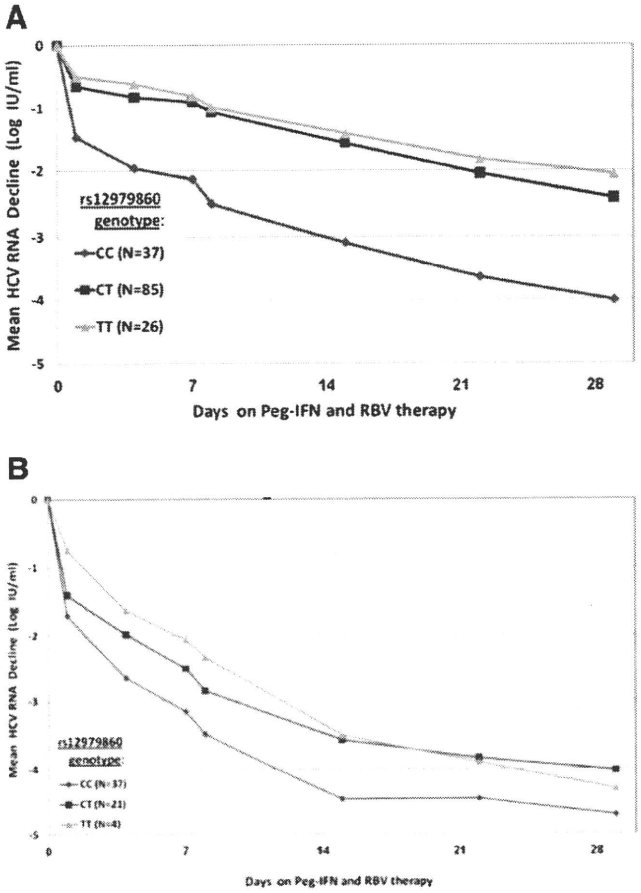


Fig. 2. Kinetics of viral suppression on the basis of SNP rs12979860. After initiation of PEG-IFN and RBV therapy, patients with a C/C genotype at rs12979860 had a greater HCV RNA decline from days 0-28 than patients with C/T or T/T genotype. In Caucasian patients, the differences between the C/C, C/T, and T/T alleles were more substantial in HCV genotype 1 patients (A) than in patients with HCV genotypes 2 or 3 (B).[12]

with the C/C genotype, Caucasians but not African Americans have greater HCV RNA declines than the other genotypes during the second phase (days 7-28).

## Mechanisms of Action of Lambda IFNs and Role of *IL28B*

The specific mechanisms of how variations in *IL28B* SNPs affect HCV suppression remain unknown. However, IL28A, IL28B, and IL29, also called type 3 or lambda IFNs, are induced by viral infection and have antiviral activity.[13] All three interact with a heterodimeric class II cytokine receptor that consists of IL10R$\beta$ and IL28R$\alpha$ (IFN$\lambda$R1)[14,15] (Fig. 3). Lambda IFNs inhibit HCV replication *in vitro*[16,17] and may protect against other RNA-containing viruses *in vivo*.[13,18] Lambda IFNs are thought to produce intracellular responses similar to those of IFN-$\alpha$ but are more specific in their tissue targets because of restricted receptor expression. This has led some to hypothesize that lambda IFNs have similar antiviral activity as IFN-$\alpha$, but with fewer adverse effects. Supporting this hypothesis are results from an open-label study of PEG-IFN-$\lambda$1 (IL29) in patients with genotype 1 HCV, in which weekly dosing had antiviral activity and was well tolerated.[19] However, larger, blinded studies are needed to further evaluate the safety and efficacy of lambda IFNs.

As for type 1 IFNs, expression of lambda IFNs occurs predominantly in antigen-presenting cells such as macrophages and dendritic cells.[13,20] Within the liver, the receptor for lambda IFNs is predominantly expressed in hepatocytes.[21] The kinetics of signal transduction appear to differ between type 1 and type 3 IFNs, with type 3 IFN showing slower activation onset and prolonged duration of activity compared with type 1.[16] However, type 1 and type 3 stimulate similar pathways, with receptor binding resulting in phosphorylation of the kinases JAK1 and Tyk2, activation of the transcription factor complex containing STAT1, STAT2, and IFN regulatory factor 9, and up-regulation of a similar set of interferon-stimulated genes (ISGs).[16,18]

Improved viral clearance could result from alterations in *IL28B* expression, messenger RNA splicing, half-life, or cytokine-receptor affinity or specificity. The responder haplotype of rs8099917 has been weakly associated with higher expression levels of *IL28A* and *IL28B* in peripheral blood mononuclear cells.[4,5] However, in the SNP expression database http://humangenome.duke.edu/software, no difference in peripheral blood mononuclear cell expression of *IL28B* on the basis of rs12979860 genotype has been noted. In addition, in two independent studies,[22,23] no differences in levels of intrahepatic *IL28B* gene expression on the basis of *IL28B* genotype were observed. Further studies are needed to elucidate the causal variants and the biological mechanisms underlying the association between *IL28B* genotype and HCV treatment response.

The expression of hepatic ISGs has been associated with treatment response and has more recently been strongly associated with genetic variation in *IL28B*. In one study, gene expression profiles were analyzed in liver tissue from 91 patients with chronic hepatitis C who received PEG-IFN and RBV combination therapy.[22] Genetic variation in host rs8099917 was determined, and the expression of ISGs was evaluated in all samples. Hepatic ISGs were associated with the *IL28B* polymorphism (OR 18.1; $P < 0.001$), and their expression was significantly higher in patients with the minor genotypes (T/G or G/G), which were associated with nonresponse to treatment, than in those with the major genotype (T/T). Because rs8099917 strongly

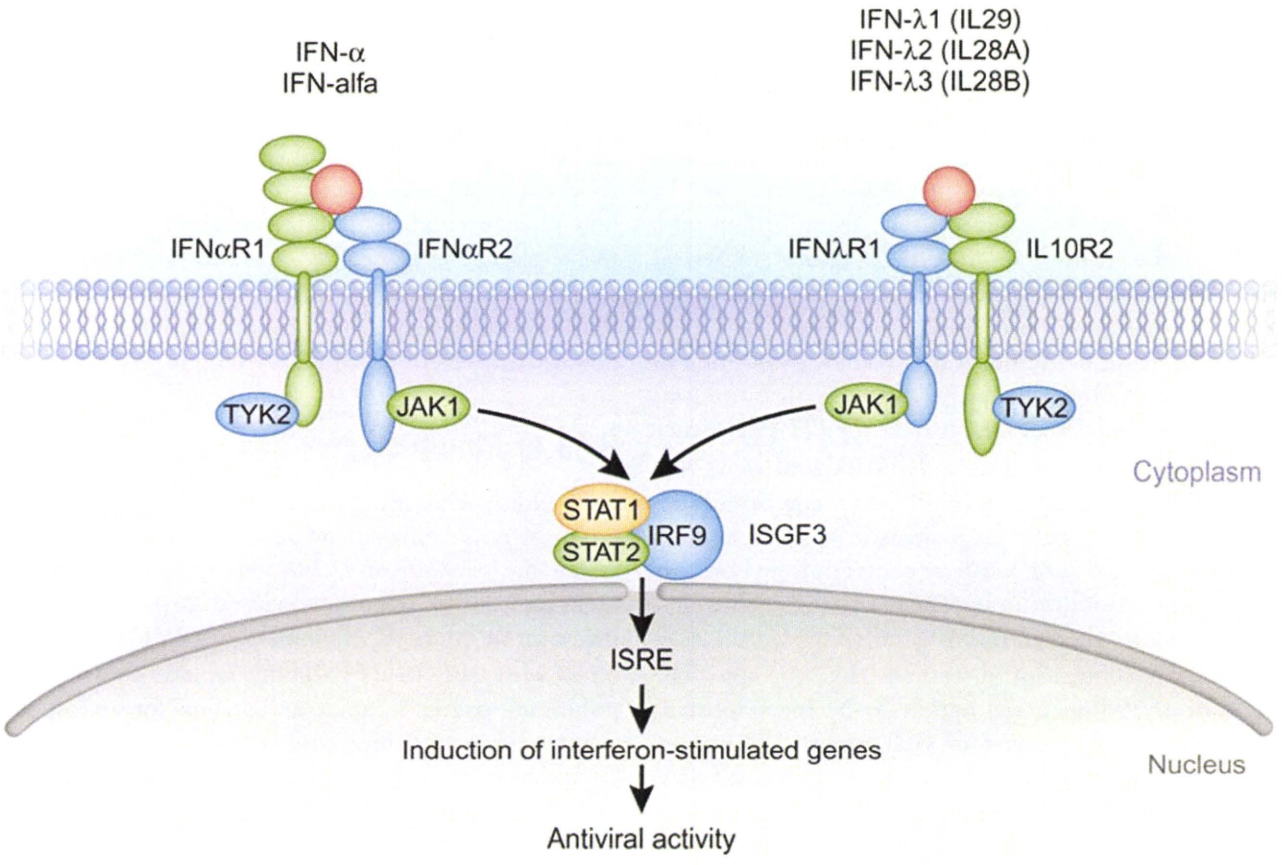


Fig. 3. Signal transduction through type 1 and type 3 IFNs. The three type 3 IFNs IL28A, IL28B, and IL29 all interact with a heterodimeric class 2 cytokine receptor that consists of IL10R2 and IL28Rα (IFNλR1). Although the kinetics of signal transduction are distinct, type 1 and type 3 IFNs stimulate similar pathways, with receptor binding resulting in phosphorylation of the kinases JAK1 and Tyk2, activation of the transcription factor complex containing STAT1, STAT2, and IFN regulatory factor 9, and up-regulation of a similar set of ISGs. Reprinted from O'Brien et al.[27] with permission from Macmillan Publishers Ltd.

correlates with rs12979860, this implies that the poor-response minor allele T at rs12979680 is associated with higher ISG expression than the good-response C allele. (It is important to note that which alleles are associated with good and bad response depends on which marker variant is considered). Similarly, in RNA expression analyses from liver biopsies of 61 North American patients with chronic HCV, 164 transcripts were differentially expressed on the basis of rs12979860 genotype.[23] The IFN signaling pathway was the most enriched canonical pathway with differential expression ($P < 10^{-5}$), and most genes had higher expression in livers of individuals carrying the poor-response non-C/C genotypes.

## Clinical Role for Pharmacogenetics in Hepatitis C

*IL28B* genotyping has multiple potential roles for current practice. For example, treatment-naïve patients with the C/C genotype at rs12979860 may decide to undergo PEG-IFN and RBV therapy given their relatively high likelihood of SVR. Patients with the T/T genotype at rs12979860 and no indications of serious liver problems may wait for new direct antiviral agents to become available, because T/T genotypes have a poor likelihood of IFN responsiveness. *IL28B* genotype may also be considered in conjunction with virological response at week 4; patients with poor viral kinetics and T/T genotype at rs12979860 may decide to stop therapy.

Although *IL28B* genotyping is highly predictive of SVR at the population level in HCV genotype 1 patients, its predictive power at the individual patient level is far from absolute. Therefore, *IL28B* genotyping should not be the sole factor in deciding on a treatment strategy. Some patients have SVR despite having an unfavorable genotype. In addition, although evidence suggests that *IL28B* genotyping is the strongest pretreatment indicator of response, the on-treatment

variable of RVR at week 4 is an even stronger predictor.[8] These factors suggest that if *IL28B* genotyping is used, it should be considered along with other baseline predictors of response and virological status at week 4.

An attractive scenario is that patients with favorable *IL28B* genotyping but HCV genotype 1 virus may be able to reduce treatment time from 48 weeks. Although this issue is a priority of future studies, at this time, there is insufficient data to recommend shortening the duration of standard of care.

Another clinical advance is the recent identification of two inosine triphosphatase (*ITPA*) polymorphisms known to be functionally responsible for *ITPA* deficiency and strongly protective against RBV-induced hemolytic anemia.[24] *ITPA* genotyping could help guide clinical decision-making, especially for patients in whom treatment with RBV is avoided or relatively contraindicated because of the high risk for developing anemia.

It is unclear whether *IL28B* genotyping will be relevant in the context of direct antiviral therapy. The effects of *IL28B* genotype appear to be most substantial during the first phase of viral decline. Therefore, direct antivirals that have swift, potent effects on viral load may diminish the influence of *IL28B* genotyping in predicting SVR. However, direct antivirals achieve reductions in viral load by a variety of mechanisms, and it should not be assumed that *IL28B* genotyping will have the same implications with different therapies or treatment strategies. The lack of data regarding whether *IL28B* genotype is predictive of response when directly acting antivirals are added to IFN and RBV makes a definitive statement on combination treatment difficult. Knowledge of *IL28B*'s effect in patients taking directly acting antivirals is a priority for research and clinical care.

## Pharmacoeconomics: Implications of Tailored Therapy

Both the process of *IL28B* genotyping and the possibility of tailored therapy affect the pharmacoeconomics of hepatitis C therapy. An important consideration regarding *IL28B* genotyping is whether it will be covered by health care plans. Coverage of genotyping could enhance clinical decision-making. However, coverage of treatment should not be based on genotyping alone. It is hoped that clinical and patient advocacy groups will insist that *IL28B* status is not used to deny treatment, especially given that its informative value is not absolute.

In the context of drug development, tailored therapy has several potential implications. Segmenting the treatment population may reduce the overall size of the market; however, this may not necessarily limit profit. Throughout the world, treatment for HCV has poor patient uptake, often because of patient concerns about efficacy and tolerability. Pharmacogenetics could make a drug more appealing to a specific group of patients, such as African Americans with a C/C genotype at rs12979860. Knowing how pharamacogenetic testing could affect the size of the marketplace, the segmenting of the treatment population, and the clinical need is critical for important considerations such as pricing, therapeutic substitutions, competition, and orphan drug status.

## Role of Pharmacogenetics in Clinical Trials

In North America, a commercial test for *IL28B* genotyping is now available and costs approximately $300.[25] Given the strength of *IL28B* genotyping as a pretreatment indicator of response to current hepatitis C therapy, investigators of trials of novel therapeutic agents combined with a PEG-IFN backbone would be advised to at minimum collect samples at baseline for retrospective genotyping. Establishing study designs with stratification on the basis of *IL28B* genotype can prevent enrichment of favorable or unfavorable genotypes in comparator cohorts. In such cases, a novel therapeutic agent is at risk for failing to reach noninferiority or superiority claims against standard of care with PEG-IFN and RBV.

Obtaining informed patient consent for genetic information is essential in elucidating relationships between genotype and response to therapy; however, patients and institutional review boards can have concerns regarding providing consent. Given the increasing clinical significance of pharmacogenomics, the US Food and Drug Administration is in the process of developing a clinical pharmacogenomics guidance, which will be available online. The panel recognized the importance of educating institutional review boards on the critical role and potential patient benefits of pharmacogenomic testing in clinical trials.

## Perspective of the US Food and Drug Administration

From the perspective of regulatory agencies, pharmacogenetics can be a factor in drug development, labeling, and eventual clinical use in the marketplace. The potential applications of pharmacogenetics-informed HCV trials are listed in Table 2. At present, it is recommended that samples for pharmacogenetic testing be stored at the outset of a clinical trial.

There are two avenues for obtaining pharmacogenetic testing information on a product label: the first

**Table 2. Applications for Incorporating *IL28B* Pharmacogenetics in Hepatitis C Trials**

| Trial Stage | Strategy |
|---|---|
| Proof of concept | Enrich with the nonresponder genotypes to increase estimate of experimental drug effect size |
| | Enrich with responder genotype for conservative estimate of experimental drug effect to inform pivotal study design |
| | Study novel direct antiviral combinations (without standard of care) in responder genotype, as a less vulnerable population that could be salvaged with standard of care if they had viral rebound with resistance |
| Phase 2a | Ensure balance of *IL28B* genotypes among treatment arms in dose-finding studies (might prevent reaching spurious conclusions regarding efficacy between treatment doses or regimens) |
| | Consider stratifying all phase 2 studies |
| | Collect samples for future genetic analyses |
| Phase 3 | Enroll or stratify for specific genotypes |
| | Establish genetically defined unmet medical need |
| | Note racial or regional differences in response |
| | Future pharmacogenetics testing of other polymorphisms may help to manage potential adverse reactions and favorably alter the risk/benefit profile |

is through codevelopment of drug and test, and the second is through postapproval label updates. Linked codevelopment provides the best opportunity to obtain evidence of clinical use for both test and drug. In this case, the evidence in support of product labeling often comes from prospective hypotheses, randomized controlled trials, and replication. The sponsor assumes primary responsibility for generating evidence.

For postapproval label updates with genetic information, evidence of clinical use often comes from observational analyses, case-control or cohort studies (versus randomized controlled trials), and retrospective analyses. The data are not always generated by a pharmaceutical sponsor and are often added to labeling because of a safety issue, such as the occurrence of an adverse event that becomes apparent with widespread product use. Common occurrences leading to label updates include frequent or severe clinical events, the further understanding of disease pathology and drug target (biological plausibility), clinical validation in prospective studies, or the presence of actionable recommendations. An example of this would be genetic testing prior to abacavir in human immunodeficiency virus therapy. The framework for evaluating the value of a genetic test is outlined in Table 3.[26]

**Table 3. Considering the Value of a Genetic Test[26]**

| Framework | Question |
|---|---|
| Clinical situation | What is the medical need? |
| Marker association | What is the strength of the association? |
| Clinical variables | What nongenetic variable is associated with the response? |
| Clinical course | What is the clinical course in marker-positive patients? |
| Cost | What is the value of the test? Is it cost-effective? |

**Table 4. Research Priorities**

| |
|---|
| Mechanistic studies of lambda IFNs in HCV suppression |
| Correlate clinical manifestations and *IL28B* genotype |
| Further discovery of genetics and *IL28B* in African Americans |
| HCV kinetics of posttransplant recurrence on the basis of *IL28B* genotype |
| Cost-effectiveness analyses of response-guided therapy that includes *IL28B* genotyping |

Currently, the US Food and Drug Administration considers *IL28B* genotyping in the treatment of chronic HCV as advisable but not necessary.

## Future Directions and Priorities

*IL28B* genotyping will almost certainly drive the hepatitis C treatment setting toward a more tailored approach. However, the role and importance of pharmacogenetics in hepatitis C treatment is multifaceted and evolving. Actions that would benefit research and clinical care include having a uniform and more intuitive nomenclature for the *IL28B* SNPs and the creation of a central data repository for reporting genotypic and phenotypic correlations to treatment response. Priorities for research studies are numerous (Table 4) and include understanding the mechanics of lambda IFNs in HCV suppression and detailing the cost-effectiveness of response-guided therapy that includes *IL28B* genotyping. Collaboration between academia, industry, and governing bodies will help move the priorities forward and should hasten advances in clinical care.

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## Appendix

The participants of the Pharmacogenetics and Hepatitis Meeting are as follows: Jeroen Aerssens, Tibotec BVBA, Beerse, Belgium; Nezam H. Afdhal, Beth Israel Deaconess Medical Center, Boston, MA; Steven M. Anderson, Laboratory Corporation of America/ Monogram Biosciences, Research Triangle Park, NC; Shashi G. Amur, Debra Birnkrant, Jeffrey S. Murray, Sarah M. Robertson, Kimberly A. Struble, Kathleen Whitaker, US Food and Drug Administration, Silver Spring, MD; David Apelian, GlobeImmune, Inc., Louisville, CO; Jim Appleman, Anadys Pharmaceuticals, Inc., San Diego, CA; Robert D. Arbeit, Idera Pharmaceuticals, Inc., Cambridge, MA; M. Michelle Berrey, Pharmasset, Inc., Princeton, NJ; David R. Booth, University of Sydney, Sydney, Australia; Martyn Botfield, Shelley George, Vertex Pharmaceuticals, Inc., Cambridge, MA; Clifford Brass, Merck & Co., Inc., Kenilworth, NJ; Jenny Brews, Paul Clark, John G. McHutchison, Susanna Naggie, Keyur Patel, Alexander J. Thompson, Duke Clinical Research Institute, Durham, NC; Scott C. Brun, Abbott Laboratories, Abbott Park, IL; Mary Carrington, SAIC-Frederick, National Cancer Institute, Frederick, MD; Sophia Chao, Stephen J. Rossi, Roche Molecular Diagnostics, Pleasanton, CA; Gavin Cloherty, Abbott Molecular, Des Plaines, IL; Eoin P. Coakley, Monogram Biosciences, Inc., South San Francisco, CA; Jacques Fellay, David B. Goldstein, Kevin V. Shianna, Thomas J. Urban, Duke University Medical Center, Durham, NC; Hawazin Faruki, LabCorp, Burlington, NC; Sam Hopkins, Scynexis, Inc., Durham, NC; Nigel Hughes, Tibotec–Virco BVBA, Beerse, Belgium; Christina Kish, Genentech, Inc., Hoboken, NJ; Bruce Kreter, Bristol-Myers Squibb, Princeton, NJ; William A. Lee, Gilead Sciences, Inc., Foster City, CA; T. Jake Liang, Emmanuel Thomas, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; Uri Lopatin, Roche Pharmaceuticals, Palo Alto, CA; Ven Manda, Rachael Scherer, William Van Antwerp, Medtronic, Inc., Minneapolis, MN; Alessandra Mangia, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy; Masashi Mizokami, National Center for Global Health and Medicine, Chiba, Japan; David Oldach, Gilead Sciences, Inc., Durham, NC; Jean-Michel Pawlotsky, Hopital Henri Mondor, University of Paris EST, Creteil, France; Gastón Picchio, Tibotec, Inc., Titusville, NJ; Kevin A. Schulman,