Prediction of Treatment Response in Patients Infected with Hepatitis C Virus

Keywords: HCV prediction; Direct-Acting Antivirals (DAAs) treatment outcome; Interferon (IFN)

Abstract

As a major world health problem, Hepatitis C Virus (HCV) infection causes various liver diseases. In the past decades, great efforts have been made in the development of novel therapy for HCV infection. Although newly developed Direct-Acting Antiviral Agents (DAAs), such as HCV NS3/4A protease inhibitors Telaprevir and Boceprevir increased the response rate up to 80% for the most prevalent HCV genotype 1 infected patient, a significant proportion of patients still do not respond. Combination therapy with pegylated IFNα and Ribavirin (PEG-IFN/RBV) remains the first choice to treat chronic HCV infections in many developing countries. In consideration of the fact that resistance is now the unmet challenge in anti-HCV therapy, prediction of treatment outcomes becomes an important goal in HCV research. Patients would spare high cost and extensive side effects if prediction of the likelihood of treatment response prior to initiating therapy, or at least soon after starting therapy, could be made. In this chapter, we reviewed several host and viral factors affecting the likelihood of achieving sustained virological response (SVR) in patients receiving stranded PEG-IFN/RBV treatment or new DAAs- PEG-IFN/RBV triple therapy.

Introduction

Hepatitis C Virus (HCV) infection is a major world health problem with a prevalence of about 170 million worldwide, representing 3% of the world’s population [1,2]. In most cases (60-85%), HCV infection progresses to chronic liver diseases, which are associated with cirrhosis and hepatocellular carcinoma (HCC) [3]. HCV remains the most common newly diagnosed cause of liver diseases and the most common reason for a liver transplant in North American and Europe [4].

HCV has been discovered in 1989 and great progress has been made in the understanding of its virology, viral life cycle and host immunity. Unfortunately, antiviral therapy is still suboptimal. The current standard of care (SOC) is the combination therapy with pegylated Interferon-α and ribavirin (PEG-IFN/RBV) [5]. Although a sustained virological response (SVR), defined as undetectable HCV RNA 6 months after treatment completion, can be achieved in most (>90%) acute HCV infections, the SVR rates drop dramatically in chronic HCV infection. This combination therapy can cure HCV in only 30%-70% of patients chronically infected with HCV depending on different genotypes [6-9]. In addition to unfavorable response rates, high cost, long duration and extensive side effects inherent to the IFN-based regimen urge us to explore new antiviral strategies. Most recently, with the increased understanding of the HCV life cycle and the solution of crystal structures of HCV proteins, Direct-Acting Antiviral Agents (DAAs) against HCV viral protease or RNA polymerase were developed, and this opened a new era for HCV therapy. At present, DAAs mainly consist of HCV protease inhibitors, polymerase inhibitors and HCV non-structure protein 5A (NS5A) inhibitors [10]. Two NS3/4 protease inhibitors (PI), Telaprevir and Boceprevir, were already approved to treat HCV genotype 1 patients [11] in 2011. Although DAAs in combination with PEG-IFN/RBV (triple therapy) improve the SVR to some extent, the apparent limitations include low genetic barrier to resistance. Genetic barrier is used to define the number of amino acid substitutions required to confer full resistance to a drug, DAA with a low genetic barrier usually requires only one or two amino acid substitutions to result in drug-escape mutants of HCV during treatment. Moreover, new medications, such as host-targeting agents which are involved in various HCV life-cycle, target HCV receptor-mediated entry (e.g.: CD81 [12], SR-B1 [13-15]), RNA replication (e.g.: miR122 and virion assembly (e.g. Glucosidase [16]). Considering the cost issue in most developing countries, less expensive natural compounds including Chinese herbal formulations are being explored but they are still in early development.

In consideration of the apparent limitations of current HCV therapy, especially high failure rate, prediction of treatment outcomes prior to the initiation of treatment and understanding of the molecular mechanism of HCV resistance are two important goals in HCV research. Patients would benefit if prediction of the likelihood of a treatment response prior to initiating therapy, or at least soon after starting therapy, could be made. In fact, numerous host and viral factors have been used to predict treatment response to interferon-based therapy (Figure 1).
Viral Factors to Predict IFN-based Treatment Response

**HCV genotype**

According to a recent international nomenclature of classification [17], HCV contains six major genotypes (1–6) that differ from each other by 30–35% of nucleotide sequence. Each group consists of a series of subtypes differing in 20-25% nucleotides [17]. Different areas in the world have different genotypes or subtypes distribution (reviewed in [18]). Numerous studies [7,19-22] indicated that the SVR rate in patients infected with HCV genotype 1 and 4 is 40-50%, much lower than that in patients infected with genotype 2 and 3 (about 75%). Therefore, HCV-1 and 4 are defined as difficult to treat genotypes. Why different HCV genotypes respond to IFN-based treatment differently remains elusive. One study [23] published in 1999 argued that the HCV E2 envelope protein could inhibit PKR (RNA-activated protein kinase R) activity. In vitro through the PePHD (PKR-eIFα phosphorylation homology domain) whose sequence was similar to the phosphorylation domains of PKR. This similarity is greater in HCV genotype 1 compared with that in genotype 2 or 3. As such, E2 was speculated to contribute to the resistance of HCV genotype 1 to PEG-IFN/RBV [23]. However, these findings could not be reproduced by other studies [24,25]. Besides HCV genotype 1, whose responses to the treatment are apparently improved by the recent development of protease inhibitors [26,27], HCV genotype 4 becomes the most “difficult to cure” genotype of HCV [28]. HCV genotype 4 is predominating in the Middle and East Africa, especially Egypt [29]. Although the specific mechanisms are still unclear, the slow viral dynamics of HCV genotype 4, which is similar to those of HCV genotype 1 and slower than those of HCV genotype 2 [30], indicated that the therapeutic strategies should be seriously considered in relation to the genotype.

**Viral load**

In a study of 88 HCV genotype 1 patients (from the Italian Hepatitis C Cohort Study, ITAHECS) [31], one of the independent predictors of RVR (Rapid Virological Response) and SVR was pretreatment HCV RNA <400000 IU/ml. The predictive effect of baseline viral load was confirmed by several other studies, even in the studies of patients co-infected with HCV and HIV [32] or in population with recurrent HCV genotype 1 infection after living donor liver transplantation [33]. Interestingly, the current threshold of basal HCV RNA is mainly identified in patients with HCV genotype 1. In patients with HCV genotype 2 or 3, the basal HCV RNA is less useful in predicting response. It is not surprising because the viral load reflects the complex virus-host interaction which can be affected by HCV genotypes. In addition to the pre-treatment baseline viral load, on-treatment viral variables are also useful in predicting the probability to achieve SVR. Viral response at week 4 of therapy (RVR) has been identified as an important predictor of SVR in patients infected with HCV genotype 1 [34] or 2 [35]. Recent studies are looking for much earlier viral response after initiation of therapy to predict SVR. Laufer et al. [36] found that the reduction in HCV viral load from baseline to 24h of <1.4 had a negative predictive value for achieving SVR of 100% in HIV/HCV genotype 1 patients. Using the changes in HCV viral load in the first 14 days after PEG-IFN/RBV treatment, Jun Itakura et al. [37] even derived a prediction equation to predict when the patients will achieve HCV RNA negativity. Yuki Wada et al. [38] analyzed 64 HCV genotype 2 patients with high viral load and reported that the HCV core antigen level at 1 week after treatment initiation could predict SVR. These studies indicated that viral load at baseline or viral dynamics during treatment can be used to predict treatment response in HCV patients.

**Viral gene mutations**

HCV nonstructural protein 5A (NS5A) is the most studied gene discriminating the non-responders (NRs) from responders (Rs) to PEG-IFN/RBV therapy. There are at least 3 functional domains of NS5A involved in IFN resistance: ISDR (Interferon Sensitivity-Determining Region), PKRBD (PKR Binding Domain) and V3 (Variable region 3) in the C terminus. In 1995, Nobuyuki Enomoto et al. [39] identified the COOH-terminal of the NS5A region as ISDR for the first time. Their findings argued that 4 or more mutations in the region (known as “mutant type”) were associated with high SVR rate in Japanese patients chronically infected with HCV genotype 1b. However, this association has become a subject of long controversy. Some observations [40-43] supported the findings, while others [44-47], especially those performed in Europe and United States, were conflicting. Several factors may contribute to this contradictory observation: 1) epidemiological differences, such as HCV genotypes and strains in which the frequency of ISDR mutations is relatively low; 2) treatment protocol and different types of interferon used; 3) ethnicity and host genetic background; 4) viral gene mutations in regions other than ISDR. Furthermore, not only the total number of ISDR mutations, but also the
sites of those mutations may affect the SVR rate [48]. In fact, total number and position of amino acid substitutions in the ISDR affects HCV replication *in vitro* [49]. PKRBD, consisting of ISDR and additional 26 distal amino acids, is required for the binding of NS5A to PKR. This NSSA-PKR interaction inhibits PKR activity and resulting in suppression of PKR-mediated eIF-2α phosphorylation. Recent studies [44,50,51] reported that variations in PKRBD were independent predictors of treatment outcomes to PEG-IFN/RBV.

Although the core region of HCV is conserved, mutations of amino acid (aa) 70 and aa 91 are frequently observed. Several studies [41,42,52] have reported that sequence changes in the core region were associated with SVR rate. However, most of them were performed only in patients with HCV genotype 1b, and the predictive effect of mutations in the HCV core region in other genotypes and subtypes still remained to be determined.

**Host Factors to Predict IFN-based Treatment Response**

**Pretreatment ISGs expression in the liver and blood**

Gene expression profiling studies that looked at the effects of HCV infection on the host liver have often been aimed to associate changes in the expression of a subset of genes with clinical outcomes or treatment responses. To identify intra-hepatic protein expression that may be used as predictors, MacQuillan et al. [53] scored baseline hepatic human myxovirus a protein 1 (Mxα) expression, and found that it was significantly higher in NRs compared to Rs. However, the baseline expression of another IFN-α induced protein, PKR, was not different in groups divided by treatment response. Another study [54] performed in 61 Italian patients with chronic HCV also revealed that lower hepatic PKR mRNA levels prior to therapy was associated with SVR. With the development of microarray gene-expression profiling, a high throughput method that allows simultaneously examine gene expression changes at the transcript (mRNA) level, it is much easier to look at the host response to HCV infection at the whole genomic scale. For instance, Chen et al. [55] used a 19,000 DNA microarray to study pretreatment liver biopsy specimens taken from patients with chronic HCV who were subsequently treated with PEG-IFN/RBV. They identified 18 genes whose expression levels were consistently and statistically different between treatment Rs and NRs. This response signature, including eight high expression ISGs prior to treatment, ISG15, ISG16, OAS2, OAS3, IFIT1 (IFN-induced protein with tetratricopeptide repeats), Mxα, USP18 (Ubiquitin-Specific Protease 18) and CEB1 (Cyclin E Binding Protein 1), accurately differentiate Rs and NRs in 96.8% (30/31) of patients with HCV. Similarly, Aschel et al. [56] also found 3 ISGs (IFI-6-16, IFI27 and ISG15) whose pretreatment high levels of expression were observed in two independent cohorts by using real-time PCR analysis. Therefore, significant positive association between “high ISG” phenotype and unfavorable treatment outcome was confirmed. We can postulate that up-regulation of ISGs reflects a pretreatment activation of IFN-α signaling pathway, making the NRs react bluntly to further IFN-α treatment.

Although a certain correlation between elevated pretreatment ISGs expression in liver and failure of anti-HCV therapy was demonstrated, it is necessary to develop an easier predictive test with fewer invasions than liver biopsy based test. Besides the liver tissue, it is likely that strong HCV signal can also be found in blood.

As reported by Gerotto et al. [54], baseline PKR mRNA up-regulation in Peripheral Blood Mononuclear Cells (PBMCs) was more frequently observed in future NRs, independent of age, gender, HCV load and genotype, histological activity and stage of liver disease. Lempicki et al. [57] studied the role of gene expression pattern in PBMCs in 29 patients co-infected with HIV and HCV. They reported 79 genes that identified all 10 NRs, 8 of 10 ETRs (end-of-treatment response, but not SVR), and 7 of 9 patients with SVR. In 17 post-treatment samples one hundred and five genes correctly classified all 9 ETRs and 7 of 8 patients with SVR. Among them were 20 IFN-stimulated genes whose baseline (before treatment) expression levels were higher in treatment NRs than in ETRs or SVRs. Most recently, Tao Huang and his colleagues [58] utilized the HG-U133A Gene Chip containing 22283 probes to analyze the global gene expression in PBMCs at different time points before and during the early stage of treatment. The prediction accuracy of their time-dependent diagnostic model is 100% and 85.7% in 36 Caucasian American patients and 33 African American patients, respectively. Although there is a certain limitation that the authors evaluated the response without concerning SVR, this study provided a new insight into the time series gene expression profiling of prediction. However, Sarasin-Filipowicz et al. [59] reported conflicting result of the study conducted in 16 Caucasians with HCV genotype-1 or non-genotype-1. Surprisingly, they found that the difference in the up-regulation of ISGs was not pronounced in the blood between Rs and NRs, unlike intra-hepatic ISGs expression pattern.

Although ISGs were elevated in NRs before treatment, identical ISGs expressions were observed in Rs and NRs/ ETRs after therapy [54,57,59,60], suggesting that maybe not ISG expression itself, but changes before and after treatment influenced the response. Importantly, given that patients infected with HCV genotype 2 or 3 were much easier to achieve SVR, the finding [59] that baseline ISG expression levels were significantly lower in subjects with HCV genotype 2 and 3 than with genotype 1 and 4 indicated that pretreatment activation of IFN-α system may be correlated with HCV genotypes. Whatever the reason, endogenously activated ISGs in chronic HCV may be a biomarker of immune dysfunction rather than antiviral effect of IFN-α. In addition, differential gene expression pattern in pre-treatment Rs and NRs may come from different cell types. Using immunohistochemical staining method, Chen et al [61] stained the pretreatment liver biopsies from HCV infected patients with ISG15 and Mxα antibodies. They found that increased expressions of these ISG proteins in hepatocytes predicted treatment non-response while in macrophages predicts SVR. This finding may change our traditional way to look at chronic HCV infection, and will help us further understand the role of ISG expression in HCV replication/production and virus resistance to therapy. This cell-type specific protein expression pattern may provide a new way to predict response to IFN-based therapy. Exploring why differential expression of a subset of ISGs in different cell types (hepatocytes Vs macrophages) predicts treatment outcome is critical to understand the molecular mechanism of IFN resistance in HCV.

**Host interleukin 28B (IL28B) genotype and SVR**

Genome-wide association studies (GWAS), which allow an unbiased sampling of variations in genes across the entire genome without a hypothesis, have independently revealed that some SNPs around IL28B on chromosome 19 which encodes IL28B (IFN-λ 3) contributed to both treatment-induced and spontaneous HCV clearance.

Ge et al [62] employed GWAS to study a population of patients involving 871 European, 191 African and 75 Hispanics with chronic HCV genotype 1 infection. They showed that Rs12979860 (located ~3 kb upstream of IL28B) was associated with a two fold increase in SVR rate, taking other clinic factors into account. They further compared the efficacy of PEG-IFN/RBV treatment in association with
IL28B genotype between African-Americans and European-Americans. They found that more favorable IL28B genotype was found in European than African populations, which explain better treatment outcomes in European-Americans to some extent. Tanaka et al. [63] investigated genetic predictors through a two-stage design, the first stage of GWAS in 154 Japanese with HCV genotype 1, and the second replication stage in an independent population of 172 patients. Strongest association was observed between SVR and Rs8099917/Rs12980275 in two stages. 6 other SNPs also achieved the suggestive genome-wide threshold in both the GWAS cohort and replication cohort. Suppiah et al. [64] also used a two-stage approach consisting of an initial GWAS stage in 293 Australian and a followed stage in Western European. They identified Rs8099917 as the variant most strongly associated with SVR. A distinct 6-alleles haplotype block (tagged by Rs8099917) encompassing regulatory regions of both IL28A and IL28B [64] indicated a relationship, if any, between these polymorphisms and genes expression. A fourth GWAS was conducted in European patients chronically infected with genotype 1 or 4 [65]. In addition, the research was extended to both HCV mono-infected and HCV/HIV co-infected populations. In their study, the minor allele of Rs8099917 was identified in 58% of patients who failed to respond, and defined as a risk factor associated with progression to chronic HCV infection, regardless of co-infection with HIV or not. Ochi et al. [66] tested for SNPs that were associated with SVR in Asian ancestry with HCV genotype 1b or 2a. Two SNPs (Rs8099917 and Rs12979860) in IL28B have been documented to affect the outcome of PEG-RBV combination or IFN monotherapy therapy. They also found that haplotypes, including 4 novel SNPs, showed more accurate in predicting treatment outcomes than any single variant.

Taken together, all the studies confirmed that genetic variation in the IL28B region was associated with SVR, suggesting that patients with the “bad” allele must wait for new antiviral therapy. Based on the data acquired from GWAS, targeted studies were performed using a candidate gene approach, in which particular polymorphisms of IL28B are chosen to be tested for the association with treatment efficacy. As the most widely explored genetic variant in IL28B, Rs12979860 CC genotype was associated with successful treatment outcomes in most studies. In contrast, the predictive effect of Rs8099917 is still controversial (Table 1). However, these studies confirmed a significant association between IL28B genetic variation and response to treatment. Further studies should be done to explore the mechanism underlying this close association.

### Table 1: Genetic association studies of IL28B and treatment outcomes.

<table>
<thead>
<tr>
<th>Single nucleotide polymorphisms</th>
<th>Subjects</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs12979860</td>
<td>268 Caucasian (HCV genotype 2/3)</td>
<td>CC genotype is associated with SVR, especially in patients without RVR</td>
<td>[61]</td>
</tr>
<tr>
<td>Rs12979860 and Rs8099917</td>
<td>817 Japanese (HCV genotype 1b)</td>
<td>Rs12979860 CC is associated with SVR and NVR</td>
<td>[62]</td>
</tr>
<tr>
<td>Rs12979860, Rs8099917 and Rs12980275</td>
<td>645 Germans (HCV genotype 1/2/3)</td>
<td>Rs12979860 CC is associated with SVR in HCV genotype 2/3-infected patients</td>
<td>[63]</td>
</tr>
<tr>
<td>Rs12979860</td>
<td>284 Spanish (HCV genotype 1 and non-1), 69 Spanish (spontaneous clearance), 378 healthy control</td>
<td>Rs12979860 CC is associated with SVR and spontaneous resolution</td>
<td>[64]</td>
</tr>
<tr>
<td>Rs12979860</td>
<td>1171 Caucasians, 300 African Americans, and 116 Hispanics (HCV genotype 1)</td>
<td>Rs12979860 CC is associated with SVR and RVR, especially in patients without RVR</td>
<td>[65]</td>
</tr>
<tr>
<td>Rs12979860 and Rs8099917</td>
<td>682 white patients (HCV genotypes 1/2/3/4)</td>
<td>Rs12979860 CC is associated with SVR in HCV genotype 1/4-infected patients</td>
<td>[66]</td>
</tr>
<tr>
<td>Rs12979860 and Rs8099917</td>
<td>109 Caucasian (HCV genotype 1/2/3/4)</td>
<td>Rs12979980 CC and Rs8099917 TT are respectively associated with SVR</td>
<td>[67]</td>
</tr>
<tr>
<td>Rs12979860</td>
<td>178 Caucasians (HCV genotype 1/2/3), 53 African American (HCV genotype 1)</td>
<td>Rs12979860 CC is associated with SVR only in Caucasians</td>
<td>[68]</td>
</tr>
<tr>
<td>Rs12979860 and Rs8099917</td>
<td>69 Caucasians (HCV genotype 3)</td>
<td>Rs12979860 and Rs8099917 are not associated with SVR</td>
<td>[69]</td>
</tr>
<tr>
<td>Rs8099917</td>
<td>160 Spanish (HCV/HIV-1 co-infected, HCV genotype 1/3/4)</td>
<td>Rs8099917 G allele is associated with treatment failure</td>
<td>[70]</td>
</tr>
<tr>
<td>Rs8099917</td>
<td>67 Japanese (HCV genotype 1/2)</td>
<td>Rs8099917 TT is associated with SVR</td>
<td>[71]</td>
</tr>
<tr>
<td>Rs8099917</td>
<td>719 Japanese (HCV genotype 2a/2b)</td>
<td>with PEG-IFN/RBV cure, Rs8099917 TT is associated with SVR in 2a genotype; with IFN cure only, Rs8099917 TT associated with SVR in 2a genotype</td>
<td>[72]</td>
</tr>
</tbody>
</table>


**Correlation between ISGs expression and IL28B SNPs**

Both the pretreatment baseline hepatic ISG expressions and SNPs of IL28B predict treatment outcomes to IFN-based therapy in patients chronically infected with HCV. Because IL28B encodes a type III IFN (IFN-λ3) that shares the same downstream Jak/STAT signaling pathway with type I IFNs, increased pretreatment hepatic ISG expression may have a close link with IL28B SNP [67]. Some previous studies provided convincing data for this possibility. For instance, Honda et al. [68] reported that hepatic ISGs were up-regulated in Japanese chronic Hepatitis C patients with the unfavorable Rs8099917 genotype (TG or GG). In an American population, Urban et al. [69] also validated the correlation between high pretreatment ISGs and minor Rs12979860 genotype. Because both Rs8099917 and Rs12979860 lie in upstream section of IL28B gene, it is likely that they may influence IL28B transcription and synthesis. Tanaka et al. [63] and Suppiah et al. [64] found positive correlation from two independent experiments studies in 20 HCV patients and 49 healthy volunteers, respectively. They measured IL28B mRNA in PBMCs of individuals, and found lower IL28B mRNA level in individuals with minor G allele of Rs8099917. However, both Honda et al. [68] and Urban et al. [69] failed to find any association between Rs12979860 genotype and levels of liver IL28B mRNA expression. Therefore, the data on the association between IL28B genotype and gene expression are conflicting. One possibility is that IL28B expression may be tissue-specific, but most previous studies didn’t test IL28B level in the hepatic tissues and PBMCs simultaneously. Another factor contributing to this contradiction may be that detection of IL28B mRNA levels cannot reflect mature IL28B protein. However, precise mechanism of the phenomenon remains to be elucidated. On another hand,
Dill et al. [70] measured variation of IL28B (Rs8099917 and Rs12979860) and quantified the ISGs expression in liver biopsies from 109 Caucasian patients with Chronic Hepatitis C. They concluded that IL28B genotype and ISGs level are independent predictors of SVR, indicating other potential pathways to explain the complex host immune response vs HCV.

\textbf{In vitro}, IFN-α induced early increases and rapid decrease in levels of known ISGs, whereas IFN-λ- induced ISGs peaked steadily [71]. Sirén et al. [72] found that IFN-α up-regulated TLR-dependent IL28 expression in macrophages by enhancing the expression of TLR3, TLR4, and TLR7. Ank et al. [73] generated type I IFN receptor-deficient mice, and observed a positive feedback on type III IFN expression mediated by type I IFN receptor system in the mice. Consequently, it is reasonable to hypothesize that type I IFN and type III IFN may interact with each other through a TLR-dependent pathway, which determined the complexity in ISGs expressions and genetic variations of IL28B. Although little is known about the mechanism of the association between IL28B polymorphism and ISGs, these observations had great biological significance, opening up a new field to search for the “predicting puzzle”.

Finally, we are left with the question of which one is the better predictor of treatment outcomes, the ISG expression pattern or the IL28B genotype? A study from Dr. McGilvray’s team in Toronto [74] tested the IL28B SNP rs12979860 and evaluated MxA in hepatocytes and macrophages of HCV patients with known IFN treatment outcomes. Data from this study suggested that absence of MxA staining in macrophages predicted treatment failure with higher accuracy than IL28B SNP Rs12979860. This observation was replicated by Micheal T. Dill et al. [75] from 109 patients chronically infected with HCV although L28B CC genotype appears to be associated with low ISGs, there is no causal link between them, and they are independent predictors for treatment response. However, the detailed mechanism involved in the interaction between them remain to be elucidated.

**Other host variables associated with SVR**

In addition to all the host factors discussed above, age, race, pathological situation, metabolism and biochemical and immune status have been reported to be associated with SVR. Younger age [44,76] and Asian ethnicity [77-78] independently predict better response to PEG-IFN/RBV, while advanced stage of fibrosis [79], presence and severity of liver steatosis [80], high body mass index (BMI) [81] and high homeostasis model assessment of insulin resistance (HOMA-IR) [82,83] predict poor response. Furthermore, biochemical factors, such as low ratio of serum γ-glutamyl transferase/alanine transaminase (γ-GT/ALT) [84] or low γ-GT [52], high levels of anti-NS4a and anti-NS5a [85], etoxin and macrophage inflammatory protein (MIP)-1b [86], apolipoprotein B-100 (apoB-100) [87], interleukin (IL)-12 and IL-18 [88] have been reported as positive predictors for favorable response.

**Prediction of Treatment Response to DAAs**

Both high replication rate of HCV and lack of proofreading activity of HCV-RNA-dependent RNA polymerase (RdRp, NS5B) contribute to the large population of quasispecies of HCV that include variants with suppressed susceptibility to DAAs. Therefore, a number of host or viral factors that were previously shown to be associated with outcomes of PEG-IFN/RBV treatment are further investigated to estimate their roles in predicting response to DAAs-PEG-IFN/RBV triple therapy. Unfortunately, most of them failed to predict treatment response to DAAs. For instance, protease inhibitors led to a rapidly undetectable HCV RNA in the majority of patients [26,27,89], making the on-treatment viral kinetics less useful to tailor the treatment. Although some specific parameters, such as HCV genotype 1 [reviewed in [90]], LDLC [91], IL28B [92] and pre-existing variants [93,94], are proposed as potential tools for pretreatment decision, it is impossible, in our opinion, that any of these parameters alone will be good enough to predict treatment response and for decision-making in the clinic. Therefore, optimized combination of several host, viral and pharmacologic factors need to be investigated and further validated in the clinic.

**Conclusion**

Numerous viral and host variables are reported to be associated with HCV treatment response, providing us with not only potential predictors of treatment outcome, but also evidence for possible mechanism of IFN resistance. Recent findings about ISGs and HCV treatment outcome may change our traditional way of understanding chronic HCV infection, and will help us further understand the effect of ISG expression on HCV replication/production and the paradoxical effects of type I IFN in viral persistence. Further studies need to be performed on an overall pattern taking both viral and host factors into account, which may be the more reliable method to predict HCV treatment response rather than one or two independent predictor(s).

**References**


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