

## Review article

# *IL28B* genotyping and treatment of HCV infected subjects

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**Background:** Chronic hepatitis C is a common cause of advanced liver disease and appropriate treatment has been complex and a challenge. Reaction of individual genotypes to classical pegylated interferon-ribavirin therapy differs and no success has been achieved in some even after repeated therapy cycles. New types of so called directly acting antivirals (DAAs) are hopeful, as shown in many recent clinical studies, and triple therapy regimens involving DAA are becoming the new standard of care.

**Objective:** To summarize knowledge about the relationship between HCV therapeutic regimens and the genetic background of the host represented by interleukin 28B (*IL28B*) gene polymorphisms. In the first part, the host basic mechanisms in specific and innate immunity are introduced. The *IL28B* genotype and its role in the course of HCV treatment are described in the second part.

**Methods:** We searched and summarized publications on HCV therapeutic regimens and host *IL28B* polymorphisms.

**Results:** Compared to classical regimens, the association between *IL28B* polymorphism and treatment outcome of HCV infected patients is weaker in triple therapy using first generation DAAs boceprevir and telaprevir.

**Conclusions:** The association between *IL28B* polymorphism and treatment outcome is lessened with availability of new therapeutic regimens. Nevertheless, *IL28B* genotyping may still be useful for individualization of treatment strategies.

**Keywords:** Chronic hepatitis C, direct acting antiviral, genotyping, hepatitis C virus, *IL28B* polymorphism, sustained virological response

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Chronic infection caused by the hepatitis C virus (HCV) is a global health problem. According to the World Health Organization in 2012, approximately 130–170 million individuals worldwide are infected with HCV. This represents about 2%–3% of the global population. Subjects are at high risk of progressive liver fibrosis, liver failure, and hepatocellular carcinoma. Mortality from hepatitis C infection is estimated to be more than 350,000 annually, resulting from chronic hepatitis, liver failure, or cancer [1, 2].

Chronic HCV infection (CHC) is one of the most common causes of advanced liver disease in the western world and appropriate treatment has been a challenge. The reaction of some genotypes to classical pegylated interferon (pegIFN) and ribavirin (RBV) therapy is different and no success has been achieved even after repeated therapy cycles in some patients. The prospects for new types of so-called “directly

acting antivirals” (DAAs) appear hopeful, as shown in many recent clinical studies. Triple therapy regimens involving DAA are becoming the new standard of care. There has also been a need to identify accurate predictors of treatment outcome to facilitate effective therapy decision-making. In wide-scale genomic studies, several single nucleotide polymorphisms (SNPs) were found including the interleukin 28B gene (*IL28B* or as newly designated, *IFNL3*, but referred to as *IL28B* in this review), which codes type III interferon influencing the host immune response. Different genotypes can apparently cause distinct reactions in spontaneous virus elimination, which may contribute to successful therapy. Probably the most important SNP of *IL28B*, –3176C/T (rs12979860), is located on the gene promoter and its determination seems to be useful for diagnosis. However, a practical application of knowing the *IL28B* genotype is changing with the introduction of new therapeutic regimens, and here we attempt to review this issue.

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### HCV genome variability

Single-stranded RNA of the HCV genome contains approximately 10000 nucleotides in a single long open reading frame. The 5'-untranslated regions (5'-UTR) encode structural proteins forming the virus nucleocapsid and envelope, while the 3'-translated regions determine nonstructural proteins. The main polyprotein precursor is formed by translation, and contains 3000 amino acid residues. N-terminal proteins (core protein and envelope proteins E1, E2/NS1) possess structural function and C-terminal proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) have important roles during virus replication. The NS3 protein forms the virus protease and helicase, while the NS5A phosphoprotein is crucial in the process of virus replication. The NS5B protein has RNA polymerase activity. HCV is known for its high genetic variability. Nucleotide sequence diversity is different for distinct genome regions and can lead to antigenic and biological changes. 5'-UTR and N-terminal sequences for core protein belong to conservative regions. By contrast, the sequences coding E1, E2, NS2, NS4, and NS5 proteins are highly variable. The antigenic sites of great importance, with different amino acid sequences, are located on the hypervariable segment areas E1 and E2/NS1 [3].

During the course of HCV infection, the virus attempts to evade the immune response and therefore creates unstable envelope mutants. Stable forms of the virus, called HCV genotypes, were probably formed in a similar way, but there has been a long-term process of their stabilization. Several methods for measurement and classification of HCV genotypes have been developed. At the end of the 20th century, the classification adopted by Simmonds et al. was introduced. It gave rise to an official global nomenclature [4]. In 2005, a new version of the classification, based on phylogenetic analysis of the genome regions and detection of complete genome sequences of HCV, was accepted [5]. During viral replication, frequent mutation occurs, especially in the hypervariable region E2/NS1 that gives rise to a large number of variable and unstable mutants. These genetically closely related mutants are called quasispecies. Their hypervariability, especially in envelope proteins, increases the ability of HCV to avoid immune responses, influences an adaptation of the virus during persistence in the host, and can cause drug resistance.

### Specific immune response and HCV

Specific cell immunity is a crucial factor in the course of HCV infection. It comprises the activity of both CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes specific to HCV and their cytokine secretion [6]. The early, strong, polyclonal, and multispecific cellular response can lead to elimination of HCV infection. On the other hand, a late, short, and poor cellular response directed against only a few viral epitopes, allows infection to convert into the chronic form [7].

CD4<sup>+</sup> helper T cells can recognize short exogenous antigenic peptides bounded by class II HLA molecules on the surface of antigen-presenting cells, including dendritic cells, macrophages, and B lymphocytes [8]. This immune response is directed predominantly against nonstructural proteins and highly conserved epitopes of HCV. Then CD4<sup>+</sup> T lymphocytes regulate the activity of antigen-specific B cells and CD8<sup>+</sup> T lymphocytes by production of lymphokines [9]. Th1 cytokines, such as interleukin 2, tumor necrosis factor beta, and interferon gamma, can stimulate cytotoxic CD8<sup>+</sup> T lymphocytes, whilst Th2 cytokines control antibody production by B lymphocytes. It is supposed that the intensive production of Th1 cytokines is usually associated with spontaneous elimination of HCV infection [6].

CD8<sup>+</sup> cytotoxic T lymphocytes interact with viral antigenic peptides localized on the surface of infected cells and their actions usually lead to the apoptosis of infected cells. The effects of CD8<sup>+</sup> T cells may not only be directed against infected cells, but may also affect uninfected cells in the close proximity using the secreted mediators promoting apoptosis [9]. The cell resistance to these mediators may lead to hepatocellular carcinoma [10]. If this type of cellular response is sufficiently intense, it may eliminate the infected cells. However, effects of CD8<sup>+</sup> T cells are not usually adequate, resulting in persistence of the virus and chronic damage to liver cells. Despite constant polyclonal and multispecific immune response of cytotoxic T lymphocytes, HCV infection persists and chronic liver disease may develop in most infected individuals [9]. Specific CD4<sup>+</sup> T cells may remain for years even after acute infection has subsided, while the response of CD8<sup>+</sup> T cells disappears over time [6].

### Innate immune response and *IL28B* polymorphism

Innate immunity is the first defence mechanism

directed against HCV infection. This process is initiated by endogenous interferon secretion and the action of natural killer cells (NK cells) [6]. During the acute phase of the infection, the innate immune response produces type I interferons, interferon- $\alpha$  (IFN- $\alpha$ ) and interferon- $\beta$  (IFN- $\beta$ ). Type I interferons have some antiviral and immunomodulatory effects including the activation of cellular protein kinase [11]. This process can cause inhibition of protein synthesis in infected cells, and the inhibition of viral replication by activating of 2'-5'-oligoadenylate synthetase and specific proteins. Other actions of type I interferons include the expression of major histocompatibility complex genes in antigen-presenting and infected cells, stimulation of natural killer cells, dendritic cells, and CD8<sup>+</sup> lymphocytes, and support the effect of molecules involved in the apoptosis of infected cells. HCV is interferon-sensitive in vitro. However, a variety of strategies for suppressing the antiviral effect of interferon in vivo has been developed by the HCV. The virus can reduce intracellular production of IFN- $\alpha$  through some of its nonstructural proteins (NS4A and NS5A), which affect the production of interferon regulatory factors. Furthermore, viral proteins NS5A and E2 may inhibit the activity of specific enzymes that are effective against viral replication [6]. Thus, in the early stages of infection, HCV stimulates the production of interferon, but it seems to be relatively resistant to its effect [12].

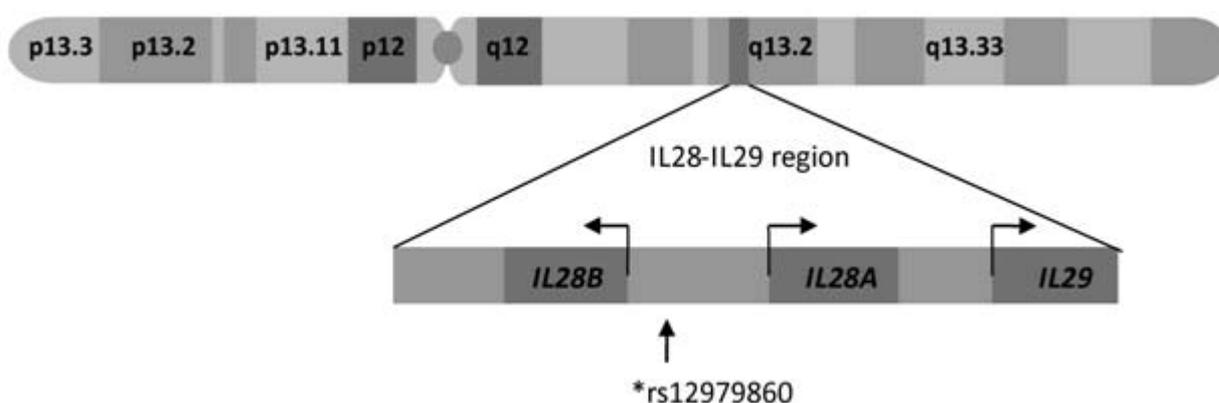
Together with IFN- $\alpha$  and IFN- $\beta$ , type III interferons are probably also involved in the immune response against HCV represented by IFN- $\lambda$ 1, IFN- $\lambda$ 2, and IFN- $\lambda$ 3, which are presumed to have the same effects as the type I interferons. These lambda

interferons are induced by viral infection showing antiviral activity in vitro [13]. The inhibitory effect of IFN- $\lambda$ 1 has been documented. It supports an induction of the interferon-stimulated gene expression, and the antiviral effects of IFN- $\alpha$  [14]. IFN- $\lambda$ 3 probably acts in the same way as IFN- $\alpha$  [15].

The gene for interleukin 28B (*IL28B*), encoding IFN- $\lambda$ 3, is located on the long arm of chromosome 19 in humans, together with the genes for IFN- $\lambda$ 1 (*IL29*, also more recently known as *IFNL1*) and IFN- $\lambda$ 2 (*IL28A*, also more recently known as *IFNL2*). The SNP rs1297860 (-3176C/T) was identified in *IL28B* [16]. It is assumed that this polymorphism determines a function of IFN- $\lambda$ 3, which subsequently affects the elimination of HCV infection. C and T alleles are known forms of rs1297860. In recent studies, the CC genotype was found to be closely associated with elimination of acute HCV infection. It is probably the most important genetic factor associated with the spontaneous clearance of HCV infection [15]. Several SNPs on *IL28B*, or those in close proximity, were identified. TT rs8099917, TT rs8105790, AA rs12980275, and CC rs10853728 homozygotes are considered to be the favorable genotypes [17].

The precise localization of *IL28B* is illustrated in **Figure 1**.

The innate immunity also involves NK cells, the activity of which can be affected by the action of HCV proteins. These proteins are able to bind to the surface of NK cells and inhibit their activity, cytotoxic effect, and cytokine secretion. Signals from the innate immune response act on the maturation of dendritic cells linking innate and specific immunities [6].



**Figure 1.** The gene for IL28B and SNP (rs12979860) localizations on the long arm of chromosome 19 (adapted with permission from Macmillan Publishers Ltd: Nature Reviews Gastroenterology and Hepatology ref. [18], copyright 2012).

### ***IL28B* polymorphisms and standard HCV therapy**

The success rate of CHC treatment is influenced by many factors originating from virus and host. Although combined therapy with pegIFN and RBV was found relatively effective, there are no signs of suppression of infection in a number of patients. Variations in the effectiveness of treatment were observed in different geographic areas and racial populations. This and other evidence led to the assumption of the existence of host genetic factors that influence the effectiveness of antiviral therapy [16]. Since 2009, several SNPs in *IL28B* have been reported. These SNPs are strongly associated with achieving sustained virological response (SVR) in patients [16, 19]. The SNPs identified in the genome-wide association studies include rs12979860 and rs8099917 [20].

The importance of SNPs in *IL28B* was first strongly noted in a study by Ge et al. [16]. It highlighted the effect of the SNP rs12979860 in combined viral therapy. This SNP localized at position -3176 occurs in two allele forms. The authors have observed that patients with the CC genotype had more than twice the success rate of combined therapy than those with the TT genotype. This fact was evident in individuals of both European and African-American or Hispanic descent. Furthermore, there were differences in the frequency of alleles and genotypes between these populations, which provided an explanation for the higher rate of SVR in European patients. The frequency of the good response allele is different in people with different ethnic backgrounds. It is more common in people from Asian and Caucasian ancestry, and less common in those with Hispanic and African ancestry. When comparing the impact of the favorable rs12979860 CC genotype on the effect of therapy with factors derived from the host (age, sex, degree of fibrosis) and other factors (initial HCV levels), very important findings were noted. The predictive value of the treatment effectiveness for genotypes of *IL28B* was higher than that for the other factors, and for the genotype 1 by comparison with genotypes 2 and 3 [16]. The relationship between the CC genotype of rs12979860 and SVR seems to be the most sensitive and the most important factor derived from the host, indicating the success of standard therapy.

In this study, other two genetic polymorphisms in *IL28B*, rs28416813 and rs8103142, were also described. These SNPs are related to rs12979860 [16]. It is assumed, that amino acid exchange caused by rs8103142 (lysine to arginine, Lys70Arg) could affect the function of IFN- $\lambda$ 3 [15]. The precise mechanism by which the variations in rs12979860 can affect therapy for HCV infection has not been fully elucidated. By contrast, the discovery that *IL28* variations are associated with the response to therapy with pegIFN- $\alpha$  and RBV (pegIFN-RVB), especially for genotype 1, was confirmed by four independent groups [15, 19, 21, 22]. All identified SNPs, that tag a haplotype block on chromosome 19 spanning *IL28B*, strongly predict the outcomes for treatment of chronic genotype 1 HCV infection. Individuals carrying the so-called "good alleles" have an approximately two-fold higher rate of SVR [20].

During the course of other research, several SNPs of *IL28B* were determined as acting on the effectiveness of HCV treatment (for example, the positive effects of TT genotype of rs8099917 were observed [19]). Nevertheless, the rs12979860 polymorphism is considered as the most important, although recent studies have questioned its role in the treatment and especially in the prediction of spontaneous elimination of HCV [23]. The meta-analysis of Shi et al. [24] aimed to derive a more precise estimation of the effects of *IL28B* SNP loci (rs12979860 and rs8099917) on SVR in patients with CHC previously naive to treatment, now receiving pegIFN-RVB. A total of 36 studies involving 10912 cases with CHC met the inclusion criteria. In genotype 1/4 patients, rs12979860 CC was associated with high SVR in CHC patients, but had no effect in genotype 2/3. In people of Caucasian and Asian descent, rs8099917 TT was associated with high SVR in both genotypes 1/4 and 2/3.

*IL28B* genotyping of patients with CHC is thus important in predicting SVR and effectiveness of therapy. This may affect the course of treatment in individual patients. Furthermore, the determination of genotypes is also useful in treatment of patients with acute HCV infection. However, it is important to note, that the predictive value of *IL28* genotypes in relation to the effectiveness of treatment is not absolute. Last, but not least, genotyping has importance in the implementation and evaluation of clinical trials with new antiviral drugs.

### **IL28B polymorphisms and new therapeutic regimens**

As discussed above, *IL28B* genotypes has been shown to be the strongest pretreatment predictor of SVR in patients with genotype 1 of CHC treated with pegIFN-RVB. However, the treatment paradigm for CHC is changing with the introduction of direct acting antivirals. *IL28B* genotypes remain relevant to treatment regimens with either boceprevir or telaprevir, although the strength of association with the virological response is less. The association between *IL28B* genotype and outcomes of treatment regimens that involve pegIFN plus combination DAA therapy, or IFN-free regimens, is currently being evaluated [25].

DAA therapy is based on the direct inhibition of HCV viral survival, targeting the HCV NS3 protease, the NS5B polymerase, and NS5A phosphoprotein, as well as host cell proteins involved in HCV replication [26]. However, monotherapy is associated with the rapid selection of resistant HCV variants and virological breakthrough. Therefore, the clinical efficiency of DAA will be dependent on its combination with pegIFN-RVB.

### **IL28B polymorphism and boceprevir**

The relationship of *IL28B* polymorphism and DAA therapy regimens is not yet clear. In 2011, the first generation of HCV protease inhibitors, boceprevir and telaprevir, were introduced in combination with pegIFN-RVB for genotype 1 HCV infection [27]. In two large retrospective studies with boceprevir, SPRINT-2 in patients naive to treatment [28] and RESPOND-2 in patients with experience of treatment [29], the main role for *IL28* genotyping was in predicting those patients who could receive a shorter duration of therapy. A total of 653 patients were recalled and consented to genetic testing in the SPRINT-2 study. Boceprevir lessened the association between the *IL28B* rs12979860 genotype and treatment outcome relative to the control arm (pegIFN and RBV therapy). However, the *IL28B* genotype remained an independent predictor of SVR. Although boceprevir was not associated with an increased rate of SVR in CC genotype patients, 89% of those had undetectable HCV RNA at week 8, and were eligible for short-duration therapy. Boceprevir treatment was associated with a greater SVR in patients with non-CC genotypes. The RESPOND-2 study in patients with treatment experience revealed the main clinical utility of *IL28B* genotyping was

prediction of the likelihood the short-duration therapy would be effective.

### **IL28B polymorphism and telaprevir**

*IL28B* genotyping in telaprevir treatment of patients naive to other treatment was evaluated retrospectively in the ADVANCE study population [30]. Rates of SVR in the telaprevir group were higher than those in the control group (pegIFN and RBV), both in CC and non-CC genotype patients. The presence of the *IL28B* CC genotype identified patients who were eligible for a shortened duration of therapy, i.e. those, who achieved an extended rapid virological response (RVR) as defined by undetectable HCV RNA at weeks 4 and 12. The major benefit was in patients with the poor response *IL28B* genotypes, where SVR rates were more than double that of the control group. The REALIZE study was performed using patients with treatment experience to evaluate the effectiveness of triple therapy based on telaprevir, pegIFN, and RBV [31]. SVR rates tended to be higher in CC patients, but the difference was not significant. Prior treatment response was the strongest predictor of treatment outcome and no difference in SVR rate was observed according to *IL28B* genotype when patients were considered on the basis of treatment history. In a subsequent study [32], the impact of *IL28* genotype on SVR in telaprevir-treated HCV genotype 1 infected patients who had previously failed treatment with standard therapy, including null responders. SVR rates were higher in patients who received telaprevir versus placebo for all *IL28* genotypes and were similarly irrespective of *IL28B* genotype for prior relapsers and prior partial responders. For null responders, SVR rates were slightly higher for the *IL28B* CC genotype. In multivariable modelling, *IL28B* genotype did not significantly affect SVR.

Thus, the results of the retrospective analyses indicate a limited role for *IL28B* genotyping [27]. However, according to other authors [25, 26], *IL28B* genotyping continues to be useful for pretreatment counselling, with a good response *IL28B* genotype identifying the likelihood of an individual being eligible for shortening therapy. Further studies to elucidate the role of *IL28B* genotyping in the era of interferon-free regimens, with other new DAAs, are taking place [33, 34].

### **Cost-effectiveness studies and DAAs**

A pair of studies have been performed with

boceprevir and telaprevir combinations. The cost-effectiveness of DAAs in combination with pegIFN and RBV in both patients with treatment experience and previously untreated CHC genotype 1 patients was evaluated, by comparison with individuals treated by classical pegIFN and RBV therapy [35, 36]. In a study with experienced subjects, higher costs and improved outcomes were associated with telaprevir combined with pegIFN-RBV related to pegIFN-RBV alone for all patients. The combination with telaprevir was cost-effective for each subgroup population (i.e. previous relapsers, partial responders, and null responders) with a high SVR advantage in relapsers. This therapy remained cost-effective regardless of *IL28B* genotype. Compared with boceprevir, telaprevir was always cost-saving, but only more effective in relapsers in combination with pegIFN and RBV [35]. Similar conclusions were observed in the study with patients previously naive to treatment: telaprevir-based therapy was cost-effective regardless of *IL28B* genotype or fibrosis stages for genotype 1 HCV patients [36].

### Second-generation DAAs

Numerous other DAAs are in clinical development, and phase 2 and 3 trials are evaluating interferon-free combination DAA therapy, both with genotype-specific, and with pan-genotypic antivirals. Interferon-free SVRs have been achieved with combinations including of asunaprevir and daclatasvir, sofosbuvir and RVB, sofosbuvir and daclatasvir, faldaprevir and BI207127 [37]. Second-generation DAAs were also tested in combination with pegIFN-RBV. In many studies, significantly increased SVR rates were achieved. Simeprevir (TMC435) is an oral once-daily NS3/4A macrocyclic protease inhibitor with the strong antiviral activity. Combined with pegIFN-RBV, it appears to be a potent and safe agent with which to treat genotype 1 HCV [38], as well as genotype 2, 4, 5, and 6 HCV infections [39]. The effect of *IL28B* polymorphisms in IFN-free regimen therapy and their potential role for clearing HCV still needs to be evaluated.

### *IFNL4* and *IL28B* polymorphism

As mentioned above, precise molecular mechanisms, by which *IL28B* influences treatment outcome, are still not yet established. There are several possible explanations for the upregulation of interferon-stimulated genes in patients with the

unfavorable genotype [40]. Recently, Prokunina-Olsson et al. have identified a novel human protein-coding gene, *IFNL4*, which is related to, but distinct from known IFNs. They have described a dinucleotide frame-shift variant upstream of *IL28B* in ss469415590 (TT or  $\Delta$ G) that is in strong linkage disequilibrium with *IL28B* rs12979860 [41]. Genetic analysis of this region in patients from several independent clinical cohorts of hepatitis C studies revealed an association of the ss469415590 [ $\Delta$ G] allele with reduced response rates to IFN treatment and reduced association with spontaneous HCV clearance. Transient expression of p179, the largest protein gene product, in hepatoma cells carrying an HCV replicon inhibited viral replication, indicating an antiviral role of p179. One of the most important findings of the study is that identified gene products may have a functional role in the innate immune response to RNA viruses [41]. Bilbert et al. reported that induction of *IL28B* expression is associated with novel TT/ $\Delta$ G polymorphism ss469415590, which is a better predictor of HCV clearance than rs12979860 [42]. Aka et al. studied HIV-positive black women, and found that HCV was cleared in 32.6% of the women with the *IFNL4* genotype TT/TT, by comparison with TT/ $\Delta$ G and G/ $\Delta$ G genotypes (11.3% and 11.9%, respectively) [43].

A recent study by Stattermayer et al. evaluated the role of *IFNL4* polymorphism on response to classical pegIFN-RBV therapy in patients with CHC by comparison with the two *IL28B* SNPs, rs12979860 and rs8099917 [44]. In that study Stattermayer et al. found 754 pegIFN-RBV treated participants of mostly Caucasian ancestry had 3 distinct SNPs. The *IFNL4* ss469415590 polymorphism was associated with SVR in patients with genotypes 1 and 4, but not genotype 3, and was only strongly correlated with rs12979860. The predictive value was almost identical to that of the SNP rs12979860 genotype. By contrast, the correlation of SNP rs8099917, which is commonly found in people of Asian descent, with *IFNL4* ss469415590 was only moderate, maybe because of its distance from ss469415590 (it lies outside *IFNL4*). Stattermayer et al. deduced that because of the strong correlation of *IFNL4* ss469415590 polymorphism with *IL28B* rs12979860, it provides no additional information for treatment prediction, at least in patients of Caucasian ancestry. However, *IFNL4* improves prediction of response to interferon-based therapies, if SNP rs8099917 is used.

## Conclusion

The association between *IL28B* (*IFNL3*) polymorphism and treatment outcome for patients infected with HCV is limited in triple therapy using first-generation DAAs boceprevir and telaprevir. Nevertheless, *IL28B* genotyping may remain useful for individualization of treatment strategy, and identification of patients, who can be successfully treated with shorter, simpler, and less expensive regimens. It provides helpful information for making clinical decisions, particularly with genotypes 1 and 4. Laboratory testing, using, for example, real-time PCR-based melting curve analysis, is relatively simple, rapid, and inexpensive. The question remains, whether *IL28B* polymorphisms will be replaced by better genetic predictors that are more suitable in clinical practice in the light of new knowledge about genetic regulation of *IL28B* and other interferons associated with HCV clearance.

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