

Genomic determinants of hepatitis C virus antiviral therapy outcomes: toward individualized treatment

Mauricio Venegas,* Javier Brahm,* Rodrigo A. Villanueva**

* Sección de Gastroenterología, Departamento de Medicina, Hospital Clínico Universidad de Chile. Chile.

** Laboratorio de Virus Hepatitis, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello. Chile.

ABSTRACT

Hepatitis C virus (HCV) is an important global health problem with an estimated prevalence of more than 170 million infected individuals worldwide. Currently, the standard antiviral therapy, based on pegylated interferon alpha and ribavirin, can achieve a virological response in only nearly 50% of the patients infected with HCV genotype 1, the most widely distributed globally. During the last years, relevant data from genome-wide association studies (GWAS) about the impact and contribution of the patient genomics on viral infection outcomes has suggested the possibility that an individualized antiviral therapy can be considered. In this review, we analyze the existing information on single nucleotide polymorphisms (SNPs) of several host genes and viral factors that influence, as a whole, the outcome of the standard antiviral therapy, and that might be used to predict an individualized antiviral response. We also discuss the clinical data within the most recent context of the triple antiviral therapy.

Key words. IL28B polymorphisms. Direct-acting antiviral. Genetic variant. PegInterferon/Ribavirin treatment (PegIFNalpha/RBV).

INTRODUCTION

Hepatitis C virus (HCV) is an important global health problem with an estimated prevalence of infection of 2.5%, which is equivalent to more than 170 million infected individuals worldwide. More than 70% of the acute infections will progress to chronicity; an estimated 27% of cirrhosis and 25% of hepatocellular carcinoma (HCC) occur in chronically HCV-infected patients.¹ HCV is mainly transmitted parenterally, e.g., between individuals subjected to transfusions or involved in intravenous drug use. On the other hand, both sexual and vertical transmissions of HCV are considered to be inefficient or infrequent.

HCV has spread worldwide with complex genetic variability.² The different genetic isolates have been classified into genotypes and subtypes. There are six

main genotypes (1–6) that each differ in their nucleotide sequence by approximately 30–35%. In addition, within the same genotype, it is possible to identify several subtypes (a, b, c, etc.), which differ in their nucleotide sequence by approximately 20–25%. Also, significant heterogeneity of different genomes in replication and circulation can coexist in an infected individual, a phenomenon called *quasispecies*, which is probably due to host immune pressure.³

The main HCV genotypes exhibit a marked difference in geographic distribution and response to antiviral therapy. Genotype 1 is widely present in North America and Europe, while genotype 2 is most frequent in Japan and China. Genotype 4 is common in Egypt, whereas genotype 5 is found almost exclusively in Southern Africa. Genotype 6 is generally limited to Southeast Asia.⁴ In Latin America, genotype 1 is the most frequent, followed by genotypes 3 and 2.⁵ The existing data indicates that HCV genotypes do not significantly influence disease progression or severity, while they are involved in determining the response to antiviral therapy. Sustained virological response (SVR) is achieved in less than 50% of patients infected with HCV genotype 1, and in 80% of those infected with genotype 2 or 3.⁶ On the other hand, genotype 3 is generally more associated with liver steatosis.⁷

Correspondence and reprint request: Rodrigo A. Villanueva, Ph.D.
Laboratorio de Virus Hepatitis, Departamento de Ciencias Biológicas,
Facultad de Ciencias Biológicas, Universidad Andrés Bello.
Av. República, Núm. 217, 3er. piso, Santiago 8370146, Chile
Tel.: (562) 770-3268
E-mail: rodrigo.villanueva@unab.cl

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HEPATITIS C VIRUS AND INFECTION

HCV is an enveloped virus that contains a single-stranded RNA genome of positive polarity. It has been classified within the genus *Hepacivirus* in the family *Flaviviridae*; its uncapped genome is proximately 9600 nucleotides-long. HCV is a non-cytopathic, hepatotropic virus that can cause acute and chronic hepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma, as previously mentioned.^{8,9}

The viral RNA genome encodes a unique, long open-reading frame for a viral polyprotein, which is flanked at both the 5' and 3' ends by relatively short, highly structured, regulatory regions, termed nontranslated regions (NTR). Both 5' and 3' NTRs bear phylogenetically conserved RNA structures essential for polyprotein translation and genome replication.¹⁰ The viral translation generates a unique viral polyprotein, which is proteolytically processed into 10 mature viral proteins, either structural (E1, E2, Core) or nonstructural proteins (NS) (p7, NS2, NS3, NS4a, NS4b, NS5a, NS5b).⁹ Whereas structural proteins are destined to encapsidate viral genomic RNA for the viral progeny, mature NS proteins assemble together to form a functional ribonucleo-multiprotein complex committed to synthesizing multiple copies of the viral RNA genome and to keeping the HCV cycle ongoing within the infected cell.¹¹ Among the viral NS proteins, two critical enzymatic activities for the viral cycle, NS3, a multifunctional protease and NTPase/helicase protein, and NS5b, a viral RNA-dependent RNA polymerase, have been targets of intense research for the design and assay of new anti-viral compounds.¹² In addition, several other targets are currently under testing for chronic HCV infection; inhibitors against HCV NS5a, cyclophilin, statins, and miR-122 are all under preclinical, and clinical testing.

Infections by HCV are highly dynamic, and it has been estimated that the half-life of the viral particle is only few hours, with a rapid turnover of about 10^{10} - 10^{12} virions/day.¹³ This enhanced replicative capacity of the virus along with the lack of proofreading of NS5b viral polymerase during genome synthesis are the basis for the high genetic variability exhibited by HCV infections.

CURRENT ANTIVIRAL THERAPY

Until now, the standard therapy for patients chronically infected with HCV, as per the NIH consensus statement, has consisted of pegylated interferon alpha (PegIFN α) in combination with the

nucleoside analog ribavirin (PegIFN α /RBV).¹⁴ However, by these days, a new standard of "triple therapy" should be considered, as discussed below.

Interferons (IFNs) are cytokines and are crucial mediators of the innate antiviral immune response because they display antiviral, antiproliferative, and immunomodulatory activities.¹⁵ The effect exerted by RBV on HCV viral load *in vivo* is minimal.^{16,17} However, ribavirin and IFN show synergistic antiviral action both *in vitro* and *in vivo*.^{18,19} PegIFN α is a recombinant form of the cytokine, which is covalently linked to a single chain of polyethylene glycol (PEG). It has been shown that this chemical conjugation significantly extends the half-life of the IFN α molecule, and therefore, it displays a more prolonged effect and a better rate of virological response.²⁰ Ribavirin is a synthetic nucleoside analog and a broad-spectrum antiviral agent. Ribavirin is a prodrug, which, when metabolized to its triphosphate form resembles purine ribonucleotides.²¹ When ribavirin is incorporated into the viral RNA strand during synthesis, it can induce nucleotide misincorporations leading to lethal hyper-mutations, and the viral sequence is finally extinguished by an *error catastrophe*. In an alternative action mechanism, it has been shown that ribavirin 5'-monophosphate is capable of inhibiting the host enzyme inosine monophosphate dehydrogenase, with the result of depleting the intracellular pools of GTP. This mechanism may be also useful in explaining the general cytotoxicity of the drug and its anti-replication effect, as well as some of its effects on viral replication.²²

Currently, the combined antiviral treatment (PegIFN α /RBV) consists of weekly subcutaneous administration of PegIFN α together with a daily oral capsule of ribavirin. For patients infected with HCV genotypes 2 or 3, the therapy takes 24 weeks. However, for patients infected with HCV genotype 1, the treatment must be extended up to 48 weeks. As a whole, the results of combined antiviral therapy indicate that for HCV genotype 1, SVR is achieved in less than 50% of the cases, whereas for HCV genotype 2 and 3, SVR is achieved in approximately 80% of chronically infected patients. On the other hand, compared to non-Hispanic whites with HCV genotype 1 (50% SVR), Latinos with genotype 1 exhibit a 34% SVR and African-Americans achieve only a 34% SVR.²³

The factors that correlate with SVR can be separated into two types:

- Viral factors, and
- Host factors.

Among the viral factors are viral genotypes 2 and 3 versus genotype 1, lower viral loads, greater *quasispecies* diversity, and acute *vs.* chronic infection, whereas among the host factors include female gender, younger age, low index of fibrosis, lower body weight and body mass index, non-African American race, and absence of significant co-morbidities such as alcohol abuse, renal disease, and HIV infection.

Recently, several additional factors associated with the genetic backgrounds of patients have been identified. Considering that the human genome consists of more than 3 billion DNA base pairs in a human haploid reference genome, and although the genome is nearly 99% identical between individuals, small differences in base sequences might influence either gene expression or function. The primary, most widespread type of genetic variation is known as a single nucleotide polymorphism (SNP), which is a difference in the nucleotide sequence occurring at a specific locus, resulting in differences between individuals in the population. More than 10 million common SNPs (or variants) exist in the human genome, where a common SNP is defined by having a minor allele frequency of at least 5%. Genetic association studies test for differences in the genotype frequency of several SNPs between two populations, one carrying a phenotype of interest and a case control.

These phenotypes of interest have been analyzed in relation to spontaneous or therapy-mediated (PegIFN α /RBV) HCV clearance and studied by genome-wide association studies (GWAS). This scheme allows the unbiased sampling of variations in the entire genome to evaluate the association between a disease, and a specific SNP that is shown to be found more frequently in affected persons compared to the control cases. These analyses have systematically found that SNPs located on chromosome 19 near the interleukin 28B (IL28B) gene are strongly associated with the events that take place during HCV infection, such as spontaneous clearance, rate of progression to chronicity, and SVR to combined therapy (PegIFN α /RBV), in patients infected with genotype 1.

IL28B POLYMORPHISM AND SPONTANEOUS HCV CLEARANCE

Some large-scale studies employing GWAS and genetic mapping have identified a number of SNPs that are in strong linkage disequilibrium, which means that they are nonrandomly related, located within or near the *IL28B* locus and are associated with the

spontaneous clearance of HCV during an acute infection.

In one analysis, a GWAS was performed searching for determinants of spontaneous HCV clearance in subjects in whom the virus had been eradicated either spontaneously or following combined treatment (PegIFN α /RBV). The study involved chronic HCV cases along with individuals with spontaneous clearance.²⁴ Seven SNPs in *IL28B* were associated with spontaneous HCV eradication with genome-wide significance. The most significant SNP, rs8099917, strongly predicted suppression of HCV infection and also treatment-induced eradication of HCV infection.

The other study investigated the association of rs12979860 SNP with spontaneous HCV clearance. This study involved individuals with persistent infections and cases of spontaneous HCV clearance.²⁵ The rs12979860 SNP highly predicted spontaneous clearance in individuals of both European and African ancestry. It was concluded that individuals with the rs12979860-CC genotype have a higher probability of clearing HCV than those with the TC or TT genotype.

Finally, in a Spanish cohort, the rs12979860-CC genotype was associated with the spontaneous eradication of infection.²⁶ Similar results were reported when a single-source outbreak in a cohort of German women infected with anti-D-contaminated immunoglobulin was analyzed.²⁷

Together, these results indicate the association of the same *IL28B* SNPs in both spontaneous and treatment-induced (PegIFN α /RBV) resolution of HCV infection.

GLOBAL STUDIES OF *IL28B* VARIANTS AND ANTIVIRAL THERAPY RESPONSE

The same *IL28B* haplotypes associated with spontaneous HCV clearance were found to also be related to treatment response (PegIFN α /RBV). The patient cases of four large studies under combined therapy were evaluated by GWAS, using comparable analytical methods, genotyping techniques, and outcome definitions.

The first analysis, concerning patients included in the IDEAL study, compared the efficacy of PegIFN α 2b *vs.* PegIFN α 2a, in individual carriers of HCV genotype 1.²⁸ The cohort involved patients from three ethnic groups, Caucasians, African Americans, and Hispanics, and compared SVR with null-responses to therapy. Seven SNPs in the *IL28B* region significantly associated with the treatment

response were identified. However, the association could be explained by a single SNP. The SNP with the strongest correlation with treatment response, rs12979860, was located 3-kb upstream from the *IL28B* gene. Individuals carrying the rs12979860-CC genotype had a two- to three-fold greater SVR than those carrying the TT genotype in all three ethnic groups. The protective rs12979860 C-allele is nearly fixed in East Asians, where the highest rates of SVR were observed. On the contrary, the protective C-allele was found to be rare in African Americans, a population with generally low SVR. This analysis is meaningful since it explains approximately half of the differences in SVR response rates between African Americans and Europeans.

The second study was looking for host genes associated with virological response to combined therapy in a cohort of Japanese individuals infected with HCV genotype 1, including responders and non-responders to the treatment (PegIFN α /RBV).²⁹ In this analysis, several SNPs strongly associated with nonresponse to treatment in a GWAS scheme were identified. All of these SNPs were located close to *IL28B* on chromosome 19. The strongest association with nonresponse was detected with two SNPs: rs12980275, 3 kb downstream, and rs8099917, 8 kb upstream of the *IL28B* gene. For both SNPs, the minor allele frequency was more than six-fold higher in nonresponders than in virological responders.²⁸ Homozygotes for the risk allele were only present in non-responders. Haplotype analyses identified rs8099917 as the strongest predictor for response to antiviral therapy.

The next analysis reported genetic variants associated with SVR to anti-HCV combined therapy (PegIFN α /RBV) in European-Australian individuals infected with genotype 1, including responders and nonresponders to the treatment.³⁰ Only rs8099917, situated in the intergenic region between *IL28A* and *IL28B*, was found to be significantly genome-wide. Homozygous carriers of the rs8099917-G allele had more than a two-fold higher risk to fail HCV therapy compared with heterozygotes and TT homozygotes. Haplotype analysis identified a distinct haplotype associated with treatment response. The responder haplotype covers a region that is likely to affect expression of *IL28B* and/or *IL28A*.³⁰

The other study covered chronically infected Caucasian individuals and screening for host genetic determinants associated with response to HCV treatment (PegIFN α /RBV). The analysis included individuals infected with HCV genotypes 1, 2, 3, or 4.²⁴ These studies found several SNPs near the

IL28B locus that were associated with chronic HCV infection at a genome-wide significance level. The strongest association with treatment null response was found with rs8099917. Individuals carrying one or two copies of the rs8099917 risk G-allele had higher risks of treatment failure compared with individuals carrying the common genotype TT.²⁴ The host genetic effect was largely observed in individuals infected with the unfavorable viral genotypes 1 and 4, with SVR rates of 28% among risk-allele carriers versus 63% among individuals homozygous for the protective allele. In individuals infected with the favorable viral genotypes 2 or 3, the differences were small.

Finally, a candidate gene study to reproduce the most significant association from the study by Ge, *et al.* (2009) was carried out in chronic hepatitis C individuals.³¹ The study included Caucasian and African American individuals infected with either HCV genotype 1 or genotypes 2 or 3. Carriage of the protective rs12979860-C allele was associated with a nearly six-fold increase in SVR rates compared with heterozygous CT and TT genotypes. No association was found in a small subgroup of African American cases. No detectable difference was observed in the effect of rs12979860 on SVR comparing subjects infected with different HCV genotypes.

Together, these GWAS independently found a consistent association between *IL28B* genetic variants and response to HCV therapy (PegIFN α /RBV). All associations that reached genome-wide significance were mapped to the *IL28* region.

LATIN AMERICAN STUDIES

The above global studies addressing the role of *IL28B* variants on HCV antiviral therapy outcome were mainly carried out within populations from North America, Europe, and Asia. However, HCV genotype 1 is also the most prevalent in Latin American countries, and the role of *IL28B* variants on therapy (PegIFN α /RBV) outcomes has been analyzed for several South American countries, including Chile, Brazil, and Argentina.

In Chile, to simultaneously analyze the association of the three main *IL28B* variants with therapy outcomes, we studied two groups of patients with chronic HCV infections (genotype 1) under standard combined therapy (PegIFN α /RBV).³² Patients with SVR and null responders were included in our study, and genotyping was performed using PCR and restriction fragment length polymorphisms. The *IL28B* genotypes rs12979860-CC, rs12980275-AA, and

rs8099917-TT were much more frequently found in patients with SVR compared to null responders: 38%, 44%, and 50% *vs.* 2%, 8.2%, and 8.2%, respectively. The differences found were highly significant in all three cases. Thereafter, our group has made available in Chile a clinical test to analyze IL28B variants in patients infected with HCV.

In Brazil, *IL28B* variants rs12979860 and rs8099917 were genotyped in HCV patients under therapy (PegIFN α /RBV), and their ethnic descent was identified utilizing genetic markers.³³ IL28B rs12979860-CC genotype was associated with SVR, whereas both CT and TT variants were associated with nonresponse to therapy. On the other hand, IL28B rs8099917-TT was associated with SVR, whereas both GG and GT genotypes were associated with nonresponse or relapse. Among cases with the rs12979860-CC genotype, ancestry does not influence the therapy outcome. Between patients with rs12979860-TT genotype, African genetic contribution was greater for nonresponders or relapsers, whereas Amerindian and European genetic descent was more common in responders. Among rs8099917-TT genotype cases, African genetic contribution was significantly greater for the non-response or relapse cases; Amerindian and European ancestry genetic contributions were greater for therapy responders.

Finally, the importance of the IL28B host genotypes rs8099917 and rs12979860 were determined in a population of Argentine HCV patients with European ancestry.³⁴ SVR rates (PegIFN α /RBV) were higher in rs8099917-TT genotypes when compared to GT/GG, and higher in rs12979860-CC genotypes than in CT/TT. The analyses showed that both rs8099917-TT and rs12979860-CC genotypes and low viral loads were significantly associated with SVR.

BIOLOGY OF IL28B

The gene product of *IL28B* gene is IFN λ 3, a cytokine involved in the innate immune response, which was discovered by bioinformatics prediction from genomic sequences.^{35,36} Functionally, IFN λ 3 is expressed at low levels by a broad variety of human tissues and is significantly induced in response to viral infection similarly to IFN α .³⁷ IFN λ 3 together with IFN λ 1 (which is encoded by *IL29* gene) and IFN λ 2 (which is encoded by *IL28A* gene) are all part of the type III or IFN λ family.³⁷⁻³⁹ In the human genome, the IFN λ genes are clustered on chromosome 19.⁴⁰ IFN λ s are structurally homologous to components of the IL10 family, and these are induced by viral in-

fections similar to type I IFNs, such as IFN α and IFN β .⁴¹ Analogous to type I IFNs, the signal transduction of IFN λ s is also carried out by the activation of the Janus kinase-signal transducers and activators of transcription (Jak-STAT) intracellular pathways, and it ultimately upregulates the expression of IFN-stimulated genes (known as ISGs) that are required to control viral infection.^{41,42} Several *in vitro* studies support a direct role for IFN λ in the control of HCV replication through the innate immune pathway. It has been shown that subgenomic and full-length HCV replicon constructs were inhibited by recombinant IFN λ 1 and IFN λ 2, which upregulated a marker ISG.⁴³ In a cell culture system, IFN λ 1 inhibited HCV replication with similar kinetics to that of IFN α . However, IFN λ 1-induced upregulation of ISGs was stronger and lasted longer.⁴² Combinations of IFN λ 1 and IFN α had the greatest inhibitory effects on HCV replication compared with the effect of the individual agents.⁴⁴ IFN λ and IFN α might therefore have synergistic effects in controlling HCV infection.

IFN λ s exert their activity through a different cell receptor than type I IFNs. All three IFN λ s interact with a heterodimeric class II cytokine receptor that consists of interleukin 28 receptor α chain (IL28R α) and interleukin 10 receptor β chain (IL10R β).⁴² Unlike IFN α and IL10 receptors, which are both found ubiquitously on various cell types, the presence of the IFN λ receptor α chain (IFN λ R1 or IL28R α) is tissue-dependent and expressed primarily in epithelial cells, liver tissue, and peripheral blood mononuclear cells.³⁷⁻³⁹ Thus, IFN λ s are thought to produce intracellular responses similar to those of IFN α but are more specific in their tissue targets due to the restricted cell receptor expression.^{35,39} Trials of IFN gamma 1 in patients with chronic HCV infections have recently shown promising results; 86% of naive patients who received combined PegIFN λ and ribavirin during 4 weeks had a higher than 2 log IU/mL decrease in HCV RNA.⁴⁵

The biological basis for the relationship between the *IL28B* polymorphism and the sensitivity of HCV infection to antiviral treatment (PegIFN α /RBV) is not yet clear. Although the two main *IL28B* SNPs in linkage disequilibrium, rs8099917 and rs12979860, were exactly the same in the majority of the studies, it is not clear how they exert their influence. It has been postulated that both SNPs, which are located upstream of the *IL28B* gene, may influence the expression of IL28B. However, the results are not completely clear: no effect on IL28B transcription was observed for rs12979860,^{28,46,47}

whereas lower IL28B mRNA levels were detected in association with the rs8099917-GG allele.^{29,30,48} It is worth noting that both rs8099917-GG and rs12979860-TT genotypes are related to the increased basal expression of ISGs in the livers of patients with chronic HCV infection,⁴⁶⁻⁴⁹ and that high hepatic ISG levels before treatment are associated with a lack of response to IFN.⁵⁰⁻⁵² This is supported by a report that identified ISG expression as the best predictor of treatment response based on multiple factors, including *IL28B* genotype.⁵³

It has been proposed that during acute infection, HCV can trigger the synthesis of IFN α , which in turn improves the production of IL28B, which is induced by the HCV itself.⁵⁴⁻⁵⁶ The rs12979860-CC and rs8099917-TT genotypes can be associated with stronger IL28B induction or with enhanced cytokine function, leading to increased ISG expression and a higher frequency of spontaneous recovery.^{29,30,48} The opposite would happen in subjects with rs12979860-TT and rs8099917-GG genotypes. On this note, it has been shown that IL28R α knockout mice, which are functionally similar to individuals with the latter genotypes, have a severely impaired antiviral response.⁵⁷ On the other hand, in chronic HCV infection, sustained HCV replication induces continuous low-level expression of endogenous IFN α and downstream activation of ISG, rs12979860-CC, and rs8099917-TT IL28B genotypes, which are associated with low basal ISG levels, allows for stronger activation of IL28B and ISG in individuals being treated with IFN α .^{46,47,49} In contrast, the high basal ISG expression observed in subjects with rs12979860-TT and rs8099917-GG genotypes impairs further induction by treatment and leads to the activation of IFN inhibitory pathways, thus decreasing the possibility of viral clearance.⁵¹

IL28B VARIANTS AND RESPONSE TO NEW DIRECT-ACTING ANTIVIRALS

Currently, the treatment for chronic HCV genotype 1 infection is developing and evolving rapidly. Triple therapy regimens concerning direct-acting antiviral agents (DAAs) in combination with pegylated interferon and ribavirin are the new standard of care. The first DAA compounds, telaprevir and boceprevir, were approved by the US Food and Drug Administration in 2011 for the treatment of chronic HCV genotype 1 infection.

IL28B polymorphisms can also exert an effect on SVR rates in individuals under combined therapy

who are also receiving the new direct-acting antivirals (DAAs) against HCV genotype 1. In these cases, the effect of the IL28B polymorphisms would be less marked since these drugs with greater antiviral potency make host factors less important. However, IL28B variants can still be utilized as a reference to decide the extent of the therapy period.⁵⁸

IL28B polymorphisms have been reported to predict therapy outcomes in patients beginning therapy with boceprevir, a recently approved DAA, which binds to the active site of HCV NS3 and inhibits the enzymatic activity of the viral protease.⁵⁹ IL28B rs12979860-CC and non-CC genotypes have been related with similar rates of SVR in previously treated individuals under combined therapy along with boceprevir.^{60,61} However, when response to combined therapy during the entrance phase was considered, IL28B variants do not independently predict SVR.^{60,61}

The other recently approved DAA against HCV is telaprevir.⁵⁹ Telaprevir is also a HCV protease inhibitor that covalently and reversibly binds the viral NS3-4A complex serine protease. In a randomized trial of individuals for which the standard combined therapy had failed, retreatment with the addition of telaprevir was more likely to induce a sustained response than repeat treatment with only the combined treatment alone.⁶² For individuals that had initiated telaprevir combination treatments, IL28B polymorphisms predicted SVR rates.^{61,63,64} In IFN α -treated patients who have received telaprevir, prior response is the major predictor of SVR, with minimal impact of IL28B variants.^{61,65}

As a whole, the main benefit of DAA therapy is in poor-response IL28B genotype patients, in whom SVR rates are significantly increased. On the other hand, in good responders, the main benefit of DAA therapy is that it allows for therapy of short duration. However, it will take some more time to have DAA therapy globally available.

Numerous additional agents and viral targets are in different stages of preclinical and clinical testing. Several other HCV NS3 protease inhibitors are under advanced clinical testing such as TMC-435, naldaprevir, danoprevir, and vaniprevir among others. On the other hand, some HCV NS5B inhibitors are in different phases of clinical testing. For the class of nucleos(t)ide inhibitors, mericitabine, PSI-7977, and IDX 184 are currently being analyzed, whereas for the class of non-nucleoside inhibitors, filibuvir, tegobuvir, and setrobuvir are also being clinically tested.⁶⁶

INOSINE TRIPHOSPHATASE (ITPA)-POLYMORPHISMS

Anemia is a very common adverse effect of HCV combined therapy and is the result of ribavirin-induced hemolysis and IFN-related bone marrow toxicity. Ribavirin-induced hemolytic anemia is normally reversible and dose-related. However, it might require significant dose reductions possibly affecting the efficacy, and it is a cause of withdrawal from HCV therapy in about 15% of patients. The molecular mechanism of hemolytic anemia is unknown. Oxidative damage and erythrocyte lysis have been related to the intracellular accumulation of pharmacologically active, phosphorylated forms of ribavirin and to ribavirin-induced depletion of erythrocyte ATP content.⁶⁷

In a recent GWAS, a strong association between hemoglobin reduction after four weeks of treatment and rs6051702 SNP genotypes was found.⁶⁸ The association was explained by two genetic and functional variants in the *ITPA* gene located on chromosome 20 and encoded by Inosine triphosphate pyrophosphohydrolase, ITPase. This enzyme hydrolyzes inosine triphosphate and deoxyinosine triphosphate to the monophosphate nucleotide and diphosphate. These two genetic variants result in reduced enzyme activity and are associated with the risk of ribavirin-induced anemia.⁶⁹

The *ITPA* genotypes appear to be strongly associated with the differential risk of hemolytic anemia induced by ribavirin and thus dose-reduction. The information on *ITPA* genotypes could play a role in clinical decisions regarding the indications for treatment in patients with co-morbidities, the frequency of hemoglobin monitoring, and the need for dose adjustments.

LOW-DENSITY LIPOPROTEIN RECEPTOR (LDLR)

The low-density lipoprotein receptor, LDLR, has been proposed to promote hepatitis C virus endocytosis and thus particle entry into the cell.⁷⁰ Polymorphisms within the LDLR gene are associated with the pathogenesis of familial hypercholesterolemia, atherosclerosis, and obesity. Genetic variants of LDLR have been shown to predict SVR to combined antiviral therapy in chronic hepatitis C individuals and severity of fibrosis.⁷¹ These findings have recently been extended to HIV/HCV-coinfected individuals.⁷² In HIV/HCV coinfection, higher levels of LDL-cholesterol are found in rs12979860-CC than in non-CC

carriers.⁷³ This effect might be due to reduced LDLR gene expression induced by lower activity of endogenous IFN α in rs12979860-CC genotypes than in CT/TT carriers. As LDLR is one of the putative HCV receptors, both IL28B and LDLR allelic variants could interact to modulate HCV replication. The consideration of both IL28B and LDLR genotypes would improve the predictive value for SVR more than the evaluation of these variables separately.

GENETIC DETERMINANTS OF HCV THERAPY OUTCOME

The RNA sequence of HCV has also been shown to exhibit variability at several positions within its genome, which can influence the outcome of the antiviral therapy. The main viral genetic determinants of treatment response are located in the core and NS5a coding regions. Mutations within a stretch of 40 amino acids in the NS5a region of HCV, designated as the IFN sensitivity determining region, ISDR, are closely associated with the virological response to standard combined therapy; a lower number of mutations are associated with treatment failure.⁷⁴⁻⁷⁷ Amino acid substitutions at positions 70 and 91 of the HCV core region also have been reported to be associated in response to combined treatment: glutamine or histidine residues at core position 70, and methionine residue at core position 91 are associated with treatment resistance.^{78,79} The relevance of substitutions in the HCV core and ISDR regions was confirmed recently by a Japanese multicenter study.⁸⁰

The association of IL28B polymorphisms and HCV variants on therapy outcomes has recently been addressed by several studies. IL28B variants and mutations in the core region and NS5a ISDR were found to be associated with IFN responsiveness.⁸¹ The IL28B polymorphisms and mutations in the ISDR of HCV NS5a coding region were found to be relevant as pretreatment predictors of combined therapy outcomes.⁸² In another study, substitutions of HCV core amino acid 70 were found to contribute independently of IL28B polymorphism to SVR in combined antiviral therapy.⁸³ However, using human hepatocyte chimeric mice with different IL28B genotypes and infectious HCV genotype 1b with substitutions in core and NS5a ISDR, it was shown that replication levels and response to IFN were affected by IL28B variants and mutations in the NS5a ISDR region but not in the core region.⁸⁴ Finally, variants of IL28B and residue substitutions of the core region were found to be useful predictors

of viral dynamics during triple therapy. Considering these relatively inconsistent results, the association between IL28B variants and the viral genetic determinants on therapy response await for further systematic research.

CONCLUSIONS

During the last years, relevant data about the impact of the patient genomics on viral infection outcomes has suggested the possibility that an individualized antiviral therapy might be considered. For this, the status of single nucleotide polymorphisms (SNPs) of the host genes interleukin 28B (IL28B), inosine triphosphatase (ITPA) and low-density lipoprotein receptor (LDLR) during standard combined therapy should be taken into account. Whereas IL28B genotypes can be used to estimate the extent of the antiviral therapy period, both IL28B and LDLR allelic variants could interact to modulate HCV replication. Finally, ITPA genotypes seem to be strongly associated with the differential risk of hemolytic anemia induced by ribavirin, which is a factor that can determine adherence to the antiviral treatment as well as dose-reduction.

The treatment for genotype 1 chronic hepatitis C infection is rapidly evolving. Triple therapy concerning potent DAAs combined with pegIFN and RBV are the new standard of care. Under this new scenario, IL28B polymorphism is still associated to therapy outcomes, but the powerful antiviral effect of these drugs reduces the strength of the association. The schemes of triple therapy are functional for patients who are carriers of the poor response IL28B variant. For individuals who are carriers of the good response IL28B variant, the benefit of DAA-based therapy will be the shorter duration of therapy. The standard of combined pegIFN and RBV will still be indispensable to avoid the selection of resistant variants by DAAs, and the sensitivity to IFN is critical for successful viral suppression. The importance of IL28B variants and IFN sensitivity to outcomes of DAA therapies is not totally clear. It has been proposed that IL28B polymorphism becomes less relevant to treatment outcomes as overall efficacy increases. IL28B variants can be helpful in order to identify patients for whom an IFN treatment will remain useful, particularly allowing them for a very short-duration therapy.

As a whole, the main benefit of DAA therapy is in poor-response IL28B genotype patients, in whom SVR rates are significantly increased. On the other hand, in good responders, the main benefit of DAA

therapy is that it allows for therapy of short duration. However, it will take some more time to have DAA therapy globally available.

ABBREVIATIONS

- **HCV:** hepatitis C virus.
- **SVR:** sustained virological response.
- **IFN:** interferon.
- **SNP:** single nucleotide polymorphism.
- **IL28B:** interleukin 28B.
- **ITPA:** inosine triphosphatase.
- **LDLR:** low-density lipoprotein receptor.
- **DAA:** direct-acting antiviral.

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