

PREDICTIVE BIOMARKERS OF ANTIVIRAL TREATMENT OUTCOME IN HCV INFECTION

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A genetic polymorphism near IL28B gene, which encodes for interferon lambda-3, strongly correlated with response to peginterferon/ribavirin in individuals infected with genotype 1 HCV. The two variants highly associated with rs12979860 single nucleotide polymorphism (SNP) were identified as genetic determinants of superior treatment response. This polymorphism also correlates with natural HCV RNA clearance as well as with differences in sustained virologic response (SVR) rates between race/ethnic populations. Other studies identified association between inosine triphosphatase (ITPA) genetic variants and RBV-induced anemia in cohort of patients infected with genotype 1 HCV treated with peginterferon alfa and RBV. Two functional variants (associated with SNP rs1127354 and rs7270101) that cause ITPase deficiency have been shown to protect against ribavirin (RBV)-induced hemolytic anemia during early stages of treatment. These results might allow identification of patients infected with genotype 1 HCV who have a very good chance, perhaps as much as a 70% chance, of achieving SVR with peginterferon/ribavirin. In the future, this approach also might be used to tailor peginterferon/ribavirin with new direct acting antiviral agents (DAA).

Key words: SNPs: rs12979860, rs1127354 and rs7270101, interferon lambda-3, ribavirin

INTRODUCTION

For two decades the scientific community has sought to understand why some people clear hepatitis C virus (HCV) and others do not. Recently, several large genome-wide association studies have identified single nucleotide polymorphisms (SNPs) linked to interferon lambda 3 (IFN λ 3) that are associated with the spontaneous resolution and successful treatment of HCV infection.

The single nucleotide polymorphism rs12979860, located 3 kb upstream of the IL28B gene, is associated with more than two-fold difference in the rate of SVR. This polymorphism independently predicts SVR in a cohort of HIV/HCV co-infected patients. The IL28B genotype was a strong, independent, predictor of SVR to peginterferon plus ribavirin in patients infected with both genotype

1 and non-genotype 1 HCV infection. Specifically, rs12979860 genotype CC was associated with two fold higher rate of SVR vs the TC/TT genotype. The impact of the rs12979860 genotype was greatest among patients with genotype 1 or 4 HCV infection but was also maintained in patients infected with genotype 3 HCV. A test for the IL28B genotype is now commercially available and may be used to help predict groups with a very high or very low likelihood of HCV clearance with therapy.

HUMAN INTERFERONS-LAMBDA FAMILY

IFNs lambda (IFN- λ) was discovered independently by two groups of scientists led by Kotenko and Gallagher¹ of the University of Medicine and Dentistry, New Jersey (Newark, NJ, USA) group and Klucher² of the ZymoGenetics™ (Seattle, WA, USA) group. The new IFN- λ family

has three members: IFN- λ 1, IFN- λ 2, and IFN- λ 3. The IFN- λ genomic structures resemble that of the IL-10 family³. Therefore, they have been described independently as IL-29 (IFN- λ 1), IL-28A (IFN- λ 2), and IL-28B (IFN- λ 3). On the other hand, at the amino acid level and functionally, IFN- λ s are more related to type I IFNs than IL-10. They activate IFN-stimulated responsive elements (ISRE) and induce antiviral activity. IFN- λ s are now collectively referred to as type III IFNs. Nevertheless, structurally, IFN- λ s are related to IL-10 and other members of the IL-10-like family such as IL-22, which has recently shown to confer hepatoprotection⁴. Table 1 shows current classification of human IFNs. IFN- λ s comprise three distinct genes: IFN λ 1 (IL29), IFN λ 2 (IL28A), and IFN λ 3 (IL28B) clustered on human chromosome 19 (19q13+13 region). This location differs from the type I IFN family clustered on chromosome 9. Virus infections readily activate the expression of IFN- α , IFN- β , and IFN- λ genes. IL-28A and IL-28B proteins are 95% identical while IL-29 shares only 80% amino acid identity with IL-28A or IL-28B. As IL-28A, IL-28B and IL-29 functionally resemble type I IFNs (IFN- α / β), they are also considered as a novel group of IFNs (type III IFNs)⁵.

IFN- λ s signal through a receptor complex comprised of IL-10R2 and a unique subunit, IFN λ -R1. The expression of the specific receptor IFN λ -R1 is more restricted to hepatic cells and weakly expressed on leukocytes. As signaling through the type-I-interferon receptor, signaling through the IFN- λ receptor results in the activation of signal

transducer and activator of transcription (STAT)-1 and STAT2. Together with an accessory factor, IFN regulatory factor 9 (IRF-9; p48), STAT1 and STAT2 form the transcription factor IFN-stimulated gene factor-3 (ISGF3) which translocates to the nucleus to initiate the induction of target genes. IL28A and IL28B gene expression is mainly controlled by IRF7, similar to the gene encoding IFN- α ⁶.

Both IL-28A and IL-29 are able to induce STAT1 phosphorylation in hepatic cells with IL-29 showing slightly stronger effects. The microarray analysis revealed activation of mostly identical genes by IL-28A and IL-29. Among them were numerous genes involved in interferon-mediated immunity and antiviral defense.

In summary, both IL-28A and IL-29 induce expression of antiviral proteins, especially in the liver cells, and are up-regulated during viral infection with no major differences. However, in contrast to IL-29, IL-28A has the capacity to repress gene expression. Both cytokines are promising candidates for the treatment of HCV infection with likely low side effects on leukocytes. Nevertheless, further studies are needed to clarify which of the three IFN- λ cytokines is the most potent with the least amount of side effects. Even if types I and III IFNs act through a distinct receptor system, they activate the same signaling pathway and induce common ISGs (Fig. 1). Collectively, these ISGs mediate the biological effects of IFNs, such as inhibition of viral replication, cellular growth inhibition, and apoptosis.

Table 1

The human IFN family members

Type of IFNs	Subclasses	Subtypes	Receptors	Comments
Type I	IFN- α ,	13 subtypes: α -1, α -2, α -4, α -5, α -6, α -7, α -8, α -10, α -13, α -14, α -16, α -17, α -21	Two subunits, IFNAR1 and IFNAR2.	Receptors expressed in most cell types
	IFN- β , IFN- ϵ , IFN- κ , IFN- ω	One type each		
Type II	IFN gamma	One type	two chains, IFNGR1 and IFNGR2	
Type III	IFN lambda	IFN- λ 1/IL-29	IFN- λ R1 and IL-10R β	Receptors with restricted pattern of expression
		IFN- λ 2/IL-28A		
		IFN- λ 3/IL-28B		

Table 2

Comparison of type I IFNs and IFN- λ s

Properties	Type I IFNs	IFN- λ
Receptors	IFNAR1/IFNAR2	IFNLR1 ¹ /IL-10R2
Receptor's distribution	ubiquitous	Hepatocyte restricted
Signaling pathways	STAT 1 & 2	Almost all STAT molecules
Regulatory functions	Broad	Cell-type specific
Clinical effects	Antiviral non specific, immunomodulator, antitumoral	Anti HBV and HCV infection
Side-effects	Significant	Less hematological and neurological toxicity

IFNLR1¹ – has a hepatocytes restricted pattern of expression

IFN- λ S EXHIBIT ACTIVITY AGAINST HCV REPLICATION

Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Zhu *et al.*⁷ demonstrate that IFN- λ 2 effectively inhibits HCV subgenomic RNA replication. Treatment of human hepatoma cells with IFN- λ 2 activates the JAK-STAT signaling pathway and induces the expression of some ISGs. IFN- λ 2 also induces the expression of HLA class I antigens in human hepatoma cells. Moreover, IFN- λ 2 appears to suppress specifically HCV internal ribosome entry segment-mediated translation.

There is a distinction between IFN- λ - and IFN- α -induced antiviral states. IFN- λ mediated dose- and time-dependent HCV inhibition, independent of types I and II IFNRs. The kinetics of IFN- λ -mediated STAT activation and induction of potential effectors genes were also distinct from those of IFN- α . IFN- λ induced steady increases in levels of known ISGs, whereas IFN- α ISGs peaked early and declined rapidly⁸. IFN- λ inhibited the replication of HCV genotypes 1 and 2 and enhanced the antiviral efficacy of subsaturating levels of IFN- α ⁹. IFN- λ s efficiently inhibit HCV replication *in vitro* with potentially less hematopoietic side-effects than IFN- α because of limited receptor expression in hematopoietic cells. Despite antiviral properties of IFN- λ s, their efficacy as antiviral agents may have similar

limitations as IFN- α as a result of inhibition by SOCS proteins¹⁰.

In addition to their antiviral and antiproliferative activities, IFN- λ s exert immunomodulatory effects that overlap type I IFNs in innate and adaptive arms of the immune system. These activities include increasing NK and T cell cytotoxicity, promoting Th1 responses, up-regulating MHC class I molecule expression on tumor cells to promote antigen presentation, and mediating cell apoptosis¹¹.

The immunoregulatory actions of IFN- λ are cell type-specific, which depend on the distribution of receptors IFN- λ Rs, the nature of the signal transduction, and genes activated. IFN- λ s are capable of signaling through almost all STAT molecules, and therefore, they exhibit broader functions as compared with type I IFNs¹².

However, treatment with type I IFNs also causes significant side-effects, such as fatigue, fever, anorexia, depression, and myelosuppression. When prolonged, for instance in the case of hepatitis C treatment, type I IFN treatment can lead to neurological or neuropsychiatric adverse effects¹³. The major challenge to the treatment of HCV is to improve antiviral efficacy and to reduce the side-effects typically seen in IFN- α -based therapy. Phase I clinical trials are currently sponsored by ZymoGeneticsTM to assess the safety and antiviral activity of pegylated IFN- λ 1 (or PEG-recombinant IL-29) in subjects with relapsed, chronic HCV infection¹⁴ (ClinicalTrials.gov, identifier: NCT00565539).

Table 3

Comparison of anti HCV actions of type I IFNs and IFN- λ s

Properties	type I IFNs	IFN- λ
In vitro activity	No effect	Inhibits HCV subgenomic RNA replication
In vivo antiviral states	peaked early and declined rapidly	dose- and time-dependent
Synergic effects	Overlapping antiviral efficacy with IFN- λ	enhanced subsaturating levels of IFN- α

GENETIC VARIATIONS IN IL28B PREDICT HEPATITIS C TREATMENT-INDUCED VIRAL CLEARANCE

Peginterferon/ribavirin yields sustained virologic response (SVR) only in a subset of patients chronically infected with HCV. Predictors of nonresponse to peginterferon/ ribavirin in patients with chronic hepatitis C infection comprise:

- Increased hepatic expression of interferon-stimulated genes (ISGs),
- IL28B (IFN λ 3) GG genotype.

Induction of ISGs in liver correlates with treatment no response^{15,16}. Analysis of receiver operating characteristic (ROC) curves for ISG expression found the 3 strongest gene predictors of treatment response according to multivariate analysis (IFI27, RSAD2, and HTATIP2 expression) and yielded a classifier with excellent test performance: area under curve (AUC): 0.94 and error rate: 15%. Addition of IL28B genotype information to classifier does not improve predictive power.

Allelic variants near IL28B gene linked with rs12979860 polymorphism were associated with treatment response and spontaneous viral clearance^{17, 18}. IL28B genotype is independently associated with virologic response and preliminary data show the following SVR rates according to IL28B genotype: TT - 41%; TG - 26%, and GG - 0%. IL28B minor allele (GG) was detected in only 4% of patients (4 of 93). Decreased IL28B mRNA expression indicates reduced IL28B protein in liver. However, expression of ISGs also significantly increased in nonresponders vs. responders across IL28B genotypes.

In conclusion, multivariate analysis identified several genes strong predictors of treatment response: IFI27 expression, RSAD2 expression, HTATIP2 expression^{19, 20}. Addition of other predictors to this 3-gene classifier did not improve predictive power. Addition of IL28B genotype information also did not improve predictive power of classifier: AUC - 0.90 and error rate, 18%. ROC curve of IL28B SNPs showed mediocre sensitivity and specificity: AUC - 0.66 and error rate, 38%.

One concern raised by study of Dill *et al.*²¹ is that the predictors identified by univariate and subsequently by multivariate analysis in rather small group of patients were not fully consistent with previous findings from other studies. Two presentations at EASLD 2010 (European

Association for Study of Liver Diseases) confirmed that higher ISG expression levels in the liver are associated with lower response rates^{20, 21}. It seems that the data supporting this finding are now sufficient to accept the association; however, other conclusions regarding the relationship between and relative contributions of IL28B and ISG expression are going to require further study.

It was suggested that IL28B genotyping could be used in the future before the approval of direct-acting antiviral (DAA) agents in order to classify individuals according to their likelihood of response to peginterferon/ribavirin. This strategy might allow identification of patients infected with genotype 1 HCV who have a very good chance, perhaps as much as a 70% chance of achieving SVR with peginterferon/ribavirin. This approach also might be used to tailor peginterferon/ribavirin with DAA agents. One can hypothesize that patients with the favorable CC genotype might be the most likely candidates for a noninterferon-based DAA regimen, whereas patients who have the TT or heterozygous genotype might require more intensive therapy.

It is important that the implications of IL28B are not misunderstood. This marker could not be used to refuse treatment in some patients – this is certainly not the case and would be an unethical practice.

In Caucasians and Africans, IL28B SNP rs12979860 CC genotype and C allele are associated with spontaneous resolution of HCV infection, however, no differences in genotype and allele distributions were observed for IL28B SNP rs12979860 between HCV patients and normal controls in Taiwanese. Paradoxically, rs8099917 GG genotype and rs28416813 G allele that associated with low response in Australians and Japanese were significantly enriched in Taiwanese normal male population. On the other hand, eight SNPs with strong linkage disequilibrium demonstrate significant associations with SVR on single point analysis. However, haplotype analysis failed to increase the significance of association, which is different from the results of previous studies²³.

The SNPs associated with the clinical outcome of HCV infection are located some distance from the IFN λ 3 gene itself, and contributory genetic variants have yet to be clearly defined. Locating these causal variants, mapping in detail the IFN λ 3 signaling pathways, and determining the downstream genetic signature so induced, will clarify the role of IFN λ 3 in the pathogenesis of

HCV. Clinical studies assessing safety and efficacy in the treatment of HCV with exogenous IFN λ 3 are currently underway. Early results suggest that IFN λ 3 treatment inhibits HCV replication and is associated with a limited side effect profile. However, hepatotoxicity in both healthy volunteers and HCV-infected patients has been described^{24,25}.

THE PROTECTIVE ROLE OF THE VARIANT ALLELE AGAINST RIBAVIRIN-INDUCED HEMOLYTIC ANEMIA

Ribavirin is a critical component of combination regimens with peginterferon alfa for the treatment of chronic HCV infection and is essential for optimizing the rate of SVR. Studies of emerging DAA agents against HCV indicate that ribavirin will also be necessary for maximizing virologic response in future treatment regimens. One limitation of ribavirin is the development of hemolytic anemia during treatment – an adverse reaction that may require transfusion therapy or treatment with erythropoietin analogues. Management of anemia may also require dose reduction or discontinuation of ribavirin. These dose modifications, in turn, may compromise a patient's chance to achieve SVR. Therefore, methods to define the risk of ribavirin-induced hemolysis could be useful in patient management.

The enzyme inosine triphosphatase (ITPase) hydrolyzes inosine triphosphate to inosine monophosphate, which plays an important role in purine synthesis because it is ultimately converted to adenine and guanine nucleotides. SNPs in the gene encoding ITPase (ITPA) have been associated with reduced ITPase activity²⁶. ITPase deficiency appears to be a benign condition, although it has been associated with adverse drug reactions from the thiopurines used to treat inflammatory bowel disease²⁷. Recently, study of samples from persons with chronic HCV infection being treated with peginterferon alfa and ribavirin through the IDEAL study²⁸ identified 2 SNPs (rs1127354 and rs7270101) in the ITPA gene that were independently associated with protection from ribavirin-induced anemia (as measured by quantitative change in hemoglobin from baseline to week 4 of treatment). The purpose of the study by Thompson and colleagues²⁹ was to replicate these findings using data from another clinical trial of persons being treated for HCV (ViraHep-C) to examine the impact of the ITPA variants on

anemia throughout 48 weeks of treatment, to determine the association between the variants and need for ribavirin dosage reductions, and to determine the effect of ITPA genetics on virologic response.

One third of patients (n = 92) had polymorphisms associated with reduced ITPase activity. The findings from the IDEAL study were replicated in that each ITPA SNP was significantly and independently associated with protection against declines in hemoglobin by week 4 of treatment. This study also extended previous findings to show that ITPase deficiency was associated with delayed hemoglobin declines during the first 12 weeks of treatment and less absolute reduction in hemoglobin over the full 48-week treatment course²⁹. Furthermore, the odds of ribavirin dose reductions due to anemia in those with the ITPA variants were 47% lower (odds ratio: 0.53; 95% confidence interval: 0.33–0.84; P = .006) than those with wild-type genetics, and this relationship held after adjustment for possible confounders. No participants with moderate or severe ITPase deficiency discontinued treatment due to anemia.

Importantly, no association was found between ITPA genetics and SVR or week 4 rapid virologic response, which may indicate that the mechanism(s) by which ITPase protects against ribavirin-induced anemia is independent from the mechanism(s) by which ribavirin exerts activity against HCV, although this requires confirmation in larger studies.

Recently, the protective role of the variant allele for the rs1127354 SNP was confirmed in 923 Japanese persons with HCV (the rs7270101 SNP is not polymorphic in Asians)³⁰. In total, 83% of patients with the variant rs1127354 A allele completed HCV treatment vs. only 37% of those with homozygosity for the wild-type allele (P < 0.01).

Nearly 600 genomewide association studies covering 150 distinct diseases and traits have been published, with nearly 800 SNP–trait associations reported as significant (P < 5 × 10⁻⁸). An interactive map of the results is available in the paper of Manolio TA, 2010 at the site www.nejm.org³¹. Such associations are consistent with the common disease – common variant hypothesis, which posits that genetic influences on susceptibility to widespread diseases are attributable to a limited number of variants present in more than 1% to 5% of the population. The expected outcomes have rarely been made explicit utility to biology or medicine, such as clinical prediction of disease or, of treatment response.

There are several problems with the interpretation of these studies. First, the great majority of genetic effects that have been identified have been so small (odds ratios, 1.1 to 1.3) that they generally do not explain more than a few percent of the heritability of disease. Second, an unexpectedly large number of variants have been associated with most analyzed traits, suggesting that many of these variants have only peripheral effects on the trait (that is not the case with HCV treatment response). However, the studies resumed in this review revealed that a pharmacogenomic treatment approach for HCV can now be envisaged, with the incorporation of host genetic variants into a predictive treatment algorithm.

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