Genetic Variation in *IL28B*: Impact on Drug Development for Chronic Hepatitis C Infection

PJ Clark[1], AJV Thompson[1] and JG McHutchison[1]

As more pharmacogenomic insights into diseases and their treatments and toxicities are published each year, the challenge arises to incorporate such insights into clinical practice and drug development.[1] For instance, recent genomic discoveries related to hepatitis C offer a challenge to clinicians, researchers, and health administrators to translate this information into knowledge in order to develop safer and more effective therapeutic strategies for all patients.

INTERFERON-λ, *IL28B* GENETIC POLYMORPHISM, AND HCV INFECTION

Five recent genetic association studies have conclusively identified single-nucleotide polymorphisms (SNPs) close to the *IL28B* gene region as the strongest pretreatment predictor of response (sustained viral response (SVR), no detectable virus 24 weeks after the end of treatment) to pegylated interferon-α (IFN-α) and ribavirin (pegIFN/RBV) therapy for patients with genotype-1 chronic hepatitis C virus (HCV).[2–6] These same *IL28B* polymorphisms are also strongly associated with spontaneous clearance of the virus after acute HCV infection.[6,7] The *IL28B* polymorphism influences viral kinetics and is associated with an early decline in viral load with treatment.[8,9] Two SNPs (rs12979860 and rs8099917) in linkage disequilibrium have consistently provided the strongest signals and are likely to share a haplotype with the causal variant that has yet to be identified.

An important finding from these studies is that the genotype associated with a good response to treatment (C/C for SNP rs12979860) is less frequent in African Americans. To a large extent, these differences explain the poorer clinical response rates observed in African-American patients.[2] Conversely, the favorable C/C genotype is more frequent in Asian populations, corresponding to the higher efficacy rates that have been observed in this group.[10] However, the frequency of the *IL28B* allele does not entirely explain heterogeneity in treatment response between racial groups. African Americans have lower SVR rates as compared with Caucasians even when matched across each *IL28B* genotype.[2] Despite very high frequencies of the allele in Asians, clinical trials in Asian patients have not universally demonstrated high SVR rates. Although *IL28B* polymorphism has been one of the most significant steps toward advances in understanding differences in response, other host and viral factors remain important.

The mechanism through which *IL28B* variation causes IFN sensitivity remains unclear. The SNPs thought to be most closely associated with the mechanism lie upstream of the *IL28B* gene, and it is possible that they effect *IL28B* transcription. Ge *et al.* observed no relationship between *IL28B* mRNA levels and *IL28B* polymorphism in peripheral blood mononuclear cells from non-HCV-infected volunteers in the SNPExpress database.[2] However, Tanaka *et al.* observed that lower levels of *IL28B* mRNA were associated with the poor-responder rs80999917 genotypes in peripheral blood mononuclear cells in a small group of HCV-infected patients.[3] Suppiah *et al.* found lower constitutive expression of *IL28A* and *IL28B* in peripheral blood mononuclear cells from healthy controls carrying the rs8099917 poor-responder allele ($P = 0.044$). The data on gene expression are therefore conflicting. Several other potentially functional SNPs have been identified as being highly linked to the discovery SNPs, including the nonsynonymous SNP (rs8103142, Lys70Arg) in exon 2 of the *IL28B* gene.[2] This polymorphism potentially affects protein function, including receptor binding and protein stability.

The IFN-λ family or type III IFNs include *IL28A*, *IL28B*, and *IL29*. IFN-λ shares many functional characteristics (e.g., downstream signal transduction) with type I IFN but has a more restricted receptor (IFN-λ-R) distribution, and it is expressed at high levels in hepatocytes but not in hemopoietic cells. IFN-λ has a direct antiviral effect on HCV that is additive with that of type 1 IFN.[9,11–17] Teleologically, IFN-λ may have evolved to provide a more tissue-specific IFN response.[14] The IL28B discovery opens up new pathways to understand pathogenesis and develop therapies, not only in the treatment of hepatitis C–related liver disease, but for other chronic viral infections and immune-related injury in other organ systems.

[1]Duke Clinical Research Institute and Division of Gastroenterology, Duke University Medical Center, Durham, North Carolina, USA. Correspondence: PJ Clark (paul.clark@duke.edu)

Received 29 April 2010; accepted 7 July 2010; advance online publication 29 September 2010. doi:10.1038/clpt.2010.185

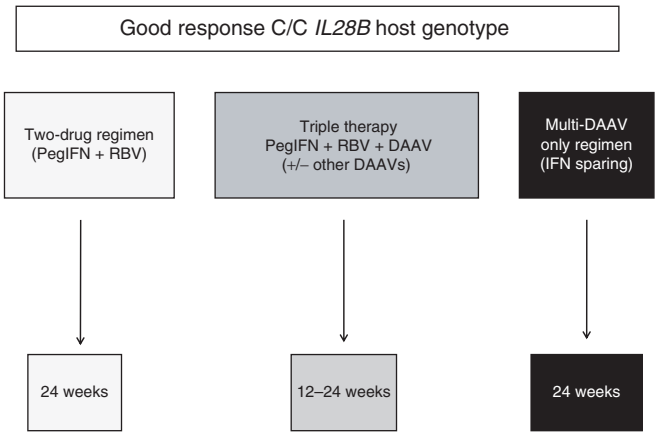

Figure 1 Possible future treatment strategies for good-response C/C *IL28B* genotype patient profile. Patients will be profiled on the basis of *IL28B* genotype and on viral response to treatment in order to allow tailored strategies. Although the host genotype is the fulcrum of such strategies, additional pretreatment predictors of response (such as fibrosis, viral load, age >40 years) will also contribute to the patient profile. Profile-specific strategies are designed before treatment, then "filtered" according to on-treatment viral kinetics (rapid viral response (RVR) and early viral response (EVR)), which are in turn strongly influenced by host *IL28B* genotype[8] and remain the critical variables in response-guided treatment decisions during therapy. Patients such as those with C/C *IL28B* genotype have response rates of ~70–80% with conventional treatment, and a reduced treatment duration of 24 weeks with two drugs may lead to comparable sustained viral response (SVR) rates. It may be possible to reduce treatment duration with the addition of an antiviral to the 24-week treatment regimen and possibly even reduce the duration to 12 weeks for C/C patients who demonstrate favorable on-treatment response (e.g., RVR). Patients with the likelihood of intermediate levels of response as estimated from their genotype (heterozygous C/T allele patients), but having otherwise favorable predictive factors, might be offered similar regimens. With a favorable pretreatment profile, some patients may achieve SVR with DAAVs only (i.e., without pegIFN/RBV) or be "rescued" with a conventional interferon-based regimen in case of treatment failure. DAAV, directly acting antiviral; pegIFN, pegylated interferon; RBV, ribavirin.

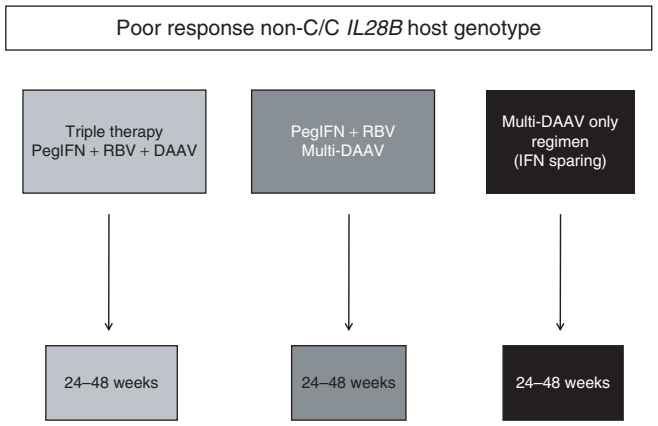

Figure 2 Possible future treatment strategies for the poor-response non-C/C *IL28B* genotype patient profile. For patients who are likely to have lower response to treatment as estimated from their genetic profiles (T/T allele), or for those whose genotype indicates a potential of an intermediate response (heterozygous C/T allele) but with other negative prognostic factors (e.g., age >40 years, or significant fibrosis), DAAVs will be required for achieving adequate sustained viral response (SVR) rates. Whether inclusion of DAAVs will also allow reduced treatment durations for these patients is not yet known. As more agents and classes of DAAVs become available, an IFN-sparing multi-DAAV regimen with potent antiviral effect may eventually permit reduced duration of duration while preserving SVR rates. These possibilities suggest the need to characterize the host genotype (e.g., *IL28B* genotype) more fully and broadly in the future so as to allow for the selection of optimal individualized treatments. DAAV, directly acting antiviral; pegIFN, pegylated interferon; RBV, ribavirin.

For chronic hepatitis C, an ideal treatment must be efficacious, simple, safe, and well tolerated, and also of short duration and cost-effective. It is critical to develop a better understanding of how recent findings related to host *IL28B* genotype will interact and potentially influence drug discovery and development. In this context, some relevant questions arise.

CAN THE DURATION OF pegIFN/RBV THERAPY BE SHORTENED ON THE BASIS OF *IL28B* GENOTYPE?

Host *IL28B* polymorphism allows identification of ~40% of genotype-1 HCV-infected Caucasians who have a 70–80% chance of SVR with current standard-of-care treatment, which consists of 48 weeks of pegIFN plus RBV. A commercial test is now available to guide clinicians and patients in the treatment decision-making process. We are of the opinion that knowledge of the effect of host *IL28B* genotype, in conjunction with viral kinetics during treatment, will be useful in helping to individualize drug regimens and treatment durations while preserving SVR rates (**Figures 1** and **2**).

Numerous prospective studies have found that patients infected with HCV genotype-1 and who achieve rapid viral response (RVR, viral negativity after 4 weeks of pegIFN/RBV) subsequently have a high rate of SVR after receiving only 24 weeks of pegIFN/RBV.[18–20] It is likely that this "responder" group is enriched with patients who carry the favorable *IL28B* C/C alleles,[8] and that 24 weeks of treatment may be sufficient to maintain high SVR rates (**Figure 1**). Theoretically, even a shorter duration (perhaps 12 weeks) may be possible in the future, using combination therapies with directly acting antivirals (DAAVs).

In poor response IL28B genotype patients (**Figure 2**), for whom current standard-of-care therapy provides only a 20–30% chance of a cure, strategies to improve efficacy will remain a major focus. Extending pegIFN/RBV therapy beyond 48 weeks has generally proved to be disappointing.[16,17] Improved efficacy will therefore be achieved by the introduction of DAAVs and other novel agents working through alternative mechanisms of action (e.g., cyclophilin inhibitors). As shown conceptually (**Figures 1** and **2**), DAAVs may permit a shorter duration of therapy in certain patients, whereas more aggressive strategies with quadruple or alternative regimens may be necessary for others.

HOW WILL *IL28B* AND DAAVs INTERACT AND FIT INTO FUTURE HEPATITIS C TREATMENTS?

DAAVs inhibit HCV replication by specifically targeting viral enzymes such as nonstructural (NS) 3 protease and NS5A protease. Agents such as the NS3 protease inhibitors telaprevir and boceprevir will likely soon be approved.[21,22] Phase II randomized clinical trials with these agents and 24–48 weeks of

Table 1 Important research priorities related to *IL28B* host genotype

Basic research priorities
To identify the causal variant and its biological functionality
To pursue novel drug design and development strategies based on these pathways and targets
To characterize the biosignatures of response to different treatment regimens (e.g., with 2, 3, and 4 drugs)
Next important clinical studies[a]
Prospective: 24 vs. 48 weeks of pegIFN and RBV therapy for *IL28B* C/C genotype, treatment-naive patients infected with HCV genotype 1
Prospective: reduced-duration, triple-drug regimens for treatment-naive patients with favorable *IL28B* C/C genotype
Prospective: novel immunomodulator therapies and their interaction with host *IL28B* genotype
Prospective: all-oral DAAV regimens for patients with favorable *IL28B* C/C genotypes in small trials, followed by a period of observation. If such regimens fail, these patients can be effectively rescued with pegIFN and RBV
Retrospective: evaluation of the interaction of *IL28B* genotype with treatment response in well-characterized cohorts of patients currently in phase I–III protease and polymerase inhibitor trials
Determine frequencies of *IL28B* polymorphisms in different populations globally

DAAV, directly acting antiviral; HCV, hepatitis C virus; pegIFN, pegylated interferon; RBV, ribavirin.

[a]Prospective clinical trials should include DNA collection at baseline after appropriate informed consent, allowing IL28B analyses and/or stratification so as to avoid bias in interpreting results.

pegIFN/RBV demonstrate overall SVR rates of 67–74%, suggesting that DAAVs may overcome the effect of a "poor-response" *IL28B* genotype to some extent.[21,23] Subgroup analyses demonstrated the benefit of adding DAAVs for patients with other "difficult-to-treat" characteristics and showed excellent response rates in patients who had previously failed treatment.[24,25] Recently, a small study of a heterogeneous Japanese cohort suggested that the *IL28B* polymorphisms remain a critical pretreatment predictor of response to telaprevir-based treatment.[26] Therefore, careful analysis of the interaction of *IL28B* type and responses in different groups of patients who have been exposed to these novel protease inhibitors is the critical next step.

Genotype-1 patients who are homozygous for the *IL28B* good-responder allele have observed SVR rates of 70–80% on standard pegIFN/RBV. Two phase II trials of telaprevir—one in the United States and the other in Europe—compared standard-of-care therapy to 12 weeks of telaprevir combined with three different treatment arms of 12, 24, or 48 weeks of pegIFN/RBV.[18–27] SVR rates were similar (67% vs. 61%) when duration was decreased from 48 to 24 weeks. Interestingly, in the European study's 12-week arm, 60% of the patients (49 of 82) achieved SVR.[27] This suggests that 12 weeks of therapy may be feasible with a three-drug regimen for selected patients (the corresponding arm in the US study had only 17 patients, with an SVR rate of 35%). For these patients, DAAVs may be included not with the primary objective of increasing SVR rates but with the aim of reducing treatment duration (**Figure 1**). Whenever possible, we also need to revisit such large clinical trials and, with informed consent of the subjects, collect DNA to reevaluate the results in different treatment arms in the light of the *IL28B* genotype. Prospective studies stratified for host *IL28B* genotype are needed in order to definitively identify patients in whom DAAV-containing shorter treatment regimens may be equally effective.

The *IL28B* polymorphism is associated with a decline in viral load in the first 12 weeks of therapy.[8,9] How this knowledge will interact with the introduction of DAAVs will become evident in time. It may be relevant to the risk of drug resistance. Poor-response non-C/C host *IL28B* genotypes, possibly associated with slower viral suppression, may be at higher risk for the emergence of resistant variants and subsequent viral breakthrough.

The scene is therefore set to diversify HCV treatment regimens with multiple new classes of potent DAAVs, drug combinations, and durations based, in part, on host *IL28B* genotype. The specific details will unfold as more data become available in the next 2 years. Shorter, highly potent multidrug regimens may provide the optimal "cocktail" for patients with good-response *IL28B* genotypes. Patients with poor-response *IL28B* genotypes will likely be tied to longer-duration courses of pegIFN/RBV combined with the newer agents (**Figure 2**). Economic modeling and formal, independent cost-effectiveness analyses will clearly be important to evaluate the relative costs and benefits of these varied strategies.

IS THERE A GROUP OF PATIENTS WHO MAY BE CURED WITHOUT IFN?

PegIFN and RBV are likely to remain the backbone of HCV therapy for the foreseeable future. The addition of a DAAV does not permit the exclusion of RBV without compromising RVR rates, viral breakthrough, SVR, and relapse rates.[27]

The limited data available on combination DAAV therapy without IFN and RBV suggest that there is potent additive antiviral action and that the oral combination regimens are well tolerated.[28] Five such exploratory "all-oral" trials are under way, but most will receive these DAAV combinations for a short period of time and then start pegIFN/RBV. Such trials might be most appropriate initially in patients with the responder C/C IL28B genotype, given that patients who fail an exploratory all-oral regimen could be "rescued" with pegIFN/RBV, safe in the knowledge that they have an excellent chance of cure. If, on the other hand, the virus can no longer be detected after stopping such oral regimens, it provides a basis to assess efficacy and cure rates with such strategies. This would be a major milestone denoting progress in the field. Finally, such "game-changing" regimens might need to include two, three, or four oral antivirals, acting at different targets so as to increase the likelihood of success in different populations of patients. *IL28B* profiling and a broader understanding of the "biosignatures" predictive of cure with all-oral drug regimens will be a priority.

WHAT ARE THE IMPLICATIONS FOR RESEARCH IN THIS FIELD?

A knowledge of host *IL28B* genotype opens exciting new areas of basic research and clinical inquiry (**Table 1**). There is an

important need for basic research to improve our understanding of the fundamental biology and clinical observations related to *IL28B* polymorphisms and how these polymorphisms are related to IFN-λ and other IFNs to affect innate and cell-mediated immunity.[12,13,15,17] Identifying the causal variant and characterizing its effect on gene function and expression may provide new biological insights of therapeutic relevance.

An obvious implication for researchers is the development of more refined and targeted IFN therapies. Because of its more restricted receptor distribution, specific targeting of the IFN-λ-R may avoid some of the systemic side effects associated with pegIFN/RBV therapy. A phase I trial of IFN-λ-1 (*IL29*) has recently demonstrated potent antiviral activity, with reduced systemic side effects in patients with genotype-1 chronic hepatitis C.[29]

There is a clear need for prospective clinical research trials to understand how existing agents (pegIFN/RBV) and future strategies will integrate with host *IL28B* genotype. The goal will be to optimize efficacy, patient convenience, and safety. The *IL28B* genotype now has major implications for HCV research protocol design in terms of stratification of trials involving novel agents and treatment strategies. This will be particularly important for smaller conceptual studies, for which ascertainment bias may have a significant impact on the interpretation of results.

CONCLUSION

Knowledge related to host *IL28B* genotype has altered the HCV treatment landscape from a clinical and research perspective. Effective translation of this emerging knowledge will require engagement on the part of patients, clinicians, researchers, health administrators, and the pharmaceutical industry. The outcome, we hope, will be shorter, more efficacious, and better tolerated treatments for patients, with individualized strategies based on the host genotype. All would benefit in such a system.

ACKNOWLEDGMENTS

P.J.C and A.J.T. received funding support from the Duke Clinical Research Institute and the Richard B. Boebel Family Fund. A.J.T. received funding from the National Health and Medical Research Council of Australia and the Royal Australasian College of Physicians. The IDEAL study was funded by the Schering-Plough Research Institute, Kenilworth, New Jersey.

CONFLICT OF INTEREST

J.G.M. has received research and grant support from Schering-Plough, Merck, and Roche. J.G.M. has received consulting fees and acted in an advisory capacity for Schering-Plough and Merck. J.G.M. and A.J.V.T. are co-inventors of a patent application based on the *IL28B* discovery. P.J.C. declared no conflict of interest.

1. Goldstein, D.B. Pharmacogenetics in the laboratory and the clinic. *N. Engl. J. Med.* **348**, 553–556 (2003).
2. Ge, D. *et al*. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* **461**, 399–401 (2009).
3. Tanaka, Y. *et al*. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat. Genet.* **41**, 1105–1109 (2009).
4. Suppiah, V. *et al*. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat. Genet.* **41**, 1100–1104 (2009).
5. McCarthy, J.J. *et al*. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* **138**, 2307–2314 (2010).
6. Rauch, A. *et al.*; Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* **138**, 1338–1345, 1345.e1 (2010).
7. Thomas, D.L. *et al*. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* **461**, 798–801 (2009).
8. Thompson, A.J., Muir, A.J., Sulkowski, M.S. *et al*. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 Hepatitis C Virus. *Gastroenterology*; **139**, 120–129, e118 (2010).
9. Neumann, A. *et al*. IL28B Polymorphism is significantly correlated with IFN antiviral effectiveness already on first day of pegylated interferon-a and ribavirin therapy of chronic HCV infection [poster 2011]. 45th Annual Meeting of the European Association for the Study of the Liver, Vienna, Austria, 14–18 April 2010.
10. Liu, C.H. *et al*. Pegylated interferon-alpha-2a plus ribavirin for treatment-naive Asian patients with hepatitis C virus genotype 1 infection: a multicenter, randomized controlled trial. *Clin. Infect. Dis.* **47**, 1260–1269 (2008).
11. Gad, H.H., Dellgren, C., Hamming, O.J., Vends, S., Paludan, S.R. & Hartmann, R. Interferon-lambda is functionally an interferon but structurally related to the interleukin-10 family. *J. Biol. Chem.* **284**, 20869–20875 (2009).
12. Sheppard, P. *et al*. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol.* **4**, 63–68 (2003).
13. Kotenko, S.V. *et al*. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol.* **4**, 69–77 (2003).
14. Mennechet, F.J. & Uzé, G. Interferon-lambda-treated dendritic cells specifically induce proliferation of FOXP3-expressing suppressor T cells. *Blood* **107**, 4417–4423 (2006).
15. Doyle, S.E. *et al*. Interleukin-29 uses a type 1 interferon-like program to promote antiviral responses in human hepatocytes. *Hepatology* **44**, 896–906 (2006).
16. Maher, S.G. *et al*. IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol. Ther.* **7**, 1109–1115 (2008).
17. Marcello, T. *et al*. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology* **131**, 1887–1898 (2006).
18. Jensen, D.M. *et al*. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* **43**, 954–960 (2006).
19. Ferenci, P. *et al.*; Austrian Hepatitis Study Group. Peginterferon alfa-2a and ribavirin for 24 weeks in hepatitis C type 1 and 4 patients with rapid virological response. *Gastroenterology* **135**, 451–458 (2008).
20. Zeuzem, S. *et al*. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *J. Hepatol.* **44**, 97–103 (2006).
21. McHutchison, J.G. *et al.*; PROVE1 Study Team. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N. Engl. J. Med.* **360**, 1827–1838 (2009).
22. Berman, K. & Kwo, P.Y. Boceprevir, an NS3 protease inhibitor of HCV. *Clin. Liver Dis.* **13**, 429–439 (2009).
23. Kwo, P. *et al*. Interim results from HCV SPRINT-1:RVR/EVR from phase 2 study of boceprevir plus peginterferon alfa-2b/ribavarin in treatment-naive subjects with genotype-1 CHC. *J. Hepatol.* **48** (suppl. 2), S46 (2008).
24. Everson, G.T. *et al*. Telaprevir, Peginterferon Alfa-2a and Ribavirin improved rates of sustained virological response (SVR) in "difficult to cure" patients with chronic hepatitis C (CHC): a pooled analysis form the PROVE1 and PROVE2 trials. *Hepatology* **50** (4 suppl.), abstract 1565 (2009).
25. McHutchison, J.G. *et al.*; PROVE3 Study Team. Telaprevir for previously treated chronic HCV infection. *N. Engl. J. Med.* **362**, 1292–1303 (2010).
26. Akuta, N. *et al*. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* (2010); e-pub ahead of print 26 March 2010.
27. Hézode, C. *et al.*; PROVE2 Study Team. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N. Engl. J. Med.* **360**, 1839–1850 (2009).
28. Gane, E.J. *et al*. Combination therapy with a nucleoside polymerase (R7128) and protease (R7227/Itmn-191) inhibitor in Hcv: safety, pharmacokinetics, and virologic results from inform-1. *Hepatology* **50**, 394a–395a (2009).
29. Muir, A.J. et al. Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. *Hepatology* (2010); e-pub ahead of print 14 May 2010.