In the past 2 years the ability to successfully eradicate chronic HCV infection has been substantially enhanced by two landmark scientific breakthroughs. Firstly, the advent of HCV-specific direct-acting antiviral (DAA) compounds has resulted in a significant increase in sustained virological response rates in HCV genotype-1-infected treatment-naive patients and in non-responders to prior pegylated interferon and ribavirin therapy. Secondly, the identification of single nucleotide polymorphisms near the interleukin 28B (IL28B) gene that potently influence spontaneous and treatment-induced recovery from HCV infection has improved our understanding of hepatitis C pathogenesis, has helped spur the development of novel antiviral therapies and has provided a tool for pretreatment decision making in the context of genotype 1 HCV infection. In light of these advances, we review the interaction between these two discoveries and discuss the role of IL28B genotyping prior to treatment initiation in the context of both standard therapy and the newly approved DAA-based regimens. The role of IL28B genotyping is particularly relevant as DAAs are more expensive and carry a higher risk for anaemia and other drug-related side effects, while decision-making based on IL28B genotype may permit non-DAA-based treatment algorithms.

The natural course of HCV infection results in chronic disease in 70–80% of cases [1], representing one of the leading causes for chronic liver disease worldwide. It is estimated that >170 million people are currently infected with the virus [2]. Chronic infection is associated with a risk for progressive liver disease and the subsequent development of liver cirrhosis and liver failure, as well as hepatocellular carcinoma [3]. In those with decompensated liver disease, the only effective treatment is liver transplantation. However, this is associated with a high risk of reinfection and accelerated progression to fibrosis and cirrhosis [4,5].

HCV virology

HCV is a member of the *Flaviviridae* family with a positive-sense, single-stranded RNA genome of approximately 9,600 nucleotides [6]. The viral genome is translated into one polyprotein that is subsequently cleaved by viral and cellular proteases and processed into 10 structural and non-structural proteins [7–9]. Among these, the NS3/4 protease that is essential for viral replication, and NS5B, an RNA-dependent RNA polymerase, have recently become targets for new direct-acting antiviral (DAA) agents. HCV shows marked genetic and antigenic diversity with six major genotypes identified that have significant nucleotide sequence divergence (>30%) [10]. Genotypes 1, 2 and 3 are predominant in the Western world and East Asia (HCV genotype 1 being the most common), genotype 4 is limited to the Middle East, Egypt and Central Africa, and genotypes 5 and 6 are rare and usually limited to South Africa and Southeast Asia [11].

Standard therapy for chronic HCV infection

The standard of care (SOC) treatment for chronic HCV infection in many parts of the world is a combination of pegylated interferon (PEG-IFN)-α and the adenosine/guanosine analogue ribavirin (RBV), as per the current guidelines of the American Association for the study of Liver Diseases (AASLD) and European Association for the study of the Liver (EASL) [12,13]. Treatment success following therapy is defined as an undetectable
HCV RNA in blood 24 weeks following the cessation of treatment, and is referred to as a sustained virological response (SVR). This is usually associated with normalization of liver tests in non-cirrhotic patients and, in the majority, represents permanent viral eradication. There are two time points during therapy when the viral load should be assessed to guide clinical decision making: rapid virological response (RVR) if HCV RNA is undetectable at week 4 and early virological response (EVR) if HCV RNA is undetectable at week 12 [13]. HCV genotypes 1, 4, 5 and 6 infections are more difficult to treat with SVR rates of 40–50% despite a longer duration of treatment (48 weeks) and with higher doses of RBV (15 mg/kg bodyweight/day) [13]. Genotypes 2 and 3 have better SVR rates (approximately 75%) and require only 24 weeks of therapy with a fixed lower dose of RBV (800 mg/day) [13], particularly in non-cirrhotic patients.

Recently, this SOC has been subject to a major re-evaluation due to two major breakthroughs in the field, namely the advent of HCV-specific DAAs and the identification of single nucleotide polymorphisms near the interleukin 28B (IL28B) gene, which strongly predict spontaneous and treatment-induced recovery from infection [14–18]. Of note, according to the current AASLD guidelines, triple therapy with PEG-IFN, RBV and either of the new DAAs (boceprevir [BOC] or telaprevir [TPV]) should be considered the new optimal therapy for HCV genotype 1 infections [12].

Direct-acting antivirals for the treatment of HCV infection

Numerous DAAs are currently at various stages of development with their aim being to improve the SVR rates. Only two of these, TPV and BOC, have been approved in Europe and North America by the regulatory authorities for the treatment of genotype 1 HCV infection. Both drugs are orally available and act as reversible inhibitors of the viral NS3/4 protease, which is essential for HCV replication. Importantly, both drugs are relatively genotype-specific, with poor efficacy against non-1 HCV genotypes and rapidly evolving viral resistance during monotherapy, well-described in both drugs [19,20]. Nevertheless, triple therapy with PEG-IFN/RBV and TPV or BOC not only improves SVR rates by approximately 20–30% in previously untreated patients, but also in prior unsuccessfully treated patients, that is, relapsers, partial responders (showing a 99% decline in HCV RNA by week 12, but never HCV-RNA-negative) and null-responders (those with <2 log drop in viral load at week 12 of therapy) [21–24]. TPV is given as a thrice-daily dose in combination with PEG-IFN/RBV for the first 12 weeks of treatment and depending on the response, PEG-IFN/RBV dual therapy is continued further for a total of 24–48 weeks. SVR rates range from 75% in treatment-naive patients to 83% in relapsers, 59% in partial responders and 29% in null-responders [25,26]. By contrast, BOC, also given thrice daily, is commenced after a 4-week lead-in with PEG-IFN/RBV dual therapy and recommended to be continued for up to 44 weeks [12]. Triple therapy is continued for 24 weeks and then, based on response and regimen, additional dual therapy of up to 24 weeks may be given. SVR rates range from 66% in treatment-naive patients to 83% in relapsers and 52% in partial responders [22,23]. Both DAAs have also been examined in the context of response-guided therapy, with good success and non-inferior SVR rates. However, triple therapy comes at the cost of increased drug-related adverse effects, such as anaemia, skin rash, peri-anal discomfort and dysguesia, compared to dual therapy [27].

**Role of IL28B polymorphisms in the treatment of chronic HCV infection**

Four large multicentre studies published in late 2009 and early 2010 sought host genetic markers that may predict treatment responsiveness in patients with chronic genotype 1 HCV infection [14–17]. A unique aspect of these reports was that they all employed genome-wide association methodology instead of a candidate gene approach; all the studies identified the same gene region near the IL28B gene, that is, the polymorphisms rs8099917 [14,15,17] and rs12979860 [16,17], to be strongly associated with treatment responsiveness. Of note, rs8099917 is in partial linkage disequilibrium with rs12979860 in Caucasian populations [16,17], and in near-complete linkage disequilibrium in East Asians. Ethnic differences in the frequency of these polymorphisms were subsequently shown to explain, in part, the inferior response rates in African Americans as compared to Caucasians and, conversely, the increased response rates in Asians as compared to Caucasians [16,18]. Subsequent reports have shown that these polymorphisms, as would be expected, also predict spontaneous recovery from HCV infection [18,28]. In terms of viral kinetics, the rs12979860 protective genotype is associated with better first- and second-phase viral load declines during treatment of genotype 1 infection [29,30] and with significantly enhanced RVR and complete EVR rates across different ethnic groups [31]. While achieving a RVR is the strongest on-treatment predictor of SVR, even if an RVR is not achieved, the rs12979860 protective genotype may still have some effect on treatment outcome [31]. The clinical impact of this is unclear. As expected, this was later also confirmed for rs8099917 [32].
Role of \textit{IL28B} polymorphisms in the context of DAA-based regimens

The addition of the currently approved DAAs, TPV or BOC, to PEG-IFN/RBV greatly improves SVR rates in the relevant genotype 1 HCV Phase III registration studies [22–26], while \textit{IL28B} genotyping consistently enables better prediction of SVR in PEG-IFN/RBV-based regimens. Hence, the obvious question is whether the benefit of certain \textit{IL28B} variants persists during the more potent DAA-based triple-therapy regimens. Only limited data is currently available on this subject, mostly in the form of conference abstracts and presentations.

To date, the TPV–\textit{IL28B} interaction is the best studied. Jacobson \textit{et al}. [33] and Pol \textit{et al}. [34] examined TPV-based triple therapy in the context of \textit{IL28B} genotype in two large genotype 1 registration trials, that is, the ADVANCE trial for treatment-naive patients and the REALIZE trial for previous non-responders. A combined total of 981 patients were available for this analysis [33,34]. In the ADVANCE trial, patients were either treated with PEG-IFN/RBV/TPV triple therapy for 12 weeks and then continued PEG-IFN/RBV for another 12 or 36 weeks based on their extended RVR (eRVR; HCV-RNA-negative at weeks 4 and 12), or received triple therapy for 8 weeks and subsequently received PEG-IFN/RBV for a further 16 or 40 weeks based on their eRVR [25]. Only Caucasian patients from the ADVANCE trial (\(n=454\)) were chosen for this subanalysis.

Overall, SVR rates were substantially higher in the TPV-treated groups with 78% and 67% in the TPV arms versus 38% in the PEG-IFN/RBV arm (Table 1). Importantly, addition of TPV improved SVR rates across all \textit{IL28B} genotypes, with \textit{IL28B} rs12979860 CC responder genotype achieving a remarkable 90% SVR rate with a 12-week course of TPV as compared to 64% with PEG-IFN/RBV. Even the CT and TT non-responder genotypes achieved improved SVR rates of 71% and 73% as compared to 25% and 23% with PEG-IFN/RBV. In total, 72% of the patients with the \textit{IL28B} rs12979860 CC responder genotype and 54% and 48% of the CT and TT ‘non-response’ genotypes achieved an eRVR with TPV compared with 16%, 3% and 0% of the control arm patients receiving PEG-IFN/RBV, respectively (Figure 1A) [33]. Among eRVR patients receiving TPV, 97% of \textit{IL28B} CC and 88% of CT/TT had an SVR with 24 weeks of treatment as compared to 67% of \textit{IL28B} CC and 38% of CT/TT non-eRVR TPV-treated patients, treated for 48 weeks (Figure 1B). In summary, TPV improved response rates.

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<th>\textbf{Table 1. Sustained virological response rates with telaprevir and boceprevir depending on \textit{IL28B rs12979860} genotype}</th>
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<td>\textbf{Telaprevir: treatment-naive}</td>
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Data are % (\(n/total n\)). Data based on [33,34,40]. *Pooled treatment arms with and without lead-in. †Pooled non-responders and relapasers as subgroup data not available. BOC, boceprevir; ND, not determined; PEG-IFN, pegylated interferon; RBV, ribavirin; RGT, response-guided therapy; TPV, telaprevir.
across all IL28B genotypes, but particularly so in those with either the IL28B non-response genotypes (CT or TT) or with the IL28B responder genotype (CC) that failed to reach eRVR. Similar results were observed in a smaller study of Japanese patients that included treatment-naive and previously relapsed patients, where the IL28B rs8099917 genotype seemed to predict EVR and SVR to triple therapy with TPV [35,36]. These studies also demonstrated that viral factors such as amino acid substitutions in the HCV core region might independently influence the response to triple therapy with TPV [35–37].

The REALIZE trial examined re-treatment with TPV in a mixed group of relapsers, partial responders and null-responders [24]. The majority of patients included were Caucasian (94%) and, as expected, the IL28B responder genotype was most common in the relapser population, whereas the IL28B TT non-response genotype was enriched in the non-responders cohort. This population was therefore not in Hardy–Weinberg equilibrium due to the selection bias from prior PEG-IFN/RBV non-response [34]. Although the numbers were too small for detailed statistical analysis, it appears that TPV strongly improved SVR across all patient groups in the pooled analysis. Interestingly, IL28B genotype did not significantly alter SVR in previous relapsers and partial responders. There was, however, a trend towards higher response rates in the IL28B CC responder genotype in prior null-responders with an SVR rate of 40% versus 29% and 31% in CT and TT non-responder genotypes, respectively. This may be due to the fact that IL28B is a good indicator in treatment-naive patients of interferon
responsiveness and their chances of achieving an eRVR and subsequent SVR, but in a group negatively selected for relapse or non-response, other mechanisms associated with previous treatment failure, such as resistance, may predominate.

Gellad et al. [38] recently presented cost-effectiveness analysis regarding TPV treatment with respect to IL28B genotypes. They suggested that if a genotype 1 treatment-naive patient has the IL28B CC responder genotype, then the addition of TPV is unlikely to be cost-effective [38]. Notably, although Jacobson et al. [33] reported a benefit of TPV even in the IL28B responder genotypes (Table 1), the numbers for the IL28B responder genotype subanalysis were small and larger studies are required to elucidate the role of TPV in IL28B responder genotypes.

With respect to BOC and IL28B polymorphisms, data from the registration trials were recently presented with retrospective genotyping for IL28B rs12979860, rs12980275 and rs8103142 [39] in treatment-naive (SPRINT-2; 563/1048) and previously treated patients (RESPOND-2; 259/394) [22,23]. This included a treatment arm with full-length treatment (48 weeks) and a response-guided therapy arm. As shown in Table 1, BOC improved response rates across all IL28B genotypes in all groups, except for the IL28B responder genotype in treatment-naive patients. The latter result is likely due to the near optimal SVR rate of 78% in that group [39]. By contrast, in African American patients, SVR rates were improved in all IL28B genotypes [39]. If patients did not achieve a ≥1 log drop by week 4, BOC improved response rates, especially in IL28B non-responder genotypes. Of note, this >1 log drop of HCV RNA by week 4, which is the length of the BOC lead-in with PEG-IFN/RBV, was recently shown to be strongly associated with the IL28B CC responder genotype (Figure 1C) [40]. Accordingly, the IL28B CC responder genotype is more likely to achieve HCV-RNA-negative status by week 8 of triple therapy as compared to the IL28B non-responder genotypes (89% versus 52%; Figure 1D). In previous relapers or partial responders, IL28B genotypes helped identify patients likely to be eligible for a shorter course of therapy. Thus, IL28B genotype influences SVR rates on triple therapy with BOC, though this effect was attenuated as compared to that seen with SOC treatment.

A disadvantage of the currently approved NS3/4 inhibitors TPV and BOC is that they have to be administered three times per day. Newer agents that inhibit this protease, such as BI201335, given once daily may simplify treatment regimens with potentially very good response rates in proof-of-concept trials. In the SILEN-C1 trial, which examined triple therapy with BI201335, addition of a DAA seemed to ameliorate the effect of the poor responder IL28B genotype [41]. A general conclusion therefore appears to be that as SVR rates improve, the effect of IL28B genotype will diminish and larger cohort sizes will be required to demonstrate a role for IL28B genotype on viral kinetics and SVR rates.

TMC435, another NS3/4 inhibitor given once daily, has been shown in the Phase Ib PILLAR trial to significantly improve response rates in treatment-naive, HCV genotype 1 patients [42]. Although only published as an abstract, the data suggests that TMC435 may improve SVR rates especially in IL28B non-responder genotypes (CC 97%, CT 80% and TT 67%) as compared to PEG-IFN/RBV dual therapy (CC 100%, CT 50% and TT 50%).

Preliminary data is also available on mericitabine, a potent inhibitor of the HCV NS5B RNA-dependent RNA polymerase, as part of the PROPEL study. Although a small study with just 96 patients, the report suggested that IL28B genotype may influence the effect of NS5B polymerase-inhibitor-based triple therapy regimens, particularly in terms of achieving undetectable HCV RNA at week 4 [43].

In another small study (n=45) using the NS5A inhibitor, BMS-790052, in combination with PEG-IFN/RBV, IL28B CT non-responder genotype likely contributed to treatment failure [44].

With respect to combination therapy with NS3/4 and NS5B inhibitors and IL28B genotype, only a small Phase II trial has been published [45]. This study investigated the non-nucleoside NS5B polymerase inhibitor, tegobuvir, and the NS3 inhibitor GS-9256, in various combinations with PEG-IFN and RBV [45]. Although the numbers per group were too small (n=15) to perform a meaningful analysis with respect to the IL28B genotype, there was a trend towards a greater magnitude in the reduction of HCV RNA among IL28B responder genotype patients [45].

Overall these data regarding DAAs in combination with PEG-IFN and RBV suggest an ameliorated but persistent impact of IL28B genotype on treatment responses. Of note, a recent study suggests that IL28B polymorphisms may affect viral kinetics even in the context of interferon-free regimens, in this case a 13-day course of a combination of the NS5B inhibitor mericitabine and the NS3/4A protease inhibitor danoprevir [46].

Conclusions

The studies discussed above clearly outline the remarkable effect DAAs and IL28B polymorphisms have had on treatment-induced clearance of chronic HCV genotype 1 infection. More importantly, it appears that despite the potency of the DAAs, host genetic factors, that is, IL28B genotype, still determine to variable degrees SVR rates on DAA-based regimens. This
is likely due to the strong association of favourable *IL28B* genotypes with first phase viral decline as well as with RVR observed in the course of PEG-IFN/RBV dual therapy [29,31,47]. Of note, RVR itself is a stronger predictor of SVR than *IL28B* genotype, but this is partially explained by the RVR being strongly selected for by the *IL28B* genotype. *IL28B* non-responder genotypes seem more likely to benefit from the addition of DAA, irrespective of prior treatment failure or choice of DAA [33,34,39]. Obviously, most of the studies discussed here are still preliminary and the numbers are too small to reach a definitive conclusion on this matter. Second- and third-generation DAAs with higher potency will likely weaken the effect of host genetics such as the *IL28B* polymorphism.

Nevertheless, we propose the following decision model (Figure 2). Treatment-naive patients with HCV genotype 1 should be genotyped for the *IL28B* polymorphism (*rs12979860* or *rs8099917*). If the patient has an *IL28B* responder polymorphism, dual therapy with PEG-IFN/RBV may be considered as a more cost-effective regimen with better tolerability and less severe adverse effects [38]. This needs to be weighed against the potential to reduce treatment duration by the addition of a DAA. If the patient has a non-responding genotype or previous treatment failure, triple therapy with a DAA-based regimen should be offered in order to provide the patient with an optimal chance for achieving an SVR. *IL28B* responder genotype patients that have relapsed or failed prior PEG-IFN/RBV therapy should be encouraged to consider re-treatment with triple therapy as they show the best SVR rates, but this decision needs to be considered in light of the likely future improvements in HCV therapy. Sensitivity to interferon and suppression of pre-existing resistant viral variants by PEG-IFN and RBV are a major determinant of treatment response. Patients with previous treatment failure are at risk of being negatively selected for both. Thus, although most in need for the new DAAs, these patients are at risk of being unable to prevent evolution or selection of DAA-resistant variants and subsequent triple therapy failure [48].

In terms of the best *IL28B* polymorphism to test, both *rs12979860* and *rs8099917* are likely to provide similar information, except for African Americans where *rs12979860* seems to predict SVR better [16]. Other *IL28B* polymorphisms have now been identified with similar or better predictive value for current SOC [49], and combinations of *IL28B* polymorphisms with HLA-C genotypes further improves prediction of treatment failure [28]. These genetic variants are likely to predict response to DAA regimes slightly better than either *rs12979860* or *rs8099917* alone. In clinical practice, ideally, at least these two polymorphisms should be tested as recent evidence suggests that in carriers of the *rs12979860* non-responder allele the additional determination of *rs8099917* improves response prediction [50].

Despite the improvements in SVR by using the currently available DAAs, we still do not have a clear algorithm for those individuals with prior null-response to therapy. Although better than PEG-IFN/RBV therapy, the addition of BOC increases SVR rates in prior non-responders to only 23–30% [23] and with TPV to 37% in previous null-responders and 55% in partial responders [51]. This leaves the majority of previous non-responders, that is, up to 70%, facing repeat treatment failure and the evolution of viral mutations, which affect the choice of future DAA-based therapies. Although the currently available data regarding the role of *IL28B* polymorphisms in this group is insufficient, it is plausible that *IL28B* genotyping, especially for some of the newer polymorphisms [49], might aid in decision-making. An alternative consideration for this patient group may be to hold off on therapy until newer drugs become available, especially in those patients with a non-responder *IL28B* genotype.

Future studies will need to address other interconnected issues, such as the effects of ethnicity, liver
fibrosis, the optimal DAA regimen and the value of response-guided therapy in the context of IL28B genotype. Another issue will be the higher risk for the development of viral resistance in HCV genotype 1a as compared to 1b [52].

The currently available DAs, TPV and BOC, are fairly specific for HCV genotype 1 and are unlikely to be relevant for other HCV genotypes, especially in the difficult-to-treat HCV genotype 4 patients, where SVR rates seem to be IL28B dependent [17,53,54]. Thus, determining a patient’s IL28B genotype will remain a useful tool, even in non-1 HCV genotypes, at least until interferon-free treatment regimens become available.

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DRB and JG have applied for patent protection for genetic tests to predict response prediction in hepatitis C. GA declares no competing interests.

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1. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral genetic tests to predict response prediction in hepatitis DRB and JG have applied for patent protection for the University of Sydney Medical Foundation.

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