

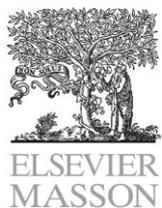
**Criteria Grid**  
**Best Practices and Interventions for the Diagnosis and Treatment of Hepatitis C**

<b>Best Practice/Intervention:</b>	Chevaliez S. et al. (2011) Mechanisms of non-response to antiviral treatment in chronic hepatitis C. Clinics & Research in Hepatology & Gastroenterology, 35(Suppl 1):31-41.			
<b>Date of Review:</b>	February 8, 2015			
<b>Reviewer(s):</b>	Christine Hu			
<b>Part A</b>				
<b>Category:</b>	Basic Science <input type="checkbox"/> Clinical Science <input type="checkbox"/> Public Health/Epidemiology <input type="checkbox"/> Social Science <input type="checkbox"/> Programmatic Review <input checked="" type="checkbox"/>			
<b>Best Practice/Intervention:</b>	<b>Focus:</b> Hepatitis C <input checked="" type="checkbox"/> Hepatitis C/HIV <input type="checkbox"/> Other: _____ <b>Level:</b> Group <input checked="" type="checkbox"/> Individual <input type="checkbox"/> Other: _____ <b>Target Population:</b> <u>people with HCV infection</u> <b>Setting:</b> Health care setting/Clinic <input checked="" type="checkbox"/> Home <input type="checkbox"/> Other: _____ <b>Country of Origin:</b> <u>France</u> <b>Language:</b> English <input checked="" type="checkbox"/> French <input type="checkbox"/> Other: _____			
<b>Part B</b>				
	<b>YES</b>	<b>NO</b>	<b>N/A</b>	<b>COMMENTS</b>
<i>Is the best practice/intervention a meta-analysis or primary research?</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Overview of the mechanisms involved in non-response (lack of sustained virological response) to the current and future standard treatment of chronic hepatitis C infection through the use of published data
<i>The best practice/intervention has utilized an evidence-based approach to assess:</i>				
<i>Efficacy</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
<i>Effectiveness</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
<i>The best practice/intervention has been evaluated in more than one patient setting to assess:</i>				

Efficacy	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Effectiveness	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	

	YES	NO	N/A	COMMENTS
<i>The best practice/intervention has been operationalized at a multi-country level:</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Articles referenced may originate from various countries.
<i>There is evidence of capacity building to engage individuals to accept treatment/diagnosis</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
<i>There is evidence of outreach models and case studies to improve access and availability</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
<i>Do the methodology/results described allow the reviewer(s) to assess the generalizability of the results?</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	No methodology was given
<i>Are the best practices/methodology/results described applicable in developed countries?</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
<i>Are the best practices/methodology/results described applicable in developing countries?</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Telaprevir and Boceprevir are relatively new drugs and studies on them may not have been done in developing countries
<i>Evidence of manpower requirements is indicated in the best practice/intervention</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
<i>Juried journal reports of this treatment, intervention, or diagnostic test have occurred</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<i>Clinics and Research in Hepathology and Gastroenterology</i>
<i>International guideline or protocol has been established</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>The best practice/intervention is easily accessed/available electronically</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Require subscription to download from <a href="http://www.sciencedirect.com">http://www.sciencedirect.com</a>
<i>Is there evidence of a cost effective analysis? If so, what does the evidence say?</i> <b>Please go to Comments section</b>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>How is the best practice/intervention funded?</i> <b>Please got to Comments section</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Not funded

<i>Other relevant information:</i> <hr/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
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# Mechanisms of non-response to antiviral treatment in chronic hepatitis C

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### Key points

- Viral genotype should be assessed before the start of antiviral treatment by means of a molecular method allowing an accurate determination between subtype 1a from 1b.
- HCV RNA levels should be performed regularly but the ideal times points, frequency need to be further evaluated in the context of protease inhibitor-based therapies.
- Resistant variants to DAAs preexist in virtually all HCV infected patients who had never been exposed to drugs before.
- Genotypic resistance testing at baseline is not recommended in clinical practice.
- Accurate methods to assess HCV treatment adherence remain to develop.
- In order to prevent HCV resistance, combination of DAAs with at least additive effects and no cross-resistance should be used.

## Introduction

Hepatitis C virus (HCV) chronically infects approximately 120-130 million individuals worldwide [1]. Approximately 20% of HCV-infected patients develop cirrhosis, which in turn exposes to life-threatening complications [2]. HCV infection has become the main indication for liver transplantation, and is becoming the leading cause of hepatocellular carcinoma in industrialized areas [2]. Mortality related to HCV infection has been estimated at approximately 300,000 deaths per year.

HCV infection is curable by therapy. The current standard-of-care (SOC) treatment is based on a combination of pegylated interferon (pegIFN) alpha-2a or alpha-2b and ribavirin. In patients infected with HCV genotype 1, by far the most frequent HCV genotype worldwide, such treatment leads to a cure of infection in only 40% to 50% of cases, versus approximately 80% in patients infected with HCV genotypes 2 and 3. Failure of IFN alpha-based treatments to eradicate HCV infection has been shown to be at least partly related to virological and genetic determinants. The HCV genotype, viral genetic diversity, the baseline viral load and on-treatment viral kinetics have been identified to play a role in the outcome of therapy. In addition, single nucleotide polymorphisms (SNPs) located in the region upstream of the *IL28B* gene in chromosome 19 have been recently identified to be strongly associated with the ability of SOC to cure HCV infection.

In 2011, new treatments will be available for chronic HCV genotype 1 infection. They will be based on the combination of pegIFN, ribavirin and a specific protease inhibitor, telaprevir or boceprevir. Phase III clinical trials recently presented at The Liver Meeting 2010 have shown that approximately 25% to 35% of treatment-naïve patients, and 50% to 60% of patients who failed to respond to a first course of treatment with pegIFN and ribavirin are not able to cure HCV infection on such triple combination [3]. The emergence of viral variants that are resistant to the

protease inhibitor appears as a major feature of treatment failures in these patients. The selection of resistant variants by direct acting antiviral (DAA) drugs is under the influence of several factors, including viral, pharmacological and host-related parameters. Treatment failure with the triple combination of pegIFN, ribavirin and a protease inhibitor is principally due to an insufficient antiviral response to pegIFN alpha and ribavirin, that favors the growth of resistant viruses selected by telaprevir or boceprevir [4]. Therefore, a strong antiviral response to pegIFN alpha and ribavirin is an absolute prerequisite in order to achieve a cure of infection with these therapies without selecting resistant viruses. Combinations of DAAs that include pegIFN and/or ribavirin or, in the future, without IFN should include DAAs that bear at least additive antiviral effects and no cross-resistance, in order to minimize the risk of treatment failure and resistance. Treatment responses and adherence should be monitored carefully to avoid the development of drug resistance.

The goal of this review is to discuss the mechanisms involved in non-response to the current and future standard treatments of chronic HCV infection.

## Definition of the “non-response”

Treatment failure is defined by the lack of a sustained virological response (SVR), defined by an HCV RNA, which is still detectable with a sensitive molecular assays 24 weeks after the end of therapy. The SVR corresponds to a cure of infection in more than 99% of cases [5]. These definitions apply to both SOC therapy (pegIFN and ribavirin) and to new therapies including telaprevir or boceprevir. Classically, the non-response to SOC treatment is defined by a less than 2 Log<sub>10</sub> HCV RNA level decrease 12 weeks after the start of treatment, or a less than 1 Log<sub>10</sub> HCV RNA level decrease at week 4. A retrospective analysis of the IDEAL study, which included more than 3,000 genotype-1 infected patients, showed a strong correlation between the virological responses at week 4 and week 12 in their ability to predict treatment failure, i.e. a lack of SVR [6].

During therapy, HCV RNA levels should be monitored by means of a molecular method with a lower limit of detection of the order of 10-15 IU/mL, ideally a real-time PCR method. Viral kinetics assessment is essential for evaluating the virological response to SOC in order to optimize the duration of treatment.

## Interferon alpha and ribavirin treatment failure

### Antiviral mechanisms of IFN alpha and ribavirin

Interferons are natural cellular proteins with a variety of actions, including induction of an antiviral state, cytokine secretion, recruitment of immune cells and induction of cell differentiation [7]. After subcutaneous administration, IFN alpha binds specifically to heterodimeric receptors that are present at the surface of most cells, including hepatocytes. IFN alpha fixation to its receptor activates a transcription factor, IFN-stimulated gene factor 3 (ISGF3),

*via* the canonical Jak/Stat pathway. ISGF3 in turn induces the expression of a large number of IFN-stimulated genes (ISGs). The best-known ISGs produced as a result of the IFN cascade induction include 2'-5' oligoadenylate synthetase (2-5'OAS), double-stranded RNA-activated protein kinase (PKR) and myxovirus (Mx) proteins [7]. The products of the ISGs mediate the cellular actions of IFN alpha. They are responsible for the antiviral effects of IFN alpha through two distinct, complementary mechanisms: an antiviral effect resulting in direct inhibition of viral replication; and immunomodulatory effects that enhance the host's specific antiviral immune response and may accelerate the clearance of infected cells [7].

Ribavirin, modestly and transiently inhibits HCV replication *in vivo*, but it efficiently prevents relapses during IFN-ribavirin combination therapy and during triple combination with a protease inhibitor [8-11]. The underlying mechanisms are largely debated. Several mechanisms including immunomodulatory properties (enhancement of Th1 responses), inhibition of the inosine monophosphate dehydrogenase (i.e. depletion of intracellular pools of GTP and dGTP), direct inhibition of RNA-dependent RNA polymerase (RdRp), enhanced mutagenesis leading to "error catastrophe", or modulation of IFN intracellular response are currently proposed [12].

Failure of pegIFN alpha and ribavirin to eradicate HCV is not due to IFN alpha and/or ribavirin-resistant variants. Instead, it is determined by a conjunction of several factors, including the drug regimen, host factors (in particular genetic factors), disease characteristics, and viral factors.

## Factors affecting pegIFN alpha-ribavirin treatment failure

### Viral factors

#### *Viral genotype*

PegIFN alpha and ribavirin fail to eradicate HCV in 50% to 60% of patients infected with genotypes 1 and 4 and in about 20% of patients infected with HCV genotypes 2 and 3 [13-16]. HCV genotypes 1 and 4 are intrinsically more resistant to the antiviral action of IFN alpha than genotypes 2 and 3. In addition, clearance of infected cells in patients who responded to IFN alpha often occurs later and more slowly in patients infected with HCV genotype 1 or 4 than in those infected by genotype 2 or 3 [17,18]. The molecular mechanism underlying the genotype-specific sensitivity to IFN alpha and the final outcome of SOC therapy are unknown.

#### *Genetic diversity*

The role of the genetic diversity of HCV strains in the outcome of therapy is debated. Various studies have focused on the envelope protein hypervariable region, the non-structural (NS) 5A coding region and, more recently, the NS3 region. More than 10 years ago, Enomoto et al. suggested the existence of an "IFN-sensitivity determining region" (ISDR) in the NS5A gene [19]. A large number of reports, mostly from Asia and Japan, supported a link between the number of amino acid changes in the ISDR and the final outcome of therapy, whereas a similar body of literature, mainly originating in Europe and the United States, reported no such correlation [20-23].

### *Baseline HCV RNA level*

Several studies demonstrated that the chance to respond to IFN-based treatment is significantly related to the baseline HCV RNA level. Patients with a high viral load, >800,000 IU/mL, are less likely to clear HCV than those with a low baseline viral load [24-30]. The optimal cut-off (400,000-800,000 IU/mL) to discriminate among patients with a low or a high baseline HCV RNA level remains to be clarified [31].

### *On-treatment viral kinetics*

The kinetics of HCV RNA levels during the first weeks of therapy are the best indicators of subsequent outcome. The presence of a rapid virological response (RVR, defined by an undetectable HCV RNA at week 4 of therapy), an early virological response (EVR, defined by an HCV RNA level which is detectable at week 4 but undetectable at week 12), a delayed virological response (defined by a more than 2 Log<sub>10</sub> HCV RNA drop with detectable HCV RNA at week 12), or no significant decrease of the viral load (less than a 2 Log<sub>10</sub> drop at week 12) helps predict the likelihood of achieving an SVR. Indeed, patients with an RVR have the greatest chance to achieve an SVR (>85%) [32], whereas patients who do not display a substantial viral load decrease are unlikely to respond to therapy [27,33-35].

### Host factors

#### *Non-genetic factors*

Responsiveness to HCV therapy strongly depends on host factors. Age, gender, the presence of cirrhosis, steatosis, insulin resistance, or diabetes, the ethnicity and body weight are predictors of the response to pegIFN and ribavirin. Patients' adherence is also a strong predictor of the outcome of therapy [36].

Co-morbidities such as HIV and/or HBV co-infection, excessive alcohol intake and intravenous or nasal drug use, are associated with lower SVR rates [37]. It was also recently shown that cannabis intake is associated with lower response to IFN treatment [38]. Patients with a history of depression not taking antidepressants and active intravenous drug users are more likely to fail on treatment when they are infected with genotypes 2 or 3 and need additional support [39]. Insulin resistance has been shown to reduce the chance to achieve an SVR [40,41]. Impaired fasting glucose and type 2 diabetes mellitus (T2DM) are both associated with lower rates of response in patients treated with pegIFN and ribavirin. The use of insulin-sensitizing agents, such as pioglitazone, has been reported to increase both RVR and SVR rates [42].

#### *Genetic factors*

Large scale genome-wide association studies (GWAS) have been used to identify a molecular pattern associated to HCV responsiveness to IFN treatment. A number of studies have reported genes that could be involved in various aspects of IFN therapy, including IFN-induced pathways or the pharmacodynamics of IFN alpha [43-45]. However, signature models constructed by multiple logistic regression happened to be poorly sensitive and specific.

In 2009, four independent GWAS studies identified single nucleotide polymorphisms (SNPs) upstream of the *IL-28B* (IFN-lambda3) coding region that were strongly associated with the response to pegIFN and ribavirin treatment [46-49].

Ge *et al.* analysed 1,137 patients with HCV genotype 1 infection. They identified several SNPs upstream of the *IL-28B* gene on chromosome 19 that were significantly associated with the SVR to SOC therapy [46]. Thomas and colleagues showed that the same *IL-28B* variants were associated with the likelihood of a spontaneous clearance of HCV during acute infection [50]. Three other studies identified another, partly overlapping set of SNPs strongly associated with the SVR to pegIFN and ribavirin [47-49]. Although all of the identified variants in these studies were located in close vicinity to the *IL-28B* gene, none of them has an obvious effect on its function and the mechanisms underlying the relationship between *IL-28B* polymorphisms and the response to IFN-based therapy remain unknown [51].

#### *Transcript gene expression*

Liver gene expression has been used to determine response to the treatment. Differential expression of genes directly and indirectly implicated in the mechanism of response to PEG-IFN and ribavirin can explain the variation of the treatment efficiency. Gene expression analyses in liver biopsies have been assessed by real-time polymerase chain reaction or microarray studies. Gene expression in responders and non-responders has been compared, in a study, it reported to ISGs upregulated in non-responder patients. This observation suggests a possible rationale for treatment resistance. The expression profile of a selection of genes related to liver dysregulated during HCV infection has been analyzed according to the response to the treatment. A two gene signature has been successfully identified, IFI27 and CXCL9 [52]. This signature predicts the response to the treatment in 79% of the patients, with a predictive accuracy of 100% in non-responders and 70% in sustained virological responders [52]. The results also suggest that ISGs are upregulated in non-responders before treatment. Gene expression analysis in peripheral blood mononuclear cells are actually lacking for HCV. Analyzing genes expression in PMBC rather than in liver biopsies represents an insight for patients because it does not need any invasive exploration. Many of the genes found to be upregulated between non-responders and responders encode molecules secreted in the serum (cytokines) [53,54]. Thus, they could be used in the development of serums markers as predictors of response to HCV treatment. In a recent study, authors demonstrated that an early expression of interferon-dependent genes could help to predict response rates to PEG-IFN plus ribavirin treatment. Blood samples of 68 patients were collected and results showed that SVR could be predicted by the gene expression of the signal transducer and activator of transcription-6 (STAT-6) and suppressor of cytokine signalling-1 (SOCS-1) in pretreatment samples. Interestingly, even after 24 h of treatment, interferon-dependent genes expression can help to predict the probability of achieving an SVR [55].

#### *CXC chemokine ligands*

A recent study investigated the binding of various ligands to the CXC chemokine receptor 3 (CXCR3). Binding of CXCL10 and CXCL9 were observed to decrease during successful anti-HCV treatment, while CXCL11 binding was not significantly modified [53]. In addition, recent studies identified the chemokine CXCL10 (also known as IP-10) as an important negative prognostic biomarker. Indeed, the plasma CXCL10

level and the number of circulating CXCR3-positive cells were significantly higher in non-responders patients than in either responders or healthy individuals. The NH<sub>2</sub>-truncated CXCL10 form and dipeptidyl peptidase IV activity responsible for cleavage of two amino acids of CXCL10 were significantly higher in non-responders patients than in those including responders and healthy individuals. In vitro studies showed that the truncated form of CXCL10 was unable of the recruiting of CXCR3-positive cells, mediating receptor internalization, and triggering a calcium flux [56]. Two recent studies showed that the combination of *IL28B* genotype and baseline IP-10, the predictive value for discrimination between SVR and nonresponse was significantly improved [57,58].

In a proteomic study, serum protein expression has been assessed in 96 patients with chronic hepatitis C receiving antiviral treatment [59]. Using logistic regression, two protein peaks have been identified. Using the resulting algorithm to predict the response to pegIFN and ribavirin treatment in an independent group of patients has yielded a correct prediction in 81% of the patients [59].

## Resistance to specific HCV inhibitors

### Molecular mechanisms of HCV resistance to DAAs

Viral resistance is defined by the selection of viral variants bearing amino acid substitutions that alter the drug target and thereby confer reduced susceptibility to the drug's inhibitory activity [60]. In infected patients, viral populations exist as complex mixtures of genetically distinct, but closely related variants, referred to as quasispecies [61]. Given that approximately 10<sup>12</sup> viral particles on average are produced every day, with a estimated half-life of approximately 3 hours [62], all possible single and double mutants and a large number of triple mutants are predicted to be generated multiple times each day [63]. Many of these might not be observed because they are lethal or confer reduced fitness and are eliminated [64], but still, a substantial number of them are likely to efficiently replicate and persist.

Typically, in an untreated infected patient, a dominant viral variant (sensitive-type) is detectable within the quasispecies along with viral variants that are present at lower frequencies and may include DAA-resistant ones. Indeed, HCV variants bearing amino acid substitutions that confer primary resistance to DAA drugs generally have reduced replicative capacity compared to sensitive viruses in the absence of drug. In the presence of the drug, replication of the sensitive virus is profoundly inhibited, widely opening the replication space to resistant variants that are not or poorly inhibited by the drug and may grow, sometimes helped by the accumulation of new mutations that improve their fitness.

### Factors affecting the selection of DAA-resistant viral variants

The selection of resistant variants during DDA treatment is under the influence of a number of factors, including viral, pharmacological and host-related factors (Fig. 1).

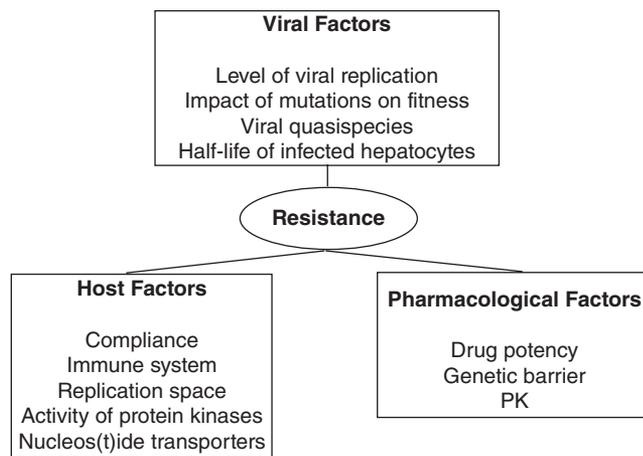


Figure 1 Viral, pharmacological and host factors able to affect the selection of resistant viral variants

### Adherence to therapy

Adherence to therapy and its impact on treatment responses has been studied in several medical conditions. Studies with highly active antiretroviral therapy (HAART) in HIV-infected patients showed that a high degree of medication adherence is required to achieve or maintain the virological response [65]. Poor adherence to HIV and HBV antiviral treatments has similar consequences including an increased risk of drug resistance and treatment failure [66,67]. In chronic hepatitis C, adherence has not been evaluated in clinical trials assessing the efficacy of DAAs alone or in combination with pegIFN and ribavirin. Nevertheless, adherence probably plays an important role. Accurate methods to assess HCV treatment adherence and investigate the determinants of non-adherence remain to be developed, while strategies that optimize adherence must be implemented [68].

### Drug pharmacokinetics

The need to achieve drug concentrations at the primary site of viral replication that are able to suppress viral replication is a key factor for successful antiviral therapy and prevention of resistance. The relative concentration of a DAA drug in the liver can be inferred by measuring the plasma drug levels. Indeed, trough plasma levels of NS3 protease inhibitors were shown to correlate with the antiviral response in Phase 1 studies [69]. The concept of protease inhibitor boosting has been developed to overcome the relatively short half-life and narrow therapeutic index of some of these drugs. Indeed, ritonavir has been shown to increase exposure of a concomitantly administered protease inhibitor [70]. Results with narlaprevir and danoprevir, two protease inhibitors in Phase 2 development, administered in combination with low doses of ritonavir and pegIFN, justified and guided further clinical investigation of once daily dosing regimens [71,72].

### Potency of the DAA

DAAs with a number of viral targets, including NS3 protease inhibitors, nucleoside/nucleotide analogue and non-nucleoside inhibitors of the RNA-dependent RNA polymerase

(RdRp), NS5A inhibitors and cyclophilin inhibitors have been shown to induce significant viral suppression (Fig. 2). These drugs display different antiviral potencies, depending on the class of molecule and the drug in the class. Antiviral potency plays a major role in HCV resistance, as a certain extent of suppression of sensitive variants is needed to allow for the outgrowth of resistant variants. Conversely, highly potent drugs may retain some antiviral activity against resistant viruses.

### Genetic barrier to the resistance

The genetic barrier to the resistance is defined as the number of amino acid substitutions required to confer full resistance to a drug or drug regimen. DAAs with a low genetic barrier to resistance typically require only one or two amino acid changes for high-level resistance to occur. Regimens with a higher genetic barrier to resistance require a greater number of amino acid changes on the same genome to render the treatment ineffective. Apart from cyclophilin inhibitors, all of the drugs currently in development have a low genetic barrier to resistance. Single and multiple variant viruses selected by NS3 protease, non-nucleoside RdRp and NS5A inhibitors have relatively high replication capacities *in vivo*, explaining the rapid outgrowth of resistant HCV variants when these drugs are administered alone. In contrast, variants selected by nucleoside/nucleotide analogues have altered replication capacities. Therefore, their capacity to grow in the presence of the drug is low [4].

### Viral fitness

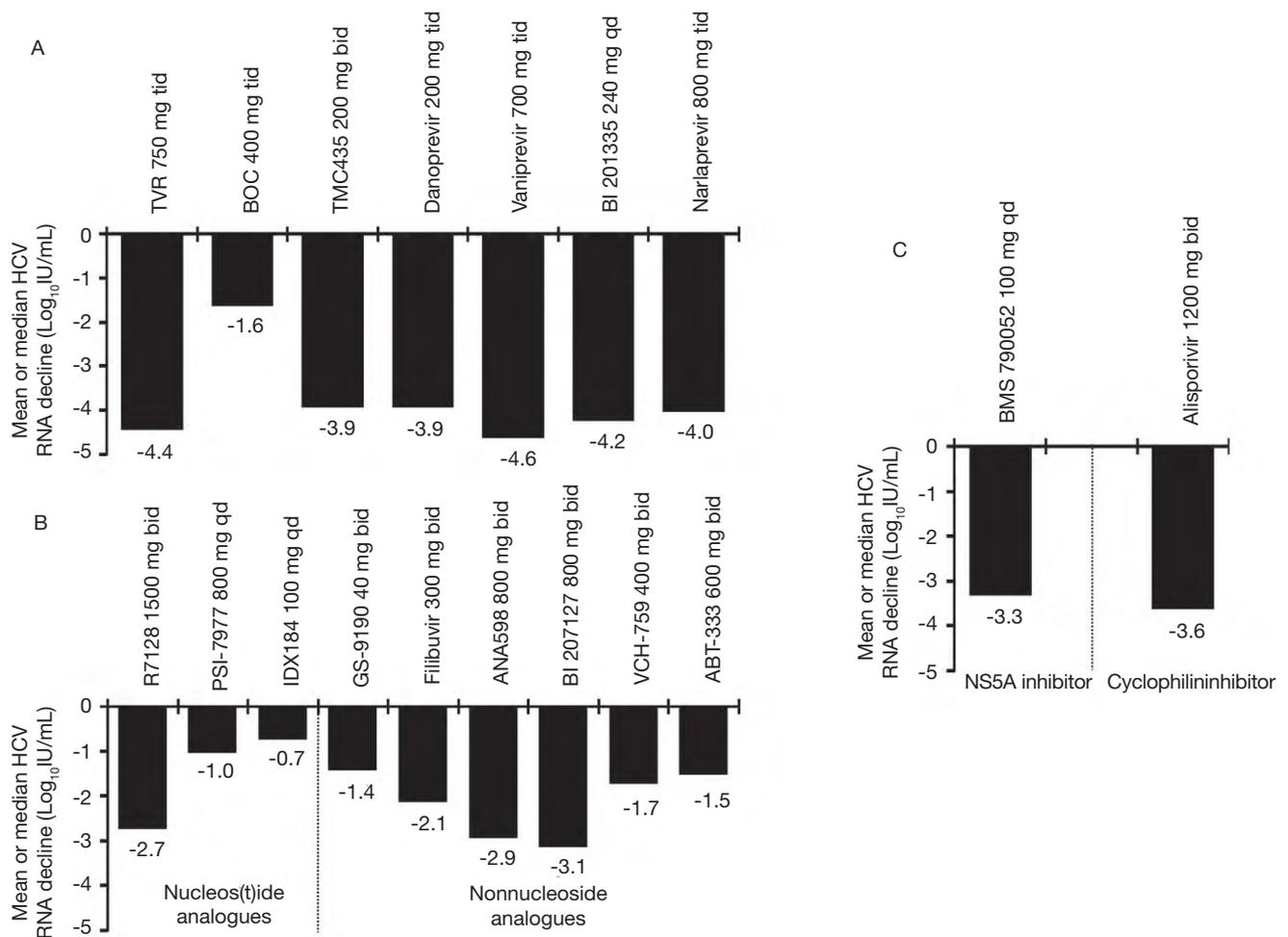
Viral fitness is defined as the capacity of a viral strain to propagate in a given environment. Viral fitness is influenced by several parameters, such as the intrinsic capacity of the virus to replicate, its capacity to escape host immune responses, and the available replication space which cannot be directly measured. The dominant viral population in a quasispecies is, by definition, the most fit one in the host's replicative environment. Viral variants with reduced susceptibility to a given class of DAAs often have reduced fitness compared to the sensitive virus in the absence of the drug [69,73]. When the drug is administered, resistant variants become more fit relative to sensitive variants. Among them, relative fitness is a major determinant of subsequent outgrowth and dominance.

### Patterns of HCV resistance to DAAs NS3 protease inhibitors

A number of peptidomimetic inhibitors of the NS3/4A serine protease are in clinical development, including telaprevir (VX-950) and boceprevir (SCH503034) for which Phase III clinical trial results have been recently reported [3,74].

#### *Telaprevir*

Telaprevir monotherapy selects resistant viral populations within days to weeks. Substitutions conferring telaprevir resistance are located principally at 4 positions within the NS3 protease (positions V36, T54, R155 and A156). The level of resistance conferred *in vitro* varies according to the mutated positions: V36, T54 and R155 substitutions confer



**Figure 2** Antiviral activities of DAA agents, as determined from phase I monotherapy studies in patients infected with HCV genotype 1. Data from different clinical trials (characteristic of enrolled patients, HCV RNA levels at baseline and different molecular methods used for HCV RNA quantification and detection) cannot be compared. (A) NS3 protease inhibitors. (B) Nucleos(t)ide and nonnucleoside inhibitors of RdRp. (C) NS5A inhibitor and cyclophilin inhibitor.

low- to medium-level resistance to telaprevir, whereas A156 and V36+ A156 substitutions confer high-level resistance [69]. Three-dimensional modelling showed that the principal resistance substitutions are located near the protease catalytic triad. Differences between HCV-genotype 1 subtypes 1a and 1b have been described in clinical studies of telaprevir alone or in combination with pegylated interferon (with or without ribavirin). The difference was shown to result from differences in nucleotide frequencies, for instance those that encode amino acid position 155 [69,75]. After termination of telaprevir treatment, the proportion of wild-type variants rapidly increases and, after a median follow-up of 3-25 months, no more resistant variants are observed in a majority of patients who failed in Phase I, I and III clinical studies [69,76,77]. In patients from extend study who failed to achieved a SVR, viral variants associated with decreased sensitivity to telaprevir were no longer detectable in a vast majority of patients after long-term follow-up of 25 months [77].

HCV resistance to telaprevir is significantly less frequent when telaprevir is administered with pegylated interferon and ribavirin [9,78,79]. The recent PROVE 2 phase II clinical

trial showed that ribavirin is needed to efficiently prevent treatment failure associated with telaprevir resistance in patients receiving pegIFN alpha and telaprevir. In patients who failed to eradicate HCV after a triple combination of pegIFN, ribavirin and telaprevir, the viral population at the time of failure is considerably enriched in telaprevir-resistant variants. A number of additional changes at positions not known to be associated with telaprevir resistance, generally located in close vicinity to the catalytic site of the NS3 protease, have been observed [80].

#### *Boceprevir*

During monotherapy, viral variants with substitutions at 6 main positions (positions 36, 54, 55, 155, 156 and 170) within the NS3 protease have been selected. Phenotypic analyses of resistance based on in vitro replicons showed different levels of resistance conferred by substitutions at these different positions. Indeed, changes at positions 36, 54, 55, 155, 156 conferred low to moderate resistance (3.8 to 17.7-fold-change in IC<sub>50</sub>) [73]. Cross-resistance studies have shown that most of the known resistance mutations confer resistance to both boceprevir and telaprevir,

as well as to most of the other protease inhibitors in development [73,81].

HCV resistance to boceprevir is significantly less frequent when boceprevir is administered in combination with pegIFN and ribavirin. The recent SPRINT-1 phase II clinical study showed that the use of standard-dose ribavirin in combination with pegIFN and boceprevir is crucial to improve the SVR rates in genotype-1 infected patients [10]. Ribavirin is also needed to efficiently prevent the emergence of boceprevir resistance. The resistance profiles in patients receiving boceprevir in combination with PegIFN and ribavirin also differ between subtypes 1a and 1b [82].

#### *Others NS3 protease inhibitors (TMC435, danoprevir, vaniprevir, BI 201335, narlaprevir, MK5172)*

TMC435 has an in vitro resistance profile that is partially overlapping with resistance to telaprevir and boceprevir. The resistance pattern of danoprevir showed that all virological rebounds carried a treatment-emergent substitution at position 155 (R155K), while a subset of patients carried an additional D168E substitution in the NS3 protease. Both mutations (R155K and D168E) conferred reduced susceptibility to danoprevir in vitro [83]. Genotypic analysis in patients receiving BI201135 who experienced a virological breakthrough revealed the selection of resistant viral variants bearing amino acid substitutions at positions 168 (D168V) and 155 (R155K). Both variants conferred reduced susceptibility to BI201335 in vitro [84]. Resistance to narlaprevir is characterized by substitutions at positions 36, 155 and 156 [85]. MK5172 is a second-generation protease inhibitor with pan-genotype activity and efficacy against most variants resistant to the first-generation protease inhibitors, except variants at position 156. Resistance patterns in treated patients have not been reported.

#### **NS5B polymerase inhibitors**

Several molecules, including nucleoside/nucleotide analogues targeting the active site of the RdRp and a number of nonnucleoside inhibitors binding to different RdRp allosteric sites, are currently in clinical development.

#### *Nucleoside/nucleotide analogues*

2' - methyl nucleosides select substitutions at position S282 in vitro, which confer low-level resistance and have a poor fitness in vivo. No amino acid substitutions known to confer reduced susceptibility to RG7128, a nucleoside analogue in clinical development, have been selected thus far in patients receiving this drug alone or in combination with pegIFN and ribavirin in the interim analysis of the PROPEL study [86]. Similarly, no resistance has been observed in patients treated with either PSI-7977 or IDX-184, a nucleoside and a nucleotide analogue in development, respectively [87, 88].

#### *Nonnucleoside inhibitors (NNI)*

At least 4 different allosteric binding sites have been identified as targets for inhibition of the RdRp by nonnucleoside inhibitors (NNI). A large number of amino acid substitutions conferring resistance to these drugs have been identified in vitro. Most of them cluster close to their binding sites. No virological breakthrough has been observed over 5 days of

treatment with BI207127, an NNI-site 1 inhibitor, but longer-term studies are needed [89]. Filibuvir (PF-00868554), an NNI-site 2 inhibitor, selects principally M423T substitutions in vivo [90]. VCH-759 is another NNI-site 2 inhibitor with low antiviral activity in monotherapy. Clonal sequence analysis revealed the emergence of resistant variants associated with a viral rebound in a majority of subjects treated with VCH-759 alone, with amino acid substitutions at positions 419, 423, 482 and 494, near the VHC-759 binding pocket [91]. Although resistant variants were previously described from in vitro replicon studies with ANA598, an NNI-site 3 inhibitor, no resistance data in treated patients have been presented so far [92]. GS-9190 and ABT-333 are NNI-site 4 inhibitors with medium antiviral activity in monotherapy. No resistance data in patients with virological breakthroughs have been presented so far.

In combination studies assessing the clinical efficacy of protease inhibitor and a nucleoside inhibitor, no evidence of resistance in INFORM study (danoprevir and RG7128) was noticed [93]. However, another study assessing the clinical efficacy of combination of GS-9256 and tegobuvir with or without SOC showed that ribavirin is also needed to efficiently prevent treatment failure associated with resistance to protease and nonnucleoside inhibitors [77].

#### **NS5A inhibitors**

Although amino acid substitutions have been observed in vitro in the replicon system with BMS-790052, no clinical data on resistance to this class of compounds have yet been presented in vivo when used as part of double combinations of oral drugs with a protease inhibitor or quadruple combinations with a protease inhibitor, pegIFN and ribavirin [94].

#### **Detection and monitoring of HCV resistance HCV RNA quantification assays**

Measurements of HCV RNA levels in blood are indispensable for monitoring and confirming drug resistance. HCV RNA assays based on real-time PCR are currently used for RNA quantification and detection; their results are expressed in IU/mL. As factors other than drug resistance (such as for instance poor or non compliance) can affect HCV RNA levels, it cannot be automatically assumed that rising HCV RNA levels are indicative of drug resistance. Nevertheless, a viral breakthrough in a patient receiving DAAs who is compliant is very likely to be due to drug resistance selection.

#### **Resistance testing**

Direct sequence analysis (population sequencing) and reverse hybridization methods are generally used to identify amino acid substitutions known to confer resistance to antiviral drugs before the viral level increases. Ultra-deep pyrosequencing (UDPS) is a new, highly sensitive method able to detect minor populations of resistant variants (down to 0.1% of the quasispecies) early on therapy [95]. The clinical indications of genotypic resistance testing are currently debated. As resistant variants preexist at various levels prior to therapy in virtually all patients, resistance testing at baseline is not recommended in clinical practice.

The value of early detection of resistance by means of genotypic assays is not clear for patients in whom serum HCV RNA level monitoring may be adequate to diagnose treatment failure due to antiviral drug resistance. In the setting of virological breakthroughs defined by an increase of at least 1 Log<sub>10</sub> compared to the nadir or an HCV RNA that becomes detectable in a patient who previously cleared it, genotypic assays could be used to distinguish between medication noncompliance and the selection of resistant variants. Systematic testing for genotypic HCV resistance is mandatory in clinical trials.

## Prevention of resistance

Within the next few months, a new standard-of-care treatment will be available for the treatment of patients with chronic HCV genotype 1 infection combining pegIFN, ribavirin and a protease inhibitor, either telaprevir or boceprevir. Although an increase in SVR rates is expected, the main clinical issue will be treatment failure associated with selection of viral variants harboring amino acid substitution known to confer resistance to either protease inhibitor. The spread of drug-resistant mutants can be reduced by avoiding unnecessary drug use in rapid virological responders with a favorable *IL28B* genotype. Combination therapy with pegIFN and ribavirin or, in the near future, with other DAAs with at least additive antiviral effects and no cross-resistance should be used. Promising results with double, triple or quadruple combinations including two DAAs, with or without pegIFN and ribavirin, have been recently presented and further studies are ongoing. Virological responses and adherence should be monitored carefully to avoid the development of drug resistance. Assays for serum levels of HCV RNA should be performed regularly but the ideal time points, frequency, and the feasibility in real-life practice need to be further evaluated. It is also needed to establish accurate methods to assess adherence, investigate determinants of non-adherence and develop strategies to optimize adherence.

## Acknowledgment

The authors are grateful to Professors Patrick Marcellin and Jean-Michel Pawlotsky for their helpful comments on the manuscript content and presentation.

## Conflict of interest statement

S. Chevaliez: No conflict of interest.

T. Asselah: Hasn't submitted his conflict of interest

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