Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs)

Tara L. Kieffer1*, Ann D. Kwong1 and Gaston R. Picchio2

1Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, USA; 2Tibotec, Inc., 1020 Stony Hill Road, Yardley, PA 19067, USA

*Corresponding author. Tel: +1-617-4446849; Fax: +1-617-4446210; E-mail: Tara_Kieffer@vrtx.com

Promising results have been observed with an investigational drug class for hepatitis C (HCV), the specifically targeted antiviral therapies for hepatitis C (STAT-Cs), when combined with peginterferon plus ribavirin (Peg-IFN/RBV). This class has the potential to increase sustained virological response (SVR) rates and reduce therapy duration in genotype 1 chronic HCV patients compared with Peg-IFN/RBV alone. However, because of the remarkable sequence variation in HCV (resulting from the high viral replication rate and intrinsically error-prone nature of HCV polymerase), variants with reduced susceptibility to STAT-Cs can occur naturally before treatment, usually at low levels, and can be selected in patients not responding to potent STAT-C treatment. This review first describes how resistance to a STAT-C can develop and then provides an overview of mutations that confer varying levels of resistance to STAT-Cs, which have been identified and characterized using both genotypic and phenotypic tools. We will discuss why an understanding of the selection of variants with reduced susceptibility to a treatment regimen may be important in optimizing the use of this new class of HCV therapy. Strategies for optimizing treatment regimens to increase response rates, and thereby minimize resistance, will be discussed. Finally, although resistance can be a consequence of not achieving an SVR on an initial regimen, there may be alternative treatment options for patients to achieve an SVR in the future. Future potential therapeutic strategies to address patients who do develop resistance to STAT-Cs are discussed, including combination therapy with multiple STAT-Cs with non-overlapping resistance profiles.

Keywords: protease inhibitors, polymerase inhibitors, variants, mutations

Introduction

Chronic infection with hepatitis C virus (HCV) presents a significant healthcare burden, with an estimated 180 million people worldwide currently infected and 3–4 million new HCV infections each year.1 Chronic hepatitis C is progressive in nature such that a proportion of affected individuals are at significant risk of developing severe, life-threatening hepatic conditions. In the developed world, HCV accounts for 50%–76% of all primary liver cancer cases and >30% of all liver transplants,1 and has been estimated to result in a reduction in overall life expectancy in infected individuals of between 8 and 12 years.2

The currently recommended treatment, consisting of a peginterferon plus ribavirin (Peg-IFN/RBV), can result in sustained viral responses (SVR; achieved when HCV RNA is no longer detectable in plasma at 6 months after treatment end). However, response rates vary considerably with HCV genotype.3 While in patients infected with HCV genotype 2 and 3, the combination of Peg-IFN/RBV for 48 weeks results in SVR rates between 76% and 80%,4,5 in patients infected with genotype 1, the most common genotype worldwide,6,7 the SVR rates with 48 weeks of Peg-IFN/RBV only reach 42%–46%.4,5 In addition, these agents are not without significant side effects, including influenza-like symptoms, cytopenias and depression.4,5 More effective therapeutic options with shorter treatment durations are needed to reduce the impact of HCV infection and its associated complications.

Several new therapeutic approaches are being assessed. These include host-targeted inhibitors (such as cyclophilin inhibitors, which may also have an impact on viral replication complex formation or RNA-dependent RNA polymerase regulation), antifibrotic agents, novel ribavirin-like drugs and interferons with improved pharmacokinetics (such as albumin-bound interferon). One therapeutic approach that appears to have a significant potential for improving treatment success is the development of agents that specifically target the replication cycle of the virus [specifically targeted antiviral therapies for hepatitis C (STAT-Cs)]. Theoretically, any step of the HCV life cycle could be targeted for therapeutic intervention. To date, direct inhibition of the NS3/4A serine protease (which processes the HCV polyprotein to generate mature viral proteins), the NS5B polymerase (which replicates the viral RNA genome) and NS5A (which functions as a part of the replicase complex) has demonstrated antiviral proof of concept or efficacy in clinical trials.8

© The Author 2009. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
Of the STAT-Cs currently in clinical trials, inhibitors of the HCV NS3/4A serine protease are the furthest in development. Recent Phase II clinical data have shown that the use of protease inhibitors, either telaprevir or boceprevir, in combination with peginterferon alfa and ribavirin, increased the rate of SVR by at least 20% compared with peginterferon alfa and ribavirin alone in patients infected with HCV genotype 1.9–11 In some cases, increased SVR rates were achieved with a shorter duration of 24 weeks with telaprevir-containing regimens, compared with peginterferon alfa-2a and ribavirin treatment of 48 weeks. In these Phase II trials, the frequency and type of adverse events observed with these STAT-Cs were generally similar to those observed with current standard of care, except for anaemia (up to 37% with telaprevir and up to 56% with boceprevir), skin events for telaprevir (rash and pruritus) and dysgeusia for boceprevir.

However, given the high genetic diversity and rapid mutation and turnover rates of HCV, emergence of viral variants with reduced susceptibility has been observed with STAT-Cs.12 This review will discuss the development of antiviral resistance and provide an overview of mutations associated with reduced susceptibility to STAT-Cs that have been characterized genotypically and phenotypically. We will then describe how an understanding of viral resistance may be important in optimizing STAT-C treatment regimens to increase SVR rates and minimize the clinical impact of resistance. Finally, we will discuss future treatment strategies that may minimize resistance and address future potential treatment strategies for patients who develop resistance to a STAT-C. Given the lack of data in other genotypes, the focus of this review will be on genotype 1 HCV. However, the principles that are discussed should also apply to other genotypes.

Development of resistance to STAT-Cs

Variants with decreased susceptibility to STAT-Cs pre-exist

Similar to some other RNA viruses, HCV replication is characterized by a high rate of replication (up to $1 \times 10^{12}$ particles produced per day).13 In addition, HCV replication is inherently error prone because the RNA-dependent RNA polymerase lacks a proofreading function. The error rate is $\sim 10^{-4}$ for a single mutation,14 thus resulting in one mutation for every genome copied. This inaccurate replication mechanism, coupled with the high rate of replication, results in HCV having extremely high sequence diversity. As a result, in chronic HCV infection the virus exists as a multitude of genetically distinct but closely related viral variants, termed quasispecies (Figure 1). Thus, new variants are constantly being generated, and it has been estimated that every possible point mutation along the HCV genome occurs at least once and probably many times each day.15 Furthermore, variants with $\geq 2$ mutations are also likely to be produced each day. It is not surprising, then, that natural

![Figure 1](https://academic.oup.com/jac/article-abstract/65/2/202/684341)
variants with mutations at sites conferring resistance to STAT-Cs exist in untreated patients.

However, the mixed population of HCV in a patient is usually wild-type (WT) virus (virus fully susceptible to a STAT-C agent) because many drug-resistant variants are less fit. Still, a small proportion of variants with decreased susceptibility to STAT-Cs are present, by chance, in most patients, even before treatment is administered. This is why treatment with a STAT-C alone (monotherapy) can rapidly select for resistant variants in some patients, as has been demonstrated by the selection of variants with resistance after monotherapy with a number of STAT-Cs, including protease inhibitors (BI-201335, boceprevir, ITMN-191, MK-7009, and telaprevir) and polymerase inhibitors (HCV-796, PF-00868554, and VCH-916). STAT-C-resistant variants have been shown to be susceptible to peginterferon or ribavirin in vitro and in vivo, and the addition of these agents has been shown to suppress the emergence of variants in clinical trials of STAT-Cs, including BI-201335, boceprevir, ITMN-191, and telaprevir.

Factors affecting the selection of resistant variants during antiviral treatment
Resistance is not an all or none phenomenon. The presence of resistant variants does not necessarily mean that clinical resistance leading to virological failure will occur. Selection of resistant variants can occur if the virus population is able to evolve in the presence of the drug regimen. Variants with mutations that allow the variant to grow better in the presence of a drug are amplified. This is determined by several factors including: (i) drug selective pressure (potency and concentration at the site of replication); (ii) the genetic barrier to resistance (number of mutations required for complete loss of activity); and (iii) the rate of replication of resistant strains (viral fitness). In this section, each of these factors is examined in the context of emergence of HCV resistance.

Drug selective pressure: potency and concentration at the site of replication
The STAT-C monotherapy trials mentioned above demonstrate that sufficient drug selective pressure must be achieved to inhibit both WT virus and variants with different levels of resistance to a drug. Drug selective pressure depends on the combination of a number of factors, including the drug potency, concentration of the drug at the site of viral replication and adherence to treatment. For HCV, in particular, the concentration of a drug that is present at the primary site of viral replication, the liver, is probably important. Unfortunately, there is no easy way to measure drug levels in the liver of patients. Thus, the relative concentration of a drug in the liver must be inferred by measuring plasma drug levels in patients and correlating it with response.

Genetic barrier to resistance: number of mutations required for loss of activity
The number of mutations (nucleotide changes) required for a virus to be resistant to a drug (or drug regimen) contributes to the genetic barrier of the drug. Regimens with lower genetic barriers to resistance typically require only one or two amino acid changes for high-level resistance to occur, as was seen with most of the first-generation HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs). Regimens with a higher genetic barrier to resistance require a greater number of mutations to be present in the same virus to render the treatment less effective. Variants that have multiple mutations are less likely to be present in a patient before treatment than variants with a single mutation. Thus, regimens with a higher genetic barrier such as those involving combinations of antiviral agents have a greater chance for success in preventing the selection of resistance.

Relative rate of replication of resistant strains: viral fitness
The dominant quasispecies in a viral population is, by definition, that which is best adapted (most fit) to replicate in the current host environment. Viable variants that are less susceptible to a STAT-C often have reduced fitness compared with WT virus. Variants with multiple mutations are less likely to be present in a patient before treatment than variants with a single mutation. Thus, regimens with a higher genetic barrier may differ from those of both HIV and HBV resistance (recently reviewed by Soriano et al.). Like HIV and HBV, HCV has a high replication rate and replicates via an error-prone mechanism (RNA-dependent RNA polymerase). However, HCV has two steps at which mutations can be introduced: first during the production of the negative strand RNA intermediate, and secondly during the synthesis of the new positive strand RNA genome from the negative strand template. This results in the rapid generation of HCV variants in the population. In theory, this may mean that viral variants with reduced susceptibility will be able to gain prominence more rapidly with HCV than HIV, where mutations are introduced to resistance in HCV and resistance in HIV and hepatitis B virus (HBV)
Although HCV shares similarities with both HIV and HBV, distinctive characteristics exist. As a result, the implications of HCV resistance may differ from those of both HIV and HBV resistance (recently reviewed by Soriano et al.). Like HIV and HBV, HCV has a high replication rate and replicates via an error-prone mechanism (RNA-dependent RNA polymerase). However, HCV has two steps at which mutations can be introduced: first during the production of the negative strand RNA intermediate, and secondly during the synthesis of the new positive strand RNA genome from the negative strand template. This results in the rapid generation of HCV variants in the population. In theory, this may mean that viral variants with reduced susceptibility will be able to gain prominence more rapidly with HCV than HIV, where mutations are introduced to resistance in HCV and resistance in HIV and hepatitis B virus (HBV).
only in one step (reverse transcription). However, it may also mean that viral variants are lost from the overall viral pool more rapidly once the drug is withdrawn. In practice, the treatment used will also influence the rate of emergence of resistance.

Probably the most important difference between HCV, HIV and HBV is the goal of antiviral treatment (Figure 2). Treatment of HCV can result in an SVR, suggesting that the virus can be completely eradicated, whereas HIV and HBV cannot be successfully cleared, and the goal of treatment is lifelong suppression with antiviral treatment. This is because for HCV, there appears to be no mechanism for the virus to be physically archived, a mechanism which has evolved very efficiently in HIV and HBV. In the case of HIV, for example, proviral DNA (both WT and resistant) is integrated within the chromosomes of long-lived infected cells, and consequently HIV genetic material can persist for the lifespan of the infected host cell and is replicated along with the host genome upon cell division. HBV genetic material can also persist as a stable covalently closed circular DNA (cccDNA) that forms within the nucleus where it persists as a stable episome. The stable cccDNA persists within the cell and serves as an archive of HBV genetic material acting as a template for the transcription of viral genes. Given that hepatocytes have a long half-life, the limiting factor in eliminating infection is thus clearance of cccDNA reservoirs from these cells.

In contrast, for HCV, there is no known stable reservoir of genetic material; the only source of viral material for producing new HCV virions seems to be the HCV RNA strands present in the cytoplasm of infected hepatocytes. HCV RNA strands only have a half-life of ~3.5 h, and therefore suppression of replication can lead to rapid loss of viral genetic material. This is one of the reasons why in the majority of patients who achieve an SVR, HCV can no longer be detected up to 5 years after stopping therapy, suggesting that the virus has been eradicated. These critical differences from HIV and HBV will hopefully result in resistance being more manageable in HCV treatment.

Tools to monitor and characterize the appearance of variants with varying levels of decreased susceptibility to a drug

Characterizing resistance to STAT-Cs in clinical trials is essential for understanding how to optimize the use of a drug regimen and to provide insight into strategies aimed at maximizing SVR rates and thereby minimizing resistance. Currently complementary methods are used to characterize viral resistance: genotypic and phenotypic assays. However, there are no commercial assays to characterize STAT-C resistance that can be used in routine clinical practice.

**Genotypic (sequence analysis) assays**

Genotypic assays examine the genetic sequence of a target region involved directly or indirectly in the interaction of a drug with its target. Initial characterization of the resistance profile for a drug requires comparing viral sequences before, during and after treatment to detect changes from baseline (pre-treatment) that occur with drug treatment. Genotypic assays can be subdivided according to their level of sensitivity. For example, although population sequencing methods are relatively simple to conduct, they cannot determine linkage between different mutations in a single variant, or detect variants with mutations that are present in less than 25% of the population. The level of resistant variants present before treatment is initiated may be below this limit of detection. More sensitive methods include clonal sequencing or the TaqMan mismatch amplification mutation assay (TaqMAMA), but these may be more costly and time consuming.

Additionally, given the variability of HCV and the possibility that HCV populations may evolve, not all substitutions observed on treatment should be considered drug-selected changes. Appropriate statistical tests for a sequence data set should therefore be employed to identify significant drug-selected substitutions.

**HCV viral fitness assays**

Although not a measure of resistance per se, the viral fitness (replicative capacity) of resistant variants is an important factor, with implications for clinical resistance. Viral fitness is defined as the replication efficiency of resistant variants as a ratio of the replication efficiency of WT HCV. Because the most commonly available infectious virus system is based on a genotype 2a virus (most new HCV drugs target genotype 1), the replication capacity of HCV variants is typically assessed in vitro using a transient replicon system, or can be examined by comparing

---

**Figure 2.** Different virus replication strategies in HBV, HIV and HCV lead to different treatment goals.

cccDNA = covalently closed circular DNA.
colony formation efficiency of the mutant replicon RNA with that of WT variants in co-culture growth competition assays. Additionally, fitness has been determined in vivo by using HCV RNA levels and clonal sequencing to calculate the frequency of a given variant over time after the end of dosing to assess the growth rate compared with WT in the absence of drug-selective pressure.

**HCV phenotypic assays**

In clinical research, viral variants identified by genotypic testing should be tested with a phenotypic assay, both to confirm that the mutation confers resistance to the drug and to quantify the degree of resistance that it confers. Phenotypic assays assess the degree of decreased susceptibility conferred by a substitution(s) by measuring the IC₅₀ (the concentration of drug required to inhibit replication by 50%) in an enzyme or replicon assay. By testing the HCV variants for drug susceptibility *in vitro*, the fold change in sensitivity can be calculated as the IC₅₀ value of the isolate/IC₅₀ value of the reference strain (e.g. WT). Biological and/or clinical cut-offs will be necessary to interpret the clinical significance of these shifts in phenotypic fold change.

**Characterization of STAT-C resistance**

As discussed above, selection of resistant HCV variants during STAT-C treatment is not unexpected. Drug-resistant HCV variants have been observed both *in vitro* and *in vivo* for protease and polymerase inhibitor classes of STAT-C compounds. Some examples of these are summarized in Table 1. *In vitro* resistance studies do not necessarily predict the emergence of variants in patients treated with the relevant drug, which is dependent on the drug concentration and other pharmacokinetic parameters, as described above. Detection of drug-resistant mutations *in vitro* also depends on the particular *in vitro* system used (e.g. a genotype 1b replicon may not predict mutations in genotype 1a patients). *In vivo* data are sparse except for telaprevir and boceprevir, which have been in clinical development for longer than the other STAT-Cs and are consequently the most widely studied.

Table 1 shows mutations that have been shown to confer resistance to a drug at varying levels, typically measured by the fold change in IC₅₀ from WT. For example, the R155K variant confers low levels of resistance (<10-fold increase in IC₅₀ compared with the WT) to boceprevir and telaprevir *in vitro*, and higher levels of resistance to BILN2061 (~250-fold increase) and ITMN-191 (~70-fold increase). However, what is more relevant in the clinic than a fold change is the protein-adjusted fold change and drug exposure. Drug concentrations (Cₐvg) in a patient need to achieve levels that exceed the protein-adjusted IC₅₀ value of resistant variants in order to effectively control these variants clinically. Therefore, clinical resistance will always be relative to the level of resistance conferred by a variant and the drug exposure in the patient. Characterizing the variants that arise clinically can give further insight into the drug levels that may be present in patients and the ability of the regimen to suppress both WT and resistant variants.

Protease inhibitors can be subdivided into macrocyclic (ITMN-191 and TMC435) and linear (telaprevir and boceprevir) inhibitors, both of which bind at the active site of the protease. Therefore, there is some overlap in resistance profiles. For example, the R155K mutation has been shown to confer resistance to both linear and macrocyclic inhibitors *in vitro*, albeit at different levels. Based on the different structures of macrocyclic and linear inhibitors, the selection of variants with changes at different sites outside of the active site may be expected. For example, the 168 position has been shown to be important for macrocyclic protease inhibitors, while the 36 or 54 positions are important for linear inhibitors.

Polymerase inhibitors can be classified into either nucleoside (active site) inhibitors (e.g. NM283 and R7128) or non-nucleoside (allosteric) inhibitors. Resistance profiles for the active site inhibitors have some overlap (e.g. S282T for NM283, R7128 and MK-0608); however, they are quite distinct from the allosteric inhibitors. Allosteric, or non-nucleoside inhibitors can be further grouped into four categories depending on where they bind the polymerase: palm I (e.g. HCV-796), palm II (e.g. GS-9190), thumb (e.g. VCH-759) or finger-loop (e.g. JTK-109). Cross-resistance between these four subcategories is limited, although there is much overlap between inhibitors that bind in the same pockets. For example, the MA414/T mutation has been shown to confer resistance to the palm II class of inhibitors (A-782759, A-837093 and GS-9190).

Cross-resistance occurs when resistance mutations are selected that are common to more than one drug within each class. This is typical for inhibitors that bind the same pocket but not necessarily for inhibitors with the same mechanism of action. Cross-resistance has also been shown for classes of compounds in HIV treatment, and will probably limit the combined use of HCV drugs within certain classes of inhibitors. However, as was the case in HIV with darunavir and tipranavir, two protease inhibitors, new HCV compounds within the same class of inhibitors that have distinct resistance profiles may eventually be developed. Importantly, there is no cross-resistance between classes of compounds that target different mechanisms of action. Thus future drug regimens in HCV are likely to include combinations of multiple classes of inhibitors (NS3/4A protease, NS5B polymerase nucleoside, NS5B polymerase non-nucleoside and NS5A inhibitors).

In addition to conferring different levels of resistance, variants also vary significantly in fitness compared with WT virus. In one study of telaprevir-resistant variants, an inverse correlation between resistance and fitness was found: the most resistant variant had the lowest fitness. Similarly, *in vitro* studies of the polymerase inhibitor R1626 showed that the S96T variant is highly resistant but highly unfit. Interestingly, in an analysis of 21 patients, S96T was not detected after treatment with R1626; only WT virus was present in these subjects. This finding was probably due to the low fitness of the S96T variant, which may have been rapidly outcompeted by WT virus after drug selective pressure was removed.

**Characterizing pre-existing resistance at baseline**

Variants with decreased susceptibility to STAT-Cs are likely to pre-exist at low levels since they are typically fitness impaired (see below) and thus are rarely detected by population sequencing. However, if their fitness approximates that of WT virus they can be present at higher levels and thus can be detected more.
Table 1. HCV variants with decreased susceptibility to STAT-Cs

<table>
<thead>
<tr>
<th>NS3/4A protease inhibitors</th>
<th>NS5B polymerase inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of data</strong></td>
<td><strong>macrocyclic</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
frequently in the untreated population. This has recently been observed in the case of some variants (M414T, C316Y and C316N) with decreased susceptibility to non-nucleoside HCV polymerase inhibitors.\textsuperscript{36} In contrast, a population sequence analysis of the NS3/4A protease in 570 treatment-naïve patients with chronic HCV infection revealed that variants that confer resistance in varying levels to protease inhibitors occur naturally at a very low frequency in the HCV-infected patient population (<1% each).\textsuperscript{36,37} Another study using the more sensitive TaqMAMA assay demonstrated that two variants (T54A and V170A) with reduced susceptibility to boceprevir existed at low levels in the viral population before boceprevir treatment was initiated (in 1 or 2 clones per 90 clones sequenced).\textsuperscript{28}

Although the clinical impact of pre-existing viral variants on treatment outcome requires further study, their presence may not necessarily predict treatment failure. In fact, in one analysis, even patients who had 100% V36M low-level telaprevir-resistant variant at baseline achieved an SVR on a telaprevir-based treatment (3 out of 4 patients with V36M achieved an SVR), suggesting that the presence of resistant variants may not necessarily preclude successful treatment.\textsuperscript{36}

**Characterizing antiviral response to STAT-C treatment**

As shown in Table 2, STAT-C regimens can result in different antiviral response patterns leading to various treatment outcomes. Successful treatment results in an SVR, in which case resistance is not an issue. In contrast, patients who ultimately do not achieve an SVR with a STAT-C regimen can be categorized according to the course of HCV RNA response into virological breakthrough, non-response (null or partial) or relapse. The outcome in terms of development of resistance and strategies to minimize resistance differ between these categories.

**SVR**

Phase II trials of the protease inhibitors telaprevir and boceprevir have shown that a significantly greater proportion of treatment-naïve patients achieved an SVR with triple therapy (STAT-C, peginterferon and ribavirin) than with Peg-IFN/RBV alone.\textsuperscript{9–11} In Phase II studies of boceprevir or telaprevir triple therapy in genotype 1, treatment-naïve subjects, approximately two-thirds of patients achieved an SVR.\textsuperscript{9–11} Therefore, in most treatment-naïve patients treated with triple therapy, resistant variants are eradicated. Even in patients who were treated previously with Peg-IFN/RBV, STAT-C-based combination therapy has shown the potential to increase SVR rates.\textsuperscript{68}

**Virological breakthrough**

In the SPRINT-1 study of boceprevir, 4%-12%\textsuperscript{10} of the patients who received boceprevir, peginterferon and ribavirin experienced virological breakthrough. Similar breakthrough rates were observed in the PROVE1 study of telaprevir, in which 7% of the patients who received telaprevir, peginterferon and ribavirin had virological breakthrough.\textsuperscript{11} Viral breakthrough was more frequent in patients infected with subtype 1a than subtype 1b, most probably due to a difference in genetic barrier.\textsuperscript{69} In the PROVE2 study, the frequency of virological breakthrough was eight times higher in subjects receiving the dual combination of telaprevir and peginterferon (without ribavirin) compared with those who received a triple combination regimen (telaprevir plus Peg-IFN/RBV).\textsuperscript{10} These results suggest that ribavirin plays a significant role in suppressing viral replication and decreasing the rate of breakthrough. To minimize the implications of developing resistance, treatment with a STAT-C should be stopped upon detection of viral breakthrough, to prevent further evolution of resistant variants. A stopping rule to discontinue STAT-C treatment in subjects who did not achieve viral response at week 4 was used in telaprevir clinical trials to help minimize the evolution of resistance.

**Non-response**

Non-response is a term that includes patients with either null or partial responses during treatment (Table 2). Based on clinical studies of patients treated with the STAT-Cs in development, complete absence of response appears unlikely; therefore, the term null response may not be applicable to STAT-C therapy.\textsuperscript{11,70} In contrast, with Peg-IFN/RBV alone, ~15% of patients have a null response.\textsuperscript{71}

**Relapse**

Relapse is likely to arise from the re-emergence of low levels of resistant variants that have not been completely eradicated within the duration of the treatment regimen.\textsuperscript{10} Although it is unknown what constitutes the viral population at the end of treatment since HCV RNA levels are undetectable, sequencing of virus immediately after relapse can give a window into the virus that remained and caused the rebound.

<table>
<thead>
<tr>
<th>Table 2. Classification of outcomes of treatment for chronic HCV infection\textsuperscript{67}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SVR</strong></td>
</tr>
<tr>
<td>Undetectable HCV RNA at the end of treatment and for at least 24 weeks after completion of treatment</td>
</tr>
<tr>
<td><strong>Virological breakthrough</strong></td>
</tr>
<tr>
<td>HCV RNA levels initially decrease (even to undetectable levels), followed by a clinically relevant increase while on treatment (usually 1–2 log\textsubscript{10}), but specific definitions vary between clinical trials</td>
</tr>
<tr>
<td><strong>Non-response</strong></td>
</tr>
<tr>
<td>null response</td>
</tr>
<tr>
<td>HCV RNA levels decrease slowly and to different extents, but never become undetectable</td>
</tr>
<tr>
<td>partial response</td>
</tr>
<tr>
<td>&lt;2 log\textsubscript{10} decrease in HCV RNA levels by week 12</td>
</tr>
<tr>
<td>≥2 log\textsubscript{10} decrease in HCV RNA levels by week 12, but never undetectable</td>
</tr>
<tr>
<td><strong>Relapse</strong></td>
</tr>
<tr>
<td>Undetectable HCV RNA levels at completion of treatment, but becoming detectable during follow-up</td>
</tr>
</tbody>
</table>
Optimizing STAT-C regimens to maximize SVR and minimize resistance

The best way to minimize resistance is to increase SVR rates. Multiple factors are important in optimizing SVR rates with STAT-C treatments. These include the potency of the inhibitor(s), pharmacokinetics (intracellular concentration of the inhibitor), genetic barrier to resistance for the treatment regimen, fitness of resistant variants, host factors, treatment durations, dosage of the drug, dosing interval and adherence to treatment. Breakthrough rates can be decreased by selecting the optimal dose of the STAT-C agent to potently inhibit WT and lower level resistant variants, and using a second (or third) drug with a different mechanism of action to suppress the higher level resistant variants. Additionally, for those patients who do experience breakthrough, treatment with the STAT-C agent should be discontinued to avoid further evolution of resistant variants. Relapse rates can potentially be decreased by optimizing the duration of treatment with all agents in the regimen.

Treatment adherence is also an important element to achieve success when treating chronic hepatitis C infection. Poor treatment adherence is a frequent cause of subtherapeutic drug levels, which in turn have been shown to be a factor in the development of resistance. In HIV, poor adherence to antiretroviral therapy has been strongly correlated with failure to achieve HIV viral suppression, increased rates of resistance, an increase in mortality and a decreased quality of life. Consequently, to reduce the development of viral resistance and the likelihood of virological failure, US and European HIV treatment guidelines encourage physicians to take steps to prevent non-adherence.

Combination therapy has been effectively applied to many viral infections, including HIV and HBV. Variants resistant to a specific STAT-C remain susceptible to peginterferon, ribavirin and drugs from other classes of inhibitors. Telaprevir- and boceprevir-resistant variants can be suppressed by subsequent treatment with Peg-IFN/RBV. Future strategies for treating hepatitis C may involve a combination of STAT-Cs that target several steps within the HCV life cycle.

Future treatment strategies

For patients who do develop resistance, minimal data exist on the persistence of viral variants that can emerge. Unlike HIV and HBV, a lack of archiving in HCV may mean that resistant variants could disappear once the drug is withdrawn. Following cessation of STAT-C-based treatment, the selective drug pressure on the HCV will be removed and the population of viral quasispecies present in the host will be dependent on the viral replicative fitness of each of the variant populations, and the possible pathways to improved fitness. In the absence of drug selective pressure, less fit viral variants are likely to be lost from the viral pool, and WT virus, or more fit variants, will emerge as the predominant variant. What is important is that the virus remains susceptible to the next treatment regimen.

A number of options for managing antiviral resistance remain to be explored in the clinical setting. However, there may be future alternative options for managing patients to achieve an SVR. For example, a patient who does not respond to an initial treatment could still potentially achieve an SVR with a new regimen in the future: by targeting a different protein or binding site; by using combinations of inhibitors against different targets; by using longer treatment durations; or through increased adherence. Treatment with a combination of STAT-Cs that have non-overlapping resistance profiles is a promising strategy for increasing the number of mutations necessary for a virus to achieve resistance. In vitro studies have shown that cross-resistance is possible within a specific class of inhibitors, implying that using two inhibitors with overlapping resistance profiles would be unsuitable. However, several combinations of different classes of STAT-C agents have been examined in vitro, with varying degrees of activity. In addition, initial results from the first clinical trial to investigate the combination of a protease inhibitor with a polymerase inhibitor were recently reported. For patients who do not achieve an SVR with an initial regimen consisting of a STAT-C, peginterferon and ribavirin, combinations of STAT-Cs may provide future therapeutic options.

Conclusions

Clinical trials have demonstrated the significant potential of STAT-C therapies in the future treatment of chronic hepatitis C. STAT-C agents are being developed in combination with peginterferon and ribavirin to inhibit the selection of resistant variants and increase SVR rates. In some patients, resistance does emerge with STAT-C therapies. Clinical trials will determine the best management strategies to minimize the evolution of resistant variants in patients failing STAT-C-containing regimens, such as determining the optimal dose and duration of a regimen and implementing stopping rules in patients who have virological breakthrough. Additionally, programmes to increase adherence to treatment should be implemented. Ultimately, unlike the goal of treatment for HIV, HCV is potentially curable, and therefore resistance will probably be addressed with future treatment options, such as the combination of two or three STAT-Cs with or without peginterferon or ribavirin.

Acknowledgements

We would like to thank Mark Namchuck, Valerie Philippon, Camilla Graham, Isabelle Lonjon-Domanec, Sarah Cowherd and Karen Eisenhauer for critically reviewing the manuscript prior to submission. We also thank Patrick Hoggard PhD and Tom Westgate PhD, from Gardiner-Caldwell Communications, for editorial assistance.

Funding

Financial support for editorial assistance was provided by Tibotec and Vertex Pharmaceuticals Incorporated.

Transparency declarations

T. K. and A. D. K. are current employees of Vertex Pharmaceuticals Incorporated and hold stock in this company. G. R. P. is an employee of Tibotec Incorporated and holds Johnson and Johnson stock.

Patrick Hoggard PhD and Tom Westgate PhD, from Gardiner-Caldwell Communications, provided editorial assistance.
References


28 Curry S, Qiu P, Tong X. Analysis of HCV resistance mutations during combination therapy with protease inhibitor boceprevir and PEG-IFN


