Significant scientific advances have enabled the development of new classes of antivirals for the treatment of HCV. Protease inhibitors were the first approved, achieving substantially higher response rates, with shorter treatment durations, in the majority of genotype 1 infected patients. However, in patients who fail treatment, drug resistant variants frequently emerge. The pattern of resistant variants observed is a result of the specific inhibitor, viral subtype, and level of drug selective pressure. Data suggest the replacement of these variants over time; however, retreatment of these patients is an area of needed investigation. As multiple drug classes progress in development, combinations of agents improve treatment success, increase the genetic barrier to resistance, and provide shorter treatment durations for diverse patient populations.

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Introduction
Worldwide, approximately 170 million people are infected with chronic hepatitis C (CHC), which can lead to significant liver-related health outcomes such as hepatocellular carcinoma (HCC) and cirrhosis [1,2]. Decades of intense research to understand HCV replication and targeted drug discovery efforts have led to significant medical advancements in the treatment of this disease. By targeting HCV proteins or enzymes essential for viral replication, numerous potent direct-acting antivirals (DAAs) have been developed. Currently, there are 3 major classes of DAAs approved or in clinical development for the treatment of HCV. These include inhibitors of the HCV NS3-4A protease, NS5A protein, and NS5B polymerase (nucleoside analogs and non-nucleoside inhibitors). Drugs that bind and inhibit the HCV NS3-4A protease were the first to be approved for the treatment of HCV infection in 2011 (boceprevir and telaprevir). Recently, another protease inhibitor (simeprevir) and the first HCV NS5B polymerase inhibitor (sofosbuvir) were also approved.

Initially being utilized in combination with pegylated interferon and ribavirin, these DAAs have significantly increased sustained viral response (SVR) rates and shortened treatment durations in many patient populations. In patients with genotype 1 HCV infection, protease inhibitor-based regimens achieve SVR rates of 63–80% after 24 or 48 weeks in treatment-naïve patients and 40–60% after 48 weeks in treatment-experienced patients [3–5]. The first protease inhibitors were designed to treat genotype 1 HCV; however, the next generation of protease inhibitors, as well as other classes of inhibitors in development, has shown broader activity against other HCV genotypes [6,7**].

With any DAA treatment, selective pressure can result in the emergence of viral variants that are resistant to the DAA in patients who do not fully eradicate the virus. This is especially true of HCV infection, due to a high genetic diversity resulting from its rapid replication rate and intrinsically high mutation rate [8,9]. Indeed, the emergence of viral variants with reduced susceptibility to DAAs has been observed during treatment in patients with HCV infection [10]. It is for this reason that the DAAs approved as of early 2014 have been used in combination with pegylated interferon and/or ribavirin, which together are able to suppress the drug-resistant variants [11]. Because drugs targeting different HCV enzymes have different resistance profiles [12**], treatment utilizing combinations of DAAs from different classes, thereby obviating the need for pegylated interferon and ribavirin, is the ultimate goal of future therapies.

This review will provide an overview of viral resistance associated with HCV protease inhibitors. Furthermore, it will discuss the importance of taking viral resistance into consideration when selecting regimens and in determining the ultimate clinical impact of resistance on successful treatment of HCV infection. Implications of resistance testing as well as future strategies to avoid the emergence of resistance will be discussed. Because the field of HCV drug development is rapidly evolving, with numerous DAAs and combinations of DAAs in clinical development, this review is not intended to be comprehensive and will focus on protease inhibitors approved as of early...
2014 and those most clinically advanced in clinical development for genotype 1 HCV.

Resistance profiles
The essential role of the HCV NS3-4A protease is to cleave the viral polyprotein into its individual functional protein components. Compounds that bind to the active site of the protease and block this function prevent subsequent downstream steps in the viral replication cycle, ultimately blocking viral replication from proceeding. As of early 2014, there are currently three protease inhibitors approved for the treatment of HCV infection: telaprevir, boceprevir, and simeprevir. Protease inhibitors in late-stage clinical development include asunaprevir, ABT-450, and faldaprevir. Additionally, MK-5172, while in Phase 2 of clinical development, will be mentioned briefly here. Telaprevir [13] and boceprevir [14] are both covalent linear inhibitors; asunaprevir [15] and faldaprevir [16] are non-covalent linear inhibitors; and simeprevir [17], ABT-450 [Pilot-Matias T et al.: abstract in J Hepatol 2011 54(Suppl 1):S485–S486], and MK-5172 [6] are non-covalent macrocyclic inhibitors (Table 1).

While all of these inhibitors bind the active site of the HCV NS3-4A protease, each class differs in structure and in the way they bind to the active site of the protease. As a result, they have significantly overlapping, yet slightly distinct resistance profiles. Substitutions at position R155 (and some substitutions at A156) affect all of these protease inhibitors. The next generation inhibitor, MK-5172, seems to retain activity against the R155 variants, though it is affected by variants at position A156 [6]. The covalent linear inhibitors additionally select for substitutions at positions V36, and T54, A156 (and V55 and V170 for boceprevir). Non-covalent inhibitors primarily select for substitutions at position D168. Additionally, simeprevir selects for substitutions at positions Q80 and S122 (Tables 2 and 3).

Viral subtype differences
Variants observed at each of the aforementioned positions can differ according to viral subtype (1a or 1b) due to the genetic barrier resulting from the wild-type virus consensus sequence. This difference is readily apparent for linear covalent inhibitors (telaprevir and boceprevir), which frequently select for V36M and R155K variants (alone or in combination) in genotype 1a HCV (Table 2) but select for V36A, T54A/S, and A156S in genotype 1b HCV (Table 3). This variability in selection is due to the fact that only a single nucleotide substitution is required to generate the V36M and R155K variants in genotype 1a HCV, whereas two changes are required in genotype 1b HCV (Table 4). It then follows that the V36M+R155K combination variant would need two nucleotide changes to occur in genotype 1a HCV, whereas four would be required for this variant to develop in a genotype 1b background. The R155K variant confers resistance to all protease inhibitors and the V36M+R155K variant confers higher levels of resistance, and may also be more fit than other higher-level resistant variants [18]. Boceprevir can also select for V55A in genotype 1b HCV and V170A in genotype 1a. For the macrocyclic inhibitor simeprevir, the Q80K and S122R variants are observed in patients with genotype 1a HCV (Tables 2 and 3). Because these variants are more frequently found in subtype 1a patients, virologic failure associated with protease inhibitor treatment was more often seen in patients with genotype 1a than with genotype 1b HCV [19] [additional reference: Brass C et al.: abstract in J Hepatol 2011 54:S471–S472].

Level of resistance
Viral resistance variants confer a spectrum of decreased sensitivity to protease inhibitors, with a range of fold-change in IC50 compared with wild-type virus in vitro (Tables 2 and 3). Phenotypic analyses in the replicon system have shown that most single variants at V36(A/M), T54(A/S), and V55A confer lower-levels of resistance (<10-fold change in susceptibility) to covalent linear inhibitors (telaprevir and boceprevir), whereas a little to no resistance is observed with these variants for non-covalent inhibitors. Single variants with certain substitutions at residue 155 (e.g., R155K/T) show cross-resistance to all protease inhibitors (ranging from 4-fold to 360-fold change), and variants with mutations at both positions V36M+R155K generally confer higher-levels of resistance than either substitution alone. Similarly, higher-levels of resistance (6-fold to 270-fold change) to most of these protease inhibitors are achieved with A156 variants. Substitutions at position D168 convey a higher-level of resistance (17-fold to 2591-fold change) to non-covalent inhibitors but confer no resistance to telaprevir or boceprevir. Additionally, a V170A variant confers a 12-fold increase in resistance to boceprevir. Variants at position Q80(K/R) and S122R appear to decrease sensitivity to simeprevir by approximately 7-fold or 9-fold and 20-fold respectively (Tables 2 and 3).

Interestingly, MK-5172, a second-generation non-covalent macrocyclic protease inhibitor, shows improved

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td><strong>HCV protease inhibitors in clinical development</strong></td>
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<tr>
<td>Protease inhibitor</td>
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<tr>
<td>Boceprevir</td>
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<td>Telaprevir</td>
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<td>Asunaprevir</td>
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<td>Faldaprevir</td>
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<td>ABT-450</td>
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<td>Simeprevir</td>
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<td>MK-5172</td>
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activity against R155K and D168Y variants \textit{in vitro}, but the A156T variants remain resistant [6].

**Clinical impact of antiviral resistance**

Viral variants resistant to protease inhibitors typically have a lower replication capacity than wild-type virus \textit{in vitro}, and this lower fitness correlates with the low frequency at which they are observed in untreated patients. Most studies have reported a low natural prevalence of single variants V36L/M, T54A/S, and V55A/I, a very low prevalence of variants D168, and V170, R155K variants, and an absence of the A156V variant (Table 4).

In contrast, the Q80K variant is observed in approximately 30% of patients with genotype 1a HCV infection, with somewhat higher levels (48%) observed in patients with genotype 1a HCV infection who were enrolled in the

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**Table 2**

<table>
<thead>
<tr>
<th>HCV Variant (Fold-Change in IC(_{50}) compared to WT 1a replicon)</th>
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<tbody>
<tr>
<td>Protease Inhibitor</td>
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<tr>
<td>Boceprevir [26]</td>
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<td>Telaprevir [18]</td>
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<td>Asunaprevir [27]</td>
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<td>Faldaprevir [28]</td>
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<td>ABT-450*</td>
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<td>Simeprevir [29]</td>
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</table>


Value from Reference [29]

4Fold-Change in replicon 1b

Value from Reference [30]

5Value from G Lenz \textit{et al.}, abstract presented at the 64th Annual Meeting of the American Association for the Study of Liver Diseases, Washington, DC, USA, Nov 1-5, 2013.

6Value from Reference [18]

7Fold-Change in 1a NS3 in replicon 1b

8Value from Reference [31]

9Value from G Kukolj \textit{et al.}, abstract in J Hepatol 2012, 56(Suppl. 2):S469.


na = not available

Yellow highlighted cells indicate variants that were observed in \textgreater 10% of treatment failure genotype 1a patients

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**Table 3**

<table>
<thead>
<tr>
<th>Resistance profiles in genotype 1b HCV</th>
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<tbody>
<tr>
<td>HCV Variant (Fold-Change in IC(_{50}) compared to WT 1b replicon)</td>
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<tr>
<td>Protease Inhibitor</td>
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<td>Boceprevir [26]</td>
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<td>Telaprevir [18]</td>
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<tr>
<td>ABT-450*</td>
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<td>Simeprevir [29]</td>
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</table>


Value from Reference [29], in replicon 1b

Value from G Kukolj \textit{et al.}, abstract in J Hepatol 2012, 56(Suppl. 2):S469.


na = not available

Yellow highlighted cells indicate variants that were observed in \textgreater 10% of treatment failure genotype 1b patients
US phase 2 and 3 clinical studies of simeprevir. This variant confers a 11-fold increase in resistance to simeprevir and, in turn, impacts the SVR rates in this population [3]. Results from clinical studies showed that treatment-naïve patients with the Q80K variant at baseline achieved SVR rates of 58%, whereas those without it achieved an SVR of 84% with simeprevir treatment [3]. Similarly, in patients previously nonresponsive to pegylated interferon and ribavirin, those with the Q80K variant at baseline achieved an SVR rate of 25% as compared to 75% in those without the variant [20]. The FDA recommends testing for this variant in patients with genotype 1a HCV infection before starting treatment with simeprevir. Outside of this case, baseline screening for protease inhibitor resistance appears unnecessary.

During treatment with protease inhibitors, emergence of resistant variants has been observed in the majority of patients who do not achieve an SVR [21*,22*], particularly in those with virologic failure due to viral breakthrough, meeting stopping rules, or relapse. Virologic failure and the consequent selection of resistance are dependent on patient, viral, and treatment regimen factors. The potency and genetic barrier to resistance will play major roles in the ability of the regimen to inhibit viral variants and prevent virologic failure.

The evolution of these variants over time after treatment is an important question in considering future treatment options for these patients. As mentioned previously, protease inhibitor resistant variants tend to have a reduced fitness level compared with wild-type virus [18], suggesting that they may be outcompeted by wild-type virus. Clinical studies that have monitored the evolution of resistance after treatment show that indeed this appears to be the case. Viral populations predominantly resistant at the end of treatment are replaced by wild-type virus over time in the absence of drug selective pressure [21*,22*]. The timing of this reversion appears to correlate with the fitness of each variant. In the follow-up period of telaprevir phase 3 studies, a Kaplan–Meier analysis showed that the median time to loss of resistance by population sequencing was 10.6 months in subtype 1a and 0.9 months in subtype 1b HCV infections [23]. A separate study utilizing deep sequencing to further understand the disappearance of variants, reported that within 1–4 years after therapy, levels of resistant virus were similar to levels detected before treatment [24].

The ultimate clinical significance of failing a DAA regimen with resistant virus will be understood only upon eventual retreatment of these patients. Thus far, no prospective trials have been conducted to re-treat patients with protease inhibitors who have previously failed a full course of protease inhibitor based treatment. However, a small study investigated patients who had detectable resistant variants present after short-term (14-day) telaprevir therapy and were subsequently retreated with a full course of telaprevir therapy after an interval of about 6 years. Although a small number of patients (n = 9), the SVR rates in this study appeared to be consistent with the results obtained for similar patient populations in phase 3 telaprevir studies [Sarrazin C et al.; abstract in J Hepatol 2013 58:S369–S370]. Another small study looked at 5 patients who had detectable resistant variants after receiving 5 days of simeprevir dosing and were subsequently retreated with a full course of simeprevir therapy after about 1.5 years. Results suggested that resistant variants declined over time to levels below the limit of detection by population sequencing, and did not necessarily preclude successful re-treatment [25*]. While promising, these studies are small, and do not rule out the possibility of resistant variants existing at levels below the limit of detection for deep sequencing assays that may impact re-treatment.

Conclusions
Patients with genotype 1 HCV infection who are treated with protease inhibitor-based regimens achieve significantly higher SVR rates, with shorter treatment durations in many patients, than the previous standard of care (pegylated interferon and ribavirin) [3–5]. However, there are still some patient populations who achieve lower SVR rates, even with these improved regimens, such as patients with other viral genotypes (i.e. 2–6), previous non-response to treatment, cirrhosis, and/or severe liver or kidney disease. Furthermore, resistance to protease inhibitors is an important factor to consider with these treatment regimens.

A low natural prevalence of protease inhibitor resistant variants has been observed, with the exception of the
Although variants successfully. Virologic failure and the consequent selection of resistance are dependent on patient, viral, and treatment regimen factors. Indeed, potency, drug exposure, and genetic barrier to resistance play major roles in the ability of a regimen to inhibit viral variants and prevent virologic failure. The resistance profile of protease inhibitors is highly dependent of viral subtype, and the pattern of variants observed is quite different for patients with genotype 1a compared to genotype 1b infection. The viral populations which are predominantly resistant at the end of HCV protease inhibitor treatment tend to be replaced by wild-type virus over time in the absence of drug selective pressure. Although resistant variants are eventually replaced by wild-type virus, a treatment strategy for patients in whom resistance may still be present is an important issue to address with future regimens.

As new treatment regimens emerge, the next goal of HCV therapy will be to maintain and improve on SVR rates and adverse event profiles, further shorten treatment duration, and omit the pegylated interferon and ribavirin component of the regimen for all patient populations. Significant progress has been made to this end in ongoing clinical studies that are evaluating combinations of DAAs with different mechanisms of action, which have shown to have additive to synergistic potency and no cross-resistance. Additionally, the likelihood of selecting resistant variants may decrease with a rapid and profound viral suppression, which can be achieved by combining several drugs with potent antiviral activity and a high barrier to resistance. Furthermore, all oral DAA combination regimens with good tolerability profiles and convenient dosing intervals are likely to increase patient adherence, thereby also decreasing the likelihood of resistance selection. Indeed, interim results have suggested much improvement in reducing breakthrough and the emergence of resistance with these combination regimens. As these therapies advance further in clinical development, the clinical impact of drug resistance will be minimized, and it is likely that combination regimens will be available in the near future to ultimately treat all patient populations successfully.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


First description of the efficacy of a protease inhibitor based regimen in re-treating patients who developed resistance after previous dosing with protease inhibitor monotherapy.


