Barrier to Resistance: Lessons From 2 Direct-Acting Hepatitis C Virus Inhibitors, MK-5172 and Sofosbuvir

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(See the Major Articles by Howe et al on pages 1657–65 and by Svarovskaia on pages 1666–74.)

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Hepatitis C virus (HCV) is a positive-strand RNA virus that infects >170 million people worldwide, including approximately 3.2 million people in the United States. Chronic HCV infection can result in chronic inflammation, liver damage, and death, with 350 000 deaths per year globally. The most exciting advance in antiviral therapy in the last decade has been the development of direct-acting antivirals (DAAs) against HCV [1, 2]. The studies by Howe et al and Svaroskaia et al, in this issue of Clinical Infectious Diseases, describe sequence and phenotypic analysis of baseline samples and samples from patients with virologic failure to MK-5172, a third-generation HCV protease inhibitor (PI), and sofosbuvir, an HCV polymerase nucleotide active site inhibitor, respectively, and are important contributions to our understanding of how sofosbuvir and MK-5172 can be used in future therapy.

We have entered an interferon-free treatment era in which the majority of HCV patients in the United States and European Union can obtain a sustained virologic response (SVR) or cure with 12 weeks of off-label treatment with 2 approved oral drugs—sofosbuvir, an HCV NS5B polymerase nucleotide (active site) inhibitor, and simeprevir, an HCV NS3 PI. As shown in Table 1, the advanced HCV combinations can be divided into those that are based on an HCV polymerase nucleotide inhibitor (sofosbuvir) vs those that are based on an HCV PI (simeprevir, asunaprevir, MK-5172). When an NS5A inhibitor alone, or in combination with an NS5B polymerase non nucleoside (allosteric site) inhibitor is added to sofosbuvir or PIs, the majority of patients can be cured with 6–12 weeks of dosing.

Properties that affect the ability of an inhibitor to be successfully combined into an HCV combination include (1) potency and pan-genotypic activity, (2) pharmacokinetic properties compatible with co-dosing, (3) safety and tolerability, (4) prevalence of baseline resistance, and (5) the barrier to treatment-emergent resistance. Sofosbuvir is the only approved nucleotide polymerase inhibitor; its high barrier to resistance as well as its efficacy, excellent safety and pharmacokinetic profile, and minimal drug–drug interactions has made sofosbuvir the most desirable drug to include in a combination. MK-5172 is an investigational once-daily pan-genotypic PI that is active against the major known resistant variants to other PIs in vitro [13]. The resistance profile of sofosbuvir and MK-5172 is important to evaluate the role that they might play in combination regimens, the potential to shorten treatment duration by rapidly shutting down the replication of wild-type and resistant variants, and the potential to re-treat patients who have failed an initial round of therapy.

Because of the high genetic variability of HCV due to a replication rate of approximately $10^{12}$ virions/day and an error-prone mode of viral replication, resistance-associated variants with amino acid changes that confer decreased susceptibility to antiviral drugs are continuously being made. The barrier to the development of resistance is determined by multiple factors including, first, the genetic barrier to resistance, which is affected by the number of nucleotide changes required to create a single amino acid substitution that confers resistance. This is exemplified by the effect of genotype/subtype on SVR rates with some PIs. Second, similarly, the barrier to resistance is increased by the number of linked amino acid changes that are required to confer resistance. This is exemplified by single amino acid changes in subtype 1a HCV protease such as R155K and V36M, which confer a low-level decrease in susceptibility in in vitro assays to the first-generation PI
telaprevir. However, the combination of R155K and V36M was associated with increased clinically relevant resistance [14, 15]. Third, the pharmacological barrier to resistance is the ability to achieve drug levels that are above the 90% inhibitory concentration (IC90) of relevant resistant variants. Fourth, the fitness barrier to resistance is an important and often overlooked component of the barrier to resistance. When the resistant variants are highly unfit, they are less likely to grow in the presence of drug, and will rapidly revert back to wild type when drug pressure is withdrawn. Sofosbuvir is the poster child for a high barrier to resistance due to the extremely poor replicative fitness of sofosbuvir-resistant variants. Finally, combinations of HCV inhibitors with different mechanisms of action significantly increase the barrier to resistance because resistant variants must contain linked mutations in ≥2 genes to break through the antiviral pressure of the combination treatment.

In this issue, Howe et al analyzed 264 treatment-naive noncirrhotic patients in a phase 2 study who received MK-5172 (100–800 mg/day) plus pegylated interferon alfa/ribavirin (peg-IFN/RBV). The SVR rate in genotype 1a was 84.8% (134/158), which is lower than that observed in genotype 1b (97.2% [103/106]). More importantly, of the 27 patients who did not achieve SVR, 6 (2.3% [6/266]) met the predetermined criteria for virologic failure, and all were infected with genotype 1a viruses. It has been proposed that subtype genotype 1b has a higher genetic resistance barrier to PIs than genotype 1a due to the requirement of 2 nucleotide changes to generate the R155K mutation in the genotype 1b background [15]. In replicon assays, MK-5172 is highly active against R155K. Therefore, it is surprising to see a subtype difference in the virologic failure rate in this study as well as the detection of relatively high levels of R155K/S in 1 patient. Larger clinical studies are required to clarify these findings.

It is likely that inadequate pharmacokinetic coverage is the primary reason why the 6 patients with virologic failure did not achieve SVR—most were in the low-dose MK-5172 plus peg-IFN/RBV dose group and had measurably low plasma MK-5172 levels. Sequencing of N53 protease and phenotypic analysis were performed to evaluate the emergence of resistant variants. D168 variants associated with a range of 2- to 95-fold loss in susceptibility to MK-5172 were detected. R155 and D168 variants were also detected in the same patients, but linkage information was not reported. Presumably an R155 + D168 variant would be more resistant than either single variant alone; more clinical studies will reveal whether this might be a resistance pathway for MK-5172. Interestingly, baseline sequencing did not detect preexisting MK-5172-resistant variants and instead detected resistance-associated variants to other PIs. This is an encouraging finding that suggests that baseline resistance to MK-5172 might be low; however, the selection of resistance variants during virologic failure suggests that other HCV DAAs are needed.

In the future, it would be helpful to obtain sequencing information from all patients who received a dose of MK-5172 and did not achieve SVR, and not just the subset who met a strict definition of virologic failure. In this case, it would be interesting to know what happened to the 16 patients who did not achieve SVR and were not analyzed for resistance, most of whom were in the high-dose MK-5172 plus peg-IFN/RBV groups. Sequencing of viral samples from patients with short exposures to HCV drugs can be very informative. This is illustrated in this study by a report on 1 patient who discontinued treatment prematurely and in whom D168E and R155K were detected after 3 days of therapy, similar to what was observed with short duration dosing with telaprevir [15].

Sofosbuvir is a potent chain-terminating nucleotide analogue inhibitor of the HCV

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**Table 1. Combinations of Hepatitis C Virus (HCV) Drugs With Different Mechanisms of Action That Confer >95% Sustained Virologic Response in HCV Genotype 1–Infected Patients**

<table>
<thead>
<tr>
<th>Drug</th>
<th>12 wk</th>
<th>8 wk</th>
<th>6 wk</th>
</tr>
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<tbody>
<tr>
<td><strong>Polymerase inhibitor (nuc)</strong></td>
<td></td>
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<tr>
<td>Sofosbuvir + simeprevir (Pl) [8, 9]</td>
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<tr>
<td><strong>Protease inhibitor</strong></td>
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<tr>
<td>Asunaprevir</td>
<td>Asunaprevir (Pl) + daclatasvir (5A) + BMS-791 325 (non nuc) [10]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABT-450/ritonavir</td>
<td>ABT-450 (Pl) + ritonavir + dasabuvir (non nuc) + ombitasvir (5A) + ribavirin [11]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-5172</td>
<td>MK-5172 (Pl) + MK-8742 (5A) [12]</td>
<td></td>
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</tbody>
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Abbreviations: non nuc, non nucleoside inhibitor; nuc, nucleotide inhibitor; PI, protease inhibitor.
NS5B RNA polymerase with a high barrier to resistance, which is primarily derived from the highly conserved binding site in the active site of HCV polymerase.

Also in this issue of CID, Svoraokai and colleagues present the combined analysis of the NS5B sequencing and phenotypic results of 1645 patients from phase 2 and 3 sofosbuvir clinical trials. All patients had an initial response (decrease in HCV RNA) to sofosbuvir-containing regimens; all were sequenced at baseline and no known sofosbuvir resistance-associated variants were detected. The amino acid substitution S282T in NS5B was detected in more than 99% of the viral population in 1 patient who relapsed 4 weeks after sofosbuvir monotherapy. By 8 weeks postinfection, the S282T variant had decreased to 27% and became undetectable by 12 weeks. Phenotypic analysis of the S282T variant at 4 weeks postinfection revealed a approximately 13.5-fold reduction in susceptibility to sofosbuvir and a >98% decrease in replicative fitness.

Analysis of the entire database identified L159F and V321A as treatment-emergent variants; however, they did not confer resistance to sofosbuvir in the replicon system. It has been shown that the double mutant L159F + L320F, but not L159F alone, confers resistance to sofosbuvir in vitro [16]. Taken together, sofosbuvir is unique among DAAs because HCV variants conferring resistance to sofosbuvir have not been detected before, or during treatment, and were rarely seen in follow-up periods in patients who were not cured, all of which support its use as the backbone for a variety of combination therapies.

In the future, it would be helpful if sub-analysis by genotype and by treatment regimen (IFN-contained vs oral DAAs) and analysis of linkage of other amino acid changes to L159F or V321A were evaluated to help understand factors that may contribute to the development of resistance to sofosbuvir. It is important to not be complacent about S282T, as additional mutations can be acquired under combination treatment that confer dual resistance to DAAs. This is illustrated by a recent report of 2 patients who acquired dual-resistance variants containing R155K in NS3 plus S282T in NS5B in a study with mericitabine (nucleotide polymerase inhibitor) plus danoprevir (PI) [17].

Note
Potential conflict of interest. Both authors: No potential conflicts.
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References