Interferon-Free Strategies with a Nucleoside/Nucleotide Analogue

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Abstract

A key to effective interferon- (IFN-) free therapy for hepatitis C virus (HCV) infection is a direct-acting antiviral (DAA) with a high barrier to resistance that can act as the backbone to any regimen. Ideally, this agent should also be active against all HCV genotypes, be well tolerated and have few drug interactions. Nucleoside/nucleotide analogues (NAs) that inhibit the function of the HCV RNA-dependent-RNA polymerase fit these requirements and thus hold promise as a cornerstone for new IFN-free regimens. To date, the issue with this class of agents has been toxicity. Numerous NAs in early clinical development have led to significant toxicity leading to their abandonment. However, sofosbuvir, a prodrug of a uridine NA, has moved through development with a clean-safety profile leading to its recent approval. When combined with ribavirin (RBV) alone, sofosbuvir is effective against genotype 2 and even genotype 3 if duration is extended. There are currently limited data with this combination in genotype 1; however, when sofosbuvir is combined with other DAAs of different classes, it is highly effective in almost all patients. To date, sofosbuvir has been studied with protease, NS5A, and nonnucleoside HCV polymerase inhibitors, including as part of a fixed-dose combination single tablet with the NS5A inhibitor ledipasvir, with very high rates of SVR with as little as 8 weeks of therapy. Combining two DAAs to sofosbuvir may shorten therapy even further. Because of the poor replicative fitness of the S282T sofosbuvir-resistant variant, resistance to sofosbuvir has not been a significant clinical issue in trials thus far. In addition to sofosbuvir, other NAs are in early-stage development. Provided unanticipated toxicity does not emerge, NAs are likely to play a major role as a backbone for future HCV therapy. The rationale for using this class of agents and the clinical data available to date are reviewed.

Keywords

► hepatitis C virus
► interferon
► nucleoside inhibitors
► direct-acting antivirals
► sofosbuvir

The Importance of a Backbone

With improved understanding of the viral life cycle, it became apparent that there were many potential targets to inhibit HCV replication. Through compound screening and rational
drug design, compounds inhibiting the NS3/4A protease, the NS5B RNA-dependent RNA polymerase (RdRp) and the NS5A protein, were identified. Although these novel agents inhibited the virus, often quite potently, with continued treatment, resistant variants began to emerge. HCV replicates at an enormous rate, producing upwards of $10^{12}$ virions per day. With an error-prone polymerase, a random mutation occurs somewhere in the genome approximately once per replication cycle. The high rate of viral production means that mutations occur at every site in the viral genome every day. Rather than a single virus, each infected individual has a large swarm of related but slightly different virions, collectively known as the viral quasispecies, representing an enormous degree of diversity. Most of these mutations are deleterious, providing no replicative advantage to the virus and thus they will remain uncommon among the quasispecies. However, just by chance, some of these variants will be inhibited less potently by antiviral compounds and will therefore have a selective advantage in the presence of drug treatment that inhibits wild-type virus effectively.

During single-drug treatment, the resistant variants will outgrow the wild-type virus and become the dominant viral species in the population. The key to successful DAA therapy is to have a strategy to deal with the selection of these resistant variants. The first approach to deal with DAA resistance was to combine DAAs with pegylated interferon (PegIFN) and RBV. Peginterferon activates a host of genes collectively known as interferon-stimulated genes (ISGs), many of which have antiviral activities. It is difficult for the virus to evade the collective action of all of these genes together and thus IFN provides an effective backbone to prevent the selection of DAA resistance. The IFN effectively controls the pre-existing DAA resistant virus and the DAA potently suppresses all other viral quasispecies. Ribavirin also plays an important role in terms of delaying or preventing the emergence of DAA resistance; however, the mechanisms underlying its effects remain elusive. The difficulty of using an IFN backbone is the toxicity associated with the therapy as well as the fact that some individuals respond poorly to IFN with limited or no ISG induction with treatment, leading to effective DAA monotherapy with a high chance of virological failure.

An alternative to using IFN is to find a DAA or group of DAAs that provide a backbone with a high barrier to resistance. The barrier to resistance may be genetic such that more than one nucleotide change is required for resistance to emerge, which explains the more frequent emergence of the R155K protease inhibitor (PI) resistant variant in those infected with genotype 1a (1 aa substitution required) compared with genotype 1b (2 aa substitutions required). The barrier may be due to high drug exposures that inhibit low-level resistant virus or alternatively the barrier to resistance may relate to fitness of the resistant variant. Although all single (and likely all double) nucleotide variants are produced every day, not all variants are created equal. Mutations at certain sites in the viral genome render the virus very unfit to replicate and hence such variants are very unlikely to replicate to high levels even in the setting of a selective advantage during drug therapy. A substitution of serine for threonine at position 282 (S282T) in the active site of the RdRp confers resistance to many of the NA HCV polymerase inhibitors; however, this variant is extremely unfit, with an $\sim 11$-fold reduction in the efficiency of the polymerase enzyme leading to a replication rate of 3 to 15% that of wild-type virus in in vitro assays. Therefore, although the S282T variant likely pre-exists, at least in some patients, and can be selected for during NA-based therapy, its poor fitness does not allow this variant to expand to any significant degree, explaining the very infrequent identification of this variant even in patients who have failed NA-based therapy. This makes NAs ideal backbones for DAA-based therapy (Table 1). The NA will potently inhibit HCV and if the S282T variant emerges, its poor fitness renders it an easy target for inhibition by any other DAA used in combination therapy.

By contrast, nonnucleoside inhibitors (NNIs) of the NS5B HCV polymerase target the same enzyme as NA polymerase inhibitors but target something other than the active site in the enzyme. Nucleotide substitutions that confer resistance to NNIs generally have minimal effect on viral fitness and therefore are rapidly selected for and quickly emerge as the dominant variant when these agents are used. The difference in NNI and NA polymerase inhibitors highlights that an ideal backbone is not necessarily based on the antiviral target, but is very specific to the characteristics of the variants that confer resistance and potentially to the specific agent. Protease and NS5A inhibitors similarly have a low genetic barrier to resistance due to relatively fit resistance-variants to both classes.

Does this mean that only NAs can be used as a backbone to IFN-free therapy? To date, this class of DAAs is the only one to have a very high barrier to resistance, making it the first

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Potent antiviral activity</td>
<td>History of severe toxicity with NAs for hepatitis C virus and other diseases</td>
</tr>
<tr>
<td>High barrier to resistance</td>
<td></td>
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<tr>
<td>Pan-genotypic activity</td>
<td></td>
</tr>
<tr>
<td>Little potential for drug interactions</td>
<td></td>
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<tr>
<td>Few adverse effects</td>
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Table 1 Advantages and disadvantages of nucleoside/nucleotide analogue- (NA-) based therapy
choice as a backbone currently. This very high barrier allows NAs to be used with only one other class of DAA, even if that other class has a low barrier to resistance. However, any agent that provides a higher barrier to resistance, whether due to the fitness of the resistant variant, the number of substitutions required for resistance or even due to marked potency that overcomes modest resistance, would be an effective backbone. Ideally, such an agent would also be pan-genotypic to allow it to be used to treat all patients with HCV. Newer PIs such as MK-5172 have higher barriers to resistance and more broad genotypic coverage that make them potentially good backbones for therapy. Other approaches such as ritonavir boosting to increase exposure and thus the pharmacologic barrier to resistance may prove useful as well. Host-targeted antivirals (HTAs) inhibit a host function necessary for HCV replication. Because they target the host, it is generally thought that HTAs will have a higher barrier to resistance. This has been borne out with alisporivir, a cyclophilin inhibitor, and miraviren, a microRNA 122 sequestron, both of which have been evaluated in phase 2 studies without IFN, with little or no selection for resistance. The alternative to a backbone with a high barrier to resistance is to use multiple low-barrier DAAs. However a high-barrier backbone potentially allows for shorter therapy, fewer drug interactions and perhaps most importantly, a lower likelihood of persistent resistant variants in those who fail therapy. To date, NAs lead the way, but other classes may be effective backbones as well.

Mechanism of Action

Like most NAs, HCV NAs act as chain terminators to inhibit the production of nascent viral genomes. Nucleosides are sugars bound to one of the bases used for DNA or RNA synthesis, while nucleotides are nucleosides with the addition of a phosphate group. Many nucleotide analogues are prodrugs linked to a molecule that is cleaved preferentially by hepatic enzymes to increase delivery of the nucleotide monophosphate to hepatocytes. Once taken up by cells, nucleosides or nucleotides must be mono-, di-, and triphosphorylated to become active. The triphosphate is then incorporated by the HCV RdRp as a false substrate during RNA replication, leading to chain termination and thus inhibition of viral replication.

Safety Profile

Although mimicking a natural nucleotide is an effective antiviral strategy, nucleotides are also required by the host cell for replication. NAs are designed to serve as specific substrates for the viral polymerase; however, if they also are incorporated by host polymerases, they can lead to toxicity. Incorporation of NAs by the gamma polymerase used to replicate mitochondrial DNA caused several deaths from multiorgan failure with one of the first HBV NAs, aluridine, as well as side effects with some of the early HIV NAs (D4T, DDI), as a result of mitochondrial toxicity. To date, this specific pattern has not been seen with HCV NAs; however, other toxicities have been reported (Table 2). The first HCV NA to reach clinical evaluation was valopicitabine, which although moderately effective, caused significant gastrointestinal toxicity leading to its abandonment. R1626, which progressed to phase 2a trials, was discontinued due to lymphopenia, including two fatal cases. PSI-938, which looked very promising particularly due to its activity against the S282T variant, was found to cause hepatotoxicity and development was suspended. More recently, BMS-986094 was found to cause severe cardiac toxicity in a phase II clinical trial. The agent appeared safe in healthy volunteers and in a 7-day study in HCV-infected patients. However, when treatment was extended to 12 weeks, one individual taking the highest dose in the study (200 mg) presented with severe biventricular heart failure and subsequently died. Eight other patients were hospitalized. The study was suspended and subsequent close cardiac evaluation revealed echocardiographic changes in several other study participants. The precise mechanism of toxicity from this agent is not known and it is unclear whether it was specific to this compound or potentially a characteristic of all 2-methyl guanosine NAs. Notably PSI-938, which caused hepatotoxicity, was also a guanosine-based NA. With this uncertainty, the U.S Food and Drug Administration (FDA) suspended development of other agents with a similar structure and has carefully scrutinized all NAs. VX-135, a uridine NA, was also recently put on partial

Table 2 Nucleoside/nucleotide analogues for hepatitis C virus

<table>
<thead>
<tr>
<th>Agent</th>
<th>Based mimicked</th>
<th>Toxicity</th>
<th>Clinical development</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valopicitabine</td>
<td>Guanosine</td>
<td>GI</td>
<td>Phase 2a</td>
<td>Discontinued</td>
</tr>
<tr>
<td>R1626</td>
<td>Guanosine</td>
<td>Lymphopenia</td>
<td>Phase 2a</td>
<td>Discontinued</td>
</tr>
<tr>
<td>PSI-938</td>
<td>Guanosine</td>
<td>Hepatotoxicity</td>
<td>Phase 2a</td>
<td>Discontinued</td>
</tr>
<tr>
<td>BMS-986094</td>
<td>Guanosine</td>
<td>Cardiac</td>
<td>Phase 2a</td>
<td>Discontinued</td>
</tr>
<tr>
<td>Mericitabine</td>
<td>Cytidine</td>
<td>None known</td>
<td>Phase 2b</td>
<td>In development</td>
</tr>
<tr>
<td>Sofosbuvir</td>
<td>Uridine</td>
<td>None known</td>
<td>Phase 3</td>
<td>Approved</td>
</tr>
<tr>
<td>VX-135</td>
<td>Uridine</td>
<td>Hepatotoxicity at 400 mg dose</td>
<td>Phase 2a</td>
<td>Clinical hold by FDA</td>
</tr>
<tr>
<td>IDX-20963</td>
<td>Uridine</td>
<td>None known</td>
<td>Phase 2a</td>
<td>Clinical hold by FDA</td>
</tr>
</tbody>
</table>

Abbreviations: FDA, U.S. Food and Drug Administration
clinical hold by the FDA because of liver enzyme elevations seen at the highest dose studied (400 mg daily).26 The concern of toxicity has plagued development of new NAs.

However, despite these initial concerns, sofosbuvir, a uridine NA has now been given to over 3,000 patients, including patients with cirrhosis27 and patients awaiting liver transplantation,28 with a remarkably clean safety profile. Specifically, sofosbuvir is a prodrug of 2’deoxy-2’-fluoro-2’-C-methyluridine monophosphate, which must be phosphorylated twice after entering the hepatocyte.29 Sofosbuvir is absorbed intact through the gastrointestinal tract leading to high liver exposure and no significant food effect. It is largely renally excreted and although the main metabolite GS-331007 accumulates in patients with impaired renal function, it is unclear if this has any clinical consequence.15 Despite concerns about NA-safety, sofosbuvir seems to be well tolerated with few treatment-associated adverse effects and no significant toxicity signal to date.15 Importantly, sofosbuvir and other NAs do not interact with the cytochrome P450 system or other major drug metabolizing enzymes and therefore have few if any significant drug-drug interactions, including with calcineurin inhibitors, opioid substitution therapy, and antiretrovirals, allowing for great expansion of their use into populations who have traditionally been difficult to treat.15 The efficacy and safety profile of sofosbuvir has led to its rapid clinical development as a cornerstone of IFN-free DAA therapy.

## Clinical Data with Sofosbuvir

Sofosbuvir is a uridine NA with potent activity against HCV genotypes 1 to 6. Sofosbuvir has been studied with PegIFN and RBV, with RBV alone and with other classes of DAAs, leading to its recent approval with different indications by viral genotype. The clinical data on NAs as part of IFN-free regimens is summarized below (Table 3).

### Genotype 1

**Sofosbuvir plus Ribavirin**

Sofosbuvir has been evaluated with RBV for the treatment of patients with genotype 1 HCV in several relatively small studies, with somewhat conflicting results. In the initial ELECTRON trial, sofosbuvir 400 mg daily with weight-based RBV was given to 25 treatment-naïve patients with HCV genotype 1 and mild fibrosis for 12 weeks. All patients suppressed virus on therapy and 84% (21 of 25 patients) achieved SVR.30 In contrast, in the QUANTUM study, 38 noncirrhotic patients were randomized to 12 or 24 weeks of sofosbuvir plus weight-based RBV and only 10 of 19 (53%) in the 12-week arm and 9 of 19 (47%) in the 24-week arm achieved SVR for an overall 50% response rate.31 The differences in outcome between the studies are not entirely clear, but may relate to a higher percentage of patients with the IL28B favorable CC genotype in the ELECTRON study (44% vs. 16%).

The SPARE study evaluated the importance of RBV dose and studied a population of patients with traditionally poor treatment-response characteristics.32 In this largely African American population, patients were randomized to sofosbuvir 400 mg daily with either weight-based RBV (< 75 kg, 1000 mg daily or ≥ 75 kg, 1200 mg daily) or RBV 600 mg daily for 24 weeks. In the weight-based RBV group, the SVR rate was 68% (17 of 25 patients), compared with 48% (12/25 patients) in those receiving low-dose RBV, highlighting the importance of RBV, at least with sofosbuvir alone. Notably 7 of the 13 patients with advanced fibrosis in this study relapsed, including all 4 patients with cirrhosis.

Sofosbuvir plus RBV alone has also been studied in other contexts including HIV/HCV coinfection and in the pre- and postliver transplant setting, with fairly good results, which are reviewed by Sulkowski and Lens et al, respectively, in this issue. Because of the small numbers and the disparate results, it is difficult to assess the true efficacy of this regimen in genotype 1 patients. As such, sofosbuvir with PegIFN and RBV for 12 weeks was the preferred indication in the recent FDA approval for treatment in patients with genotype 1 infection (see Aronsohn and Jensen in this issue); however, sofosbuvir plus RBV alone for 24 weeks was proposed as an alternative approach in patients who cannot take PegIFN.33

**Sofosbuvir with Other DAAs**

Because sofosbuvir plus RBV alone proved somewhat suboptimal in patients with genotype 1 infection, it seemed natural to combine it with another class of DAA. To date, sofosbuvir has been studied with PIs and NS5A inhibitors, either alone or with additional DAAs (PI or NNI), with impressive results.

**Sofosbuvir plus Protease Inhibitors**

The COSMOS trial evaluated sofosbuvir (400 mg daily) in combination with the second-wave, first-generation PI simeprevir (150 mg daily) with or without weight-based RBV for 12 or 24 weeks.34 The study was separated into two cohorts: 1/ prior null responders to PegIFN and RBV with fibrosis stage F0–2 (n = 80) and 2/ treatment naïve or prior null responders with fibrosis stages F3–4 (n = 87). In cohort 1, the results with 12 and 24 weeks of treatment and with and without RBV were similar (12 weeks: with RBV 96% vs without RBV 93% and 24 weeks: with RBV 79.3% vs. without RBV 93%). These impressive results appear to be valid in patients with advanced fibrosis as well, with SVR4 rates of 100% with and without RBV with 12 weeks of therapy for treatment-naïve patients and 100% and 93% for prior null responders. Data from the 24-week arms have not yet been reported. Based on these studies, 12 weeks of simeprevir and sofosbuvir without RBV appears to be a very efficacious regimen and will likely be used in clinical practice now that both agents have been approved, albeit not specifically for use together. Notably, although no patients experienced viral breakthrough on therapy, the four patients who have relapsed to date with this combination all harbored genotype 1a infection with the Q80K mutation that leads to reduced susceptibility to simeprevir at baseline.35,36 Of the total of 38 patients across treatment arms with the Q80K mutation at baseline, 34 (89.5%) achieved SVR4 or SVR12, compared with the 100% (76 of 76 patients) in those without this mutation.34 Clearly,
Table 3 Summary of clinical trial data with sofosbuvir in interferon-free regimens

<table>
<thead>
<tr>
<th>Population</th>
<th>Study</th>
<th>N</th>
<th>Agents studied</th>
<th>Duration (wk)</th>
<th>SVR* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1 naive</td>
<td>ELECTRON</td>
<td>25</td>
<td>SOF/RBV</td>
<td>12</td>
<td>84%</td>
</tr>
</tbody>
</table>
|                     | QUANTUM       | 38  | SOF/RBV        | 12 vs. 24     | 12 wk: 53%  
|                     |               |     |                |               | 24 wk: 47%  |
|                     | SPARE         | 50  | SOF/RBV        | 12            | WB: 68%  
|                     |               |     | (WB vs LD RBV) |               | LD: 48%   |
|                     | COSMOS        | 19  | SOF/SMV +/- RBV| 12            | SOF/SMV: 100% 
| (Cohort 2 F3/4)     |               |     |                | SOF/SMV/RBV: 100% |
|                     | AI444–040     | 126 | SOF/DCV +/- RBV| 12 vs. 24     | 12 wk: SOF/DCV: 98% 
|                     |               |     |                | SOF/DCV/RBV: 95%  
|                     |               |     |                | SOF/DCV/RBV: 100% |
|                     | LONESTAR      | 60  | SOF/LDV +/- RBV| 8 vs. 12      | 8 wk: SOF/LDV: 95%  
|                     |               |     |                | SOF/LDV/RBV: 100% |
|                     | ION1          | 431 | SOF/LDV +/- RBV| 12            | SOF/LDV: 98%  
|                     |               |     |                | SOF/LDV/RBV: 97% |
|                     | ION2          | 647 | SOF/LDV +/- RBV| 8 vs. 12      | 8 wk: SOF/LDV: 94%  
|                     |               |     |                | SOF/LDV/RBV: 93% |
|                     |               |     |                | SOF/LDV: 95%  |
|                     | SYNERGY       | 60  | SOF/LDV +/- GS9669 (NNI) or GS9451 (PI) | 12 vs. 6 | 12 wk: SOF/LDV: 100% 
|                     |               |     |                | 6 wk: SOF/LDV/9669: 90%  
|                     |               |     |                | 6 wk: SOF/LDV/9451: 100% |
| Genotype 1 treatment failure | COSMOS (Cohort 1 F0–2) | 80  | SOF/SMV +/- RBV| 12 vs. 24     | 12 wk: SOF/SMV: 93%  
|                     |               |     |                | SOF/SMV/RBV: 96%  
|                     |               |     |                | SOF/SMV/RBV: 93% |
|                     |               |     |                | SOF/SMV/RBV: 79% |
|                     | COSMOS (Cohort 2 F3/4) | 22  | SOF/SMV +/- RBV| 12            | SOF/SMV: 100%  
|                     |               |     |                | SOF/SMV/RBV: 93% |
|                     | AI444–040 (PI Failures) | 41  | SOF/DCV +/- RBV| 12            | SOF/DCV: 100%  
|                     |               |     |                | SOF/DCV/RBV: 100% |
|                     | LONESTAR (PI Failures) | 40  | SOF/LDV +/- RBV| 12            | SOF/LDV: 95%  
|                     |               |     |                | SOF/LDV/RBV: 100% |
|                     | ION3 (Including PI Failures) | 440 | SOF/LDV +/- RBV| 12 vs. 24     | 12 wk: SOF/LDV: 94%  
|                     |               |     |                | SOF/LDV/RBV: 96%  
|                     |               |     |                | SOF/LDV/RBV: 99% |
| Genotype 2 naive    | FISSION       | 140 | SOF/RBV vs PegIFN/RBV | 12 vs 24 | 12 wk: SOF/RBV: 94%  
|                     | POSITRON      | 109 | SOF/RBV        | 12            | 93%      |
| Genotype 2 treatment failures | FUSION | 68  | SOF/RBV        | 12 vs. 16     | 12 wk: 86%  
|                     |               |     |                | 16 wk: 94%    |
| Genotype 3 naive    | FISSION       | 496 | SOF/RBV vs PegIFN/RBV | 12 vs 24 | 12 wk: SOF/RBV: 67%  
|                     | POSITRON      | 98  | SOF/RBV        | 12            | 61%      |
|                     | VALENCE       | 105 | SOF/RBV        | 24            | 93%      |
| Genotype 3 treatment failures | FUSION | 127 | SOF/RBV        | 12 vs. 16     | 12 wk: 30%  
|                     |               |     |                | 16 wk: 62%    |
|                     | VALENCE       | 145 | SOF/RBV        | 24            | 79%      |
| Genotype 4 naive    | FISSION       | 28  | SOF/RBV        | 12 vs. 24     | 12 wk: 79%  
|                     | POSITRON      | 32  | SOF/RBV        | 12 vs. 24     | 12 wk: 100%  

Abbreviations: DCV, daclatasvir; LD, low-dose ribavirin; LDV, ledipasvir; NNI, nonnucleoside polymerase inhibitor; PI, protease inhibitor; PegIFN, peginterferon; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; WB, weight-based ribavirin; wk, weeks.

*SVR (sustained virological response) includes SVR4, 12, and 24 data as available.
this combination is a very promising regimen for patients with genotype 1 infection. Because of the high prevalence of the Q80K mutation in North American populations infected with genotype 1a (up to 47%), combinations of other PIs or other classes of DAAs with sofosbuvir may prove superior even to sofosbuvir and simeprevir in this subgroup.

Sofosbuvir plus NS5A Inhibitor

The potency of the NS5A inhibitors combined with the very high barrier to resistance of NAs, makes this an appealing combination. Sofosbuvir was evaluated in combination with daclatasvir (60 mg daily), a potent NS5A inhibitor, with or without RBV for 12 or 24 weeks in 126 genotype 1 treatment-naive patients. All patients treated for 24 weeks achieved SVR12, with (15 of 15), or without (14 of 14) RBV. With 12 weeks of this combination, the SVR4 rate was 98% (40 of 41) without RBV and 95% (39 of 41) with RBV with no on-treatment breakthrough. This combination was also evaluated in 41 patients who had failed prior treatment with PegIFN, RBV, and either telaprevir or boceprevir. With 12 weeks of treatment, with or without RBV, all patients achieved SVR.

With these impressive results, sofosbuvir was then combined with ledipasvir (90 mg daily), another potent NS5A inhibitor, into a single, fixed-dose combination (FDC) pill. The phase II LONESTAR trial evaluated the FDC regimen with or without RBV for 12 or 8 weeks duration in treatment-naive patients. A single patient relapsed in each of the 8- and 12-week arms, both of whom were randomized to no RBV. All 21 patients treated with the FDC and RBV for 8 weeks achieved SVR. This approach was then extended to patients who failed prior triple therapy with telaprevir or boceprevir, including 50% with compensated cirrhosis. All 21 patients treated with the FDC and RBV for 12 weeks achieved SVR compared with 18 of 19 (95%) who were treated with the FDC alone.

To clarify the importance of RBV and the optimal duration of therapy, the ION1, ION2, and ION3 phase 3 trials were undertaken. To date, the results have been reported in press releases only (Fig. 1); thus, the trials have not been peer-reviewed. In ION1, genotype 1, treatment-naive patients were randomized to sofosbuvir/ledipasvir FDC with or without RBV for 12 weeks, leading to a 97% (211 of 217 patients) SVR12 rate with RBV and 97% (209 of 214 patients) with the FDC alone. In ION2, treatment-naive patients were randomized to 8 weeks of the FDC with or without RBV or 12 weeks with the FDC alone. Ribavirin had no effect with a 94% (202 of 215 patients) SVR12 rate without RBV or 93% (201 of 216 patients) with RBV in the 8-week arms and 95% (206 of 216 patients) in the 12-week arm with the FDC alone. Finally, in ION3, patients who failed prior treatment with PegIFN and RBV with or without a PI, were randomized to 12 or 24 weeks of therapy with the FDC with or without RBV. In this population, 20% of whom had compensated cirrhosis, 94 and 96% achieved SVR in the 12-week arm with and without RBV respectively, while 99% with or without RBV achieved SVR with 24 weeks of therapy. Collectively, these data suggest that only 8 weeks may be sufficient in treatment naive patients and RBV adds no additional benefit beyond the FDC alone with the treatment durations tested.

Sofosbuvir with Multiple DAAs

With near 100% SVR rates with sofosbuvir combined with either a PI or an NS5A inhibitor, what would be the rationale of adding a third DAA? Although shortening therapy from 48 to 12 weeks has been a dramatic improvement, 12 weeks of any type of treatment is quite long for most patients. If a third DAA would significantly shorten therapy without adding toxicity, it may well be worthwhile. The SYNERGY trial evaluated sofosbuvir/ledipasvir, daclatasvir (60 mg daily), or a PI (GS-9451 80 mg once daily) or a NNI (GS-9669 500 mg once daily) for only 6 weeks of total duration. All 20 patients with the FDC plus GS-9451 and 18 of 20 (90%) with the FDC plus GS-9669 achieved SVR4. Although these data are very preliminary, they suggest that adding additional DAAs may allow shortening of therapy, which will be important for...
compliance and for expanding treatment to difficult-to-reach populations.

**Genotype 2**

Although genotypes 2 and 3 have traditionally been grouped together, something that was done again in the sofosbuvir trials, the results differ markedly between the genotypes and it is therefore useful to discuss the data from the three phase III trials (FISSION, POSITRON, and FUSION) separately.

In the FISSION trial, treatment-naïve patients were randomized to receive PegIFN and RBV for 24 weeks ($n = 67$) or sofosbuvir 400 mg daily and weight-based RBV for 12 weeks ($n = 73$). The sofosbuvir/RBV regimen was superior with an SVR rate of 94% compared with 78% in the control arm ($p < 0.001$). Sofosbuvir and RBV was similarly effective in genotype 2 patients with cirrhosis (91% 10/11) as in those without cirrhosis (98% 58/59) and was very well tolerated. In a similar trial of treatment-naïve patients who could or would not take IFN-based therapy (POSITRON), sofosbuvir and RBV for 12 weeks led to an SVR rate of 93% (101/109), again with similar results in patients with and without cirrhosis at baseline. In the FUSION trial, patients who had failed previous PegIFN and RBV therapy were randomized to sofosbuvir and RBV for 12 or 16 weeks. Overall, 86% of genotype 2 patients treated for 12 weeks achieved SVR compared with 94% who were treated for 16 weeks. The difference in the arms appeared to be related to the presence of cirrhosis with 25 of 26 (96%) non-cirrhotic patients achieving SVR in the 12-week arm compared with 6 of 10 (60%) patients treated similarly with cirrhosis. With 16 weeks of therapy, 7 of 9 (78%) cirrhotic patients achieved SVR suggesting that perhaps longer therapy would be helpful in cirrhotic treatment-experienced genotype 2 patients, however the numbers are too small to draw strong conclusions. Based on the results of these trials, the FDA has approved sofosbuvir and weight-based RBV for 12 weeks for all patients with genotype 2 infection, however it may be prudent to consider extending therapy in patients with cirrhosis. Hopefully as more data emerge, the optimal duration for genotype 2 patients with cirrhosis will be better clarified.

**Genotype 3**

The results from the Phase III trials of sofosbuvir and RBV in patients with genotype 3 were somewhat less impressive than those for patients with genotype 2 infection. Among treatment-naïve patients, 67% (170/253) and 61% (60/98) achieved SVR with 12 weeks of sofosbuvir and RBV in the FISSION and POSITRON trials, respectively. The presence of cirrhosis had a major effect on outcome with only 21% (3/14) to 34% (13/38) of patients with cirrhosis achieving SVR with 12 weeks of therapy. In the FUSION trial, treatment-experienced patients received 12 or 16 weeks of treatment. With 12 weeks of treatment, the SVR rate was 37% (14/38) in patients without cirrhosis and only 19% (5/26) in those with cirrhosis at baseline. Extending treatment to 16 weeks increased the SVR rate to 63% (25/40) in noncirrhotics and to 61% (14/23) in patients with cirrhosis. The apparent benefit of extending therapy raised the question of whether longer durations would further improve the results.

The VALENCE trial evaluated 24 weeks of sofosbuvir and RBV in patients with genotype 3 infection. For treatment-naïve patients, this added clear benefit, with 93% (98/105) achieving SVR, including 12 of 13 (92%) with cirrhosis. For treatment-experienced patients, 87% (87/100) without cirrhosis achieved SVR, however only 60% (27/45) of those with cirrhosis went on to SVR with 24 weeks of therapy, almost identical to the 61% seen in a similar population with 16 weeks of treatment. Although 24 weeks seems to be preferable to 12 weeks of treatment, for treatment-experienced patients with cirrhosis, sofosbuvir plus RBV leaves some room for improvement. The small LONESTAR-2 trial reported an 83% (10/12) response rate with the use of PegIFN, RBV and sofosbuvir in a genotype 3 population with cirrhosis, the majority of whom were treatment-experienced.

The data from these Phase III trials clearly show that genotype 3 is more difficult to clear with NA-based therapy. The reasons for this are not clear. In vitro susceptibility to sofosbuvir is similar with genotype 3 isolates compared with those of other genotypes. Keeping with this observation, patients with genotype 3 suppressed virus on therapy, with similar early viral kinetics to patients with other genotypes. Virological failure occurred exclusively due to relapse and to date, no genotype 3 sofosbuvir-resistant clinical isolates have been identified. Whether treatment failure has something to do with genotype 3-related steatosis or another virus-specific factor is still unknown. There is the lack of resistance means that NA-based regimens that include other genotype-3 active DAAs are likely to be effective. With the data available to date, sofosbuvir with RBV for 24 weeks is likely adequate for most genotype 3 patients but for those who have failed previous treatment and have cirrhosis, adding PegIFN may be a worthwhile consideration. Ongoing studies will hopefully clarify the optimal management strategy for this group of patients.

**Genotype 4, 5, and 6**

To date there are very few data on genotype 4 patients treated with sofosbuvir without PegIFN. In a small study of Egyptian patients in the US, 11 of 14 (79%) achieved SVR12 with 12 weeks of sofosbuvir plus weight-based RBV compared with 14 of 14 (100%) treated with this regimen for 24 weeks. There are no data currently on treatment-experienced populations or any patients with genotypes 5 and 6.

**Resistance**

Because of the high barrier to resistance of sofosbuvir, resistance has not been a significant issue in studies to date. In the LONESTAR trial, one patient who relapsed after 8 weeks of therapy with the FDC alone was found to have virus harboring both NS5A resistant mutations (L31M and Y93H), and the S282T signature sofosbuvir resistant mutation. This patient had detectable L31M NS5A resistant HCV prior to starting any therapy. When he relapsed, the S282T mutation was present in 91% of the quasispecies population. Exemplifying the poor
fitness of this variant, by 2 weeks later, the frequency of the S282T variant had gone down to just 8% due to the outgrowth of wild-type HCV in the absence of drug pressure. However, it is notable that even the 8% figure represents a very large absolute quantity of virus, suggesting that even this relatively unfit variant is able to replicate. This patient was then retreated with sofosbuvir/ledipasvir FDC with the addition of RBV and extension to 24 weeks and achieved SVR. This case is instructive in many ways. It demonstrates that although unfit, the S282T variant can emerge and replicate and it suggests that this may be more likely when variants resistant to the second DAA (in this case NS5A) are present at high frequency at baseline. Whether this would have been less likely to occur with the use of RBV is hard to say, but the ION data suggest that most patients do not need RBV.40 Reassuringly, even though this resistant variant was present upon relapse, even without treatment, the prevalence of the S282T mutant diminished markedly and then was effectively cleared with retreatment for a longer duration with the addition of RBV. The S282T mutation confers only 3.5–10 fold resistance in vitro, and therefore although it is less susceptible to sofosbuvir, its replication is still inhibited, particularly with the addition of a potent NS5A inhibitor that has no impairment of activity against this variant.

Beyond the S282T signature resistance mutation, a novel double mutant (L159F/L320F) was recently identified in a genotype 1b patient treated with mericitabine, an NA with similar structure to sofosbuvir.48 This mutant is also very unfit compared with wild-type virus but in a replicon system is also resistant to sofosbuvir and other NAs. The L159F variant has also been seen alone in 6 patients treated with sofosbuvir and although it does not result in resistance in in vitro systems, it is still certainly possible that it will prove to be an issue in patients. It is likely that resistance will be seen more frequently in clinical practice than in the controlled setting of a clinical trial and it is certainly possible that other resistance mutations will be identified, but the real strength of the NAs as a backbone is that resistance is unlikely to persist at high levels and can be effectively treated with an NA-containing regimen as long as a DAA with activity against the S282T or other variant is used.

**Predicting Treatment Failure of NA Therapy**

With the high success rates achieved in many of the clinical trials, too few patients have failed therapy to allow for meaningful evaluation of factors predictive of treatment failure. The SPARE and QUANTUM trial in genotype 1 and the genotype 3 studies with sofosbuvir and RBV alone had lower rates of SVR. In the SPARE study, patients who relapsed had somewhat slower early viral kinetics in the first week of therapy, which may predispose to a lower chance of complete viral eradication.15 In both the SPARE and QUANTUM trials, patients who relapsed were more likely to have characteristics traditionally associated with IFN-nonresponse such as an unfavorable IL28B genotype.32 In addition, patients who previously failed therapy with PegIFN and RBV also responded less well than treatment-naïve patients, even those with unfavorable response characteristics. Collectively, these data suggest that innate immune function may still be relevant for viral clearance with DAAs. It also raises the intriguing possibility that past treatment failure may not only relate to nonresponse to IFN, but also to RBV. The mechanism of RBV remains poorly understood. Its minimal antiviral activity and the inability to definitively select for RBV resistant HCV in cell culture makes it unlikely that prior nonresponders have true RBV-resistant HCV; however, it is possible that like IFN, some individuals are predisposed to respond poorly to RBV.49 For such patients, if RBV has little or no effect, treatment with sofosbuvir and RBV may approximate sofosbuvir monotherapy, which is likely inadequate in most patients. As the data with combination therapy demonstrate, once a second DAA is added to sofosbuvir, baseline characteristics, even cirrhosis, become less and less important. Ultimately, it is likely that there will be regimens that are effective against all patients; however, if patients with favorable response characteristics can be identified, shorter, and thus less costly therapy may be possible.

**Other NAs**

The toxicity associated with BMS-986094 effectively stopped development of 2-methyl guanosine NAs. However, NAs of other bases continue to move forward in clinical development. Mericitabine is a uridine nucleoside analogue that has been studied in IFN-containing and IFN-free regimens. Unfortunately, the relatively low potency and surprisingly high relapse rate reported when used with PegIFN and RBV have limited its likely utility in the future.50 As a component of quadruple therapy with PegIFN, RBV, and dariprevir, a ritonavir-boosted PI, mericitabine was shown to be useful, with a significant improvement in SVR rates in a prior null responder population.51 However, given the greater potency of sofosbuvir and other NAs in development, it is unlikely that mericitabine will play a major role in HCV therapy in the future.

VX-135 is another NA in development. It has been studied in combination with RBV to date in phase I and phase 2a dose-finding studies. When combined with RBV at a dose of 100 mg or 200 mg daily in 10 patients with genotype 1 HCV, HCV RNA was suppressed in all patients during 12 weeks of therapy; however, only 1 of 10 achieved SVR12 at the 100 mg dose and 5 of 10 at the 200 mg dose.52 Based on liver enzyme elevations seen at the 400 mg dose in 3 of 10 patients, the drug was placed on partial clinical hold by the FDA limiting ongoing studies to a maximal dose of 100 mg.26 At a dose of 100 mg, VX-135 will need to be combined with other agents and studies are planned with daclatasvir (NS5A) and simprevir (PI).26

Others NAs in early stage development include a liver-targeted uridine nucleotide prodrug IDXX20963 with pan-genotypic activity and a good preclinical safety profile with no apparent cardiac or mitochondrial toxicity in animal studies.53 However IDXX20963 is on clinical hold from the FDA pending more safety data.54
The Future of NAs in HCV

The recent approval of sofosbuvir is a major advance. The potency, high barrier to resistance, pan-genotypic activity, once-daily dosing, excellent safety profile, and limited drug interactions make this an ideal backbone for IFN-free combinations. The emerging data from clinical trials support its use in combination with other DAAAs, particularly an NS5A inhibitor (ledipasvir or daclatasvir) or a PI (simeprevir). Treatment regimens as short as 6 weeks now seem likely and as additional potent DAAAs are added on, treatment of less than 4 weeks is no longer inconceivable. However, it is likely that there will also be populations of patients who will always require longer therapy, particularly those with cirrhosis. Finding the right combination, for the right duration, for the right patient will be one of the many challenges to come. The history of toxicity with NAs in the HCV field and in other areas raises a small degree of concern; however, the excellent safety profile from clinical trials, including patients with advanced liver disease, is very reassuring. By far the biggest advantage of sofosbuvir and of NAs as a class is the high barrier to resistance. Although we certainly will try to ensure compliance among our patients, the security that missed doses will not quickly lead to highly resistant HCV is a major advantage, particularly as we expand care to harder-to-reach populations.

The incredibly rapid development of new agents in HCV means that clinicians will have multiple effective regimens to treat most if not all of our patients. NAs will clearly play a major role in the short, and likely in the long-term, as we move to the next challenge of delivering these highly effective agents to those in need so that we can move to global elimination or even eradication of HCV. Hopefully availability of multiple effective regimens will bring prices down and increase access to care. At current prices, DAA treatment will likely not be an option for many people living with HCV and it will take major global cooperation to figure out how to deliver these effective treatments to those who need them, both in industrialized and in developing nations. Hopefully, we are up to this extremely important but, rather daunting challenge.

Financial Disclosures
Jordan J. Feld, MD, MPH, has received honoraria for consulting or advisory work for Abbvie, Achillion, Boehringer-Ingelheim, BMS, Gilead, Janssen, Idenix, Merck, Roche, and Vertex. He has also received research support from Abbvie, Boehringer-Ingelheim, Gilead, Roche, and Vertex.

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