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REVIEW

New Horizons in Hepatitis C Antiviral Therapy With Direct-Acting Antivirals

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Most direct-acting antivirals (DAAs) that are being developed as therapy against hepatitis C virus target the NS3/4A protease, the NS5A protein, and the NS5B polymerase. The latter enzyme offers different target sites: the catalytic domain for nucleos(t)ide analogues as well as a number of allosteric sites for nonnucleos(t)ide inhibitors. Two NS3/4A protease inhibitors have been approved recently, and more than 40 new NS3/4A, NS5A, or NS5B inhibitors are in development. These agents can achieve very high cure rates when combined with pegylated interferon-α and ribavirin and show promising clinical results when administered in all-oral combinations. In addition to the more canonical drug targets, new alternative viral targets for small-molecule drug development are emerging, such as p7 or NS4B and viral entry. Future research will need to define well-tolerated and cost-effective DAA combinations that provide the highest rates of viral eradication in all patients (including those with advanced liver disease), the broadest spectrum of action on viral genotypes showing minimal or no clinical resistance, and the shortest treatment duration. (HEPATOLOGY 2013;58:428-438)

For more than a decade, the standard treatment of chronic hepatitis C virus (HCV) infection has been based on the combination of pegylated interferon-α (PEG-IFN) and ribavirin (RBV) administered for 24 or 48 weeks. These regimens eradicate infection in 40% to 50% of treated patients with genotype 1 (HCV-1) infection and 80% of treated patients with genotype 2 (HCV-2) and 3 (HCV-3) infection. In addition, PEG-IFN/RBV regimens are poorly tolerated and contraindicated in a high percentage of patients. In order to address the shortcomings of PEG-IFN/RBV therapy, new direct-acting antivirals (DAAs) are being developed that target specific HCV functions. The recent approval of the first two NS3/4A oral protease inhibitors (PIs), boceprevir (BOC) and telaprevir (TVR), for use in combination with the PEG-IFN/RBV backbone has represented a dramatic advance in the efficacy of pharmacotherapy against chronic hepatitis C. With this new triple therapy regimen, patient cure rates for chronic HCV-1 infection have increased to 70% to 80% while significantly reducing treatment duration. However, these recently approved DAA regimens are poorly tolerated, are associated with a high pill burden and an inconvenient dosing frequency, and are not indicated for genotypes other than HCV-1. Moreover, selection of DAA-resistant viral variants occurs in patients who respond poorly to the PEG-IFN/RBV component of combination therapy. In light of these limitations, newer DAAs are being developed to identify regimens that are more convenient and efficacious, are better tolerated, are pan-genotypic, and have a low propensity to develop viral resistance. These are primarily targeted at the NS3/4A protease, NS5A protein, or NS5B RNA-dependent RNA polymerase. In addition, other less-studied viral proteins (e.g., NS4B or p7) or the virus entry steps have been recently demonstrated to be druggable. The ultimate goal in HCV research is to develop a broadly efficacious, IFN-free all-oral therapy. Toward this aim, several clinical trials combining only oral antivirals have begun to show very promising results. In this review, we summarize the

Abbreviations: ASV, asunaprevir; BOC, boceprevir; DAA, direct-acting antiviral; DCV, daclatasvir; DNV, danoprevir; FQ, ferroquine; HCV, hepatitis C virus; NI, nucleos(t)ide inhibitor; NNI, nonnucleos(t)ide inhibitor; PEG-IFN, pegylated interferon-α; PI, protease inhibitors; RBV, ribavirin; RdRp, RNA-dependent RNA polymerase; SIL, silibinin; SOF, sofosbuvir; SVR, sustained viral response; TVR, telaprevir.

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progress in the development of DAA-based therapies, with particular emphasis on those compound classes/combinations that have shown the most encouraging antiviral activity in the clinic.

Inhibitors of the NS3/4A Serine Protease

The HCV NS3/4A protease is a heterodimeric serine protease composed of the NS3 180 N-terminal amino acids and a short central hydrophobic domain of the NS4A protein, a protease coactivator[1] (Fig. 1A). The protease activity of the NS3/4A complex is responsible for the proteolytic maturation of a large portion of the nonstructural region of the HCV polyprotein, NS3 to NS5B. A number of peptidomimetic active site inhibitors have now been developed. From a chemical point of view, these can be divided into three main categories: (1) covalent linear PIs, (2) noncovalent linear PIs, and (3) noncovalent macrocyclic PIs. Macrocyclic PIs can be further classified as P_3-P_1 macrocycles or P_4-P_2 macrocycles (Fig. 1B).

In the scientific community, the terms "first-generation" and "second-generation" are used to define successive PI generations. First-generation NS3/4A PIs are defined as agents that display potent activity on HCV-1 but oppose a low barrier to selection of resistant viral variants and are not effective on all viral genotypes. First-generation NS3/4A PIs are in turn distinct in "first-wave" (i.e., BOC and TVR, both covalent linear inhibitors) and "second wave" (noncovalent linear or macrocyclic inhibitors). Conversely, second-generation NS3/4A PIs are defined as agents that pose a high barrier to the development of viral resistance, retain activity against the viral variants that are resistant to first-generation compounds, and are active across all HCV genotypes.

First-Generation NS3/4A HCV PIs. The currently approved NS3/4A inhibitors, BOC[2] and TVR,[3] contain a α-ketoamide warhead that forms a covalent reversible bond with the protease catalytic serine (Fig. 1B). Mutations associated with resistance to TVR or BOC readily occur at several positions close to the protease active site and are selected within a few days of monotherapy (Fig. 1A). These mutations include V36A/M/L, T54A/S, R155K/M/S/T, A156S (conferring low- to medium-level resistance), and A156T/Y (conferring high-level resistance).[4]

A number of second-wave, first-generation NS3/4A PIs are in advanced clinical development. These include the noncovalent linear PIs faldaprevir/BI 201335,[5] asunaprevir/BMS-650032 (ASV),[6] sovaprevir/ACH-1625,[7] and GS-9451[8]; the noncovalent P_3-P_1 macrocyclic PIs simeprevir/TMC435,[9] danoprevir/RG7227/ITMN-191 (DNV),[10] ABT-450,[11] and GS-9256[12]; and the noncovalent P_4-P_2 macrocyclic PI vaniprevir/MK-7009[13] (Fig. 1B). These agents are characterized by potent activity on genotype 1 HCV replicons (typically, low-nM EC_{50}). This translates into clinical efficacy in HCV-1 patients similar to that of BOC or TVR, leading to a decrease in circulating viral RNA of 3.5 to 4.5 log IU/mL when administered as monotherapy for a few days. Unlike their first-wave counterpart, second-wave PIs do not have the chemical reactivity needed to covalenty attack their target, leading to generally better tolerability. In addition, these agents have pharmacokinetic profiles compatible with once or once daily dosing (low-dose ritonavir boosting is used with DNV and ABT-450 in order to decrease dosing frequency). Although some second-wave NS3/4A PIs have a significantly broader spectrum of action on the different HCV genotypes compared with their predecessors, including activity on HCV-4, these agents are not pangenotypic, being invariably inactive on genotype 3.[14]

Along with the restricted genotype coverage, the genetic barrier to resistance to first-generation NS3/4 PIs is low, with extensive cross-resistance observed between the different compound classes. In particular, mutations of Arg155 have been shown to confer broad cross-resistance to all first-generation inhibitors. Conversely, mutations of Val36 or Thr54 have been observed exclusively in association with covalent linear inhibitors (first- wave), and mutations of Asp168 are specifically found to confer mutation to noncovalent peptidomimetic inhibitors (second-wave, either linear or macrocyclic).[14]

Second-Generation Active Site and Allosteric NS3/4A PIs. MK-5172 (Fig. 2B) is a second-generation NS3/4A PI with pan-genotype antiviral activity and

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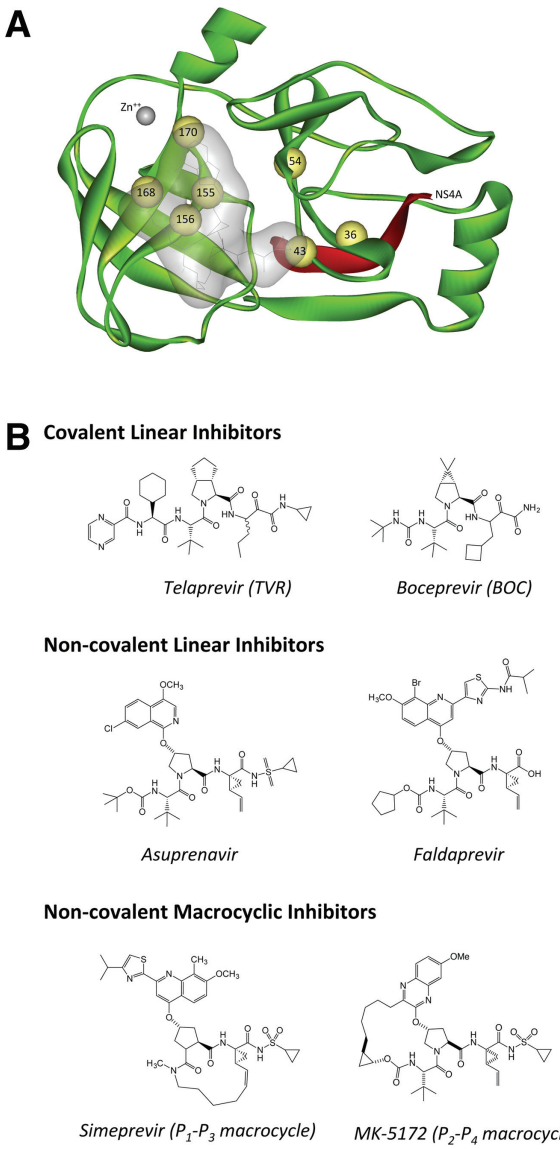

Fig. 1. Structure of the NS3/4A protease and resistance to protease inhibitors. (A) Three-dimensional structure of an NS3/4A protease domain–inhibitor complex (Protein Data Bank entry: 3M5L). NS3 is depicted in green, NS4A in red. The structural Zn^{++} ion is in gray. The amino acids corresponding to the main resistance mutations are numbered. (B) Chemical structures of selected protease inhibitors.

improved resistance profile.[15] Importantly, this agent maintains antiviral activity against most mutations that confer resistance to first-generation PIs, such as the two multidrug-resistant variants R155K and D168A. A recent crystallographic study analyzing the molecular basis of drug resistance against NS3/4A PIs revealed that TVR, DNV, and vaniprevir interact directly with the residues that confer resistance upon mutation, whereas MK-5172 interacts in a unique conformation with the catalytic triad, avoiding direct contact with R155 and D168.[16] No viral breakthrough has been observed in HCV-1 patients who received this drug alone for 7 days,[17] suggesting a higher barrier to resistance compared with first-generation inhibitors. Moreover, HCV-3 patients responded with a robust decline in viral RNA at the higher drug doses. ACH-2684, a P_3-P_1 macrocyclic inhibitor, is another second-generation HCV PI currently being tested in a phase 1 clinical trial. ACH-2684 has potent biochemical activity against HCV genotypes 1-6 and against known resistant variants.[18] It is worth noting the recent discovery of a new class of allosteric NS4/4A PIs that bind at the interface between the NS3 protease and helicase domains.[19] These agents exhibit a unique and novel resistance profile *in vitro*, implicating mutations of amino acids located at the allosteric drug binding site (M485 and V630). Comparable inhibition against genotypes 1, 3a, 5, and 6 but loss of activity against genotypes 2a and 4 were reported for these compounds.

NS5A Inhibitors

HCV NS5A is a multifunctional, dimeric protein essential for HCV RNA replication and virion assembly.[1] The NS5A protein structure consists of three

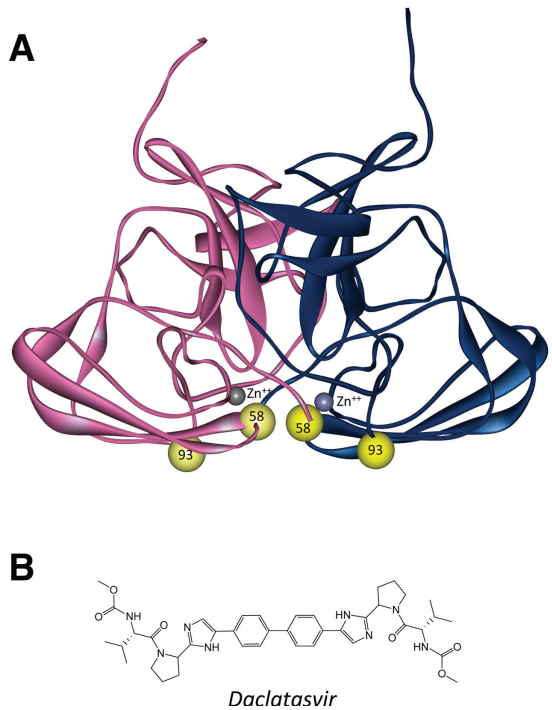

Fig. 2. Structure of the NS5A protein and resistance to NS5A inhibitors. (A) Three-dimensional structure of NS5A protein domain I (Protein Data Bank entry: 1ZH1). The structural Zn^{++} ion is in gray. Amino acids corresponding to main resistance mutations are evidenced and numbered. Substitutions of Y93 represent primary mutations, whereas mutations of position P58 act as a secondary mutation.[23] Other positions that are mutated in NS5A-resistant HCV variants are not visible in the domain I crystallographic structure. (B) Chemical structures of a selected NS5A inhibitor.

domains: domain I (amino acids 1-213), domain II (amino acids 250-342), and domain III (amino acids 356-447). The crystal structure of domain I has been crystallized in a dimeric form containing a zinc-binding and an RNA-binding motif[20] (Fig. 2A). NS5A inhibitors, initially discovered by replicon screening,[21,22] are believed to bind to domain I of NS5A and result in the suppression of viral RNA synthesis. Subsequent medicinal chemistry efforts led to the identification of extremely potent compounds characterized by a peculiar dimer-like structure (Fig. 2B). The most advanced of this "palindromic" NS5A inhibitor class is daclatasvir /BMS-790052 (DCV),[23] a compound with picomolar activity against a broad range of HCV genotypes. Clinically, single doses of DCV have been associated with a sharp and long-lived reduction in viremia.[23] In spite of the potent antiviral activity, the genetic barrier to resistance for DCV is low, especially for genotype 1a. Thus, resistant variants emerge readily, with the more relevant substitutions found at NS5A residues 28, 30, 31, and 93 for genotype 1a and residues 31 and 93 for genotype 1b.[24] DCV is currently being evaluated combination with PEG-IFN/RBV as well as in IFN-free regimens, in combination with sofosbuvir (polymerase nucleotide inhibitor), ASV (PI), and/or BMS791325 (polymerase nonnucleoside inhibitor).

Other NS5A inhibitors in clinical development include GS-5885, ABT-267, PPI-461, ACH-3102, and MK-8742. The latter two are early stage agents with a higher barrier to resistance and which retain substantial levels of potency against resistance mutations selected by early NS5A inhibitors. These novel agents can thus be viewed as second-generation NS5A inhibitors.[25]

NS5B Polymerase Inhibitors

The NS5B RNA-dependent RNA polymerase (RdRp) is the enzyme directly responsible for the synthesis of the HCV RNA genome.[1] Similar to other nuclei acid polymerases, NS5B has the typical right-hand polymerase structure, consisting of a thumb domain and a fingers domain encircling the enzyme active site located within the palm domain (Fig. 3A). Inhibitors of the NS5B RdRp are classified into nucleos(t)ide inhibitors (NIs) and nonnucleos(t)ide (NNI) inhibitors (Fig. 3B).

Nucleos(t)ide Analogues. NIs target HCV RNA synthesis at the catalytic site of the NS5B enzyme. They are mimics of the natural polymerase substrates and are incorporated by the polymerase in the nascent RNA, leading to premature chain termination.

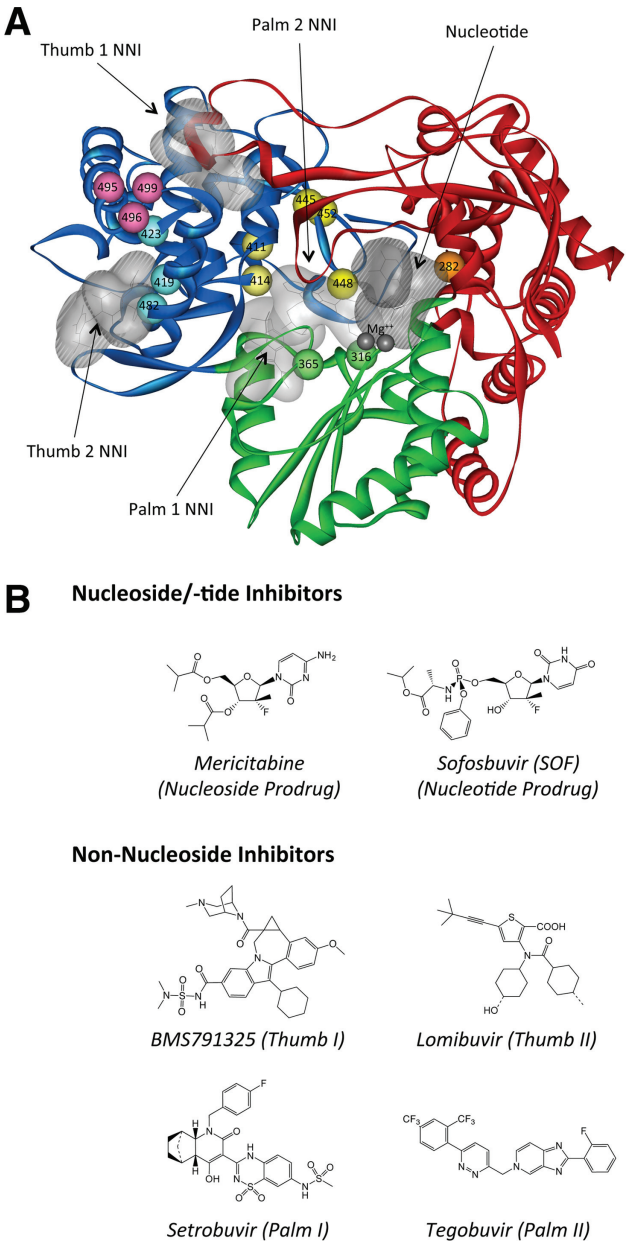

Fig. 3. Structure of the NS5B RdRp and resistance to polymerase inhibitors. (A) Structure of the HCV NS5B polymerase and binding sites for nucleos(t)ide and NNIs. The palm, finger, and thumb domains are colored green, red, and blue, respectively. Mn^{++} ions in the active site in gray. The amino acids corresponding to the main resistance mutations for different inhibitor classes are numbered. (B) Chemical structures of selected NS5B polymerase inhibitors.

Nucleoside inhibitors need three phosphorylation steps by cellular kinases to be converted to the active 5′ triphosphate form. Conversely, nucleotide polymerase inhibitors are prodrugs of nucleoside 5′ monophosphates, thus bypassing the rate-limiting step represented by the first phosphorylation step. Because of the active site conservation, NIs have similar efficacy across all HCV genotypes/isolates.[26] For the same reason, nucleos(t)ide inhibitors pose a high barrier to

development of drug resistance.[27] Virtually all NIs in development contain 2′-*C*-methyl and 2′-fluoro groups at the sugar 2′ position. The primary mutation associated with decreased susceptibility to these drugs is NS5B S282T[28] (Fig. 3A). The S282T mutation severely reduces HCV replication capacity, explaining the high barrier to resistance posed by 2′ modified NIs.[27] Sofosbuvir/GS-7977 (SOF) (Fig. 3B) is currently the most advanced NI NS5B polymerase inhibitor in clinical development (phase 3). SOF is a uridine nucleotide monophosphate analogue (beta-*D*−2′-deoxy-2′-fluoro-2′-*C*-methyluridine monophosphate). The S282T mutation is the most common SOF resistance mutation to emerge during resistance selection *in vitro*.[29] Whereas this mutation conferred resistance to SOF in genotype 1 replicons, it only caused a very minor shift in potency in genotype 2a, thus suggesting that additional mutations in genotype 2a NS5B are required for the resistant phenotype.[29] Most importantly, to date, viral resistance has been observed rarely in any SOF-based clinical study, regardless of the viral genotype.[30] SOF is currently studied in IFN-free combinations with a number of other DAAs, including NS3/4A protease inhibitors (GS-938, simeprevir) and NS5A inhibitor (DCV or GS-5885). Other nucleos(-t)ide polymerase inhibitors in active clinical development include mericitabine/RG7128 (prodrug of 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine; phase 2), and ALS-2200/VX-135 (structure undisclosed, phase 1).

NNIs. Unlike NIs, NNIs bind to less conserved allosteric sites found on the enzyme surface (Fig. 3A). In contrast to NIs, these molecules have shown a restricted spectrum of activity against the various HCV genotypes[26] and present a very low barrier to emergence of resistance.[27] The hallmark of all allosteric HCV NNIs described so far is that, in contrast to active site NIs, they are noncompetitive with nucleotide triphosphate substrates and inhibit the polymerase at a stage preceding the elongation reaction.[1] Different NNI binding sites are illustrated in Fig. 3B. These include thumb site I (benzimidazole-binding site), thumb site II (thiophene-binding site), palm site I (benzothiadiazine-binding site), and palm site II (benzofuran-binding site). Significant variability in the amino acid sequence is observed at these sites, making it difficult to achieve antiviral efficacy against different genotypes or even HCV isolates within the same genotype. As a result, most reported NNIs are rather specific for genotype 1 or 1b.[14]

Several structurally related NNIs have been shown to bind to the thumb site I.[31] This class of inhibitors interrupts the intramolecular contacts between the thumb and the finger loop, thereby preventing the formation of a productive enzyme/RNA complex.[32] These agents are also known as finger loop inhibitors and are characterized by having a common benzimidazole or indole chemical core (Fig. 3B). HCV variants resistant to these agents carry mutations at positions P495, P496, and T389[33,34] (Fig. 3A). Clinically, agents belonging to this class of NNIs display reduced activity against genotype 1a compared with genotype 1b.[35] Several thumb I NNIs are currently being investigated in phase 2 clinical trials, including BI 207127, TMC647055, and BMS791325.

Thumb II NNIs bind to a cavity located at the base of the thumb domain of NS5B (Fig. 3A). Mutations at positions L419, M423, and I482 in the viral polymerase have been shown to confer resistance to this class of compounds.[36] Lomibuvir/VX-222 (Fig. 3B), a tiophene carboxylic acid, and filibuvir/PF-868554, a dihydropyranone derivative, are currently in phase 2 clinical trials.

The palm I NNI-binding site is located at the junction of the palm and the thumb domain of NS5B, in proximity to the catalytic site. Benzothiadiazine compounds such as setrobuvir/RG7790 (formerly ANA598; Fig. 3B), ABT-333, and ABT-072 bind to this NNI site. The most frequently resistant mutations selected by these agents are C316Y, M414T, Y448H/C, and S556G (Fig. 3A).[37] These compounds are currently in phase 2 clinical trials. Acylpyrrolidines are yet another class of palm I-binding compounds.[38] In this class, GSK625433 was advanced into phase 1 clinical trials, but this study was halted because of adverse effects noted in preclinical carcinogenicity studies.[39]

The palm II NNI-binding site partially overlaps with the palm I site and is located proximal to the junction between the palm and thumb domain. A class of benzofurans was originally identified as an NNI class binding to this site. These molecules select for resistant mutants at residues L314, C316, I363, S365, and M414[40] (Fig. 3A). HCV-796 showed significant activity in early stage clinical trials[41] but was discontinued because of adverse side effects. HCV variants resistant to imidazopyridines**,** another HCV NNI chemotype, carry mutations in the same site (C316Y) as well as mutations in a β-hairpin loop (C445F, Y448H, Y452H) located in close proximity to the catalytic active site (Fig. 3A). In contrast to other NNIs, imidazopyridines do not inhibit the enzymatic activity of the purified RdRp, suggesting that these molecules target the enzyme via a unique mechanism. Recent data have revealed that imidazopyridine NNIs require metabolic activation by CYP1A for its activity. The

resulting metabolite, after forming a conjugate with glutathione, directly and specifically interacts with NS5B.[42] Within this class of imidazopyridine NNIs, tegobuvir/GS-9190 (Fig. 3B) is undergoing phase 2 clinical trials.

Presumably due to their barrier to resistance and restricted genotype/subtype coverage, HCV polymerase NNIs such as tegobuvir or filibuvir have provided disappointing clinical results when combined with PEG/RBV. Several NNIs, including setrobuvir, lomibuvir, BI207127, BMS791325, ABT-333, and ABT-072 are now being tested with more promising results in all-oral, IFN-free combination regimens.

Emerging Targets for DAA Development

While many inhibitors of HCV protease, polymerase, or NS5A are at an advanced development stage, new classes of direct-acting antivirals targeting less explored viral functions have begun to appear on the horizon.

NS4B is an RNA-binding integral membrane protein required for the biogenesis of the membranous web required for the formation of HCV RNA replication complex.[1] Several classes of NS4B inhibitors have been identified recently.[43] Clemizole, a first-generation antihistamine, inhibits NS4B RNA-binding, thereby preventing HCV RNA replication.[44] Clemizole-resistant variants carry mutations at position W55 and R214 in the NS4B protein. An ongoing proof-of-concept study is evaluating the safety and efficacy of clemizole monotherapy in treatment-naïve HCV-infected patients. Preliminary data reveal that clemizole, while inactive as a single agent, when combined with PEG-IFN and RBV may result in a more efficacious reduction in viral load than PEG-IFN/RBV alone.[45]

Unexpectedly, very recent data point to NS4B as a candidate target for the anti-HCV action of silibinin (SIL).[46] SIL is an intravenous drug that has recently been administered to HCV-positive liver transplant recipients, leading in some instances to eradication of the infection. In cell culture, SIL potently inhibits HCV RNA replication for genotype 1a and genotype 1b, but not for genotype 2a. Mutations in NS4B, obtained either by selection in cell culture (Q203R) or observed in a liver transplant recipient experiencing viral breakthrough under SIL monotherapy (F98L+D228N), were found to confer *in vitro* resistance to SIL. These new exciting data point to the possibility that an already approved agent may be added to the growing armamentarium of HCV DAAs. Unfortunately, the intravenous mode of administration will ultimately limit the use of SIL in all-oral DAA combinations.

The HCV p7 protein is a viroporin[1] critical for the release of infectious virions. When its cation channel activity is pharmacologically blocked, virus production is significantly reduced.[47] A number of HCV p7 inhibitors have been identified, such as amantadine, rimantadine, long-alkylated iminosugar, and amiloride derivatives. The *in vitro* sensitivity to HCV to these drugs is highly genotype-dependent, presumably because of the high sequence variability associated with the p7 genetic region. To date, none of these p7-directed agents has demonstrated any significant clinical activity.

Another way to potentially limit acute as well as chronic HCV infection would be to prevent virus entry into the noninfected cells. Ferroquine (FQ), a novel antimalarial currently undergoing clinical evaluation, has been reported recently to inhibit HCV entry in cell culture at the membrane fusion step.[48] FQ-resistant HCV was selected with a single resistance-conferring mutation in the E1 envelope protein (S327A). FQ may therefore represent a novel direct antiviral agent ready to be combined with other DAAs for all-oral therapy.

Future Treatment Strategies

Although there are still some concerns regarding how many of the anti-HCV drugs currently in development will actually hit the market, it is clear we are on the verge of a revolution in the treatment of chronic hepatitis C. This revolution, at least for the hard-to-cure HCV genotypes 1 and 4, is likely going to consist of a two- to three-step process that will ultimately lead us to the holy grail of an all-oral, pan-genotypic, IFN-free therapy. The first step forward in anti-HCV therapy will be the introduction of a second-wave PI to be used in combination with PEG-IFN/RBV. This will be followed by NS5A and NS5B inhibitors to be used with PEG-IFN/RBV in triple therapy regimens or in quadruple therapy regimens in combination with a second-wave PI. Finally, several all-oral combinations will enter the market, likely becoming the standard of care first therapeutic option for all HCV genotypes.

PIs in Combination With PEG-IFN/RBV. One of the main limitations of the first-wave PIs BOC and TVR is tolerability when they are used with PEG-IFN/RBV. This stems both from the induction of specific side effects as well as from the rather impractical assumption mode that both compounds require.[49]

These first-generation, first-wave PIs need to be taken every 7 to 9 hours with food, causing a significant pill burden that may lead to suboptimal adherence and suboptimal efficacy. First-generation, second-wave PIs such as simeprevir, faldaprevir, and ritonavir-boosted DNV will be able to bypass this issue, as they are being studied in phase 3 trials with once-daily dosing.[50] For HCV-1 patients, dosing convenience is likely to be one of the main advantages of these PIs, as phase 2b sustained viral response (SVR) rates of second-wave PIs in combination with PEG-IFN/RBV do not exceed those seen with first-wave PIs. Second-wave PI triple therapies, in fact, achieve suboptimal response rates in poor responders to PEG-IFN/RBV, patients with cirrhosis, or HCV-1a patients.[51,52] Moreover, their resistance profile is largely similar to that of BOC or TVR, meaning that second-wave PIs cannot be considered as a rescue therapy. These drugs, however, can be a clinical breakthrough for patients with non–genotype 1 infection, as they are active against genotypes 2, 4, 5, and 6.[53] This is especially significant for HCV-4 patients that not only are on the rise in many countries due to immigration from endemic areas but also currently represent a large unsatisfied medical need, given that TVR and BOC show little efficacy and are not reimbursed in this patient population. Importantly, in a phase 2b study of HCV-4 patients receiving PEG-IFN/RBV and ritonavir-boosted DNV, 100% achieved an SVR following a course of 24 weeks of triple therapy.[54]

NS5A or NS5B Inhibitors in Combination With PEG-IFN/RBV. NS5A inhibitors and NS5B polymerase inhibitors will enter the HCV market in a second phase and will probably be, at least for a short time, associated with PEG-IFN/RBV therapy in substitution of first-wave PIs and in competition with second-wave PIs. Whether they will provide a true innovation in terms of viral cure rates, safety profile, or patient tolerability is still to be demonstrated. A 24-week treatment of PEG-IFN/RBV plus DCV in HCV-1–naïve patients has been shown to attain SVR rates that range from 87% for HCV-1b patients to 58% for HCV-1a. These rates are similar to TVR or BOC triple-combination regimens, and also confirm the low barrier to resistance of first-generation NS5A inhibitors in the 1a subtype.[14,55] NS5A inhibitors seem better fit as partners of other DAAs[56] as shown by the very promising data obtained by a 24-week quadruple regimen of PEG-IFN/RBV plus DCV and ASV (PI) in HCV-1 patients with a previous null response to PEG-IFN/RBV. This regimen was associated with a 100% SVR rate in a small pilot study and is now being explored in phase 3 studies.[57] Although this is an impressive performance gain compared with TVR/BOC, which reach subpar SVR rates (30%-35%) in this population, this quadruple regimen is still relatively complex for patients, has a largely unknown safety profile, and still needs to be explored in patients with cirrhosis.

Equally impressive SVR rates have been seen with a 12-week regimen of PEG-IFN/RBV plus the NS5B nucleotide inhibitor SOF, as 90% of 51 HCV-1–naïve patients (and 77% of HCV-1a–naïve patients) achieved SVR12 in the phase 2 ATOMIC study.[58] This regimen will improve SVR rates in HCV-1a patients, as NS5B NI activity is not influenced by HCV-1 subtype, but is unlikely to revolutionize the field in HCV-1b patients. In fact, when comparing the SVR rates of the ATOMIC study to phase 3 trials of BOC/TVR in HCV-1b patients with similar viral and disease characteristics, the actual SVR gain quantifiable in the 10% to 15% range. Importantly, equally high SVR rates have been achieved by the PEG-IFN/RBV plus SOF combination in HCV-4 patients (82%).

All-Oral IFN-Free Regimens. The first all-oral anti-HCV regimen will be likely available in 2014 for HCV-2 and HCV-3 patients. Phase 3 studies investigating a 12-week course of the NS5B inhibitor SOF in combination with RBV are already fully enrolled and completed, and final results are expected for the second semester of 2013. This regimen has proven to be particularly effective in the phase II ELECTRON study, where 100% rates were obtained by this combination in HCV-2 and HCV-3 patients.[59] For HCV-1 patients in the ELECTRON study, this regimen turned out to be less effective, as SVR rates ranged from 84% (naïve patients) to a disappointing 10% in the treatment-experienced patients.[59] In the National Institutes of Health–sponsored SPARE study, 25 HCV-1–naïve patients were treated with SOF and RBV for 24 weeks. The SVR12 rate was 72%,[60] not dissimilar from the current TVR/BOC-based standard of care. This study should not be overlooked, as it was obtained in a cohort of patients enriched in known predictors of treatment failure such as advanced fibrosis (24% of patients), African American ethnicity (72%), and interleukin-28B CT/TT (84%). Taken together, these data indicate that this regimen might be an effective treatment option only for easier-to-cure patients, including those infected with HCV-1b and interleukin-28B CC and patients with mild disease, while probably being suboptimal in patients with harder-to-cure HCV disease, especially those who have failed previous PEG/IFN therapy.

Table 1. Developing HCV Treatment Regimens

Therapeutic Regimen	Advantages Over SOC 2013	Open Issues
IFN-containing regimens		
PEG-IFN + RBV + second-wave PI	Improved tolerability Reduced pill burden Effective in non–HCV-1 patients	Cost-effectiveness Specific side effects Limited efficacy in HCV-1a Drug-drug interactions
PEG-IFN + RBV + NS5A inhibitor	Improved tolerability Reduced pill burden	Cost-effectiveness No data in patients with cirrhosis Limited efficacy in HCV-1a Drug-drug interactions
PEG-IFN + RBV + NS5B NI	Improved tolerability Reduced pill burden Effective in HCV-1a patients Effective in non HCV-1 patients	Cost-effectiveness No data in patients with cirrhosis
PEG-IFN + RBV + second-wave PI + NS5A inhibitor	Effective in HCV-1a patients Effective in non HCV-1 patients	Tolerability Pill burden No data in patients with cirrhosis Drug-drug interactions
IFN-free regimens		
NS5B NI + RBV	Improved tolerability Effective in HCV-2 and HCV-3 patients Effective in HCV-1a patients	Cost-effectiveness HCV-2/3 Pill burden (RBV component) Limited efficacy in difficult-to-cure patients
Second-wave PI + NS5A inhibitor	Reduced pill burden Improved tolerability	Drug-drug interactions Limited efficacy in HCV-1a
Second-wave PI + NS5A inhibitor + NS5B NNI±RBV	Improved tolerability Effective in HCV-1a patients	Pill burden Drug-drug interactions Limited data in patients with cirrhosis
NS5B NI + NS5A inhibitor	Improved tolerability Reduced pill burden Fixed-dose combination Effective in HCV-1a and in non HCV-1 patients	Limited data in patients with cirrhosis

The combination of two or more DAAs is fundamental to achieve more potent and broad HCV RNA suppression and avoid IFN in HCV-1 patients. Several regimens meeting these requirements are in advanced phase of development.[61] The optimal regimen should combine a drug with potent antiviral activity (PI or NS5A inhibitor) with a drug with a high genetic barrier to resistance (NS5B NI); however, high SVR rates have been achieved by regimens that are driven more by the drug portfolio of the various pharmaceutical companies than by rational mixing and matching of DAAs. A quadruple therapy regimen consisting of 12 weeks of a ritonavir-boosted PI (ABT-450/r) plus an NS5B NNI (ABT-333) and an NS5A inhibitor (ABT-267) obtained SVR rates of 97.5% in 79 HCV-1–näive patients and 93.3% in 45 previous null-responders to PEG-IFN/RBV, with no significant differences in HCV-1a or HCV-1b patients.[62] A similar 12-week regimen of ASV (PI) plus DCV (NS5A inhibitor) plus BMS791325 (NS5B NNI) reached 94% SVR in 16 HCV-1–naïve patients.[63] These impressive numbers compare well with what today could be considered the optimal IFN-free regimen (i.e., the combination of the NS5A inhibitor DCV and the NI NS5B inhibitor SOF). This regimen, when given for 12 weeks, achieved an SVR4 of 98% in 41 HCV-1–naïve patients.[64]

Remaining Issues

Although treatment of chronic HCV infection is advancing at such a fast pace that keeping a strong grip on drug development can be problematic at times even for experts, some common themes surface from most if not all ongoing studies (Table 1). First, there is a widespread perception that most investigational agents for the treatment of chronic hepatitis C are being explored in easy-to-cure populations, at least partially devoid of negative prognostic factors.[65] The lack of consistent safety and efficacy data in patients with advanced fibrosis/cirrhosis represents a major drawback in most, if not all, regimens. Although cirrhosis is likely going to lose its negative predictive power as a response moderator once potent anti-HCV regimens are available, it might nonetheless remain a

key determinant of reduced safety with some regimens. Indeed, some drugs have side effects that might be worrisome in patients with cirrhosis, such as increased bilirubin with simeprevir,[66] while others (such as ASV) show significant pharmacokinetic modifications in patients with impaired liver function,[67] and thus need to be managed with caution in this group of patients. The same safety questions need to be ascertained in post–liver transplantation patients as well as those on the transplant waiting list. To date, we only have two very preliminary case reports of posttransplant fibrosing cholestatic hepatitis C patients who reached an SVR with either a triple therapy regimen of PEG-IFN/RBV and DCV[68] or an IFN-free regimen of DCV plus SOF,[69] in each case without any significant safety issue and without any clinically relevant drug-drug interaction with the ongoing immunosuppressive regimen. Real-life studies of IFN-free regimens have shown surprisingly low rates of adherence to the correct treatment schedule and lower SVR rates compared with sponsored studies, meaning that once we move drugs into more difficult-to-cure patients, we might not completely replicate the data obtained by phase II trials.[60,70]

Affordability of some of these innovative regimens will also be an issue. Whether anti-HCV regimens that provide small benefits in terms of SVR but radically improve patient tolerability will be deemed cost-effective by national health systems and hence be reimbursed universally is unclear. Given that cost-effective drugs such as TVR/BOC[71,72] are still not reimbursable in many countries, it is possible that these innovative regimens will be confined to groups of patients in whom TVR/BOC or PEG-IFN/RBV are either ineffective or unsafe. This might create a paradox where pharmaceutical innovation might not translate into clinical innovation, with some patients receiving marginally less effective and less tolerable drugs for cost-containing issues.[73] This might reduce the penetration of treatment into key cohorts of infected patients, preventing anti-HCV therapy from playing a major role in containing the spread of the disease and in reducing the rate of progression to cirrhosis in resource-constrained countries.

The last open issue that often goes unnoticed when discussing future anti-HCV drugs is whether drug resistance will emerge as a clinical problem with all oral IFN-free regimens.[4] Resistance to TVR/BOC has limited clinical significance as HCV quasispecies reverts to wild-type virus in a relatively short period.[74] This is explained by the lack of a stable genetic reservoir for HCV and by the replication unfitness of most resistant variants to TVR/BOC. Whether this last point holds true for other classes of DAA needs to be discussed. NS5B NIs are characterized by a high barrier to resistance, as the S282T mutation associated with decreased susceptibility to this class of compounds dramatically reduces HCV replication capacity. This means that this mutation is very rarely found as a pretreatment naturally occurring variant and is also seldom found at the time of relapse.[30] However, NS5B NIs require compounds from other classes to achieve maximal SVR rates. Resistance to first-generation NS5A inhibitors, ideal partners for an NS5B NIs, have been shown to occur naturally and in some cases to persist as the dominant viral strain for at least 48 weeks following treatment failure.[75,76] In a Japanese study of HCV-1 patients treated with 24 weeks of ASV and DCV, DCV-resistant variants were found in 20% of patients at baseline. In virological failures, when NS3 and NS5A resistance-associated variants were detected together (NS3: D168A/V; NS5A: L31M/V-Y93H), DCV-resistant substitutions persisted through 48 weeks, whereas ASV-resistant substitutions were no longer detectable.[77] It is too early to tell if this finding should alarm us, since the ASV-DCV combination is considered suboptimal in terms of genetic barrier to resistance, but it shows that not all we have learned from TVR/BOC can be safely translated to future anti-HCV drugs.

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