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Future Classes of HCV Therapeutic Agents

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Introduction

More than 170 million people are infected with hepatitis C virus (HCV), which is responsible for approximately 350,000 deaths per year. [1] Of those infected with HCV, up to 80% develop chronic infection, which frequently progresses to cirrhosis, hepatocellular carcinoma, and death. [2, 3]

Cure of HCV, or achievement of sustained virologic response (SVR), defined as an undetectable serum HCV RNA measured 24 weeks after the end of treatment, is associated with reduction in the risk of liver-related complications. [4, 5] For the past two decades, treatment of HCV has centered on the use of pegylated interferon alfa (peginterferon alfa, PEG) in combination with ribavirin (RBV). Therapy with PEG/RBV has produced overall SVR rates of 54%–56%, with a rate of 45%–50% in patients with HCV genotype 1 (GT1, the most common genotype of HCV) and a rate of 80% in patients with HCV genotype 2 or 3. [6–8] Long-term follow-up studies have revealed that a sustained virologic response is maintained indefinitely and corresponds clinically with cure. [9–11]

However, HCV treatment is associated with numerous side effects, which have led to lack of adherence and premature treatment discontinuation. More specifically, peginterferon alfa is associated with flulike symptoms, cytopenia, autoimmunity, and depression, and ribavirin causes hemolysis. This multitude of side effects, numerous absolute and relative contraindications, as well as the less than 50% cure rate achieved among among patients with HCV genotype 1, have limited the real-world effectiveness of peginterferon and ribavirin. The vast majority of the 4 million persons in the United States who are infected with HCV have never been treated, let alone cured.

For the first time since the discovery of HCV, recent advances in the understanding of the molecular characteristics of the virus have led to the development of novel antiviral therapeutics. Direct-acting antivirals (DAAs) are designed to inhibit viral targets, while host-targeted antivirals block host factors that are utilized by the virus for its own life cycle. In 2011, the first DAAs, telaprevir and boceprevir, were approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of chronic HCV genotype 1 infection, representing an important milestone in HCV therapy. Moreover, the rapid development of agents in multiple classes has led to the promise of

shorter therapy duration, an improved side effect profile, and eventually interferon-sparing regimens. In this article, we review novel HCV therapeutics in development, including mechanism of action, efficacy, and adverse effects.

HCV Virology and Life Cycle

The development of in vitro HCV replication models has greatly increased the understanding of viral structure, entry, replication, and host factors utilized for virus propagation. [12, 13] These advances have enabled the discovery of new strategies aimed at interrupting key steps in the HCV life cycle.

Within the family *Flaviviridae*, Hepatitis C virus is a linear, positive-sense, single-stranded RNA virus with a 9.6-kb genome. The HCV genome contains an open reading frame that encodes a single polypeptide of approximately 3000 amino acids.

HCV enters hepatocytes through endocytosis and uncoats in a pH-dependent fashion (Figure 1). [14] After uncoating, the positive-sense strand RNA within the virion is used as a template for cytoplasmic translation into the single polypeptide. The polypeptide is cleaved by both host and viral proteases, including NS3/4a protease, into ten proteins: three structural proteins (core, E1, and E2), and seven non-structural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) (Figure 1).

Viral replication occurs at an endoplasmic reticulum membrane-derived replication complex, which includes the nonstructural proteins NS4B and NS5A. Through the activity of the NS5B RNA-dependent RNA polymerase and the NS3 helicase, positive-sense RNA strands are copied into negative-antisense strands in a cyclophilin A- and microRNA-122-dependent fashion. The negative-antisense strands act as a replicative intermediate for copies of positive-sense RNA strands.

Newly produced viral RNA genomes are then encapsidated, and are released by the host plasma membrane. Viral secretion occurs in association with very low-density lipoproteins (VLDL), through a process that remains incompletely understood.

The genetic variation of HCV cannot be underestimated. There are six known major genotypes and more than 100 subtypes of HCV. Moreover, the virus produces approximately 10 infectious virions each day in an infected adult, and the error-prone RNA-dependent RNA polymerase has no proofreading capability, resulting in a mutation rate of 10^{-4} per nucleotide. [15] The risk for development of resistance poses a significant challenge to the development of novel HCV therapeutics.

Direct-Acting Antivirals (DAAs)

NS3/4A Serine Protease Inhibitors

Discovery of the NS3/4A crystal structure, in conjunction with mutagenesis studies, has enabled the development of multiple NS3/4A serine protease inhibitors. Because the protease has a shallow peptide-binding groove, few molecules were identified that blocked

NS3/4A activity. Subsequently, peptidomimetic NS3/4A inhibitors were developed, which inhibit enzymatic activity by mimicking the cleavage end product of the proteolytic reaction.

The first drugs in this class approved by the FDA, telaprevir (TPV) and boceprevir (BOC), are structurally similar linear molecules that are primarily active against genotype 1 (GT1) HCV. Telaprevir and boceprevir are recommended for use in combination with PEG and RBV. Overall, the efficacy of telaprevir and boceprevir appear similar, and are associated with a significant increase in SVR rates in both treatment-naïve and treatment-experienced subjects with GT1 infection. [16–20] However, TPV and BOC have a low barrier to resistance [21–23], variable activity against genotypes other than GT1, adverse side effects including rash and anemia, and numerous drug-drug interactions, particularly with drugs metabolized principally by CYP3A4 of the cytochrome P450 pathway.

Additional inhibitors of the NS3/4A serine protease are in clinical development, and can be divided into two categories according to their structure. Linear ketoamide derivatives (such as telaprevir and boceprevir) interact with the catalytic site and lead to reversible, covalent inhibition. Macrocytic inhibitors block the catalytic site via a reversible, noncovalent mechanism, and have a potentially more favorable resistance profile than most linear protease inhibitors. [24, 25]

BI 201335 is a linear tripeptide PI that has been studied in treatment-naïve patients and treatment-experienced patients in two phase IIb trials, SILEN-C1 and SILEN-C2, respectively. [26, 27] In the SILEN-C1 study, the addition of BI 201335 dosed at 240 mg daily to PEG/RBV was associated with an increased SVR rate compared to standard of care (SOC, PEG/RBV combination therapy) among treatment-naïve patients with GT1: 73%–83% versus 56%, respectively. [26] In the SILEN-C2 study, BI 201335 was studied in combination with PEG/RBV among prior partial and null responders with GT1 HCV. [27] Rates of SVR were 27% to 41%, which were lower than those observed among treatment-experienced patients who received telaprevir and boceprevir in prior trials. [20, 28] Furthermore, BI 201335 has been studied as part of an interferon-sparing regimen. Preliminary data from SOUND-C2, a phase IIb trial, revealed an SVR rate of 68% among treatment-naïve GT1 patients who received BI 201335 in combination with BI 207127 (a non-nucleoside polymerase inhibitor) and RBV. [29] These data illustrate the efficacy and tolerability of an all-oral combination regimen that includes drugs with different viral targets. Advantages of BI 201335 include its once-daily dosing, and side effects include jaundice, rash, nausea, vomiting, and diarrhea.

TMC-435 is a macrocyclic inhibitor whose improved pharmacokinetic properties, including a 40-hour half-life, allows once-daily dosing. [30] It has antiviral effects against HCV genotypes 1, 2, 5, and 6, but limited efficacy against genotype 3. A phase IIb study revealed an increased efficacy of TMC-435 in combination with PEG/RBV: more than 90% of patients given TMC-based therapy achieved RVR and EVR, and rates of SVR were 75%–85%. [31, 32] Side effects of TMC-435 include asymptomatic hyperbilirubinemia, and data suggest an improved resistance profile compared to the linear protease inhibitors. [33]

Danoprevir (ITMN-191/RG7227) is a macrocyclic PI with high specificity and antiviral efficacy against GT1 HCV, and is dosed 2 to 3 times daily. [34] Results from ATLAS, a phase IIB study, revealed that danoprevir in combination with PEG/RBV was associated with SVR rates of 68% to 85%, compared to 42% among those who received SOC. [35] Danoprevir is generally well-tolerated, but associated with increased risk of neutropenia and elevation in alanine aminotransferase. The addition of ritonavir reduces overall systemic exposure to danoprevir (ie AUC and Cmax) than with danoprevir alone while maintaining potency against HCV. The combination, along with PEG and RBV, has been shown to suppress HCV without increasing levels of aminotransferases. [36]

A number of other protease inhibitors are in clinical development (Table 1), including vaniprevir (MK-7009), asunaprevir (BMS-650032), ABT-450, GS-9451, and GS-9256. In addition, MK-5172 has demonstrated potent activity against many of the first-generation protease inhibitor resistance-associated variants in biochemical assays, and has broad genotypic activity. [37, 38]

As with TPV and BOC, a major limitation of PIs has been the rapid development of resistant variants of HCV. When given as monotherapy, PIs exert selective pressure on the population and allow resistant variants to become the dominant viral species. Amino acid changes primarily occur in the NS3 protease catalytic domain and prevent binding of the peptidomimetic inhibitor. However, in vitro studies have demonstrated that HCV variants that are resistant to PIs remain susceptible to other antiviral agents, suggesting the importance of combination therapy with agents from different antiviral classes.

NS5B RNA-dependent RNA polymerase inhibitors

The HCV NS5B protein is an RNA-dependent RNA polymerase (RdRp), which plays a pivotal role in viral replication. The protein has a 3-dimensional, right-handed motif in which the palm is the active site of polymerase activity and chain elongation, the thumb plays a significant role in initiation of RNA synthesis, and the fingers and thumb encircle the active site (Figure 2). [39]

Inhibitors of the HCV RdRp are divided into two groups: nucleos(t)ide inhibitors (NIs) and non-nucleoside inhibitors (NNIs). Nucleos(t)ide inhibitors bind to the NS5B active site, compete with nucleotide triphosphates for incorporation into nascent HCV RNA, and once incorporated, prevent chain elongation. Because nucleos(t)ide inhibitors are already phosphorylated and bypass host phosphorylation steps, they are able to achieve high levels of nucleotide triphosphate in the liver. Non-nucleoside inhibitors bind to one of at least three allosteric pockets outside the active site, and induce a conformational change in the active site that blocks chain elongation.

Nucleos(t)ide Inhibitors

Nucleos(t)ide inhibitors have shown in vitro potency against multiple HCV genotypes, and offer a particularly high barrier to the development of viral resistance since the NS5B active site is intolerant to amino acid substitutions. Thus, active site NS5B mutations that confer resistance to this antiviral class are also more likely to inhibit RdRp activity, rendering the mutant virus less “fit” compared with wild-type virus. Cell culture experiments have

confirmed that nucleos(t)ide analogues are less likely to select for resistance mutations than NNIs and protease inhibitors.

PSI-7977 is a uridine NI administered once daily, and has been studied in several phase II trials. The PROTON study reported SVR rates of 88% to 91% among treatment-naïve HCV GT1 patients who received PSI-7977 plus PEG/RBV. [40] Of note, the high barrier to resistance of PSI-7977 was shown in this study: no virologic breakthrough was observed during 12 weeks of triple therapy with PSI-7977 at different dosing levels (200 mg and 400 mg once daily). Furthermore, PSI-7977 may be effective in regimens that do not contain peginterferon. In the ELECTRON study, among 25 treatment-naïve patients with GT1 infection who received PSI-7977 and RBV, 88% achieved undetectable HCV RNA four weeks after completion of treatment. [41] In the QUANTUM trial, 59% of treatment-naïve GT1 patients were HCV RNA undetectable at four weeks after treatment with PSI-7977 and RBV. [42] Similarly, among treatment-naïve patients with HCV genotype 2 or 3 who received 12 weeks of therapy with PSI-7977 and RBV, an SVR of 100% was observed. [43]

Mericitabine (RG7128) is an oral cytosine NI dosed twice daily, and has shown efficacy against genotypes 1, 2, and 3 in phase I trials. [44] Results from the JUMP-C study, a phase II trial, highlighted the efficacy of mericitabine in combination with PEG/RBV: at week 24, 91% of patients who received mericitabine plus PEG/RBV had undetectable HCV RNA compared to 62% who SOC. [45] In addition, no viral rebound or resistance mutations were observed by week 12.

Non-Nucleoside Inhibitors of HCV Polymerase

In contrast to nucleos(t)ide analogues, NNIs have moderate to low antiviral potency and a low barrier to resistance. Because NNIs induce allosteric conformational changes that limit access of the nucleotides to the growing viral RNA chain, mutations that block binding of NNIs do not necessarily reduce viral fitness. Thus, NNIs generally have a low barrier to resistance.

NNIs are categorized according to the site of interaction with the NS5B protein. There are several NNIs in clinical development (Table 2), and they include tegobuvir and VX-222. The results of a phase II study of tegobuvir in combination with PEG/RBV were recently reported: similar SVR rates of 56% were observed among treatment-naïve chronic GT1 patients who received tegobuvir plus PEG/RBV with those who received SOC. [46] Similarly, when VX-222 was combined with telaprevir in the ZENITH study among treatment-naïve GT1 patients, the results at week 12 were disappointing, with 17% to 31% virologic breakthrough rates. [47] However, quadruple therapy consisting of VX-222, telaprevir, PEG and RBV appeared to be more effective in the study, with 83% to 90% achieving an undetectable HCV RNA at week 12.

NS5A Inhibitors

In addition to direct antiviral therapy targeting the NS3/4A serine protease and NS5B RNA-dependent RNA polymerase, novel therapeutics have been developed to inhibit the NS5A protein. The NS5A protein is important for viral replication and for the assembly of virions, and is thought to impair the innate immune response, but the precise mechanisms have yet to

be characterized. [48–51] The protein consists of three domains: domain I contains an amphipathic domain important for membrane attachment; domain II is thought to have interaction sites for cyclophilin; and domain III is involved in viral assembly. [52]

Daclatasvir (BMS-790052) is an oral NS5A inhibitor with potent in vitro activity. It appears to bind complement domain I of NS5A, and has in vitro activity against all 6 major viral genotypes, though its in vivo activity against genotypes 2–6 has not yet been demonstrated. A phase II study of daclatasvir revealed high efficacy in treatment-naïve GT1 patients treated with this drug combined with PEG/RBV, with SVR12 rates of 83% to 92%. [53] When studied in combination with GS-7977 (NS5B Inhibitor) in a phase IIa trial, the once-daily, interferon-free combination therapy demonstrated a 100% response rate at posttreatment week 4 (SVR4) in treatment-naïve patients with GT1 infection. [54]

Moreover, daclatasvir has been studied among patients with GT1 who had not responded to or were intolerant to prior therapy with PEG/RBV: dual therapy with daclatasvir and asunaprevir (an NS3 PI) was associated with an overall 77% SVR rate at 24 weeks. [55] These findings demonstrate that alternatives to interferon-based regimens are feasible and can cure even the most difficult to treat patients.

Additional NS5A inhibitors in clinical development include ABT 627 and GS-5885.

Alternate Interferons

The type I interferon alfa is a cytokine that induces hundreds of genes which collectively generate an antiviral state, and has been a cornerstone of HCV treatment. In 2009, the identification of the association between possession of single nucleotide polymorphisms (SNP) near the *IL28B* gene locus on chromosome 19 and HCV clearance has had widespread implications on the understanding of the pathogenesis of HCV and potential therapeutic options, including antiviral drug development. Host *IL28B* genotype has been shown to be an important predictor of SVR after treatment with IFN-based regimens (Ge, Thompson) and even IFN-sparing regimens (Chu). The mechanism for the contribution of *IL28B* genotype to regimens that do not include IFN is unclear, but suggests a host innate component contributes to SVR induced by direct acting antiviral therapy.

The *IL28B* gene encodes IFN- λ -3, a type-3 IFN (along with IFN- λ 1 and IFN- λ 2). In contrast to IFN alfa, type 3 IFNs bear more semblance to IL-10 in structure, and signal through a heterodimeric receptor (IFN- λ R and IL-10R). [56–58] After receptor binding, IFN- λ shares the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway with IFN alfa and has anti-HCV effects. [59, 60]

Type 3 interferons are attractive therapeutic targets. IFN- λ 1 and the IFN- λ 1 receptor are expressed at high levels by hepatocytes, but not all tissues. [61] This limited distribution of the IFN- λ 1 receptor offers the potential for a more targeted delivery of interferon therapy with decreased systemic toxicity. In a phase Ib study, PEG-IFN- λ 1 inhibited HCV without significant toxicity. [62] In the phase IIb EMERGE study of treatment-naïve patients with HCV GT1, PEG-IFN- λ 1 plus RBV produced a complete EVR in 30% patients compared with 28% of patients who received pegylated interferon-alfa plus RBV. [63] Although

delivered as a weekly subcutaneous injection, similar to PEG-IFN- α , there were generally fewer side effects such as flu-like complaints and cytopenia, as well as fewer dose reductions among those who received PEG-IFN- λ 1 and RBV.

Host-Targeted Agents

An additional approach to HCV therapy includes targeting host proteins utilized by the virus for its own life cycle. Novel therapeutics within this category include cyclophilin inhibitors, miRNA122 inhibitors, and HMG-Co-A-Reductase inhibitors.

Cyclophilin A Inhibitors

The cellular protein cyclophilin A is required as part of the HCV replication complex, and appears to modulate NS5A function by unclear mechanisms. [64, 65] Cyclophilins were identified as therapeutic targets after cyclosporine, an inhibitor to cyclophilin, was found to suppress HCV in vitro and in vivo. [66] However, because cyclosporine also inhibits calcineurin, it exerts immunosuppressive effects that limit its use as anti-HCV therapy. Additional compounds that inhibit cyclophilin but not calcineurin are in clinical development, and include alisporivir and SCY-635.

Alisporivir (DEB025) blocks the isomerase activity of cyclophilin A, preventing the interaction between NS5A and cyclophilin A, and is active against genotypes 1–4. [64, 67–69] A phase IIb study, ESSENTIAL, compared alisporivir in combination with PEG/RBV to SOC in treatment-naïve patients with HCV GT1. [70] There was a significantly higher rate of SVR among those who received 48 weeks of alisporivir-based triple therapy compared to SOC (76% vs 55%, respectively); however, there was no statistical difference in SVR rate between those who received alisporivir-based therapy for 24 weeks or response-guided alisporivir therapy compared to SOC (53%, 69%, and 55%, respectively). Alisporivir has also been studied in combination with RBV (without PEG) among treatment-naïve patients with genotype 2 or 3 infection: at posttreatment week 12, SVR rates of greater than 80% were observed. [71] The FDA has placed alisporivir on clinical hold because of pancreatitis experienced by a number of patients who received alisporivir in combination with PEG/RBV.

Preliminary studies suggest that cyclophilin inhibitors in general have a high barrier to resistance, and the resistant variants that do occur remain susceptible to DAAs.

miRNA122 inhibitors

MicroRNAs are short, noncoding RNAs that control gene expression following transcription and are involved in translational repression. [72, 73] MicroRNA-122 is the most abundant microRNA found in hepatocytes, and interacts with the HCV genome to promote viral replication (Figure 3). [74–76]

Miravirsin (SPC-3649) is a locked nucleic acid developed to inhibit miR122, and is administered subcutaneously once weekly. It has a high genetic barrier to resistance since it targets host rather than viral RNA, and has potential pan-genotypic activity. In a phase II study among treatment-naïve GT1 patients, miravirsin monotherapy yielded a mean 2–3 log

HCV RNA reduction from baseline that persisted for weeks beyond treatment and was well-tolerated. [77]

HMG CoA reductase inhibitors

HCV replication appears to rely on host cholesterol biosynthetic pathways, in particular with the mevalonate pathway. [78] The HMG-Co-A-reductase and synthase enzymes are involved in HCV replication [79, 80], and inhibitors of HMG-Co-A-reductase (statins) have antiviral effects in vitro [81] but limited antiviral effects in vivo.

When given as monotherapy, statins have not been effective against HCV [82]; however, when given in combination with PEG-IFN and RBV among treatment-naïve patients with GT1 infection, they increased both EVR and SVR rates (76% and 64%, respectively). [83]

Conclusion

Improved understanding of the HCV life cycle and virus-host interactions has led to the development of novel antiviral agents that will significantly expand options for the management of HCV. The addition of two FDA-approved protease inhibitors, telaprevir and boceprevir, to the standard of care regimen has already improved outcomes for patients with HCV genotype 1 infection.

Moreover, the development of additional classes of antiviral therapeutics, including NS5B RNA-dependent RNA polymerase inhibitors, NS5A inhibitors, alternate interferons, cyclophilin antagonists, and miR122 inhibitors, provides a unique opportunity to redefine the paradigm for the treatment of HCV. (Table 3) With a rapidly expanding selection of potent agents, it is of critical importance to determine which combination of antiviral drugs provides the most favorable resistance profile with the least toxicity. A multitude of clinical trials is under way to test combinations of agents from distinct antiviral classes with non-overlapping resistance profiles. Regimens of all-oral DAA have shown efficacy and tolerability in preliminary studies, including among patients who did not respond to prior therapy. [55] Furthermore, improved understanding of the influence of IL28 genotype on viral kinetics in response to combination DAA may allow for a more personalized approach to HCV therapy.

With the myriad of anti-HCV therapeutics in the development pipeline, we are firmly on the cusp of a new era. For the first time in twenty years, patients with chronic HCV will soon have access to oral combination therapy associated with a high resistance profile, decreased toxicity, and most importantly, higher rates of cure.

Glossary

Rapid Virologic Response (RVR)	negative HCV RNA after 4 weeks of treatment
Early Virologic Response (EVR)	at least a 2 log reduction in HCV RNA or undetectable HCV RNA after 12 weeks of treatment

	<ul style="list-style-type: none"> • Complete Early Virologic Response (cEVR): undetectable HCV RNA after 12 weeks of treatment • Partial Early Virologic Response (pEVR): at least a 2 log reduction in HCV RNA, but HCV RNA is detectable after 12 weeks of treatment
Extended Rapid Virologic Response (eRVR)	HCV RNA undetectable at both weeks 4 and 12
End of Treatment Response	undetectable HCV RNA at the end of treatment
Sustained Virologic Response (SVR)	an undetectable serum HCV RNA measured by polymerase chain reaction 24 weeks after the end of treatment.
Partial Responders	Patients who achieved a 2 log reduction in HCV RNA after 12 weeks of treatment but did not achieve an end of treatment response
Null Responders	Patients who did not achieve a 2 log drop in HCV RNA by week 12 of treatment
Prior Relapsers	Patients who achieved an undetectable HCV RNA at the end of treatment but did not achieve a sustained virologic response

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Key Points

- More than 170 million people are infected with chronic hepatitis C virus, which remains the leading indication for liver transplantation worldwide.
- Recent advances in the understanding of HCV molecular virology have led to the development of novel antiviral therapeutics.
- The HCV life cycle consists of several key steps, including polypeptide cleavage and viral replication, which represent important targets for viral eradication.
- Direct-acting antivirals (DAAs) are designed to inhibit viral targets, and include NS3/4A Serine Protease Inhibitors, NS5B RNA-dependent RNA polymerase inhibitors, and NS5A Inhibitors
- Host-targeted antivirals block host factors that are utilized by the virus for its own life cycle, and include cyclophilin A inhibitors, miRNA122 inhibitors, and inhibitors of cholesterol biosynthesis
- The development of agents in multiple classes has led to the promise of shorter therapy duration, an improved side effect profile, interferon-sparing regimens, and higher rates of cure

Figure 1. HCV viral lifecycle, HCV polypeptide structure, and cleavage sites

(A) The HCV viral lifecycle. The virus circulates as a highly lipidated lipoviral particle (LVP). Once internalized, the viral genome is uncoated, revealing the naked viral RNA and viral nucleocapsid. The viral RNA is translated by host ribosomes into the viral polypeptide (step 3), which is then cleaved by a combination of host and viral proteases into the 10 viral proteins. Replication occurs at an endoplasmic reticulum membrane-derived replication complex (the membranous web), which includes the lipid droplet (LD) and nonstructural viral proteins NS4A and NS5B (step 4). Viral replication is also dependent on the participation of key host factors, which include miR-122 and cyclophilin A (CypA). The newly synthesized viral RNA is assembled into new LVP by the Golgi apparatus and subsequently released by the cell (steps 5 and 6).

(B) HCV viral genome. The viral genome is a positive-sense, single-stranded RNA genome. The 5' untranslated region (UTR) contains 2 important domains. The internal ribosome entry site (IRES) directs translation in a cap-independent manner. The 5' UTR also contains 2 recognition sites by miR-122 that are critical for viral replication. After translation, a single viral polypeptide is generated. The structural proteins are cleaved by host proteases. The NS2/3 autoprotease cleaves the NS2-NS3 junction. The NS3/4A protease initially serves as an autoprotease and separates NS3-NS4A, but then subsequently cleaves the remaining nonstructural proteins.

NS5B RdRP binding sites: McGovern, et al. Hepatology 2008

Figure 2.
Structure of the HCV NS5B RNA-dependent RNA polymerase.

Figure 3. Sequence of miR-122

Below is shown a representation of the two miR-122-binding sites, S1 and S2, located between stem-loop (SL) I and II in the 5' nontranslated region (5'UTR) of the HCV genome. miR122 interacts with two conserved sites, which contain overlapping *cis*-acting signals involved in promoting translation of the virus. Adapted from Jangra RK et al. *Journal of Virology* 2010.

Table 1

NS3/4A Serine Protease inhibitors in advanced clinical testing

DRUG	Current Phase	CLASS	Genotype	Dosing	Clinical Data (if available) *	Adverse Effects/Notes:
Telaprevir	FDA Approved	Linear	1 (2,5,6)	3/day	SVR: 69–75% (1) SVR (SOC): 44%	Rash, anemia, pruritus
Boceprevir	FDA Approved	Linear	1 (2,5,6)	3/day	SVR: 63–66% (2) SVR (SOC): 40%	Anemia, dysgeusia
TMC-435	Phase 3	Macrocyclic	1 (2,5,6)	1/day	SVR: 75–85% (3) SVR (SOC): 65%	Elevated bilirubin
BI 201335	Phase 3	Linear tripeptide	1	1/day	SVR: 73–83% (4) SVR (SOC): 56%	Nausea, diarrhea, jaundice, rash
Narlaprevir	Phase 2	Linear	1	1/day	SVR24: 70–85% (5) SVR24 (SOC): 28%	Used with ritonavir; fatigue, myalgias
ACH-1625	Phase 2	Linear	1	1/day	RVR: 75–81% RVR(SOC): 20% (6)	Headache, fatigue, nausea
Danoprevir	Phase 2	Macrocyclic	1	2/day	SVR24: 68–85% (7) SVR24(SOC): 42%	ALT elevation decreased when used with ritonavir
Vaniprevir	Phase 2	Macrocyclic	1	2/day	SVR: 61–84% (8) SVR (SOC): 63%	Headache, nausea, vomiting
ABT-450	Phase 2	Acrylsulfonamide	1	1/day	cEVR: 92% cEVR (SOC): 18% (9)	Used with ritonavir; headache, pruritus
BMS-650032	Phase 2	Active Site	1,4	2/day	cEVR: 75–91.7% (10) cEVR (SOC): 0%	ALT elevation at higher doses
GS-9256	Phase 2	Macrocyclic	1	2/day	cEVR: 100% (11) **	Used with tegobuvir
MK-5172	Phase 2	Macrocyclic	1 (2,3,4,5,6)	1/day	75% of GT1 and 38% of GT3 achieved HCV RNA levels below the limit of quantitation with 7 days of monotherapy (12)	Headache, pruritus, nausea, fatigue; pan-genotypic, low cross resistance (13)
GS-9451	Phase 2	Macrocyclic	1	1/day	n.a.	

Abbreviations: SVR: Sustained virologic response. SVR12: undetectable HCV RNA 12 weeks after completion of therapy. RVR: rapid virologic response. SOC: standard of care. pegylated-interferon α plus ribavirin. cEVR: complete early virologic response.

* Data are for genotype 1, combined with standard of care (SOC) pegylated interferon α and ribavirin, unless otherwise indicated

** On quadruple therapy including tegobuvir plus SOC, GS-9246 plus SOC not tested

Table 2

NS5B Nucleoside Inhibitors (NI) and Non-Nucleoside Inhibitors (NNIs)

CLASS(site)	DRUG	Current Phase	Genotype	Dosing	Clinical Data (if available) *	Adverse Effects/Notes:
Nucleoside Inhibitors						
Active site	Mericitabine	Phase 2b	1–3 (4,5,6)	2/day	SVR12: 91% (14) ** SVR12 (SOC): 62%	No resistance-related breakthrough
Active site	PSI-7977	Phase 2b	1–3 (4,5,6)	1/day	SVR: 88–91% EOT (SOC): 50% *** (15)	Anemia, neutropenia; No viral breakthrough observed
Active site	IDX 184	Phase 2a	(1–6)	1–2/day	RVR: 63–73% (16)	Anemia, nausea
Non-nucleoside Inhibitors						
Thumb Domain 2	Filibuvir	Phase 2b	1	2/day	SVR12: 30–50% (17) SVR12 (SOC): 50%	High rates of relapse observed
Palm Domain 2	Tegobuvir	Phase 2b	1	2/day	SVR: 56% SVR (SOC): 56% (18)	No significant difference from SOC
Palm Domain 1	Setrobuvir (ANA-598)	Phase 2b	1	2/day	cEVR: 78% cEVR (SOC): 56% (19)	Rash more common at higher doses
Palm Domain 1	ABT-072	Phase 2a	1	1/day	EVR: 70% (20) EVR (SOC): 18%	Unfavorable IL28B genotype associated with failure to achieve cEVR
Palm Domain 1	ABT-333	Phase 2a	1	2/day	EVR: 75% (21) EVR (SOC): 18%	Rash, photosensitivity
Thumb Domain 1	BI 207127	Phase 2a	1	3/day	RVR: 100% (22) ****	Combined with RBV, and BI 201335. Headache, nausea, pruritus
Thumb Domain 2	VX-222	Phase 2	1	1/day	SVR12: 83–90% (23) ****	Fatigue, rash, anemia, pruritus
Palm Domain 2	IDX-375	Phase 2	1	1x/d	n.a.	n.a.

Abbreviations: SVR: Sustained virologic response. SVR12: undetectable HCV RNA 12 weeks after completion of therapy. RVR: rapid virologic response. SOC: standard of care, pegylated-interferon α plus ribavirin. EOT: End of treatment response. cEVR: complete early virologic response.

* Data are for genotype 1, combined with standard of care (SOC) pegylated interferon α and ribavirin, unless otherwise indicated

** SVR12 based on subset who achieved RVR. SOC data not yet available.

*** Genotypes 1 SVR (SOC) data pending. 100% SVR was observed in genotype 2/3 patients receiving PSI-7977 and RBV only (21)

**** No SOC arm for comparison.

Table 3

Classes of HCV antiviral agents

CLASS	POTENCY	BARRIER TO RESISTANCE	COMMENTS
Protease Inhibitors	High	Low	Linear and Macrocyclic inhibitors with some cross resistance
NS5B Nucleos(t)ide Inhibitors	Moderate – High	High	Activity across genotypes
NS5B Non-Nucleoside Inhibitors	Low - Moderate	Low	Several allosteric active sites and targets; variable resistance patterns, might be combined
NS5A Inhibitors	Moderate-High	Low	NS5A has pleiotropic effects
Cyclophilin Inhibitors	Moderate	High	Activity across genotypes
MiR122 Antagomir	Moderate	High	In early development