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Anti-HCV drugs in the pipeline

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Abstract

Several directly-acting and host-targeting antivirals that inhibit hepatitis C virus replication have entered clinical trials. Amongst the most advanced of these are RG7128, an inhibitor of the NS5B polymerase; BMS-790052, an inhibitor of NS5A; and alisporivir, an inhibitor of human cyclophilins. These agents have potent antiviral activity in chronic HCV patients, act additively or synergistically with inhibitors of the HCV NS3/4A protease, and improve the rate of virologic response produced by traditional pegylated interferon plus ribavirin therapy. No cross resistance has been observed; moreover, nucleoside NS5B and cyclophilin inhibitors appear to suppress resistance to non-nucleoside NS5B and NS3/4A inhibitors. Several recent reports of virologic responses produced by combinations of agents that inhibit HCV replication in the absence of interferon provide optimism that eradication of HCV will be possible without interferon in the future.

Introduction

Hepatitis C virus (HCV) is a small enveloped RNA virus that currently infects over 170 million individuals worldwide, making it a leading cause of liver disease. Infection with HCV has a high rate of chronicity, estimated to be in the range of 75-85%. Chronic HCV is associated with significantly increased risk for chronic liver disease, cirrhosis, and hepatocellular carcinoma. Pegylated interferon and ribavirin (PEG-IFN/RBV), mainstays of chronic HCV therapy and until recently the standard of care (SOC), are poorly tolerated and have variable efficacy across the seven major HCV genotypes with particularly low success

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rate against genotype 1. Unlike many other chronic viral infections, however, chronic HCV is a curable disease. Recently approved drugs targeting the HCV NS3/4A protease (PIs) are a major step toward the goals of improving the percentage of patients who experience a sustained virologic response (SVR) and decreasing treatment time. These and other directly-acting antivirals (DAAs) and host-targeting antivirals (HTA) that act via independent mechanisms are needed in order to combat protease inhibitor resistance, to improve efficacy across all HCV genotypes, and to advance antiviral therapy towards the ultimate goal of an interferon-free cure. The goal of this review is to provide an overview and summary of non-protease HCV inhibitors currently in the clinical pipeline. Due to space limitations, specific inhibitors have been chosen as foci for our discussion with the goal of highlighting the major targets, mechanisms of action, and resistance studies carried out to date.

Directly-Acting Antivirals (DAAs) and Host-Targeting Antivirals (HTAs)

The plus-sense RNA genome of HCV is translated as a single polyprotein that is processed into ten individual proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) by a combination of host and viral proteases. Replication of HCV is mediated by a combination of viral and host factors (Figure 1), and candidates in the current clinical pipeline include agents against both types of targets (Table 1). As indicated in Figure 2 and Table 1, directly-acting antivirals (DAAs) targeting the NSS3/4A protease, NS4B, NS5A, and NS5B polymerase are currently in various stages of clinical development. Agents that target host factors essential for HCV replication are also under development and may have higher genetic barriers to resistance compared to DAAs. The goal of these host-targeting drugs (*e.g.*, alisporivir, SPC3649) is to interfere with host factors that support viral replication, a therapeutic strategy distinct from agents that modulate the host innate immune response (*e.g.,* alternative interferons, nitazoxanide). Compounds targeting the NS5B polymerase, NS5A, and the cyclophilins are the most likely to be approved in the near-term for treatment of chronic HCV and are the major focus of this review.

NS5B Polymerase Inhibitors

Replication of the HCV genome is catalyzed by non-structural protein 5B (NS5B), an RNA-dependent RNA polymerase (RdRp), which forms an active replicase when complexed with other viral and cellular proteins. NS5B is highly conserved across HCV genotypes and adopts a right-handed three-dimensional structure with fingers, palm, and thumb domains (PDB IDs 1C2P and 1CSJ) analogous to those of the RdRps of other RNA viruses. Current NS5B inhibitors are classified in two major groups: the nucleoside and nucleotide inhibitors (NIs) that bind in the polymerase active site and the non-nucleoside inhibitors (NNI) that bind in one of four allosteric binding sites on the polymerase (NNI sites 1-4). Due to the high degree of conservation in the NS5B active site, NIs of NS5B generally exhibit broad spectrum activity against the HCV genotypes with high barriers to resistance due to the fitness costs associated with the mutations that confer resistance [1**-3*]. Compared to NIs, NNIs exhibit more variable activity across HCV genotypes [4], consistent with the reduced sequence conservation at the allosteric sites. Resistance to NNIs develops rapidly in vitro [2,5,6], and the presence of naturally occurring mutations that confer resistance to NNIs in treatment-naive patients [7**,8*] has been correlated with resistance in vivo [9*]. We will focus here on RG7128 (Pharmasset/Roche), the NS5B inhibitor which has progressed furthest in clinical testing to date. Although space limitations preclude an in depth discussion of the many NIs and NNIs in development, we refer readers to the excellent review by Legrand-Abravanel and colleagues [10].

RG7128 (R7128, mericitabine), a prodrug, is hydrolyzed in vivo to produce PSI-6130. [11] (Figure 3). Following activation by cellular kinases to the 5′-triphosphate, PSI-6130 acts as a non-obligate chain terminator [12]. In early results from a Phase 2b study (JUMP-C) of

RG7128 with PEG-IFN/RBV in genotype 1 and 4 patients, 60% of patients achieved an extended rapid virologic response (eRVR, defined as undetectable HCV RNA from weeks 4 to 22) versus 13% for the SOC group; moreover, of the 49 patients who achieved eRVR on RG7128 with PEG-IFN/RBV, 37 went on to achieve a sustained virologic response with undetectable HCV RNA at 12 weeks post-treatment (SVR12) [13]. In addition, of 20 genotype 2 and 3 non-responders treated with RG7128 and SOC, 18 achieved RVR and 13 achieved SVR12 [14]. In an ascending dose Phase 1 trial (INFORM-1), treatment naïve patients infected with HCV genotype 1 who were given the combination of RG7128 and RG7227, an HCV NS3 protease inhibitor, experienced a median change from baseline HCV RNA of ~5.0 $\log_{10}$ IU/mL at the highest doses tested, with 5 of 8 naïve patients achieving HCV RNA below the limit of detection (< 15 IU/mL) [15**]. Importantly, 2 of 8 patients who were null responders with prior PEG-IFN/RBV achieved undetectable HCV RNA during this short-term trial. Interestingly, the effects of RG7128 and RG7227 in combination were greater than the sum of their effects as monotherapies. This study provided the first demonstration that two DAAs can be combined safely to suppress HCV replication in HCV patients and provides the foundation for Phase 2 trials assessing RVR and SVR in patients infected with genotype 1 who have previously not responded to SOC.

In vitro studies have established that Ser282Thr confers resistance to RG7128 and other 2'C-methyl nucleoside NIs [16,17], but resistance to RG7128 and other NIs in vivo has not yet been observed [1*]. Second generation nucleotide inhibitors of NS5B, such as the purine analogues PSI-879 and PSI-938, are active against the S282T-resistant variant selected in vitro by RG7128, a pyrimidine [18*]. This suggests that it may be possible to use purine and pyrimidine NIs of NS5B in combination with each other as well as in combination with NNIs, DAAs against other viral targets, host-targeting antivirals, and PEG-IFN/RBV in the future.

NS5A inhibitors

NS5A is a multifunctional phosphoprotein required for several stages of HCV replication [19]; however, with no known enzymatic activities, NS5A's precise role in the HCV life cycle is poorly understood. Several NS5A inhibitors with picomolar potency in HCV replicon-based assays have been reported [20-27], and impressive anti-HCV effects have been observed in clinical studies with BMS-790052 (Bristol-Myers Squibb) [28**], GS-5885 (Gilead) [29], and PPI-461 (Presidio) [30]. In vivo effects have correlated well with in vitro replicon activity [31]. Of these, BMS-790052 is the most advanced in development and the focus of this review. BMS-790052 (Figure 3) is a replication complex inhibitor with broad genotypic coverage [28**]. Its development was based on a screening hit, BMS-824, that was identified in a cell-based HCV replicon assay and that was found to undergo an intermolecular, radical-mediated dimerization in cell culture [32]. Although the precise mechanism by which BMS-790052 inhibits NS5A function is not known, it is clear that this series of NS5A inhibitors can block HCV RNA replication [28**,32]. The ability of BMS-790052 to potentially inhibit both cis- and trans-functions of NS5A strongly suggests that NS5A has more than one function required for viral RNA replication [33]**.** The C2-symmetry of BMS-790052 and related compounds complements the structure of the N-terminus of domain-1 of NS5A, which crystallizes as a dimer [34,35]. The exceptional *in vitro* potency of BMS-790052 has translated to a robust anti-HCV effect in the clinic. In a single ascending dose trial conducted in patients chronically infected with HCV, a 1 mg dose of BMS-790052 resulted in a 1.8 $\log_{10}$ IU/mL reduction in mean viral load measured at 24 hours post-dose, while a 100 mg dose produced a 3.3 $\log_{10}$ reduction in viremia [28**]. In a Phase IIa study, BMS-790052 (3, 10, and 60 mg) combined with PEG-IFN/RBV generated SVR12 rates of 42% (5 of 12), 92% (11 of 12), and 83% (10 of 12), respectively, compared to 25% (3 of 12) for the placebo control [36*].

Almost all subjects treated with BMS-790052 in a 14-day monotherapy study experienced a robust initial decline in HCV RNA; however, viral RNA was detectable in all genotype 1a and some genotype 1b subjects by the end of the treatment period [37]. Genotypic and phenotypic analysis of clinical specimens showed the emergence of resistant variants [38**]. In general, the resistance profile observed in the clinic is similar to the profile observed in vitro: (i) substitutions associated with resistance map to the first 100 amino acids of NS5A; (ii) major substitutions occur at residues Met28, Gln30, Leu31 and Tyr93 for genotype 1a and at residues Leu31 and Tyr93 for genotype 1b; (iii) single amino acid substitutions in genotype 1b NS5A generally yield low levels of resistance (<30-fold), while single amino acid substitutions in genotype 1a NS5A and combinations of two amino acid substitutions in either genotype generally yield a much higher level of resistance (>300-fold) [36**,38**,39*]. Importantly, HCV variants resistant to BMS-790052 remain fully sensitive to IFN and small molecule inhibitors of the HCV protease and polymerase [38**,39**].

Host-targeting Antivirals: Cyclophilin inhibitors

The cyclophilins (CyPs), a family of highly conserved cellular peptidyl-prolyl cis-trans isomerases (PPIases), are involved in many cellular processes such as protein folding, protein trafficking, and multi-protein complex assembly. CyPs are required for HCV replication, and both pharmacological inhibition of CyPs and RNAi-mediated “knock-down” of CyPs have been shown to block HCV replication in vitro [40-44]. CyPA (and possibly CyPB) interacts directly with NS5A (and possibly NS5B) [41,42,45-49]. CyP PPIase activity is required for efficient HCV replication [40,42,43,50]; moreover, genetic [51-54]; and biochemical data [45,54-56] suggest that HCV proteins are substrates for CyP. Although the functions of CyPs in HCV replication remain to be further elucidated, roles in the correct folding and trafficking of viral proteins to replication complexes (RCs) as well as in the modulation of RNA binding and or RNA synthesis by NS5B have been proposed [40,41,46,47,49,50,52]. The most common concern of targeting host factors is the potential side effect that may be incurred by inhibiting their normal cellular functions. While Cyp A is one of the most abundant cytosolic proteins, it does not appear to be essential to cells: the main defect of Cyp A knock-out mice was allergic blepharitis, which has not been noted in humans treated with cyclophilin inhibitors.

The first known cyclophilin inhibitor, cyclosporin A (CsA), is an immunosuppressive drug used for organ transplantation. Importantly, the immunosuppressive function of CsA is mediated by calcineurin-binding but not cyclophilin-binding [57], making non-calcineurin binding, non-immunosuppressive cyclosporin analogs ideal candidates for HCV therapy. To date three non-immunosuppressive cyclophilin inhibitors have shown clinical efficacy in HCV patients [58*-60*]. Among them alisporivir (Debio-025, DEB025; Debiopharm/Novartis) (Figure 3) is the most advanced in Phase III studies. It is about ten times more potent than CsA in vitro but lacks immunosuppressive activity [61]. In a Phase I trial with HIV-HCV co-infected patients, 1200 mg twice daily of alisporivir monotherapy resulted in a 3.4 $\log_{10}$ reduction of HCV RNA after 14 days [62**]. In the Phase IIa combination study, 600 mg daily alisporivir plus PEG-IFN-α2a led to 4.6 $\log_{10}$ viral load reduction after 4 weeks in genotypes 1 and 4 patients and 5.9 $\log_{10}$ reduction in genotype 3 patients [58*]. In the Phase IIb trial genotype 1 treatment-naïve patients had a 76% SVR rate after receiving alisporivir and PEG-IFN-α2a/ribavirin triple therapy for 48 weeks [63**]. Two other cyclosporin analogs, NIM811 and SCY-635, have also shown proof-of-concept efficacy in HCV patients either alone or in combination with PEG-IFN-α2a [57,60*,64].

It appears much more difficult to develop resistance against cyclophilin inhibitors compared to DAAs both in vitro and in patients. Only low-level (~10-fold) of resistance was selected in vitro after prolonged (weeks to months) incubation of replicon cells with cyclophilin inhibitors [54,65*,66*]. Several in vitro resistance studies revealed that viral mutations are

mainly located in the domain II of NS5A [48,52,54,66*], which is consistent with the fact that this proline-rich region is a substrate of cyclophilin PPIase [45]. Asp320Glu in NS5A, which may reduce the need for Cyp A-dependent isomerization of NS5A [54], was the only mutation consistently selected in all the resistant clones. Asp320Glu confers only a modest degree of resistance to cyclophilin inhibitors in vitro (approximately three-fold), and although it has been observed clinically, it does not appear to be sufficient to permit viral breakthrough in patients [67]. Importantly, no cross-resistance has been observed in vitro between cyclophilin inhibitors and DAAs including NS5A inhibitors, which target domain I of NS5A. The combination of alisporivir with DAAs not only led to additive to synergistic antiviral effects in vitro but also helped to block the emergence of resistance [65*,68*]. In patients, very low breakthrough rates have been reported with alisporivir as monotherapy or in combination with SOC. Additional viral mutations and possibly cellular changes are likely required to confer a more significant level of resistance.

Novel anti-HCV targets in earlier stages of the drug development pipeline

Several recently reported anti-HCV targets are also worth noting although drug development efforts against them are at much earlier stages. HCV non-structural protein 4B (NS4B) [69] and host phosphatidylinositol-4-kinase IIIa (PI4KIIIa) [70-75] are required for formation of the membranous web and efficient HCV RNA replication. Although selective inhibitors of PI4KIIIa demonstrating pharmacological inhibition of HCV via this target have not yet been reported, proof of concept has been established for NS4B. Clemizole was identified in a high-throughput microfluidic screen as an inhibitor of NS4B-RNA binding (EC_{50} ~8 μM) that blocks HCV RNA replication in cell culture [76*]. Isolation of clemizole-resistant variants permitted mapping of resistance to Trp55Arg and Arg214Gln mutations in NS4B [76*]. GlaxoSmithKline very recently disclosed that GSK-8853, a potent NS4B inhibitor (EC_{50} 0.74 nM in genotype 1a replicon assays) caused a mean viral load reduction at nadir of 4.23 $\log_{10}$ when given orally at 100 mg/kg twice daily for 7 days in the human uPA/SCID mouse model [77*].

MicroRNA-122 (miR-122), which regulates cholesterol biosynthesis, is essential for the accumulation of HCV RNA in cell culture [78]. Although the underlying mechanism(s) are still under investigation, miR-122's interaction with two target sites in the 5′ noncoding region of the HCV genome are known to be critical for its positive regulation of the virus [79,80]. Demonstrating miR-122's potential as an anti-HCV target, SPC3649 (miravirsen), a locked nucleic acid-modified oligonucleotide complementary to miR-122, caused long-lasting suppression of HCV viremia with no evidence of viral resistance in infected chimpanzees [81*].

Diacylglycerol acyltransferase-1 (DGAT1) is a host factor that induces lipid droplet formation and whose interaction with core has been demonstrated to be essential for HCV assembly [82]. A small molecule inhibitor of DGAT1 was shown to block this interaction and prevent recruitment of RNA replication complexes to the site of virion assembly while having no effect on the formation of DGAT2-induced lipid droplets [82]. These findings suggest that DGAT1 inhibitors that are currently in early clinical trials for obesity-associated diseases may also have potential as anti-HCV agents.

Is there an IFN-free Cure for HCV in the Future?

Two decades following the discovery of HCV, drug development efforts are now beginning to yield a diverse set of anti-HCV agents that inhibit HCV replication via both viral and host targets. Theoretically, a collection of mechanistically distinct HCV drugs that behave additively and/or synergistically against HCV in combination and that have orthogonal resistance profiles has the potential to yield an IFN-free cure for chronic HCV. The

demonstration that HCV replicons with dual resistance to PI and NNI can be selected in vitro [83**] suggests that such a cure will require more than two agents. Recent data indicate that this goal is not yet attainable but provide reasons for optimism. First, Gilead [84] and Vertex [85] have recently reported that while combinations of two DAAs targeting NS3/4A and NS5B were not able to achieve desirable levels of RVR, the addition of ribavirin to a combination of tegobuvir (NS5B NNI) and GS-9256 (PI) significantly improved the RVR rate (38% versus 7%). -Boehringer Ingelheim [86] also showed that all 17 subjects achieved HCV RNA suppression (< 25 IU/mL) after 4 weeks of combination therapy with BI-201335 (PI)/BI-207127 (NS5B NNI)/ribavirin. A combination of two NS5B NIs, PSI-7977 and PSI-938, resulted in 94% subjects with HCV RNA <15 IU/mL at the end of 14-day treatment [87]. Finally, a combination of BMS-790052 (NS5A inhibitor) and BMS-650032 (PI) in null subjects showed promising initial results: for a total of 11 subjects, 2 of 2 GT-1b subjects and 2 of 9 GT-1a subjects achieved SVR12 [88**], providing the first clinical evidence that HCV can be eradicated by a DAA combination. Future inhibitor combinations of complementary antivirals with pan genotypic coverage certainly offer new hope for the treatment of HCV infection. As was observed with the rapid development of highly efficient combination therapy for HIV, the arsenal of modern approaches for drug discovery including well designed biochemical and cellular high-throughput screening, structure-based drug design, mutation susceptibility screening, and expeditious exploration of combination therapy in preclinical and clinical studies is likely to provide drugs that will revolutionize the treatment of HCV.

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Highlights

New antivirals in clinical trials for chronic HCV have viral and host targets

RG7128 is a classic active site inhibitor of the HCV NS5B RNA-dependent RNA polymerase

BMS-790052 targets the HCV NS5A protein to inhibit viral replication

DEB025 inhibits formation of active viral replicases by targeting host cyclophilins of active viral replicases by targeting host cyclophilins

SVR12 in patients treated with DAAs suggests that an IFN-free cure may be possible

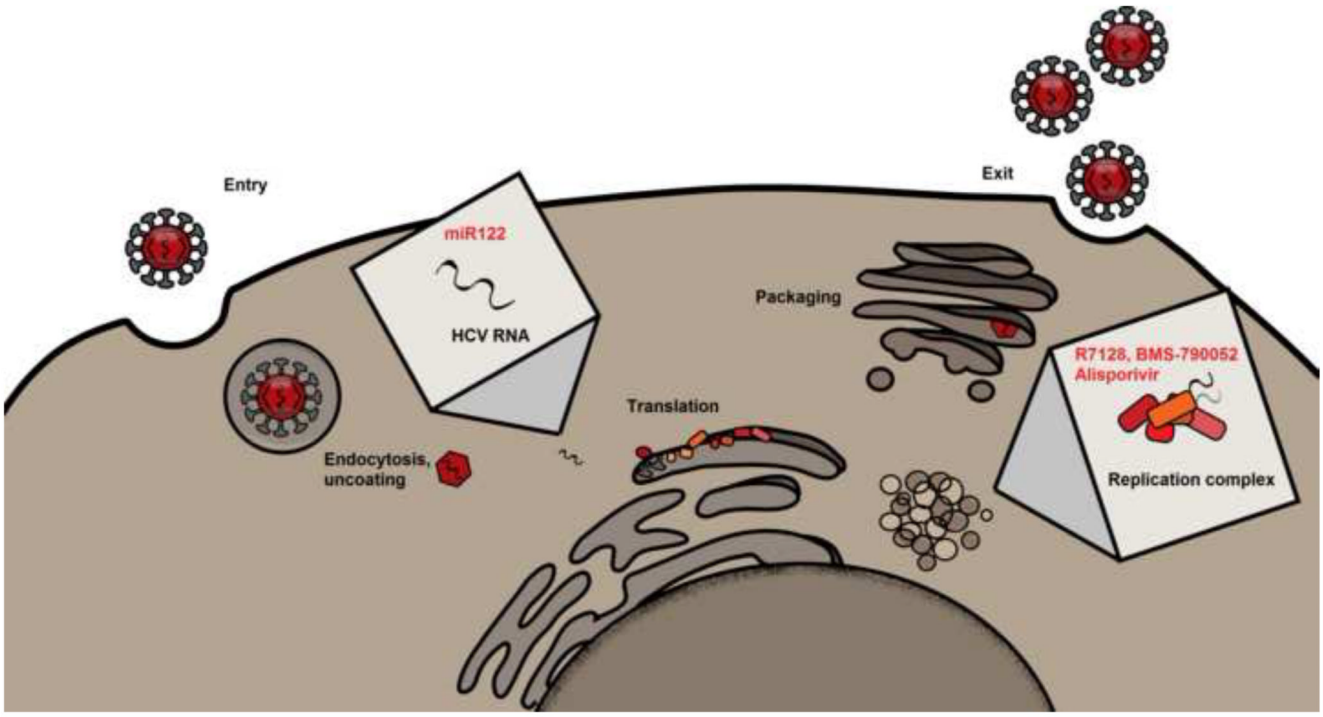

Figure 1. The hepatitis C virus replication cycle

HCV enter host hepatocytes via receptor-mediated endocytosis. HCV RNA (positive strand in black) is liberated in the cytoplasm and translated into a single polyprotein precursor that is cleaved by viral and host proteases to produce single viral proteins. Individual non-structural proteins form a replication complex to mediate replication of the positive-stranded viral RNA via a complementary intermediate (negative strand in grey). The newly-synthesized positive-stranded RNA is packaged and assembled with structural proteins to produce mature virions, which are then secreted. Each major step in the HCV life cycle is being examined to generate alternative anti-viral agents. Novel HCV therapeutics (in red text) that are currently in advanced clinical trials are discussed in detail.

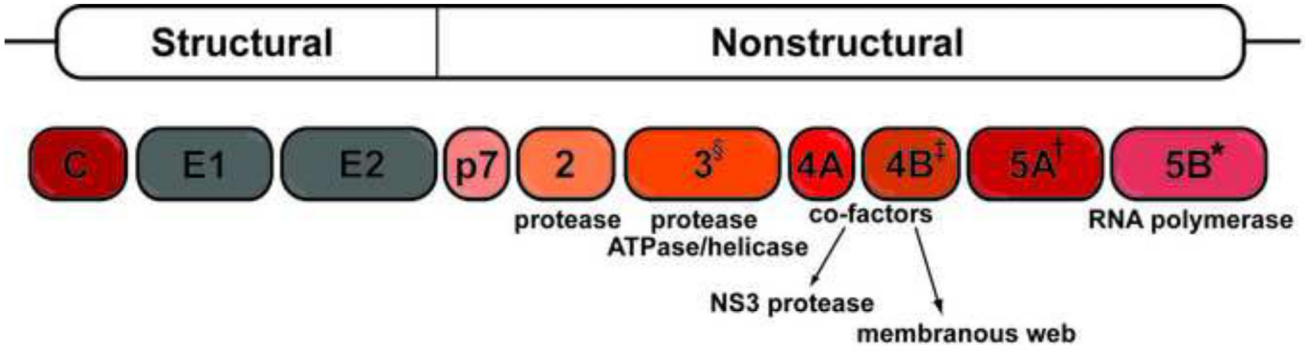

Figure 2. The HCV polyprotein prior to cleavage by viral and hosts proteases

Core (C), Envelope1 (E1) and Envelope2 (E2) are the protein components of the assembled virus and house the viral RNA. Nonstructural (NS) proteins, which are not packaged in the mature virus, assist or mediate the replication of viral RNA. P7 is a putative ion channel important for NS2 translocation. As listed above, each NS protein contributes to the propagation of viral RNA. NS5A is a multifunctional phosphoprotein. §Numerous directly-acting antivirals are currently in advanced-stage clinical trials or have been approved for use. ‡Directly-acting antivirals are currently being studied, although none are currently in advanced clinical trials. †,*Directly-acting antivirals in advanced studies are discussed in detail in this review.

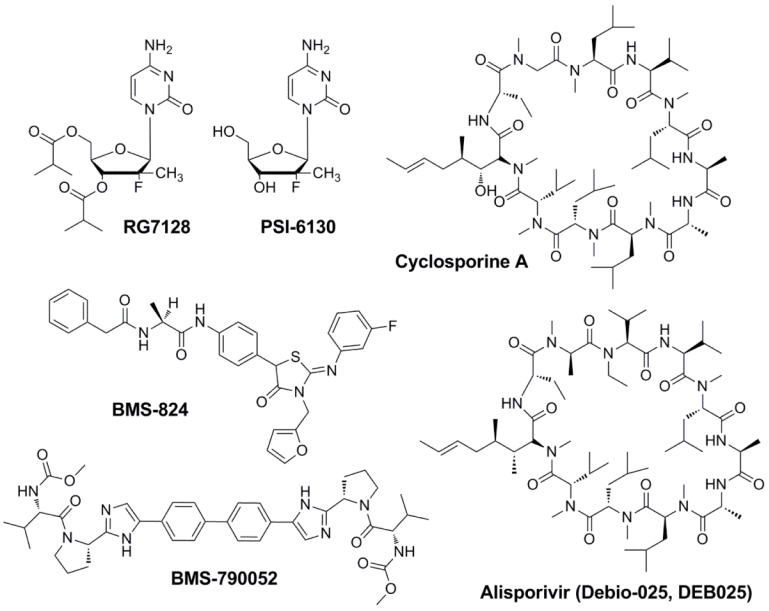

Figure 3. Structures of inhibitors and parent compounds discussed in this review.

Table 1
Current drugs in the anti-HCV pipeline

This is a list of drugs currently in phase II and phase III stages of development and testing. While we have made our best effort to make this list up to date, this is a dynamic and rapidly changing area of research.

Target	Inhibitor Name	Company	Status
Directly-acting			
NS3	Incivek (telaprevir, VX-950)	Vertex	Approved
NS3	Victrelis (Boceprevir, SCH503034)	Merck	Approved
NS3	MK-7009 (Vaniprevir)	Merck	Phase II
NS3	ITMN-191/R7227	Intermune/Roche	Phase II
NS3	TMC435	Medivir/Tibotec	Phase III
NS3	BI 201335	Boehringer Ingelheim	Phase III
NS3	GS 9256	Gilead	Phase II
NS3	BMS-650032	Bristol-Myers Squibb	Phase II
NS3	ACH-2684	Achillion	Phase II
NS3	ABT-450	Abbott/Enanta	Phase II
NS3	ACH-1625	Achillion	Phase II
NS3	MK-5172	Merck	Phase II
NS5A	BMS-790052	Bristol-Myers Squibb	Phase III
NS5A	PPI-461	Presidio	Phase II
NS5A	GS5885	Gilead	Phase II
NS5B (NI)	IDX184	Idenix	Phase II
NS5B (NI)	PSI-7977	Pharmasset	Phase II
NS5B (NI)	PSI-938	Pharmasset	Phase II
NS5B (NI)	R7128	Roche/Pharmasset	Phase II
NS5B (NI)	INX-189	Inhibitex	Phase I
NS5B (NI)	GS6620	Gilead	Phase I
NS5B (NI)	TMC-649128	Tibotec/Medvir	Phase I
NS5B (NNI; palm 1)	ABT-072	Abbott	Phase II
NS5B (NNI; palm 1)	ABT-333	Abbott	Phase II
NS5B (NNI; palm 1)	ANA598 (Setrobuvir)	Anadys	Phase II
NS5B (NNI; palm 2)	GSK2485852A	GSK	Phase I
NS5B (NNI; thumb 1)	BI 207127	Boehringer Ingelheim	Phase II
NS5B (NNI; thumb 2)	VX-222	Vertex	Phase II
NS5B (NNI; thumb 2)	VX-759	Vertex	Phase II
NS5B (NNI; thumb 2)	PF-868554 (Filibuvir)	Pfizer	Phase II
NS5B (NNI; beta-hairpin)	GS 9190 (Tegobuvir)	Gilead	Phase II
Antibody to HCV	Civacir	NABI Biopharmaceuticals	Phase II
Host targeting antivirals			
Cyclophilin	Debio 025 (Alisporivir)	Novartis/Debiopharm	Phase III
Cyclophilin	SCY-635	Scynexis	Phase II
Cholesterol	Fluvastatin	Oklahoma University	Phase II

Target	Inhibitor Name	Company	Status
Entry inhibitor (SR-BI)	ITX-5061	iTherX	Phase II
Matrix metalloprotease	CTS-1027	Conatus	Phase II
miR-122	Miravirsen (SPC3649)	Santaris Pharma	Phase II
Immunomodulators			
Anti-inflammatory	JKB-122	Jenkin	Phase II
Anti-inflammatory	Mito-Q	Antipodean	Phase II
Anti-inflammatory	PYN17	Phynova	Phase II
Anti-inflammatory	CF102	Can-Fite	Phase II
Anti-inflammatory	NOV-205	Novelos	Phase II
Immunomodulatory	SCV-07	SciClone Pharma	Phase II
Immunomodulatory	Zadaxin	SciClone Pharma/Sigmatau	Phase III
Interleukin-7	CYT107	Cytheris	Phase II
Phosphatidylserine	Bavituximab	Peregrine Pharm	Phase II
PKR	nitazoxanide (Alinia)	Romark	Phase II
TLR-7	ANA773	Anadys	Phase II
Unknown	Silymarin, Silibinin	various	Phase II, III