Novel Therapies in Hepatitis B and C

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Chronic hepatitis B and C affect approximately 500 million people in the world, with substantial disease burden including liver cirrhosis and hepatocellular carcinoma. For chronic hepatitis B, two treatment strategies are currently available, both with suboptimal response and significant side effects. Promising new drugs are approaching the stage of approval; however, these agents still need further development to control this disease. Based on the understanding of the hepatitis C virus life cycle, new treatment developments for chronic hepatitis C tend to succeed rapidly; therefore, it is only a matter of time before new therapies emerge. This review summarizes the most important new agents available for treatment of chronic hepatitis B and C.

Introduction

Chronic hepatitis B and C affect 9% of the world population; of these approximately 500 million people, 6% have chronic hepatitis B and 3% have chronic hepatitis C [1]. Worldwide, it is estimated that 57% of cirrhosis is due to either hepatitis B virus (HBV) (30%) or hepatitis C virus (HCV) (27%) [2]. In hepatocellular carcinoma, 78% of cases are attributable to both viruses, in which HBV accounts for 53% and HCV for 25%. Mortality estimation models estimate approximately 366,000 deaths are due to HCV each year and 563,000 to 620,000 deaths are due to HBV, thereby making chronic hepatitis B the ninth leading cause of death in the world [2–4].

In the past decade, therapeutic options for chronic hepatitis B and C have dramatically improved. This has resulted in more HCV patients achieving a sustained virological response and more chronic hepatitis B patients achieving a state of inactive disease. Unfortunately, treatment of both infections is not yet optimal, especially in chronic hepatitis B, for which a cure is still far away. This article summarizes new treatment options for both chronic hepatitis B and C.

Chronic Hepatitis B
Therapeutic aims and response definitions
The primary aim in treating chronic hepatitis B is reducing liver cell inflammation and progression of fibrosis before cirrhosis and hepatocellular carcinoma develop. This is done by suppressing HBV replication and inducing hepatitis B e antigen (HBeAg) seroconversion in HBeAg-positive chronic hepatitis B patients. Because the natural course in chronic hepatitis B is typically silent and asymptomatic until cirrhosis or hepatocellular carcinoma develops, the problem lies in detecting which patients need treatment. Indications for treatment include alanine aminotransferase (ALT) levels greater than two times the upper limit of normal for more then 6 months, with HBV DNA levels greater than 20,000 IU/mL. In some chronic hepatitis B patients who do not fully meet these criteria, a liver biopsy can help decide if treatment is indicated.

Two treatment strategies are available that affect different steps in the HBV life cycle. Immunomodulatory therapy consists mainly of pegylated interferon-α (IFN-α), which enhances the host immune response against HBV-infected hepatocytes. There are also nucleoside/nucleotide analogues that interfere directly with the life cycle of HBV by inhibiting reverse transcription.

Standardization of terminology and definitions for therapy end points is of utmost importance. *Biochemical responses* are generally defined as decreases in serum ALT levels to the normal range (≤ 45 U/L). *Histological responses* are usually defined as at least a two-point decrease in histological activity index with no worsening of fibrosis between pretreatment and end-of-treatment liver biopsies. A *virological response* is defined as loss of HBV DNA in serum measured by polymerase chain reaction assays, and loss of HBeAg in patients who were initially HBeAg positive. Response is *complete* when hepatitis B surface antigen (HBsAg) has disappeared and anti-HBs antibodies have appeared. A viral response is *sustained* when it has been maintained for more than 6 months after stopping therapy. *Virological breakthrough* is defined as a 1-log_{10} increase in HBV DNA during treatment, indicating virological resistance. When ALT levels during treatment also rise, it is called *biochemical breakthrough* [5–7].

Current therapeutic options
Table 1 outlines the various treatment options currently available for chronic hepatitis B and details their response outcomes [6,8–13,14••,15•,16–18].

Standard IFN-α
IFN-α has antiviral, antiproliferative, and immunomodulatory effects, and is widely used in the treatment of viral hepatitis. IFN-α is administered three times weekly in 5- to 10-MIU doses for the duration of 4 to 6 months.

In 1993, a meta-analysis demonstrated that HBeAg-positive chronic hepatitis B subjects treated with IFN-α had significantly better results in terms of HBsAg loss (8%), HBeAg loss (33%), and undetectable HBV DNA levels (37%) [8]. A more recent study demonstrated about the same results, but also reported an HBeAg seroconversion of 19% and improvement of histological activity of 46% [9].

Clinical trials with HBeAg-negative chronic hepatitis B patients have also been described, but all have had smaller numbers of patients (Table 1). About half of the patients relapsed when treatment was discontinued.

Pegylated IFN-α (peginterferon-α)
Pegylation of IFN-α with a 12- or 40-kD polyethylene glycol molecule prolongs the half-life of IFN-α. This causes an increase of systematic exposure and biological effect. Currently, only peginterferon alfa-2a (Pegasys, Hoffman-La Roche Inc., Nutley, NJ) is registered for the treatment of HBeAg-positive and -negative chronic hepatitis B. Peginterferon alfa-2a provokes a higher sustained virological response than standard IFN-α and has the advantage of more convenient administration.

A phase 2 trial comparing peginterferon alfa-2a (180 μg weekly) with standard IFN-α (4.5 MIU three times weekly) for 24 weeks in HBeAg-positive chronic hepatitis B patients showed a twofold higher response in HBeAg loss, HBV DNA suppression, and ALT normalization [19]. Two randomized controlled trials described an HBeAg seroconversion in approximately one fourth of the patients, with loss of HBsAg in about 3% of the patients [10,11]. One study showed that patients with genotypes A and B responded better than those with genotypes C and D [11]. Other predictors of HBeAg seroconversion are high pretreatment ALT levels and lower levels of HBV DNA. In HBeAg-negative patients, 48-week treatment with peginterferon-α resulted in a 36% sustained virological response at week 72 [16]. Pilot studies suggesting higher rates of sustained virological response in this group of patients with longer treatment duration (eg, for 60 weeks) are under way [20].

Lamivudine
Lamivudine is an oral nucleoside analogue with a strong HBV inhibitory effect. In a study of HBeAg-positive chronic hepatitis B patients, about 20% achieved HBeAg seroconversion and 50% had histological improvement after 1 year of treatment with lamivudine (100 mg daily) monotherapy [12]. For HBeAg-negative chronic hepatitis B patients, undetectable HBV DNA (< 2.5 pg/mL) appears in about 60% of the patients and histological improvement is nearly the same [21]. However, a great disadvantage of lamivudine is the rapid emergence of its resistance in the YMDD motif, which develops in 24%, 38%, 49%, and 68% of patients after 1, 2, 3, and 4 years, respectively. This has clinical implications for future nucleotide analogue therapy [5,12,22].

Adefovir dipivoxil
Adefovir dipivoxil is an oral adenine nucleotide analogue that inhibits HBV polymerase and reverse transcriptase. In HBeAg-positive chronic hepatitis B patients, 10 mg of adefovir daily led to HBeAg seroconversion in 12%, with histological improvement in 53% and undetectable HBV DNA (< 400 copies/mL) in 21% [13]. In HBeAg-negative chronic hepatitis B patients, 51% had undetectable HBV DNA levels and 64% had histological improvement [19]. Virological resistance after 5 years of treatment in HBeAg-negative chronic hepatitis B patients was 29% (1 year 0%, 2 years 3%, 3 years 11%, and 4 years 18%) [5]. Furthermore, adefovir is highly active in lamivudine-resistant chronic hepatitis B [23,24].

Entecavir
Entecavir is an oral deoxyguanosine analogue with potent activity against HBV. In HBeAg-positive and -negative chronic hepatitis B patients, 1-year treatment with entecavir 0.5 mg daily resulted in significantly higher virological, biochemical, and histological responses than lamivudine treatment (Table 1). Entecavir did not result in higher rates of HBeAg seroconversion [14••,18]. In a phase 3 study in lamivudine-refractory HBeAg-positive chronic hepatitis B patients, entecavir 1.0 mg daily for 1 year resulted in only 19% undetectable HBV DNA levels (< 300 copies/mL) and 1.4% entecavir resistance [22].

Telbivudine
Telbivudine is an HBV-specific L-nucleoside analogue of thymidine and has been recently approved by the US Food and Drug Administration for the treatment of HBeAg-positive chronic hepatitis B in the United States. A phase 3 clinical trial comparing telbivudine 400 and 600 mg with lamivudine 100 mg and telbivudine/lamivudine combination therapy showed that telbivudine monotherapy resulted in significantly higher mean reduction in HBV DNA levels (6.01 vs 4.57 $\log_{10}$) than lamivudine, with more patients attaining undetectable HBV DNA (61% vs 32%), ALT normalization (86% vs 63%), and greater HBeAg seroconversion rate (31% vs 22%) at week 52. After 1 year, resistance was 4.5% in the telbivudine group [15•].

Agents in late-stage development
Tenofovir
Tenofovir disoproxil fumarate, a nucleotide analogue, is licensed for the treatment of HIV at an oral dose of 300

Table 1. Current treatment options for chronic hepatitis B patients and their response

Study	Standard therapy	Loss of HBsAg, %	Loss of HBeAg, %	HBeAg seroconversion, %	Undetectable HBV DNA, %	ALT normalization, %	Histological improvement, %
HBeAg-positive chronic hepatitis B patients							
Wong et al. [8] and Schalm et al. [9]	Interferon-α	8	23/33	19	29/37	29	46*
Lau et al. [10] and Janssen et al. [11]	Peginterferon-α	3/5	30/29	27/22	25/10	39/34	38†/53‡
Dienstag et al. [12]	Lamivudine	<1	32	17	41	41	52§
Marcellin et al. [13]	Adefovir	0	24	12	21	48	53‡
Chang et al. [14••]	Entecavir	2	22	21	67	68	72‡
Lai et al. [15•]	Telbivudine	0	33	31	61	86	75‡
Marcellin et al. [13]	Placebo	0	11	6	0	16	25‡
HBeAg-negative chronic hepatitis B patients							
Lok and McMahon [6]	Interferon-α	NA	NA	NA	60–70	60–70	NA
Marcellin et al. [16]	Peginterferon-α	4	NA	NA	63	38	59†
Marcellin et al. [16]	Lamivudine	4	NA	NA	65	63	60§
Hadziyannis et al. [17]	Adefovir	<1	NA	NA	51	72	64‡
Lai et al. [18]	Entecavir	<1	NA	NA	90	78	70‡
Lok and McMahon [6]	Telbivudine	<1	NA	NA	88	74	66‡
Hadziyannis et al. [17]	Placebo	<1	NA	NA	0	29	33‡

*Treatment duration is 12–24 weeks.
†Posttreatment biopsies obtained at week 72.
‡Posttreatment biopsies obtained at week 48.
§Posttreatment biopsies obtained at week 52.
ALT—alanine aminotransferase; HBeAg—hepatitis B e antigen; HBsAg—hepatitis B surface antigen; HBV—hepatitis B virus; NA—not available.

mg daily and has a strong antiviral activity with a favorable safety profile. From studies in HIV/HBV-coinfected patients, tenofovir appeared to also have a strong inhibitory effect on HBV replication, with a mean 4- to 5-$\log_{10}$ decrease in HBV DNA after 48 weeks of treatment [25,26]. Two small phase 3 trials showed that tenofovir has stronger antiviral activity than adefovir in lamivudine-resistant chronic hepatitis B, with no resistance yet described. Results of large phase 3 trials in chronic hepatitis B patients not coinfected with HIV are under way [27••,28].

Emtricitabine (FTC)
Emtricitabine is a cytosine analogue of deoxycytidine triphosphate that is structurally similar to lamivudine and is a potent inhibitor of HIV and HBV replication. In a phase 3 trial, emtricitabine 200 mg was given daily for 48 weeks in HBeAg-positive and -negative chronic hepatitis B patients; histological improvement appeared in 63% and 59%, respectively. Sixty-five percent of both HBeAg-positive and -negative chronic hepatitis B patients had normalized ALT levels. Undetectable HBV DNA (< 400 copies/mL) was measured in 39% and 79% of patients, respectively, but there was no difference in HBeAg seroconversion rate as compared with the placebo group. Overall, due to mutations in the YMDD motif, emtricitabine treatment resulted in resistance in 13% of the chronic hepatitis B patients. Most patients in this trial were HBeAg positive and showed 17% resistance after 1 year of treatment [29].

Clevudine
Clevudine is a nucleoside analogue with potent activity against HBV and some activity against Epstein-Barr virus. Two phase 3 clinical trials have been published in which the efficacy of 24 weeks of treatment with clevudine was compared with placebo in both HBeAg-positive and -negative chronic hepatitis B patients. In the HBeAg-positive patients, 24 weeks of clevudine 30 mg/d showed a mean 5.10-$\log_{10}$ reduction in HBV DNA level, with 59% attaining undetectable HBV DNA (< 300 copies/mL), 68% attaining ALT normalization, and 8% attaining HBeAg seroconversion. No viral resistance was reported and clevudine was well tolerated [30].

In HBeAg-negative chronic hepatitis B patients, 24 weeks of clevudine 30 mg/d showed a 4.25-$\log_{10}$ reduction in HBV DNA level, with 92% attaining undetectable HBV DNA (< 300 copies/mL) and 75% attaining ALT normalization. This study also reported no viral resistance, and clevudine was well tolerated [31]. Because clevudine was studied for only 24 weeks, little can be said about the incidence of resistance development in longer treatment durations.

Pradefovir
Pradefovir is a prodrug of 9-(2-phosphonylmethoxyethyl) adenine (PMAE). PMAE is a phosphonate analogue of adenine that is effective against HBV. Pradefovir is activated by CYP3A4, which is located mainly in the liver, resulting in high intrahepatic metabolite concentrations and thereby preventing systemic side effects (eg, nephrotoxicity). A phase 1 trial showed that pradefovir was well tolerated, with high levels of PMAE but lower levels in the kidney, suggesting less nephrotoxicity. Phase 2 and 3 trials are currently in development [32].

Valtorcitabine
Valtorcitabine is a prodrug of telbivudine that has demonstrated potent suppression of serum HBV DNA in HBeAg-positive patients. In a phase 1/2 dose-escalation study that evaluated valtorcitabine 300, 600, 900, and 1200 mg/d for 28 days, valtorcitabine 900 mg/d produced a mean reduction in serum HBV DNA level of 3.04 $\log_{10}$ copies/mL after only 4 weeks of treatment. Valtorcitabine was well tolerated in all patient groups, with a safety profile comparable with that of placebo [33].

Chronic Hepatitis C
Hepatitis C antiviral therapies
The current standard of care for patients with chronic hepatitis C is a combination of peginterferon-α and ribavirin. This results in a sustained virological response in approximately 80% of patients infected with HCV genotype 2/3 and less than 50% with HCV genotype 1 [34,35]. Genotype 1 causes more than 60% of HCV infections in Europe and Asia and more than 70% of HCV infections in the United States, and is therefore the most prevalent genotype worldwide. The current standard of care is expensive, requires at least 24 to 48 weeks of treatment, and is associated with significant adverse effects. Thus, new therapies resulting in a higher rate of sustained virological response and fewer adverse effects are highly desirable.

Recently, major progression in cell culture systems and replication assays has advanced the in vitro propagation and production of infectious HCV [36,37••]. This has accelerated the development of new classes of antiviral drugs, including specifically targeted antiviral therapy for hepatitis C (STAT-C), which are expected to improve the current standard of care. These antiviral drugs inhibit different phases in the HCV life cycle or modulate the immune response [38]. Many antiviral drugs and immune modulators are being tested in preclinical settings and phase 1, 2, and 3 clinical trials (Table 2). These novel HCV therapies are reviewed below following our description of the steps of the HCV life cycle (Fig. 1) [39].

Early steps of infection
Viral attachment, entry, and fusion involve two groups of molecules and proteins. The first group comprises the HCV structural envelope glycoproteins, E1 and E2 [40]. These are transmembrane glycoproteins at the surface of the HCV virion envelope [41]. Whereas E1 is thought to

Table 2. Clinical status of HCV inhibitors categorized by phase within the HCV life cycle

Drug type	Compound	Manufacturer	Development phase
Phase 1: Early steps of infection			
Polyclonal immunoglobulins	Civacir (HCIG)	Nabi Biopharmaceuticals (Boca Raton, FL)	2
Monoclonal antibodies	HCV-AB65; HCV-AB68	XTL Biopharmaceuticals (Rehovot, Israel)	2
	XTL6865	XTL Biopharmaceuticals (Rehovot, Israel)	Halted
Phase 2: HCV RNA translation			
Antisense oligonucleotides	AVI-4065	AVI BioPharma (Portland, OR)	2
	ISIS 14803	Isis Pharmaceuticals (Carlsbad, CA)	Halted
Ribozyme	Heptazyme	Ribozyme Pharmaceuticals (San Francisco, CA)	Halted
IRES inhibitor	VGX-410C	VGX Pharmaceuticals (Blue Bell, PA)	2
Small inhibitory RNA	TT 033	Tacere Therapeutics (San Jose, CA)	Preclinical
	Sirna-034	Sirna Therapeutics (San Francisco, CA)	Preclinical
Phase 3: Posttranslational processing			
NS3 serine protein inhibitors	SCH 503034	Schering-Plough (Kenilworth, NJ)	2
	Telaprevir (VX-950)	Vertex Pharmaceuticals (Cambridge, MA)	2
	TMC435350	Tibotec (Mechelen, Belgium) and Medivir (Huddinge, Sweden)	2
	ITMN-191	InterMune Inc. (Brisbane, CA)	1
	BILN-2061	Boehringer-Ingelheim (Ingelheim, Germany)	Halted
	ACH-806 (GS-9132)	Achillion Pharmaceuticals (New Haven, CT)	Halted
	ACH-1095	Achillion Pharmaceuticals (New Haven, CT)	Preclinical
Phase 4: HCV replication			
NS5B polymerase inhibitors	HCV-796	ViroPharma (Exton, PA) and Wyeth (Madison, NJ)	2
	R1626	Roche (Basel, Switzerland)	2
	R7128; R1479	Roche (Basel, Switzerland)	1
	MK-0608	Merck (Whitehouse Station, NJ)	1
	ANA598	Anadys Pharmaceuticals (San Diego, CA)	1
	A-782759	Abbott Laboratories (Abbott Park, IL)	1
	JTK-003; JTK-109	Akros Pharma (Princeton, NJ)	Halted
	R-803	Roche (Basel, Switzerland)	Halted
	XTL-2125	XTL Biopharmaceuticals (Rehovot, Israel)	Halted
	GL59728; GL60667	GeneLabs Technologies (Redwood City, CA)	Preclinical
Cyclophilin B inhibitors	DEBIO-025	Debiopharm (Lausanne, Switzerland)	2
	NIM811	Novartis Pharma (Basel, Switzerland)	1
NS5A inhibitors	A-831	Arrow Therapeutics (London, UK)	1
	A-689	Arrow Therapeutics (London, UK)	Preclinical
NS3 RNA helicase inhibitor	QU 663	BioBlocks Inc. (San Diego, CA)	Preclinical
Phase 5: Viral assembly and release			
Glucosidase inhibitors	Celgosivir (MX-3253)	Migenix Inc. (Vancouver, Canada)	2
	UT-231B	United Therapeutics (Silver Spring, MD)	Halted

HCIG—hepatitis C antibody–enriched immune globulin; HCV—hepatitis C virus; IRES—internal ribosome entry site.
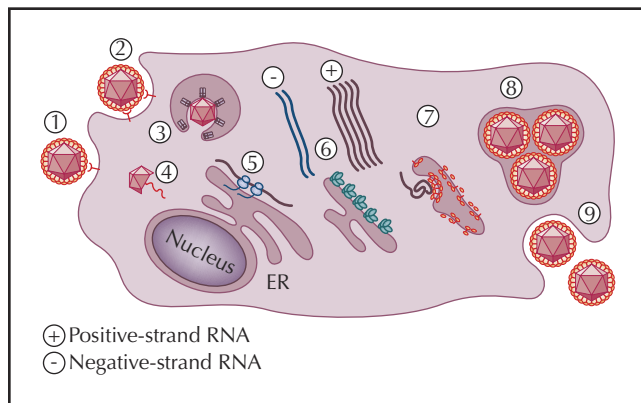
Figure 1. The hepatitis C virus (HCV) life cycle. (1) Virus binding to cellular receptor(s) (small molecule inhibitors of cell attachment, monoclonal antibodies, hyperimmune anti-HCV immunoglobulins); (2) receptor-mediated endocytosis; (3) membrane fusion and nucleocapsid release; (4) nucleocapsid uncoating; (5) translation and polyprotein processing (internal ribosome entry site inhibitors, NS3 serine protease inhibitors, NS2 zinc-dependent autoprotease inhibitors); (6) HCV RNA replication (NS5B RNA-dependent RNA polymerase inhibitors, NS5A inhibitors, inhibitors of replication complex formation); (7) virion formation and budding in intracellular vesicles; (8) virion transport and maturation; (9) virion release. ER—endoplasmic reticulum. (*From* Penin [39].)

be responsible for mediating the intracytoplasmic virus membrane fusion, E2 is involved in the initiating process of binding to target cells [42]. The second group consists of several receptor molecules on the hepatocytes. These molecules (CD81, claudine-1, scavenger receptor class B type 1, and dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin) are thought to mediate HCV binding and internalization and may act as potential targets for anti-HCV drugs.

Although many potential targets have been identified, only a few clinical trials have been performed using viral attachment as a target. HCV-AB68 (XTL Biopharmaceuticals, Rehovot, Israel), is a human monoclonal antibody against the envelope protein E2 of HCV [43]. HCV-AB68 has been tested in phase 1b and 2 clinical trials for safety, tolerability, and antiviral activity using HCV-infected liver transplant recipients.

Civacir (Nabi Biopharmaceuticals, Boca Raton, FL), a human hepatitis C antibody–enriched immune globulin, has been tested in two phase 2 clinical trials with HCV-infected liver transplant recipients. The result of one randomized, open-label study using two different dosages for 14 weeks showed that HCV RNA levels were not suppressed [44].

HCV RNA translation/polyprotein synthesis
The HCV genome translation initiation is directed by an internal ribosome entry site that recruits cellular and viral proteins. After these proteins liberate free RNAs in the cell cytoplasm by decapsidation of viral nucleocapsids, they serve as messenger RNAs (mRNA) for synthesis of the HCV polyprotein.

Several potential antiviral approaches have been developed in various in vitro systems to inhibit HCV RNA translation. Ribozymes recognize and catalyze the cleavage of target RNA molecules. Heptazyme (Ribozyme Pharmaceuticals, San Francisco, CA), a chemically modified and stabilized ribozyme, was being tested in preclinical trials; however, the trial was halted in 2000 due to animal toxicity. To date, no other trials on ribozymes have been reported.

The second group comprises antisense DNA or RNA oligonucleotides. The sequence of these oligonucleotides is complementary to the target mRNA and they prevent the construction of regulatory sequences and/or structures important for efficient translation of HCV polyprotein [45]. Recently, two antisense oligonucleotide inhibitors have been tested. A phase 1 clinical trial testing ISIS 14803 (Isis Pharmaceuticals, Carlsbad, CA) was halted because of lack of antiviral efficacy and concerns of transient elevated liver enzymes [46].

Specific small molecule inhibitors of the HCV internal ribosome entry site are the third group of drugs potentially able to inhibit HCV RNA translation. As mentioned above, the HCV internal ribosome entry site directly regulates the assembly of translation initiation complexes on viral mRNA by recruiting cellular and viral proteins [47]. Currently, two phase 2 clinical trials are under way in which HCV-infected patients are receiving VGX-410C (VGX Pharmaceuticals, Blue Bell, PA), a ribosome formation inhibitor, in various doses. No results have been published to date.

Posttranslational processing
The HCV genome encodes a single polyprotein and is then processed into 10 mature structural and regulatory proteins. The processing of the structural proteins (E1, E2, core protein, and p7) requires at least two host cellular peptidases. The regulatory proteins are processed by a virus-specific helicase, polymerase, and proteases. Polyprotein processing of positive-stranded HCV RNA by the various peptidases is important in the regulation of gene production and replication [48]. Because of the critical function of these proteins in the viral life cycle, they represent attractive targets for STAT-C.

BILN-2061 (Boehringer-Ingelheim, Ingelheim, Germany), an NS3 protease inhibitor preventing cleavage of the NS3/4 part of the polyprotein, was the first HCV NS3/4A protease inhibitor to enter human clinical trials. BILN-2061 was highly effective, inducing a rapid 3-$\log_{10}$ decline in virus load in all treated patients with HCV genotype 1 within 2 days, and reaching undetectable levels within 24 to 28 hours after administration in some patients. However, the study was halted because of myocardial toxicity in apes [49]. Telaprevir (VX-950; Vertex Pharmaceuticals, Cambridge, MA), another NS3/4A serine protease inhibitor, has recently been tested in a placebo-controlled, double-blind phase 1b clinical trial. Telaprevir was well

tolerated and had substantial antiviral effects. All 28 HCV genotype 1 patients had a decline of at least 2 $\log_{10}$ in HCV RNA from baseline, and 26 (93%) of these 28 patients showed a decline of at least 3 $\log_{10}$. The highest plasma drug concentration was reached in the group dosed with 750 mg every 8 hours, and the median HCV RNA reduction in this group was -4.4 $\log_{10}$ after 14 days [50•]. Viral breakthrough occurred in a substantial number of patients due to variant selection with decreased sensitivity to telaprevir. Resistant variants were mainly seen in lower-dose groups (450 mg every 8 hours) [51]. The rapid selection of resistant HCV isolates during telaprevir therapy indicates that combination treatment with peginterferon-α or other antiviral drugs will probably be necessary to avoid drug resistance. Subsequently, the group dosed with telaprevir 750 mg every 8 hours was continued with peginterferon-α for 14 days, which resulted in median HCV RNA decline of -5.5 $\log_{10}$. No viral breakthrough was observed in this patient group. Twelve weeks after starting off-study standard therapy, HCV RNA was undetectable in all patients in the telaprevir and peginterferon-α combination group [52•]. Subsequently, several phase 2 placebo-controlled, randomized trials (PROVE 1, 2, and 3) testing telaprevir in combination with peginterferon-α with or without ribavirin were started. These trials included treatment-naive and nonresponse patients with HCV genotype 1 who received telaprevir treatment for 12 or 24 weeks in combination with peginterferon-α and ribavirin.

SCH 503034 (Schering-Plough, Kenilworth, NJ), a novel HCV NS3 protease inhibitor, showed potential antiviral properties in vivo. In a phase 1b, randomized, two dose–level, three-way cross-over, multicenter study, virologic response and tolerability of SCH 503034 were investigated in patients with HCV genotype 1. The included patients were nonresponders to previous treatment with peginterferon alfa-2b with or without ribavirin. All patients (n = 26) received three dosing periods, each followed by a washout period of at least 2 weeks. The three dosing periods consisted of 1 week of SCH 503034 monotherapy (200 or 400 mg), 2 weeks of peginterferon alfa-2b monotherapy, and 2 weeks of SCH 503034 and peginterferon alfa-2b combination therapy. The highest decline in HCV RNA plasma levels was detected in the highest SCH 503034 dose group (400 mg) in combination with peginterferon alfa-2b after 2 weeks. SCH 503034 in combination with peginterferon-α showed a mean maximum $\log_{10}$ change of -2.88, compared with -1.61 (200 mg) and -1.26 (400 mg) changes in monotherapy [53].

More phase 1 and 2 clinical trials testing treatment-naive and treatment-experienced patients with and without combination of peginterferon alfa-2b and ribavirin and higher doses of SCH 503034 are ongoing.

HCV replication

The mechanisms by which various viral and cellular components and RNA strands regulate HCV replication are complicated and not entirely understood. Three main phases in the HCV replication process can be identified. First, negative-strand RNA is synthesized by using positive-strand RNA as a template. The newly synthesized negative RNA strands serve as templates to produce many positive RNA strands in phase 2. Phase 3 is characterized by positive polarity strands that are used for polyprotein processing, synthesis of new intermediates of replication, or packaging into new virus particles [54]. NS5B RNA-dependant RNA polymerase (RdRp) is a crucial viral component for negative- and positive-strand HCV RNA replication. The structure of this RdRp domain consists of a fully encircled active site formed by the classical three finger-palm-thumb subdomains. HCV regulatory proteins (NS5A, NS3 helicase-NTPase, NS4B) together with cellular components (cyclophilin B) form a complex mechanism to bind, activate, and catalyze NS5B RdRp. Currently two categories of RdRp inhibitors are being evaluated in clinical trials. The catalytic site of the RdRp—where nonnucleoside inhibitors target the allosteric site of the RdRp—forms an interesting target for nucleoside/nucleotide inhibitors. Valopicitabine (NM283; Idenix Pharmaceuticals, Cambridge, MA and Novartis, Basel, Switzerland) and R1626 (Roche Products, Basel, Switzerland) are both nucleoside analogues being tested in phase 1b trials. Valopicitabine monotherapy (800 mg for 2 weeks) showed an average dose-dependent HCV RNA decrease of 1.2 $\log_{10}$ in HCV genotype 1–infected patients. An ongoing phase 2 clinical trial is comparing HCV genotype 1 nonresponders with previous IFN-based therapy patients receiving valopicitabine alone, valopicitabine with peginterferon-α, or peginterferon-α with ribavirin for 48 weeks. Results at week 24 showed a dose-dependent additive effect of valopicitabine to peginterferon-α, including a significant number of patients with undetectable HCV RNA levels [55]. However, valopicitabine is no longer being studied due to its overall risk/benefit profile.

In another phase 1 study, HCV genotype 1–infected patients received R1626 1500, 3000, and 4500 mg twice daily for 2 weeks. HCV RNA levels were reduced by -1.2, -2.6, and -3.7 $\log_{10}$, respectively, making this the largest hepatitis C viral load drop for this class of STAT-C. However, both drugs were associated with significant side effects. The NM283 dosage had to be reduced due to gastrointestinal side effects and high dosages of R1626 were associated with anemia and other side effects [56].

HCV-796 (ViroPharma, Exton, PA, and Wyeth Pharmaceuticals, Madison, NJ) is a nonnucleoside allosteric site D inhibitor of the HCV RdRp. A phase 1 dose-escalation study showed a -1.4 $\log_{10}$ HCV RNA level reduction within the highest-dose group. A subsequent phase 2 study was halted due to clinically significant elevated liver enzymes, including those in two patients who had to withdraw because of serious adverse events.

In addition to the many nucleoside/nucleotide and nonnucleoside inhibitors, NS5A and cyclophilin B inhibitors are being tested in ongoing trials.

Virus assembly and release

No appropriate study model exists to assess the mechanisms of HCV assembly and release. The viral capsid is thought to be formed by structural core protein. Several structural proteins have been detected within the endoplasmic reticulum and the Golgi apparatus, and both organelles may be involved in later steps of virus assembly. The interaction between core protein and genomic RNA is probably the process that initiates viral particle formation. Targets for HCV antiviral drugs to inhibit virus assembly are based upon the assumption that a certain degree of glucosylation is needed for envelope proteins to cross cellular membranes. Iminosugars inhibit cellular glucosidases, resulting in the hyperglucosylation of envelope proteins, which inhibits their ability to cross membranes [57]. Two glucosidase inhibitors, UT-231B (United Therapeutics, Silver Spring, MD) and MX-3253 (Migenix, Vancouver, Canada), have been tested in phase 1 clinical trials. Both studies reported modest antiviral effects [58,59] and these agents have since entered phase 3 study evaluation.

Novel immunomodulatory agents

At present, peginterferon-α with ribavirin is the only combination known to permanently eliminate HCV RNA levels and improve liver histology. Therefore peginterferon-α treatment, together with ribavirin, is currently the standard of care for HCV-infected patients. Several immune modulators are being tested to improve the outcome of current standard of care, reduce side effects, and lower treatment costs. Several modifications to the IFN proteins have resulted in long-acting (Albuferon, Human Genome Sciences, Rockville, MD), controlled-release (Locteron, OctoPlus NV, Leiden, Netherlands and Biolex Therapeutics, Pittsboro, NC), and orally administered (Belerofon, Angel Biotechnology, Edinburgh, Scotland, and Nautilus Biotech, Evry, France) IFNs. These modifications should increase the IFN plasma levels and lead to longer dosing intervals and better response rates. The long-acting and oral IFNs have the potential to replace current peginterferon-α–based therapies in the future.

Ribavirin, the other component of standard-of-care treatment, is associated with significant hemolytic anemia, which may require dose reduction, discontinuation, or treatment with recombinant human erythropoietin or even blood transfusion. However, dose reduction of ribavirin is associated with a decrease of sustained virological response rates, making ribavirin analogues, with less side effects, attractive alternatives.

Viramidine (taribavirin; Valeant Pharmaceuticals International, Aliso Viejo, CA), is a ribavirin analogue that has been tested in a phase 2 clinical trial. Peginterferon alfa-2a with either ribavirin or Viramidine showed sustained virological response rates of 37% in the optimal dose group for Viramidine and 44% in the ribavirin group. Significantly fewer patients within the Viramidine group developed anemia compared with the ribavirin group (4% vs 27%) [60]. Unfortunately, two phase 3 trials (VISER 1 and VISER 2) comparing Viramidine plus peginterferon alfa-2a or peginterferon alfa-2b showed that sustained virological response rates in the Viramidine group were inferior to those in the ribavirin group [61].

Current standard HCV treatment demonstrated that HCV infection can be eliminated by agents that stimulate the host innate and adaptive immunity. Toll-like receptors are pathogen recognition receptors expressed by immune cells that initiate the innate immune response after stimulation. Signaling through Toll-like receptors induces the production of type 1 T-helper cells, promotes cytokine and chemokine production, and stimulates natural killer cells [62]. Synthetic Toll-like receptor agonists are potentially able to alter the HCV RNA load by mimicking the immune response observed during the course of acute HCV infections.

Conclusions

Currently, peginterferon-α and nucleoside/nucleotide analogues are the only treatment options for chronic hepatitis B, but they have suboptimal responses and substantial side effects. Promising drugs are in late stages of development, ready to be approved. Due to the advances in cell culture systems, our understanding of the HCV life cycle has developed tremendously over the past few years. Every phase of the HCV life cycle forms a potential target for antiviral therapy. As mentioned above, many of the STAT-C drugs are currently being tested in clinical trials. Some trials showed excellent results and form the basis of our optimism for finding more efficient therapies against HCV in the near future. However, side effects and viral resistance remain serious problems encountered in several clinical trials and continue to present a major challenge for the development of potential novel therapeutic agents.

Clinical Trials Acronyms

PROVE—Investigation of HCV Protease Inhibition for Viral Eradication; VISER—Viramidine's Safety and Efficacy vs Ribavirin.

Acknowledgments

Disclosures

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have been highlighted as:
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••   Of major importance

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