REVIEW

Gut and Liver, Vol. 5, No. 4, December 2011, pp. 406-417

The Therapeutic Approaches for Hepatitis C Virus: Protease Inhibitors and Polymerase Inhibitors

Paul Y. Kwo and Rakesh Vinayek

Division of Gastroenterology/Hepatology, Indiana University School of Medicine, Indianapolis, IN, USA

The current standard of care for hepatitis C infection is peginterferon/ribavirin (PegIFN/RBV). We are entering the era where direct-acting antiviral agents (DAAs) will be added to PegIFN/RBV, leading to higher sustained response rates in genotype 1 infected individuals. Currently DAAs are directed toward specific proteins involved in hepatitis C replication with NS3/NS4A protease inhibitors furthest in development. Telaprevir and boceprevir are both NS3/NS4a inhibitors that significantly improve sustained response when added to PegIFN and RBV. The hepatitis C virus (HCV) polymerase inhibitors are another promising DAA class. These molecules are divided into nucleoside/nucleotide polymerase inhibitors and nonnucleotide/nucleoside polymerase inhibitors. Nucleoside/nucleotide polymerase inhibitors have a high barrier to resistance and appear to be effective across a broad range of genotypes. Nonnucleoside polymerase inhibitors have a lower barrier of resistance and appear to be genotype specific. Preliminary data with these compounds are also promising. A third class, NS5A inhibitors, has also shown potent HCV RNA suppression in preliminary studies as monotherapy and with PegIFN and RBV. Combinations of these agents are also entering clinical trials and indeed a preliminary report has demonstrated that the combination of an NS3/4A protease inhibitor and NS5B polymerase inhibitor can effectively suppress virus in genotype 1 individuals. Future studies will concentrate on combinations of direct-acting antiviral agents without and with PegIFN and RBV. Clinicians will need to be familiar with managing side effects as well as resistance as we enter this new era. **(Gut Liver 2011;5:406-417)**

Key Words: Polymerase inhibitor; Hepatitis C virus; Protease inhibitor

INTRODUCTION

The hepatitis C virus (HCV) is the most common blood born infection worldwide, and is a major cause of chronic liver disease leading to death from liver failure or hepatocellular carcinoma. The current paradigm for HCV treatment relies on pegylated interferon (PegIFN) and ribavirin (RBV) as agents that enhance endogenous mechanisms for viral clearance and are dependent on host factors. In patients with genotype 1 HCV infection, which comprises the majority of patients infected in most of the world, including Asia, North America, and Europe, sustained viral response (SVR) rates remain suboptimal with less than half of genotype 1 infected individuals going on to achieve SVR. This has led to a shift in the investigational focus for treatment of HCV towards direct acting anti-viral agents (DAA) or specifically targeted antiviral therapy for HCV (STAT-C) agents. This review will focus on the HCV protease and polymerase inhibitors in development for the treatment of hepatitis C infection, discussing their mechanisms of action, therapeutic advantages and disadvantages, and current status in therapeutic armamentarium for anti-HCV therapy.

REPLICATION CYCLE OF HCV

The HCV is a single-stranded RNA molecule that is approximately 9,600 nucleotides in length.[1] The hepatitis C life cycle is similar to many positive strain RNA viruses and the replication cycle and targets for therapy are shown in Figs 1 and 2.

PROTEASES AS POTENTIAL TARGETS OF ANTI-HCV THERAPY

Preclinical data demonstrated the role of the NS3/4A protease as chimpanzees inoculated with HCV containing defective

Correspondence to: Paul Y. Kwo
Division of Gastroenterology/Hepatology, Indiana University School of Medicine, 975 W. Walnut, IB 327, Indianapolis, IN 46202-5121, USA
Tel: +1-317-274-3090, Fax: +1-317-274-3106, E-mail: pkwo@iupui.edu
Received on February 9, 2011. Revised on June 20, 2011. Accepted on June 20, 2011.
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl.2011.5.4.406

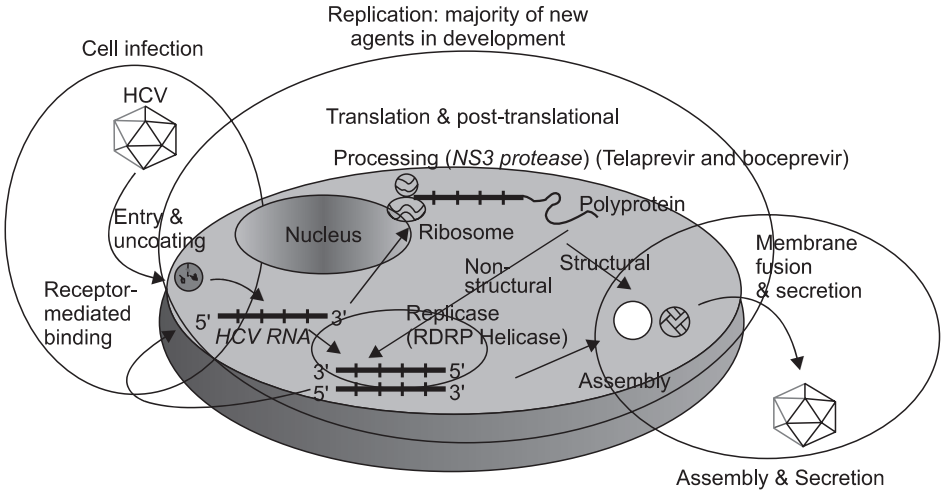

Fig. 1. Hepatitis C virus (HCV) infection and replication: targets for therapy. The virus enters the cell through attachment entry and fusion and undergoes transcription to form a complementary negative sense RNA molecule which serves as the template for positive stranded RNA molecules. This HCV RNA reading frame contains approximately 9,000 nucleotides and generates 3 structural proteins, 6 nonstructural (NS) proteins, and 2 proteins of unclear function. The most advanced therapies in the direct-acting antiviral drug development are directed toward the NS3/NS4A serine protease. The structural proteins are used to assemble new viral particles and the NS proteins support viral RNA replication. The NS3/4A is a serine protease (NS3) and cofactor (NS 4A) that catalyzes the post-translational processing of NS proteins from the polyprotein which is important for viral replication. The NS3 protease cleaves NS4A-NS4B, NS4B-NS5A and NS5A-NS5B junctions. The products released go on to form a replicative complex responsible for forming viral RNA providing an ideal target for anti-viral therapy HCV RNA replication primarily depends upon the RNA dependent/RNA polymerase (NS5B). X-ray crystallography has demonstrated that the NS5B polymerase has a classical fingers, palm, and thumb structure providing multiple areas for therapeutic targets. Another key structure in HCV replication is the NS5A protein, though the mechanisms by which NS5A regulates HCV RNA replication are less clear. It appears to have a key role in formation of the replication complex as well as interact with the NS5B polymerase. Viral assembly and release involves the HCV core protein and to date, therapeutic targets have not shown significant antiviral efficacy.

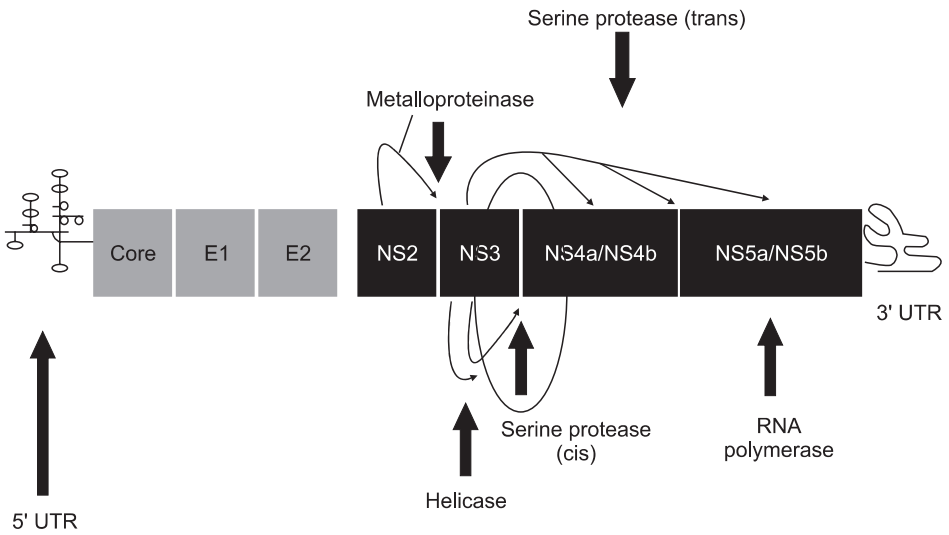

Fig. 2. Potential sites for development of inhibitors of hepatitis C virus replication.

NS3/4A activity did not demonstrate HCV RNA replication.[2] The initial proof of principle that addition of NS3/4A protease could efficiently and effectively suppress HCV RNA replication was established by administration of the NS3/4A inhibitor BILN2061 for 2 days in genotype 1 patients with chronic hepatitis C, which led to reductions of 100-1,000 fold in all individuals.[3] This molecule BILN2061 did not receive further development due to concerns over cardiac toxicity.

DAA

Telaprevir (VX950; Vertex Pharmaceuticals, Cambridge, MA, USA) and boceprevir (SCH503034; Merck, Kenilworth, NJ, USA) are both peptidomimetic inhibitors of the NS3/4A protease that are currently in phase 3 trials and other agents are in earlier phase trials (Table 1). Both show substantial potential to favorably impact SVR rates when added to PegIFN and RBV. Telaprevir, a selective peptidomimetic inhibitor of HCV NS3/NS4A

Table 1. NS3-4a Protease Inhibitors in Development

Protease compound	Company	Phase	Other
BILN 2061	Boehringer Ingelheim	1 Stop	Heart toxicity
Telaprevir	Vertex/Tibotec	3	
Boceprevir	Merck	3	
ACH-806	Achillion	1 Stop	Renal toxicity
ITMN 191/R7227	InterMune/Roche	2	
TMC435350	Medivir/Tibotec JJ	2	
BI 201335	Boehringer Ingelheim	2	
MK7009	Merck	2	

Several compounds will report sustained viral response data this year.

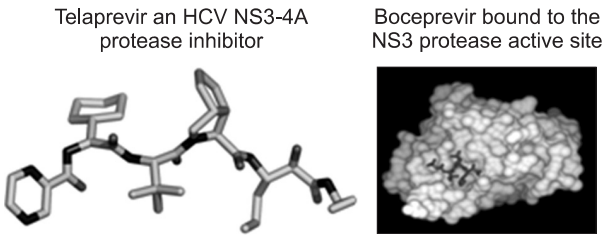

Fig. 3. The NS3-4A protease inhibitors telaprevir and boceprevir are shown. Reprinted with permission from Vertex and Merck.

protease forms a covalent, reversible complex with the NS3/4A protease (Fig. 3). *In vitro* data with genotype 1b replicons demonstrated a 4 log reduction in HCV RNA level.

1. Telaprevir

1) Phase 1 studies

An initial phase 1B dose finding study with 14 days of telaprevir monotherapy replicated the *in vitro* findings. Patients who were both naïve and had failed previous antiviral therapy with PegIFN/RBV were randomized to receive telaprevir or placebo at a dose of 450 mg q8h, 750 mg q8h, or 1,250 mg q12h.[4] The study demonstrated that the 750 mg q8h dose displayed the highest trough plasma concentrations with a median reduction in 14 days of 4 $\log_{10}$ and HCV RNA became undetectable in 2 individuals. In the other 2 dosing regimens, viral rebound was seen and was later seen to be associated with the development of telaprevir resistant variants. A second phase 1 study confirmed that PegIFN alfa-2a 180 μg could be combined with telaprevir for 14 days at a loading dose of 1,250 mg followed by 750 mg q8h. In this study, 60% of 15 participants who received telaprevir or telaprevir/PegIFN before treatment with standard HCV therapy achieved SVR.[5]

2) Phase 2 studies: treatment of naïve patients

These phase 1 studies allowed the development of phase 2 telaprevir studies in naïve HCV patients, the Prove 1 and Prove 2 studies. The Prove 1 study, the first North American multicenter telaprevir trial demonstrated the potent antiviral effects of telaprevir 750 mg q8h when given in combination with PegIFN and RBV.[6] Two hundred fifty genotype 1 HCV infected individuals were randomized to receive telaprevir 750 mg q8h weekly with PegIFN alfa-2a 180 μg and RBV1,000 to 1,200 mg for 12 weeks followed by none, 12, or 36 additional weeks of PegIFN/RBV. Patients randomized to the 12- and 24-week duration arms were eligible to stop treatment at early time points only if HCV RNA was undetectable at week 4 which was the first use of a response guided paradigm with a DAA. The control arm was PegIFN2a/RBV for 48 weeks. Twelve weeks of PegIFN/RBV, and telaprevir followed by 12 weeks of PegIFN/RBV led to an overall SVR rate of 61% vs the control 48-week PegIFN/RBV SVR rate of 41%. Extending therapy with PegIFN/RBV for an additional 24 weeks improved the SVR rate to 67% with a relapse rate of 6%. The 12-week cohort, while small, nonetheless had a SVR rate of 35%. Both the 24- and 48-week treatment arms were superior to 48 weeks of PegIFN/RBV. Similar results were seen in the European study, Prove 2.[7] In this study, 332 European patients were randomized to 1 of 4 treatment groups including 12 weeks of telaprevir, PegIFN alfa-2a 180/RBV. The 24-week therapy consisted of telaprevir plus PegIFN/RBV for 12 weeks followed by 12 additional weeks of PegIFN/RBV, and finally a RBV sparing arm consisting of 12 weeks of PegIFN and telaprevir. Similar to Prove 1 results, high RVR rates were seen in the telaprevir based arms (74% vs 14%). The SVR rate in 12-week based triple combination arm with telaprevir, PegIFN/RBV was 60% and the 24-week treatment arm which consisted of 12 weeks of telaprevir dosed in combination with PegIFN/RBV and additional 12 weeks of PegIFN/RBV alone was 69%. These regimens were superior to the control arm with PegIFN/RBV with SVR rate of 46%. This study also demonstrated an important concept in that elimination of RBV markedly reduced the SVR rate with an overall SVR of 36% with high breakthrough and relapse rates in the ribavirin-sparing arm.

3) Phase 2 studies: treatment of nonresponders

The recently published Prove 3 study evaluated the role of telaprevir-based regimens in genotype 1 HCV patients who did

not achieve SVR with at least one prior course of PegIFN and RBV and enrolled nonresponders, relapsers, and those with breakthrough.[8] This phase 2 study conducted in the United States, Canada, and Europe enrolled 453 patients with failed previous IFN-based regimens due to nonresponse, and received either 12 weeks of telaprevir/PegIFN/RBV followed by 12 weeks of PegIFN/RBV, 24 weeks of telaprevir/PegIFN/RBV followed by 24 weeks of PegIFN/RBV, as well as a 24 week PegIFN/telaprevir (RBV sparing) arm and the control (48 weeks PegIFN/RBV). Nonresponders were defined as those who never achieved undetectable HCV RNA after a course of PegIFN and RBV of at least 12 weeks duration or at the end of the treatment. Relapsers were defined as those with undetectable HCV RNA during treatment for at least 42 weeks and detectable HCV RNA levels during follow-up. Breakthrough was defined as those who achieved undetectable HCV RNA during treatment but detectable levels of HCV RNA before end of treatment. To address the issue of resistance, stopping rules were defined as those with an increase in HCV RNA of greater than 1 log compared with nadir or an increase of HCV RNA to greater than 100 IU after undetectability and anyone with detectable HCV RNA by week 24 was discontinued. In this study 40% to 49% of individuals had advanced fibrosis. The SVR was 39% in prior nonresponders with 12 weeks of triple combination therapy followed by 24 weeks of PegIFN/RBV similar to the SVR rate (38%) seen with 24 weeks of telaprevir, PegIFN/RBV followed by 24 weeks of PegIFN/RBV. Again, the elimination of ribavirin markedly reduced SVR rates with high rates of breakthrough and relapse. In relapsers, the SVR was 69% with 12 weeks of telaprevir, Peg IFN/RBV followed by 12 weeks of PegIFN and RBV and 76% with48-week treatment with 24 weeks of telaprevir, PegIFN/RBV followed by 24 weeks of PegIFN/RBV. In this study, the control group achieved an SVR of 14%. Discontinuation rates again were higher in the telaprevir based arms because of rash (7%). While the cohort was small, 53% of cirrhotic individuals treated with 12 weeks of telaprevir with PegIFN/RBV achieved SVR. Dropout rates were highest in the 48-week treatment arm with 58 of 113 individuals discontinuing therapy suggesting that the optimal duration for telaprevir is 12 weeks, not 24 weeks.

4) Phase 3 studies

Currently, phase 3 is underway and fully enrolled including the Advance trial with 1,050 individuals and Illuminate trial with 500 individuals. There is also the Realize nonresponder trial with 650 individuals. All of these trials will provide further data on the optimal use of telaprevir in those who have not been treated or naïve patients as well as those who failed to achieve SVR.

5) Emergence of drug resistance

While telaprevir and other DAA agents will substantially improve SVR rates, clinicians who treat HCV infection will have to be cognizant of the generation of drug-resistant mutations given the high rate of replication of the HCV virus. Drug-resistant variants may exist as minor viral populations or quasispecies serves as the source of drug resistance. The resistance profiles are listed in Table 2. Regardless, with the addition of most DAA agents, it is likely that genetic resistant drug resistant mutations are generated immediately but these resistant mutations are typically associated with reduced replicative fitness, and retain sensitivity to PegIFN/RBV.

Table 2. Common Resistance Variants Reported Thus Far with Protease Inhibitors

	Site of action	Common resistant variants reported a position (*in vitro* and *in vivo*)
Telaprevir	NS3/4a	A156V/T, V36M/A+R155K/T, V36M/A+A156V/T, V36M/A, T54A, R155K/T, A156S
Boceprevir	NS3/4a	V36M, T54S, R155K, T54A, V55A, R155T, A156S, V158I, V170A, V36A, V36L, I170T
Danoprevir	NS3/4a	R155K
TMC435	NS3/4a	80, 155, 156, 158
BI201335	NS3/4a	155, 156, 168

6) Safety and toxicity

Regarding the safety and toxicity of telaprevir, it is generally well tolerated, though side effects that will require careful management include gastrointestinal side effects including diarrhea, rash, pruritus, and anemia. These adverse events were the most common reasons leading to discontinuation in the telaprevir arms in the phase 2 studies. The rash appears in phase 2 trials to account for approximately 7% of all treatment discontinuations, and pruritus is common. Anemia is also noted with telaprevir as well as other DAA agents such as boceprevir.

2. Boceprevir

1) Preclinical studies

Boceprevir is peptidomimetic ketoamide HCV NS3 protease inhibitor that binds reversibly to the NS3 active site (Fig. 3). Malcolm *et al*.[9] demonstrated a robust anti-viral activity of boceprevir in HCV replicons with treatment resulting in a 1.5 to 2-log drop in RNA levels at 72 hours and a 3.5 to 4-log drop by day 1. Cells treated with boceprevir and PegIFN had a greater HCV replicon suppression than either agent alone and this effect appeared to be additive, rather than synergistic. This promising *in vitro* data allowed boceprevir to enter clinical trials.

2) Phase 1 studies

The first of these trials was a European phase 1 clinical trial comparing boceprevir monotherapy to PegIFN 2b 1.5 μg/kg weekly and PegIFN plus boceprevir therapy in a nonresponder

population.[10] Twenty-six patients with HCV genotype 1a or 1b who previously did not achieve EVR with PegIFN with or without RBV received boceprevir monotherapy (200 or 400 mg q 8 hours) for 1 week, PegIFN 2b 1.5 μg/kg weekly for 2 weeks and combination PegIFN 2b plus boceprevir for 2 weeks. Patients treated with PegIFN and boceprevir 200 mg q 8 hours, had a mean reduction in HCV RNA of -2.28 $\log_{10}$ and in those treated with PegIFN and boceprevir 400 mg q 8 hours, a mean reduction in HCV RNA of 2.68 $\log_{10}$ was observed with 4 patients clearing HCV RNA from the serum.

3) Phase 2 studies

With this preliminary data, a phase 2 dose-finding boceprevir study was initiated to determine the optimal boceprevir dose, whether ribavirin is required in combination with boceprevir, and what the optimal treatment duration would be in a null responder population.[11] In this study, 357 null responders who either failed to achieve EVR or failed to clear virus with >12 weeks PegIFN alfa-2b/RBV therapy were enrolled and treated with PegIFN alfa-2b/RBV plus placebo, PegIFN alfa-2b plus boceprevir in ascending doses (100/200/400/800 mg) t.i.d. or PegIFN alfa-2b boceprevir 400 mg t.i.d. plus RBV. After an interim analysis by the data safety monitoring board (DSMB), the protocol was amended and all responding patients (defined as less than 10,000 IU/mL on original therapy) were assigned to receive PegIFN/RBV and boceprevir 800 t.i.d. for 24 weeks. While the overall SVR rate was low, this trial established several important concepts in the treatment of HCV nonresponders with boceprevir. For treatment of null responders, ribavirin is required for optimal response. Boceprevir dose of 800 mg t.i.d. was safe when given in combination with ribavirin for 24 weeks (no patient initially received this dose). Finally, the null responders randomized to the PegIFN and RBV without boceprevir arm (control) who demonstrated interferon responsiveness (1-2 log reduction at week 13) were more likely to go on to achieve SVR with addition of boceprevir.

These preliminary results led to the phase 2 clinical trial HCV Serine Protease Inhibitor Therapy-1 (SPRINT-1) evaluating boceprevir in combination with PegIFN and RBV in HCV genotype 1 treatment naïve patients.[12] In this multi-arm trial, genotype 1 subjects were randomized to receive PegIFN alfa-2b 1.5 μg/kg (P), weight based RBV and boceprevir (B) 800 mg t.i.d. for 28 or 48 weeks, or a lead-in strategy with 4 weeks of PegIFN/RBV followed by boceprevir 800 mg t.i.d. addition to PegIFN/RBV, and these treatment arms were compared to standard therapy of PegIFN/RBV for 48 weeks. The rationale for the lead-in strategy was based on the following hypothesis: PegIFN/RBV reach steady-state concentrations by week 4, and with the lead in strategy, patients will have the protease inhibitor added when backbone drug levels have been optimized and the patient's immune system activated, minimizing the period of time with a "functional monotherapy," potentially reducing the likelihood for the development of resistance to boceprevir (or other DAA). This strategy may also reduce the likelihood of the development of resistance by identifying patients who are responders to IFN and RBV prior to their receiving a protease inhibitor or other DAA drug.

Approximately 100 subjects were enrolled in each arm and stratified for cirrhosis and African American race. Compared to PegIFN/RBV, significantly more patients in the triple therapy groups achieved SVR In the 28 week treatment arms, SVR rates were 54% and 56% in the non-lead-in and lead-in arms, and in the 48 week treatment arms, SVR rates were 67% and 75% for non-lead-in and lead-in arms. Reducing the dose of RBV reduced the hematologic toxicity (anemia), but similar to telaprevir, reduced SVR rates with high rates of breakthrough due to resistance. Those who cleared virus at week 4 of boceprevir had high rates of SVR when treated for just 28 weeks. Finally, response rates in African Americans, who typically have poor response to standard therapy, were as high as 53%. Patients with cirrhosis went on to SVR at rates as high as 67%.

4) Phase 3 trials

The recently reported phase 3 Sprint-2 and Respond-2 phase 3 trials give us further insight into the optimal use of boceprevir in combination with PegIFN/RBV in genotype 1 infected individuals. Sprint-1 enrolled 1,094 treatment naïve (938 nonblack cohort and 159 black cohort) patients into 3 treatment arms: 1) 48 weeks of PegIFN/RBV (control), a response-guided therapy (RGT) arm, with 4-week lead-in followed by boceprevir for 24 weeks with an additional 20 weeks of PegIFN/RBV if HCV RNA was detected during weeks 8 through 24.[13] In the third arm, patients received a PegIFN/RBV lead-in, followed by 44 weeks of PegIFN/RBV, and boceprevir. In both cohorts, higher sustained response rates were seen in the boceprevir-containing regimens, with the sustained response rates in the nonblack arm being 67% for the RGT arm and 68% for the 44-week boceprevir/peg/ribavirin arm. This was superior to PegIFN/RBV control of 40%. Superior sustained response rates were also seen in the black cohort where the response-guided therapy arm achieved an SVR of 42%, with the peg/ribavirin/boceprevir 44-week arm achieving an SVR of 53%, both superior to the control peg/ribavirin of 23%.

The nonresponder Respond-2 trial had a comparable design but had a longer duration of boceprevir therapy of 32 weeks in the response guided arm.[14] Patients received either PegIFN alfa-2b and ribavirin control or a 4-week lead-in followed by 32 weeks of boceprevir, PegIFN/RBV with an additional 12 weeks PegIFN/RBV in slow responders vs 44 weeks of PegIFN/RBV/boceprevir after the 4-week lead-in. This nonresponder study included historical relapsers and partial responders (>2 log reduction week 12), but historical null responders (<2 log reduction after 12 weeks of PegIFN/RBV) were excluded. Again, superior SVR rates were seen with the boceprevir-containing

regimens, with 59% of response-guided therapy individuals achieving SVR and 67% of individuals who received boceprevir for 44 weeks of peg/ribavirin after the lead-in achieving SVR. Boceprevir has now been approved for the treatment of naïve patients and nonresponders in combination with PegIFN and RBV in the United States.

5) Safety and toxicity

Anemia and dysguesia were the most significant side effects noted in the boceprevir arms, though those who became anemia had higher SVR rates. The use of erythropoietin (EPO) was permitted in this trial and higher SVR rates were noted in those who developed anemia and required EPO. The role of EPO with boceprevir is currently being studied in a randomized trial that is fully enrolled. The resistance profile of boceprevir is shown in Table 2 and is similar to that of telaprevir.

3. Other protease inhibitors are currently in development

The NS3/NS4A protease inhibitor ITMN-191 (R7227, danoprevir) (Intermune, Roche) is a selective inhibitor of the NS3/NS4 protease. As a monotherapy, ITMN-191 led to reductions in plasma HCV RNA in a phase 1B ascending dose study from 100 mg q12h to 200 mg q8h and 300 mg q12h. In this study, maximal decreases in HCV RNA were noted in a 3.9 $\log_{10}$ and 3.2 $\log_{10}$ in those receiving danoprevir 200 mg q8h and 200 mg q12h.[15] This study was followed by a preliminary presentation demonstrating robust HCV RNA decline with danoprevir PegIFN 2a/RBV over 14 days with undetectable HCV RNA in up to 57% of individuals receiving danoprevir 300 mg t.i.d.[16] Because a phase 2 study with danoprevir 900 mg twice a day demonstrated Grade IV hepatotoxicity, a pilot study has been reported, demonstrating that ritonavir-boosted danoprevir; with, PegIFN/RBV could lead to high rates of HCV RNA clearance with no hepatotoxicity. In this pilot study, 30 individuals were randomized to receive danoprevir 100 mg or 200 mg b.i.d. or daily with PegIFN/RBV. Indeed, 100% of individuals clearing HCV RNA in those who receive danoprevir 200 mg b.i.d. with ritonavir boosting 100 mg b.i.d.[17] As ritonavir boosting has been successfully used in the HIV treatment, it may also serve as a useful adjunct to reduce HCV protease exposure and minimize toxicity.

The NS3/NS4 protease, TMC-435 (Medavir Incorporated) has also been shown to be effective in treatment of genotype 1 hepatitis C when given in combination with PegIFN and RBV. The initial study of TMC-435 is a macrocyclic HCV NS3/NS4A protease inhibitor, with a favorable pharmacokinetic profile supporting once daily dosing. A small pilot study demonstrated a median of 3.9 $\log_{10}$ reduction in HCV RNA after 5 days of monotherapy in individuals who had failed previous interferon-based therapy.[18] A phase 2A study with TMC435 (Opera 1) has been reported. In this study, TMC435, was combined in ascending doses from 75 mg to 200 mg for 4 weeks, in combination with PegIFN/RBV in treatment naïve and treatment experienced individuals (nonresponders and relapsers). At week 4, 44%, 78%, and 70% of individuals in the 75, 150, and 200 mg daily treatment groups, achieved plasma HCV RNA levels of <25 IU, with relapsers responding with higher rates of HCV RNA clearance than nonresponders. TMC435 was well tolerated, though elevated serum bilirubin levels (total direct and indirect), primarily with the 200 mg dose were noted.[19] Current studies are ongoing with TMC in combination with PegIFN alfa-2a and ribavirin in the Pillar study and Aspire study, and we await further results for this promising compound which can be given daily. The preliminary resistance profile is listed in Table 2, with mutations at NS3 amino acid position 80, 155, 156, and 158 being reported.

The NS3 protease inhibitor BI201335 is a potent HCV NS34A inhibitor with preliminary results demonstrating improved viral clearance rates through week 12. In the Silen-C1 study, BI201335 was added to PegIFN2a180/RBV at doses of 240 and 120 mg daily in treatment naïve patients. In this study, RVR rates ranging from 90% to 92% (dose independent) and complete EVR ranging from 84% to 91% were noted.[20] The Silen-C2 study used higher doses of BI201335 (240 mg and 240 mg b.i.d.) in combination with PegIFN/RBV in nonresponders who failed previous PegIFN/RBV. The 12-week analysis was recently presented with RVR rates of 62% to 69% seen in the 240 b.i.d. with 3-day PegIFN/RBV lead-in and EVR rates ranging from 54% to 59%.[21] Similar to the Silen-1 study, an increased incidence of jaundice and rash were noted. The final SVR rates for these 2 studies is currently being awaited. Amino acid changes were most commonly seen as residues 168, 156, and 155.

POLYMERASES AS POTENTIAL TARGES OF ANTI-HCV THERAPY

1. NS5B polymerase inhibitors

The HCV replication process is complex and therefore offers a wide variety of targets for antiviral intervention other than the NS3/NS4 protease. As a class, the development of inhibitors of NS5b is not as mature as that of the NS3/NS4a protease inhibitors. Nonetheless, preliminary data suggest that this will be an effective class of agents in the treatment of HCV infection. In contrast to NIs, the heterogeneous class of nonnucleoside inhibitors (NNIs) bind to different allosteric enzyme sites, which results in conformational protein change before the elongation complex is formed. NNIs achieve NS5B inhibition by binding to 1 of multiple allosteric enzyme sites resulting in conformational changes of the protein-inhibiting catalytic activity of polymerase.[22-25] They have genotype-specific activity and potential for rapid selection of resistance. The rapid development of resistant mutants is possible with non-nucleoside inhibitors because they bind distantly to the active center of NS5B and mutations at the non-nucleoside inhibitor binding site may not necessarily lead to impairment of the enzyme function. Due to their distinc-

tive binding sites, different polymerase inhibitors could theoretically be used in combination to reduce the risk of development of resistance (Table 3, Fig. 4).

1) Nucleoside inhibitors

(1) RG7128

RG7128 is the oral prodrug of PSI-6130, a second cytidine nucleoside analogue under clinical development and has demonstrated potent *in vitro* activity regardless of race, ethnicity and genotype. Thus far, viral resistance has not been detected in any clinical trials with RG7128, which suggests that the nucleoside class may offer a higher genetic barrier to viral resistance than the protease class of inhibitors. In a dose-escalating phase 1b trial, a dose-dependent decrease in HCV RNA was observed in genotype 1 previous nonresponders.[26] RG7128 is well tolerated as monotherapy and no serious AEs were reported in any study arm. In treatment naïve genotype 1 patients, the combination of R7128 (1,500 mg b.i.d. in combination with PegIFN/RBV achieved a reduction in HCV RNA of approximately 5 $\log_{10}$ at 4 weeks translating into a RVR rate of 85% (vs 2 $\log_{10}$ IU/mL and 10% in control arm).[27] No virological rebound was observed during R7128 treatment to 4 weeks. Importantly, R7128 was generally well tolerated in combination with PegIFNa and RBV. The grade 3/4 hematological toxicity was rare and headache, fatigue, and chills were classified as mild AEs. Preliminary resistance testing failed to identify any variants to week 4 and this trial is ongoing. The combination of a potent anti-viral effect and satisfactory toxicity profile makes R7128 an attractive agent.

In addition, it is the first polymerase inhibitor being tested for anti-viral activity against genotypes 2 and 3 HCV. A small study done recently showed higher SVR with RG7128 plus PegIFNa/RBV in genotypes 2 and 3 HCV patients who previously failed PegIFNa/RBV treatment.[28] This randomized trial comparing RG7128 (n=20) with placebo (n=5), each in combination with PegIFN/RBV for 4 weeks followed by continued PegIFN/RBV treatment for a total of 24 to 48 weeks, depending on the patient's previous response to therapy and achievement of RVR in the current trial. The RVR rate was 95% with RG7128 triple therapy vs 60% with PegIFNa/RBV and the SVR rates were 65% vs 60%, respectively. Higher SVR rates with RG7128 treatment were associated with achieving RVR and longer duration of PegIFNa/RBV therapy, whereas HCV genotype did not impact the likelihood of SVR with 63% of genotype 2 patients with

Table 3. Polymerase Inhibitors for Hepatitis C Virus: Drugs in Development

Drug name	Company	Target/Active drug	Current phase of the trial
Nucleoside analogue NS5B polymerase inhibitors			
Valopicitabine (NM283)	Idenix/Novartis	Active site/NM107	Stopped
R7128	Roche/Pharmasset	Active site/PSI-6130	Phase 2
R1626	Roche	Active site/PSI-6130	Stopped
PSI-7851	Pharmasset	Active site	Phase 1
IDX184	Idenix	Active site	Phase 2
Nonnucleoside NS5B polymerase inhibitors (NNI)			
BILB 1941	Boehringer Ingelheim	NNI site 1/thumb 1	Stopped
BI207127	Boehringer Ingelheim	NNI site 1/thumb 1	Phase 2
MK-3281	Merck	NNI site 1/thumb 1	Stopped
Filibuvir (PF-00868554)	Pfizer	NNI site 2/thumb 2	Phase 2
VCH759	ViroChem Pharma	NNI site 2/thumb 2	Phase 1
VCH916	ViroChem Pharma	NNI site 2/thumb 2	Phase 1
VCH222	ViroChem Pharma	NNI site 2/thumb 2	Phase 1
HCV-796	ViroPharma/Wyeth	NNI site 4/palm 2	Stopped
ANA598	Anadys	NNI site 3/palm1	Phase 1
GS-9190	Gilead	NNI site 4/palm2	Phase 2
VX-222	Vertex	NNI site 2/thumb 2	Phase 1b
ABT-333	Abbott	NNI site 4/palm2	Phase 1
NS5A inhibitors			
BMS-790052	Bristol-Myers Squibb	NS5A domain 1 inhibitor	Phase 2
A832	Astra Zeneca	NS5A inhibitor	Phase 1
AZD7295	Astra Zeneca	NS5A Inhibitor	Phase 2

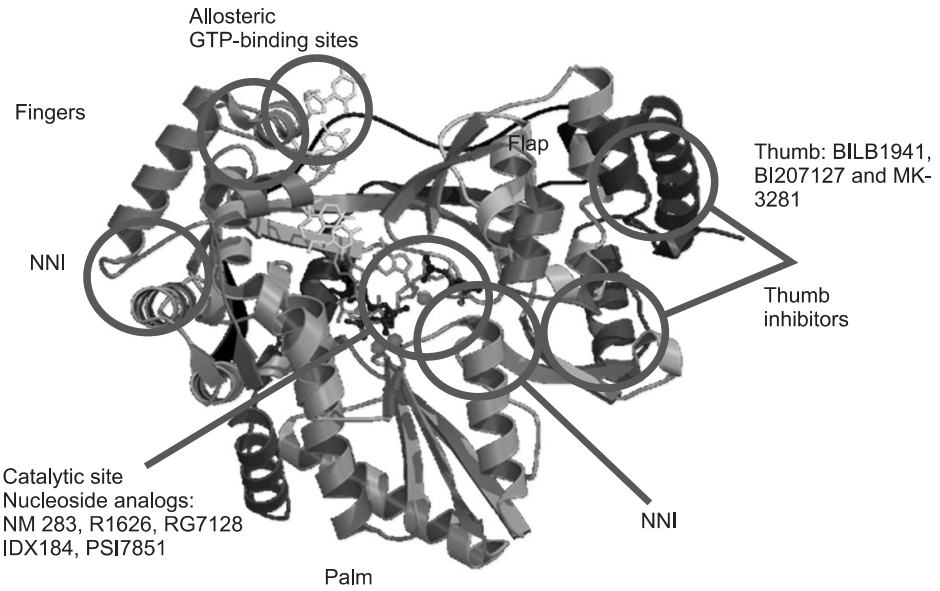

Fig. 4. Binding sites of various nucleoside and nonnucleoside inhibitors. The hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase (RdRp) is a key enzyme involved in HCV replication, catalyzing the synthesis of the complementary minus-strand RNA and subsequent genomic plus-strand RNA from the minus-strand template. The NS5B polymerase inhibitors for treatment of chronic hepatitis C can be divided into 2 distinct categories: 1) nucleoside or nucleotide inhibitors (active site inhibitors), and 2) nonnucleotide inhibitors (allosteric inhibitors). Nucleoside analogues mimic the natural substrates of the polymerase and are incorporated into the growing RNA chain, thus causing direct chain termination by tackling the active site of NS5B. As the active center of NS5B is a highly conserved region of the HCV genome, NIs are potentially effective against different genotypes, in contrast to NS3/4A inhibitors. Moreover, single amino acid substitutions in every position of the active center may result in loss of function and severely reduce replication fitness. Thus, there is a relatively high genetic barrier in the development of resistances to NIs, a desirable trait for anti-HCV direct-acting antiviral agent. Adapted from Butcher *et al*. Nature 2001;410:235-40.

achieving SVR vs 67% of patients with genotype 3. The higher RVR rates but similar SVR rates with RG7128 triple therapy vs PegIFNa/RBV in this study suggest that polymerase inhibitor treatment will need to be administered for longer than 4 weeks in previous nonresponders with genotype 2 and particularly genotype 3 infection.

The novel study INFORM-1, the first dual combination clinical trial with oral antivirals in HCV patients evaluated the safety and combined antiviral activity of RG7227, a protease inhibitor and RG7128, a polymerase inhibitor, in 14 days of combination therapy in treatment-naïve patients, experienced non-null or null-responders infected with HCV genotype 1.[29] The basis of this trial is that induction therapy with potent DAA regimens could potentially enhance the efficacy and decrease the duration of treatment with the current treatmentfor chronic hepatitis C (PegIFN/RBV). Patients receiving this combination for 14 days experienced a median reduction in viral levels of 4.8 to 5.2 $\log_{10}$ IU in the higher doses tested and this combination was equally effective in both naïve and previous nonresponders with the lowest reductions observed in treatment-naïve patients receiving the lowest drug doses and in previous nonresponders (excluding null responders). The highest end-of-treatment response rate in treatment-naïve individuals (100%) was achieved in patients treated with the highest dosage combination of RG7128/RG7227 (1,000/900 mg b.i.d.). All patients achieved an RVR at week 4 of treatment with PegIFN/RBV were assigned to an abbreviated 24-week regimen. These encouraging results provide a proof of concept that, when given at optimal doses, a short course of dual combination therapy can be highly effective in suppressing HCV RNA in the absence of PegIFN/RBV. Importantly, no drug resistant mutations emerged during the 14-day treatment period in any patient group. No treatment-related serious adverse events, dose reductions, drug-drug interactions or discontinuations were reported. Given these encouraging data, combinations of DAA agents in the absence of PegIFN and/or RBV will be undertaken.

(2) Other nucleoside/nucleotide inhibitors (NIs)

Several other new NIs are currently under various stages of clinical trials including IDX184, liver-targeted nucleotide NS5B polymerase inhibitor.[30] PSI-7851, a second generation nucleotide inhibitor and PSI-7977, an isomer of PSI-7851. A phase 2 study of PSI-7977 administered once-daily in combination with PegIFNa/RBV for 28 days in 63 previously untreated patients with genotype 1 chronic hepatitis C with PegIFN/RBVcontinued for an additional 44 weeks. PSI-7977 has been enrolled and further results are expected later this year.[31]

2. Nonnucleoside analogue inhibitors

1) NNI-site 1 inhibitors (thumb 1/benzimidazole site) (Fig. 4)

BILB1941, BI207127, and MK3281 are NNI-site 1 inhibitors which have been investigated in clinical phase 1 trials and exhibit low to medium anti-viral activities.[32-34] No selection of

resistant variants and viral breakthrough has been observed during 5 days of treatment with BILB1941 or BI207127. Gastrointestinal intolerance at higher doses, elevated liver enzymes, and its liquid formulation led to a halt in further development of BILB1941. In a recent double-blind placebo-controlled study, 7 days of MK-3281 monotherapy in genotype 1/3 HCV male patients resulted in rapid and significant HCV RNA reductions vs placebo with the greatest degree of virologic suppression in genotype 1b HCV patients and no serious clinical or laboratory adverse events were reported.

2) NNI-site 2 inhibitors (thumb 2/thiophene site) (Fig. 4)

Filibuvir (PF-00868554) is a NNI-site 2 inhibitor with moderate anti-viral activity in a phase 1 study. In a subsequent trial viral breakthrough was observed in 5 of 26 patients during combination therapy with PegIFN-a 2a and RBV for 4 weeks.[35] A phase 2, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of filibuvir plus PegIFN-a 2a/RBV in treatment-naïve, HCV genotype 1 infected subjects is currently underway. Other NNI-site 2 inhibitors which were evaluated in phase 1 trials are VCH-759, VCH-916, and VCH-222. Like during treatment with filibuvir, VCH-759 and VCH-916 application resulted in viral breakthroughs with selection of resistant variants, indicating a lower genetic barrier to resistance of these agents as opposed to the NIs. Preliminary results from a randomized, placebo-controlled phase Ib/IIa dose-escalation study of the novel nonnucleoside HCV NS5B polymerase inhibitor VX-222 were recently reported.[36] VX-222 monotherapy was associated with >3.0 $\log_{10}$ IU/mL mean decreases in HCV RNA from baseline to day 3 at all doses evaluated, suggesting that this agent represents one of the most potent nonnucleoside polymerase inhibitors tested to date. Reductions in HCV RNA levels were observed within 1 day of VX-222 initiation in all cohorts, including in patients infected with genotypes 1a and 1b HCV. This finding is important because nonnucleoside polymerase inhibitors often have differential activity toward HCV genotypes 1a and 1b. The most frequent adverse events included diarrhea headache and nausea with no serious adverse events were reported.

3) NNI-site 3 inhibitors (palm 1/benzothiadiazine site)

ANA598 is a NNI-site 3 inhibitor which displayed antiviral activity during treatment of HCV genotype 1 infected patients when combined with PegIFN/RBV.[37] A larger phase 2 trial is planned. IDX375 demonstrated strong inhibition of HCV replication (EC50=2.3 nM) in the subgenomic replicon system, with no *in vitro* cytotoxicity in rat, mouse, monkey, and human hepatocytes, and no apparent *in vivo* adverse events in monkeys and is continuing clinical development.[38]

4) NNI-site 4 inhibitors (palm 2/benzofuran site)

ABT-333, another palm site inhibitor, has demonstrated a promising *in vitro* antiviral profile, with enzyme inhibition IC50 levels of 2.2 nM against HCV genotypes 1 and 2 and EC50 values of 0.5 to 0.8 nM in the context of the replicon system against HCV genotypes 1a and 1b.[39] Recent data on the pharmacokinetic profile, safety, and efficacy of ABT-333 treatment-naïve patients infected with genotype 1 HCV is promising and is being investigated further in combination with PegIFN/RBV.

The NNI site 4 inhibitor GS-9190 displays anti-viral activity in a clinical study and variants conferring resistance were identified in the beta-hairpin of the polymerase.[40,41] Preliminary data of 23 study participants who received multiple ascending doses over 8 days suggested that GS 9190 may be associated with QT prolongation. After consultation and a separate dose-ranging study in healthy volunteers, the QT prolongation at a lower dose of the drug was determined to be "clinically manageable" (Gilead Quarterly Report, May 2, 2008). GS-9190 is currently the most advanced NS5B polymerase NNI (phase 2) and a study in combination with PegIFN/RBV is currently underway with results to be reported in the next year.

3. NS5A inhibitors

The function of HCV NS5A is not fully defined. Two potent NS5A specifically targeted antiviral therapy compounds have been evaluated in clinical trials, including compounds A-832 (phase 1) and BMS-790052 (phase 2). BMS-790052 binds to domain I of the NS5A protein, which was shown to be important for regulation of HCV replication. It is highly potent selective inhibitor of NS5A, and has shown strong activity against several genotypes in both replicon and JFH-1 systems.[42] The *in vitro* potency is extremely high with a half maximal effective concentration in the range of 9-127 pM, depending on the viral genotype. This value reflects 100- to 1,000-fold higher potency than most other drugs that are being investigated. The results of a previous single ascending-dose study of BMS-790052 in patients infected with genotype 1 HCV were striking in that patients who received a single 100-mg dose exhibited an approximately 3.6 $\log_{10}$ mean reduction in HCV RNA that was maintained 144 hours after dosing.[43] A week 12 data from a randomized, placebo-controlled, phase IIa trial investigating different once-daily BMS-790052 doses (3 mg, 10 mg, and 60 mg) in combination with PegIFNa/RBV for 48 weeks in treatment-naïve patients infected with genotype 1 HCV was recently reported.[44] The preliminary results study (n=48) demonstrated high RVR rates of 92% and 83% with BMS-790052 doses of 10 mg and 60 mg, respectively, in combination with PegIFNa/RBV. Complete EVR rates were similarly high at 83% in both the 10-mg and 60-mg dosing arms. Patients treated with BMS-790052 3 mg daily experienced lower RVR and complete EVR rates of 42% and 58%, respectively. The adverse event profile at this early stage appears favorable. This preliminary analysis suggests that this drug class may be promising for patients with genotype 1 HCV and it is hoped that similar efficacy will be

observed across other genotypes. The resistance profile of BMS-790052 is well characterized in replicon systems but there is limited data from the preliminary clinical trials. Other NS5A inhibitors such as PPI-461 and AZD 7295 are also in clinical development phase and results look encouraging.[45,46] No clinical data on resistance to this class of drugs have been presented yet and results of multiple dose and combination therapy studies have to be awaited.

CONCLUSION AND FUTURE DIRECTIONS

In summary, it is very likely that the NS3/NS4a protease inhibitors will be approved next year by regulatory agencies in the United States and Europe for use in combination with PegIFN/RBV. This will dramatically improve SVR rates but in those who are poorly interferon responsive, the risk of resistance will remain. Preliminary results suggest that the addition of nucleoside polymerase inhibitors to PegIFN/RBV will also lead to high SVR rates and the nucleoside polymerase inhibitor class may be a particularly attractive backbone therapy for the treatment of hepatitis C. Finally, preliminary data with the NS5a inhibitor class appears to be highly promising when given in combination with PegIFN and RBV. In the future, combination of DAAs including polymerase inhibitors both nucleoside, and nonnucleoside, protease inhibitors, and NS5a inhibitors to PegIFN, and RBV will likely significantly reduce resistance rates, and it is anticipated that the combinations of DAAs will improve SVR rates further in combination with PegIFN and RBV likely by reducing the risk of developing resistance. Moving forward, the ability to eliminate IFN and/or RBV in the future and still achieve SVR will be the next major goal in the treatment of hepatitis C.

CONFLICTS OF INTEREST

Dr. Kwo has received consulting fees from Abbott, Anadys, Bayer, Bristol Myers Squibb, Gilead, Merck, Novartis, Vertex; he also received fees for Non-CME/CE services directly from Bristol Myers Squibb, Gilead, Merck, and Roche; and contracted research funding from Abbott, Anadys, Bayer, Bristol Myers Squibb, Gilead, Merck, Novartis, Roche, and Vertex.

Dr. Vinayek received fees for Non-CME/CE services directly from Merck, and Roche; and contracted research funding from Pfizer, Celgene, Roche.

REFERENCES

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41-52.
2. Bartenschlager R, Lohmann V. Replication of hepatitis C virus. J Gen Virol 2000;81(Pt 7):1631-1648.
3. Hinrichsen H, Benhamou Y, Wedemeyer H, et al. Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients. Gastroenterology 2004;127:1347-1355.
4. Forestier N, Reesink HW, Weegink CJ, et al. Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C. Hepatology 2007;46:640-648.
5. Zeuzem S. Telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in chronic hepatitis C patients. J Hepatol 2008;49:157-159.
6. McHutchison JG, Everson GT, Gordon SC, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827-1838.
7. Hézode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839-1850.
8. McHutchison JG, Manns MP, Muir AJ, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010;362:1292-1303.
9. Malcolm BA, Liu R, Lahser F, et al. SCH 503034, a mechanism-based inhibitor of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral activity of alpha interferon in replicon cells. Antimicrob Agents Chemother 2006;50:1013-1020.
10. Friedrich-Rust M, Forestier N, Sarrazin C, Reesink HW, Herrmann E, Zeuzem S. Ultrasound evaluation of perihepatic lymph nodes during antiviral therapy with the protease inhibitor telaprevir (VX-950) in patients with chronic hepatitis C infection. Ultrasound Med Biol 2007;33:1362-1367.
11. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: 2008 update. Clin Gastroenterol Hepatol 2008;6:1315-1341.
12. Kwo PY, Lawitz EJ, McCone J, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. Lancet 2010;376:705-716.
13. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1195-1206.
14. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1207-1217.
15. Forestier N, Larrey D, Guyader D, et al. Treatment of chronic hepatitis C patients with the NS3/4A protease inhibitor danoprevir (ITMN-191/RG7227) leads to robust reductions in viral RNA: a phase 1b multiple ascending dose study. J Hepatol 2011;54:1130-1136.
16. Gane EJ, Roberts SK, Stedman C, et al. First-in-man demonstration of potent antiviral activity with a nucleoside polymerase (R7128) and protease (R7227/ITMN-191) inhibitor combination in HCV: safety, pharmacokinetics, and virologic results from INFORM-1. J Hepatol 2010;50(Suppl 1):S380.

17. Gane E, Rouzier R, Stedman C, et al. Ritonavir boosting of low dose RG7227/ITMN-191, HCV NS3/4A protease inhibitor, results in robust reduction in HCV RNA at lower exposures than provided by unboosted regimens. J Hepatol 2010;52(Suppl 1):S16-S17.
18. Reesink HW, Fanning GC, Farha KA, et al. Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. Gastroenterology 2010;138:913-921.
19. Marcellin P, Reesink H, Berg T, et al. Antiviral activity and safety of TMC435 combined with peginterferon alpha-2a and ribavirin in patients with genotype 1 hepatitis C infection who failed previous IFN-based therapy. J Hepatol 2009;50(Suppl 1):S385.
20. Sulkowski MS, Ferenci P, Emanoil C, et al. SILEN-C1: early antiviral activity and safety of BI 201335 combined with peginterferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 HCV infection. Hepatology 2009;50(Suppl 4):90A.
21. Sulkowski M, Bourliere M, Bronowicki JP, et al. SILEN-C2: early antiviral activity and safety of BI 201335 combined with peginterferon alfa-2a and ribavirin (PEGIFN/RBV) in chronic HCV genotype-1 patients with non-response to PEGIFN/RBV. J Hepatol 2010;52(Suppl 1):S462-S463.
22. Betzi S, Eydoux C, Bussetta C, et al. Identification of allosteric inhibitors blocking the hepatitis C virus polymerase NS5B in the RNA synthesis initiation step. Antiviral Res 2009;84:48-59.
23. Kim MS, Park C, Lee JH, Myung H. Selection and target-site mapping of peptides inhibiting HCV NS5B polymerase using phage display. J Microbiol Biotechnol 2008;18:328-333.
24. Martin Hernando JI, Ontoria JM, Malancona S, et al. Optimization of thienopyrrole-based finger-loop inhibitors of the hepatitis C virus NS5B polymerase. ChemMedChem 2009;4:1695-1713.
25. McGowan D, Nyanguile O, Cummings MD, et al. 1,5-Benzodiazepine inhibitors of HCV NS5B polymerase. Bioorg Med Chem Lett 2009;19:2492-2496.
26. Reddy R, Rodriguez-Torres M, Gane E, et al. Antiviral activity, pharmacokinetics, safety and tolerability of R7128, a novel nucleoside HCV RNA polymerase inhibitor, following multiple, ascending, oral doses in patients with HCV genotype 1 infection who have failed prior interferon therapy. Hepatology 2007;46(Suppl 1):862A.
27. Lalezari J, Gane E, Rodriguez-Torres M, et al. Potent antiviral activity of the HCV nucleoside polymerase inhibitor R7128 with PEG-IFN and ribavirin: interim results of R7128 500 mg bid for 28 days. J Hepatol 2008;48(Suppl 2):S29.
28. Gane EJ, Rodriguez-Torres M, Nelson DE, et al. Sustained virologic response (SVR) following RG7128 1500mg BID/PEG-IFN/RBV for 28 days in HCV genotype 2/3 prior non-responders. J Hepatol 2010;52(Suppl 1):S16.
29. Gane EJ, Roberts SK, Stedman CA, et al. Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet 2010;376:1467-1475.
30. Lalezari J, Asmuth D, Casiró A, et al. Antiviral activity, safety and pharmacokinetics of IDX184, a liver-targeted nucleotide HCV polymerase inhibitor, in patients with chronic hepatitis C. Hepatology 2009;50(Suppl 4):228A.
31. Lalezari J, Lawitz E, Rodriguez-Torres M, et al. Once daily PSI-7977 plus PEGIFN/RBV in a phase 2B trial: rapid virologic suppression in treatment-naïve patients with HCV GT2/GT3. J Hepatol 2011;54(Suppl 1):S28.
32. Larrey D, Benhamou Y, Lohse AW, et al. Safety, pharmacokinetics and antiviral effect of BI 207127, a novel HCV RNA polymerase inhibitor, after 5 days oral treatment in patients with chronic hepatitis C. J Hepatol 2009;50(Suppl 1):S383-S384.
33. Brainard DM, Anderson MS, Petry A, et al. Safety and antiviral activity of NS5B polymerase inhibitor MK-3281, in treatment-naïve genotype 1A, 1B and 3 HCV-infected patients. Hepatology 2009;50(Suppl 4):263A.
34. Erhardt A, Wedemeyer H, Benhamou Y, et al. Safety, pharmacokinetics and antiviral effect of BILB1941, a novel HCV RNA polymerase inhibitor, after 5 days oral treatment in patients with chronic hepatitis C. J Hepatol 2007;46(Suppl 1):S222.
35. Jacobson I, Pockros P, Lalezari J, et al. Antiviral activity of filibuvir in combination with pegylated interferon alfa-2a and ribavirin for 28 days in treatment naïve patients chronically infected with HCV genotype 1. J Hepatol 2009;50(Suppl 1):S382-S383.
36. Rodriguez-Torres M, Lawitz E, Conway B, et al. Safety and antiviral activity of the HCV non-nucleoside polymerase inhibitor VX-222 in treatment-naïve genotype 1 HCV-infected patients. J Hepatol 2010;52(Suppl 1):S14.
37. Lawitz E, Rodriquez-Torres M, Rustgi VK, et al. Safety and antiviral activity of ana598 in combination with pegylated interferon alpha2a plus ribavirin in treatment-naïve genotype-1 chronic HCV patients. J Hepatol 2010;52(Suppl 1):S467.
38. Good SS, Bu C, Camire M, et al. Preclinical pharmacokinetic and safety profile of IDX375, a novel and potent nonnucleoside HCV polymerase inhibitor. J Hepatol 2009;50(Suppl 1):S125-S126.
39. Rodriguez-Torres M, Lawitz E, Cohen D, et al. Treatment-naïve, HCV genotype 1-infected subjects show significantly greater HCV RNA decreases when treated with 28 days of ABT-333 plus peginterferon and ribavirin compared to peginterferon and ribavirin alone. Hepatology 2009;50(Suppl 4):91A.
40. Bavisotto L, Wang CC, Jacobson IM, et al. Antiviral, pharmacokinetic and safety data for GS-9190, a non-nucleoside HCV NS5B polymerase inhibitor in a phase-1 trial in HCV genotype 1 infected subjects. Hepatology 2007;46(Suppl 1):255A.
41. Shih I, Vliegen I, Peng B, et al. Mechanistic characterization of GS-9190, a novel non-nucleoside inhibitor of HCV NS5B polymerase with potent antiviral activity and a unique mechanism of action. Hepatology 2007;46(Suppl 1):859A.
42. Gao M, Nettles RE, Belema M, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 2010;465:96-100.
43. Nettles R, Chien C, Chung E, et al. BMS-790052 is a first-in-class

potent hepatitis C virus (HCV) NS5A inhibitor for patients with chronic HCV infection: results from a proof-of-concept study. Hepatology 2008;48(Suppl 1):1025A.

44. Pol S, Everson G, Ghalib R, et al. Once-daily NS5A inhibitor (BMS-790052) plus peginterferon-alpha-2a and ribavirin produces high rates of extended rapid virologic response in treatment-naïve HCV-genotype 1 subjects: phase 2a trial. J Hepatol 2010;52(Suppl 1):S462.
45. Gane E, Foster GR, Cianciara J, et al. Antiviral activity, pharmacokinetics, and tolerability of AZD7295, a novel NS5A inhibitor, in a placebo-controlled multiple ascending dose study in HCV genotype 1 and 3 patients. J Hepatol 2010;52(Suppl 1):S464.
46. Colonno R, Peng E, Bencsik M, et al. Identification and characterization of PPI-461, a potent and selective HCV NS5A inhibitor with activity against all HCV genotypes. J Hepatol 2010;52(Suppl 1):S14-S15.