Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

New developments in small molecular compounds for anti-hepatitis C virus (HCV) therapy*

Jing TONG[1,2], You-wei WANG[2], Yuan-an LU[†‡1,2]

([1]*Department of Public Health Sciences, University of Hawaii, Honolulu, Hawaii 96822, USA*)

([2]*Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry of Education, Institute of Traditional Chinese Medicine & Natural Products, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, China*)

[†]E-mail: ylu@pbrc.hawaii.edu

Received Mar. 25, 2011; Revision accepted June 10, 2011; Crosschecked May 26, 2011

Abstract: Infection with hepatitis C virus (HCV) affects approximately 170 million people worldwide. However, no vaccine or immunoglobulin is currently available for the prevention of HCV infection. The standard of care (SOC) involving pegylated interferon-α (PEG-IFN α) plus ribavirin (RBV) for 48 weeks results in a sustained virologic response in less than 50% of patients with chronic hepatitis C genotype 1, the most prevalent type of HCV in North America and Europe. Recently, reliable in vitro culture systems have been developed for accelerating antiviral therapy research, and many new specifically targeted antiviral therapies for hepatitis C (STAT-C) and treatment strategies are being evaluated in clinical trials. These new antiviral agents are expected to improve present treatment significantly and may potentially shorten treatment duration. The aim of this review is to summarize the current developments in new anti-HCV drugs.

Key words: Hepatitis C virus (HCV), Clinical trial, Small molecular compounds, Review
doi:10.1631/jzus.B1100120 **Document code:** A **CLC number:** R575.1

1 Introduction

Hepatitis C virus (HCV), first characterized in 1989 as the major cause of non-A and non-B hepatitis infections (Choo *et al*., 1989), has been described as a "silent epidemic" and a "serious global health crisis". The World Health Organization estimates that the prevalence of HCV infection is approximately 130–170 million people, with 3–4 million new infections each year (Shepard *et al*., 2005). The highest prevalence of HCV cases is in the African and Eastern Mediterranean regions (Lavanchy, 2009). The Center for Disease Control and Prevention estimates that HCV is the most common chronic blood-borne viral infection in the US. As many as 75%–85% of patients newly infected with HCV will develop chronic infection and face an increased risk of developing liver cirrhosis or hepatocellular carcinoma (HCC). Although only 25% of new infections are symptomatic, 60%–80% of patients develop chronic liver disease, of whom an estimated 20% progress to cirrhosis, with a 1%–4% annual risk of developing HCC (Pawlotsky, 2006). HCV is currently a leading cause of death in human immunodeficiency virus (HIV)-coinfected patients (Salmon-Ceron *et al*., 2005). HCV-related end-stage liver disease is the most common reason for liver transplantation today in the US and Western Europe (Tang and Grise, 2009). No vaccine is currently available to prevent hepatitis C infection (Houghton and Abrignani, 2005).

‡ Corresponding author

* Project partially supported by the Centers for Oceans and Human Health Program of the National Institute of Environmental Health Sciences (No. P50ES012740), the National Science Foundation of USA (Nos. OCE04-32479 and OCE09-11000), and the National Mega Project on Major Drug Development of China (No. 2011ZX09401-302)

HCV, the only known member of the hepacivirus genus in the family *Flaviviridae*, is a small, enveloped RNA virus transmitted by blood-to-blood contact, including drug injection and blood transfusion, or sexual exposure. Six major HCV genotypes and 100 subtypes have been identified worldwide (Simmonds, 2004). HCV genotype 1 (HCV-1), followed by genotypes 2 (HCV-2) and 3 (HCV-3), is the most common genotype found in North and South America and Europe, and has therefore been the focus of most clinical trials. Genotype 4 (HCV-4) is the most frequent cause of chronic hepatitis C in the Middle East, North Africa, and sub-Saharan Africa, and has recently spread to Southern Europe (Nguyen and Keeffe, 2005). Genotype 5 (HCV-5) was initially reported in South Africa (Ohno *et al*., 1994), and genotype 6 (HCV-6) is rare and confined to Southeast Asia (Wong *et al*., 1998).

The HCV genome is composed of a positive, single-stranded RNA of approximately 9 600 nucleotides in length, with a single open reading frame (ORF) flanked by 5′- and 3′-untranslated regions (UTRs). The 5′-UTR contains six secondary structure domains, termed stem-loops (SLs) I–VI. SLII, SLIII, and SLIV form an internal ribosome entry site (IRES) that facilitates translation of the capless HCV RNA (Wang *et al*., 1993). The 5′-UTR also contains essential replication signals for the negative-strand RNA (Friebe *et al*., 2001). The 3′-UTR is highly conserved and is essential for HCV replication (Fried *et al*., 2002; Yi and Lemon, 2003).

The HCV genome encodes a long, single precursor polypeptide of approximately 3 000 amino acids in the gene order of 5′-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3′. Three structural proteins, the capsid protein (core), two glycoproteins (E1 and E2), and a small ion channel protein (p7), reside in the N-terminal half of the polyprotein. Six nonstructural proteins (NSs), NS2, NS3, NS4A, NS4B, NS5A, and NS5B, are situated at the C-terminus.

2 Current treatment for HCV

The lack of an efficient in vitro replication system or small animal infection model has long hampered therapeutic developments for chronic HCV. The goal of anti-HCV treatment is to achieve a sustained viral response (SVR), which is the absence of serum HCV RNA up to six months after therapy is concluded. The current standard of care (SOC) approved by the US Food and Drug Administration (FDA) is a weekly injection of pegylated interferon-α (PEG-IFN α), combined with oral administration of ribavirin (RBV) twice-daily (BID). Although this combination therapy results in over 54% SVR of HCV-infected subjects, the response and rate of SVR can be vary significantly dependent on HCV genotypes (Manns *et al*., 2001; Fried *et al*., 2002; Hadziyannis *et al*., 2004). Also, current therapies are often poorly tolerated because of a plethora of treatment-associated adverse effects, including fatigue, influenza-like symptoms, gastrointestinal disturbances, neuropsychiatric symptoms, anemia, and hematologic abnormalities. Dose reduction is required in more than one third of patients, and drug discontinuation is necessary in approximately 10% of patients (Feld and Hoofnagle, 2005; Manns *et al*., 2007). With few alternatives available, more effective agents with fewer adverse effects are clearly needed.

3 Development of new drugs

The limitation of suboptimal response has driven research toward developing more specifically targeted antiviral therapy for HCV (STAT-C). The discovery of HCV's autonomous subgenomic replication system in 1999 (Lohmann *et al*., 1999) boosted knowledge of the HCV replication cycle, and a robust cell culture infection system JFH-1/HCVcc was established in 2005. This system has allowed researchers to study the HCV life cycle (Cai *et al*., 2005; Lindenbach *et al*., 2005; Wakita *et al*., 2005; Zhong *et al*., 2005) and has accelerated the development of new HCV therapies.

HCV encodes at least four enzymes required for virus replication, including NS2/3 autoprotease, NS3 helicase, NS3/4A serine protease, and NS5B RNA-dependent RNA polymerase (RdRp) (Kolykhalov *et al*., 2000). Current studies have been focused on developing specific inhibitors of NS3/4A and NS5B (Hayashi and Takehara, 2006). These targeted therapeutics have advanced to phases II and III clinical trials. The combination of current standard therapy with novel, potent, multi-target inhibitors could significantly reduce adverse effects and may prove to be

more effective. This review highlights the recent progress of clinical studies aimed at developing effective anti-HCV compounds.

4 Protease inhibitors

The C-terminal half of NS2 with the N-terminal protease domain of NS3 forms a catalytically active protease to cleave the NS2/3 junction (Grakoui *et al*., 1993). Heterodimerization of serine protease with its cofactor NS4A significantly enhances proteolytic processing efficiency. This protease is responsible for the cleavage of the viral polyprotein at four sites (NS3/4A, NS4A/4B, NS4B/5A, and NS5A/5B). Both the protease and the helicase activities are important for HCV replication (Kolykhalov *et al*., 2000; Lam and Frick, 2006). The HCV NS3/4A serine protease is not only involved in viral polyprotein processing, but also efficiently blocks the retinoic acid-inducible gen I (RIG-I) and Toll-like receptor (TLR)-3 signaling pathways and contributes to virus persistence by enabling HCV to escape the interferon antiviral response (Foy *et al*., 2005; Seth *et al*., 2006). NS3/4A protease inhibition can prevent Cardif and/or Toll/interleukin-1 receptor domain-containing adaptor-inducing interferon (TRIF) inactivation during HCV infection, thereby maintaining the innate immune response (Franco *et al*., 2008). Thus, differences in NS3/4A protease catalytic efficiency could be related to viral pathogenicity. Both its essential role in the HCV life cycle and its ability to counteract host innate immunity make the NS3/4A protease an ideal target for the development of new anti-HCV agents and control of the disease (Franco *et al*., 2007).

The development of small molecule inhibitors of NS3/4A protease as antiviral agents has been intensively pursued as a viable strategy to eradicate HCV infection. Protease inhibitors are very efficacious in lowering viral loads to undetectable levels. However, the development of effective inhibitors is a daunting task (Reiser and Timm, 2009). Inhibitors of the NS3/4A protease have been the most extensively studied direct antivirals to date. BILN 2061 (ciluprevir) was the first NS3/4A protease inhibitor to enter clinical trials; however, trials were halted due to cardiac toxicity in rhesus monkeys (Reiser *et al*., 2005). Ciluprevir has opened the door to future trials with NS3/4A protease inhibitors. Currently, a number of compounds from Boehringer Ingelheim, Vertex, ViroBay and Abbott/Enanta are in phase I clinical trials; Merck, Roche, Achillion, and Bochringer Ingelheim in phase II; and, Merck and Vertex in phase III (Chen and Njoroge, 2009). Two agents, the peptidomimetic inhibitors telaprevir and boceprevir, are most advanced; both have progressed to phase III clinical trials (Table 1).

According to their chemical structures, HCV protease inhibitors are classified as non-covalent (linear peptidic inhibitors and macrocyclic inhibitors) or covalent (linear peptidic inhibitors and macrocyclic inhibitors).

4.1 Telaprevir (VX-950)

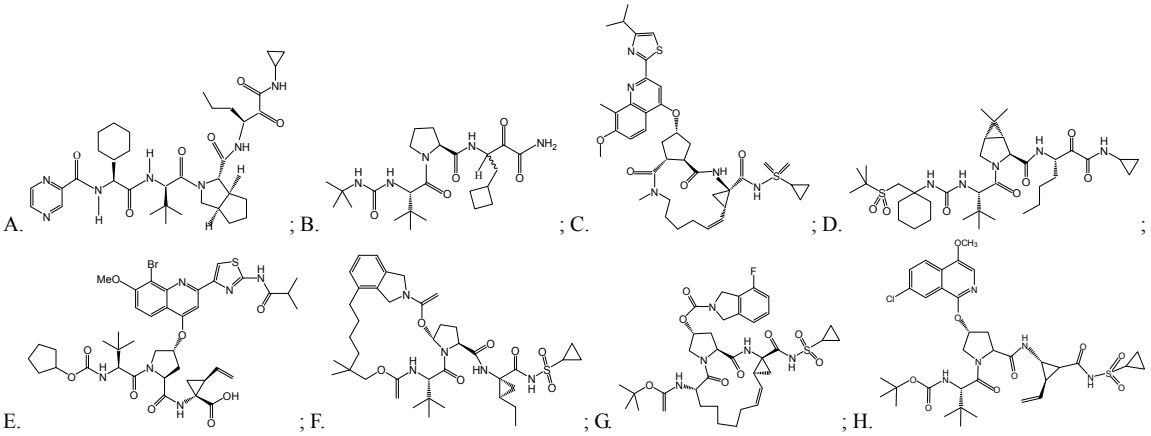
Telaprevir (VX-950) is a selective peroral α-ketoamide peptidomimetic inhibitor of the HCV NS3/4A serine protease. This drug possesses an excellent antiviral activity to both HCV-1a and HCV-1b in vitro (Perni *et al*., 2006). A time- and concentration-dependent reduction of HCV RNA in replicon cells was observed (Lin C. *et al*., 2006). Following a 9-d incubation of HCV replicon cells, a combination of 2 μmol/L telaprevir and 50 U/ml IFN-α resulted in a 3.8 $\log_{10}$ IU/ml reduction of HCV RNA level. These results indicate that the therapeutic effect of the combination can be sustained over time (Lin *et al*., 2004).

In phase I clinical trials, telaprevir showed a robust anti-HCV activity. In phase Ib clinical trials, telaprevir was able to rapidly reduce the plasma viral load of subjects chronically infected with HCV-1 by a mean of 3 $\log_{10}$ IU/ml in 2 d and 4.4 $\log_{10}$ IU/ml after 14 d (Lin C. *et al*., 2006). Furthermore, phase Ib studies suggested that combining telaprevir with PEG-IFN α with or without RBV, increases anti-HCV effect and decreases the emergence of resistance (Hezode *et al*., 2009). This drug was well tolerated at doses of 25–1 250 mg and exhibited good systemic exposure (Lin *et al*., 2004). In addition, a large phase II controlled study of 250 treatment-naive, HCV-1-infected individuals showed that triple therapy with telaprevir, PEG-IFN α, and RBV led to increased rates of rapid viral response (RVR) (74% vs. 14%) and early virologic response (EVR) (79% vs. 43%) over the standard treatment (Weisberg and Jacobson, 2009).

Table 1 Ongoing clinical trials of anti-HCV protease inhibitors (PIs)

Company	Compound	Class	Clinical phase	Structure*
Vertex	Telaprevir (VX-950)	PI	III	A
Merck	Boceprevir (SCH 503034)	PI	III	B
Tibotec Pharmaceuticals Ltd.	TMC435	PI	II	C
Merck	Narlaprevir (SCH 900518)	PI	II	D
Boehringer Ingelheim	BI 201335	PI	II	E
Merck	Vaniprevir (MK-7009)	PI	II	F
Roche	Danoprevir (ITMN-191/R7227)	PI	II	G
Bristol-Myers Squibb	BMS-650032	PI	II	H
Achillion	ACH-1625	PI	II	Undisclosed
Abbott/Enanta	ABT-450	PI	I	Undisclosed
Vertex	VX-500	PI	I	Undisclosed
Boehringer Ingelheim	BI 207127	PI	I	Undisclosed
ViroBay	VBY-376	PI	I	Undisclosed
Vertex	VX-813	PI	I	Undisclosed

* Structure:



To date, more than 2500 people infected with HCV have received telaprevir-based regimens as part of the phase III ADVANCE, ILLUMINATE, and REALIZE studies. The ADVANCE trial enrolled approximately 1095 patients at 114 clinical sites worldwide. The primary endpoint of the trial was SVR and the secondary endpoint was to evaluate the safety of telaprevir when dosed in combination with PEG-IFN α and RBV. There were 75% of the subjects achieved SVR or viral cure after receiving a 12-week telaprevir-based combination regimen, followed by treatment with PEG-IFN α and RBV alone. In total, 69% of the subjects achieved SVR after receiving an eight-week telaprevir-based combination regimen, followed by treatment with PEG-IFN α and RBV alone. In total, 44% of the subjects in the control arm achieved SVR after 48 weeks of treatment with the currently approved regimen of PEG-IFN α and RBV. Results from the phase III ILLUMINATE study showed that there was no benefit to extend telaprevir-based therapy to 48 weeks. Clinical studies of telaprevir have been completed, except for those addressed in "VX-950-TiDP24-C219: A Roll over Trial for Patients in the Control Group of the C216 Study Who Received Telaprevir Placebo" (http://clinicaltrials.gov/ct2/show/NCT01054573) and "Safety and Efficacy of Telaprevir in Combination with PEG-IFN α-2a and RBV in Subjects Co-Infected with HCV and HIV". In addition, current preliminary data suggests that telaprevir has substantial antiviral activity against HCV-2 and HCV-4 infections, but limited efficacy in HCV-3 subjects (Hezode *et al*., 2009). The combination of telaprevir and PEG-IFN α also suppressed the emergence of in vitro resistance mutations against telaprevir in replicon cells (Lin K. *et al*., 2006).

4.2 Boceprevir (SCH 503034)

Boceprevir (SCH 503034) is an HCV NS3/4A protease inhibitor, developed by Merck as a capsule formulation. Boceprevir is a structurally novel

peptidomimetic ketoamide type inhibitor that binds reversibly to the NS3 active site with robust anti-HCV activity. Boceprevir treatment resulted in a 1.5–2.0 $\log_{10}$ IU/ml drop in HCV RNA levels at 72 h and a 3.5–4.0 $\log_{10}$ IU/ml drop by Day 15. No toxic hepatocyte effects were observed. Cells treated with a combination of boceprevir and IFN-α had a greater HCV replicon suppression than agent alone (Malcolm *et al*., 2006). This promising in vitro data has allowed boceprevir to enter clinical trials.

The boceprevir phase I clinical trial compared boceprevir with PEG-IFN α-2b as monotherapy or combined therapy in a nonresponder population who previously did not achieve an EVR with PEG-IFN α-2b, with or without RBV. It revealed that combination therapy yielded much greater reductions in viral load than either drug given as monotherapy. Patients treated with PEG-IFN α-2b and 400 mg of boceprevir thrice-daily (TID) had a maximum mean change in HCV RNA of (–2.68±1.12) $\log_{10}$ IU/ml. As comparison, single-week monotherapy with 400 mg TID of boceprevir resulted in viral load reductions by (–1.61±0.21) $\log_{10}$ IU/ml. Two-week monotherapy with PEG-IFN α-2b yielded RNA reductions of –1.08 to –1.26 $\log_{10}$ IU/ml. No new adverse events were observed in the combined therapy trial. Additionally, there was no difference in safety parameters in subjects treated with 400 mg of boceprevir as compared with PEG-IFN α-2b. Pharmacokinetic phase I data did not reveal significant interaction between these two drugs (Sarrazin *et al*., 2007). These preliminary findings facilitated a phase II study which showed that RBV is required for optimal response in combination with NS3/4A protease inhibitors such as boceprevir. When added to the SOC for treatment of chronic HCV-1, the optimal dose of boceprevir, 800 mg TID (Schiff *et al*., 2008), significantly increased SVR in both 28-week and 48-week regimens, compared with the control of PEG-IFN α-2b and RBV. The use of low-dose RBV in combination with PEG-IFN α-2b and boceprevir, while reducing haematological toxic effects, did not improve SVR rates compared with those of the control group. Also, the combination was associated with a high rate of viral breakthrough and a rate of relapse similar to that of the control group. Boceprevir-based groups had higher rates of anaemia and dysgeusia compared to the control group (Kwo *et al*., 2010).

Phase III trials for boceprevir began in 2008. Results available to date have demonstrated the compound to be well tolerated, with adverse events that were within the range of current SOC therapy. The HCV RESPOND-2 and SPRINT-2 studies each evaluated two treatment strategies with boceprevir in order to assess the ability to improve SVR and potentially shorten overall treatment duration compared to standard therapy. In the first-line boceprevir study, SPRINT-2, involving subjects who are new to treatment, the 48-week boceprevir arm had an SVR of 66% vs. 38% for the control arm, and 63% for the boceprevir response-guided therapy arm. In the second-line boceprevir study, RESPOND-2, involving subjects who have previously been unsuccessfully treated, the 48-week boceprevir arm had an SVR of 66% vs. 21% for the control arm, and 59% for the boceprevir response-guided therapy arm. Clinical studies of boceprevir are now complete, except for that described in "A Phase IIb, Safety and Efficacy Study of Boceprevir in Patients Coinfected with HIV and Hepatitis C (P05411 AM3)" (http://clinicaltrials.gov/ct2/show/NCT00959699).

4.3 Narlaprevir (SCH 900518)

Narlaprevir (SCH 900518), a next-generation once-daily (QD) HCV protease inhibitor, is a novel ketoamide protease inhibitor, which forms a reversible covalent bond with the active-site serine. It has an overall inhibition constant (K_i) of 7 nmol/L and a dissociation half-life of 1 to 2 h. Narlaprevir inhibited replicon RNA at a 90% effective concentration (EC_{90}) of (40±10) nmol/L (~28 ng/ml), and a 50% effective concentration (EC_{50}) of (20±6) nmol/L. In biochemical assays, narlaprevir was active against proteases of HCV-1 to HCV-3. A two-week treatment with five times EC_{90} of the inhibitor reduced the replicon RNA level by 3 $\log_{10}$ IU/ml. Cross-resistance studies demonstrated that the majority of mutations for resistance to boceprevir and telaprevir caused similar fold losses of activity against all three inhibitors; however, narlaprevir retained more activity against these mutants due to its higher intrinsic potency. Combination treatment with IFN-α enhanced the inhibition of replicon RNA and suppressed the emergence of resistant replicon colonies. The replicon data also suggest that combination therapy with IFN-α may enhance HCV RNA reduction and also

suppress the selection of resistant HCV mutations in a clinical setting (Tong *et al*., 2010). The EC_{50} data indicate that narlaprevir is approximately 10-fold more potent in vitro than other protease inhibitors currently in phase III trials (telaprevir and boceprevir) (Lin *et al*., 2004; Malcolm *et al*., 2006).

Narlaprevir, originally developed as an HIV protease inhibitor, is a strong inhibitor of the cytochrome P450 3A (CYP3A) enzyme. In the phase IIa study, named NEXT-1, narlaprevir was tested to boost with ritonavir. Potent antiviral activity of narlaprevir was observed in combination with PEG-IFN α-2b and RBV among treatment-naive subjects infected with HCV-1 chronic hepatitis C. Across the doses tested, 53% to 87% of narlaprevir recipients achieved undetectable HCV RNA by Week 4. Nalraprevir/ritonavir demonstrated no unique or treatment-limiting adverse effects. Adverse events included gastrointestinal symptoms, lethargy, elevated liver enzymes, loss of appetite, and psychiatric symptoms. Adverse events generally occurred with similar frequency, although anemia and dizziness were more common in the narlaprevir arms.

4.4 BI 201335

BI 201335, developed by Boehringer Ingelheim, is a non-covalent, reversible, and highly optimized competitive inhibitor targeting the full-length viral NS3/4A proteases of HCV-1a and HCV-1b (Lemke *et al*., 2010). K_i values are 2.6 and 2.0 nmol/L, respectively, and have a several thousand-fold selectivity relative to host cell proteases. K_i values of 2 to 250 nmol/L of BI 201335 were measured against the NS3/4A proteases of HCV-2 to HCV-6. The EC_{50} values of BI 201335 are 6.5 and 3.0 nmol/L in HCV-1a and HCV-1b replicon assays, with an in vitro selectivity index of 4600 for HCV-1a. Its in vitro absorption, distribution, metabolism, and excretion (ADME) properties and animal pharmacokinetics are consistent with its excellent human pharmacokinetics profile (White *et al*., 2010b). Furthermore, drug levels were significantly higher in rat liver than in plasma, suggesting that distribution to the target organ may be especially favorable (White *et al*., 2010a). When resistance to BI 201335 was examined in vitro, phenotypic characterization of the mutants revealed shifts in sensitivity to inhibition by BI 201335. The HCV-1a R155K and HCV-1b D168V variants reduced sensitivity to BI 201335 by 400- and 1000-fold, respectively. BI 201335 maintained activity against NS3/4A variants, such as V36M, that confer resistance to other HCV protease inhibitors (Kukolj *et al*., 2010). BI 201335 was studied with chronic HCV-1 infection for 14 d as monotherapy in treating naive subjects, followed by combination with PEG-IFN/RBV for an additional 14 d. It has also been tested in treatment-experienced subjects for 28 d as a combination therapy with PEG-IFN/RBV. All dose groups achieved median viral load reductions of ≥ 3 $\log_{10}$ IU/ml. HCV NS3 variants that confer resistance to BI 201335 were selected during treatment. These variants do not alter the sensitivity to PEG-IFN/RBV, as the majority of treatment-naive subjects with resistant virus subsequently displayed anti-viral responses during the combination therapy (Kukolj *et al*., 2009). BI 201335 has currently advanced to clinical test and development through phase IIb trials.

4.5 TMC435 (TMC435350)

TMC435 (TMC435350), under development by Tibotec Pharmaceuticals Ltd. in collaboration with Medivir, is an oral, macrocyclic inhibitor of the HCV NS3/4A serine protease administered QD. TMC435 is currently in phase IIb development. Biochemical analysis using NS3/4A proteases of HCV-1a and HCV-1b showed K_i values to be 0.5 and 0.4 nmol/L, respectively. This compound inhibited HCV replication in an HCV-1b cellular assay with an EC_{50} of 8 nmol/L and a selectivity index of 5 875. The compound was synergistic with IFN-α and an NS5B inhibitor in the replicon model and additive with RBV. In rats, TMC435 was extensively distributed to the liver and intestinal tract, and the absolute bioavailability was 44% after a single oral administration. Compound concentration detected in both plasma and liver at 8 h post-dosing was above the EC_{99} value measured in the replicon (Lin *et al*., 2009).

TMC435350-C101 is a phase I, double-blind, randomized, placebo-controlled trial of TMC435 in both treatment-naive and treatment-experienced subjects infected with HCV-1. In this trial, there were no serious adverse effects, no grade 3 reactions, and no treatment-related discontinuations. Pharmacokinetics supported a QD dosing regimen (200 mg QD). Plasma HCV RNA levels dropped rapidly in all subjects, with a median maximal reduction of

3.9 $\log_{10}$ IU/ml and a median of 6 d to maximal reduction. The initial steep reduction of HCV RNA (median 3.5 $\log_{10}$ IU/ml at Day 3) was followed by a more gradual decline that was maintained over the dosing period. No viral breakthrough (>1 $\log_{10}$ IU/ml HCV RNA increase from nadir) was observed during treatment nor in the 3 d post-treatment; HCV RNA returned to pretreatment levels by Week 4 after treatment cessation (Reesink *et al*., 2010). Viral breakthrough was infrequent in a TMC435 phase IIa study named OPERA-1 (Manns *et al*., 2009). The global phase IIb study ASPIRE (TMC435-C205) in HCV-1 treatment-naive subjects has completed enrollment of 400 subjects from study sites in North America, Europe, and Australia/New Zealand; the clinical trial is ongoing.

4.6 Vaniprevir (MK-7009)

Vaniprevir (MK-7009) is a new class of HCV NS3/4A protease inhibitors that contain a P2 to P4 macrocyclic constraint. Vaniprevir is an inhibitor of HCV-1a and HCV-1b proteases at subnanomolar concentrations, with modestly shifted potency against HCV-2a and HCV-2b proteases at low nanomolar concentrations. The pharmacokinetic properties of vaniprevir were evaluated in multiple species and exhibited good liver exposure (McCauley *et al*., 2010). Dosed vaniprevir was administered to chimpanzees to evaluate plasma and liver pharmacokinetics at a 10 mg/kg oral dose. Excellent plasma and liver exposures were observed, with a liver concentration at 12 h 1500-fold greater than the serum-shifted replicon IC_{50} (McCauley *et al*., 2010). MK-7009 also had excellent selectivity against both a range of human proteases and a broad panel of pharmacologically relevant ion channels, receptors, and enzymes (Liverton *et al*., 2010). The evaluation of safety and efficacy of vaniprevir is still under research in Merck.

4.7 Danoprevir (ITMN-191/R7227)

Danoprevir (ITMN-191/R7227), under development by Roche, is a highly potent and selective inhibitor of NS3/4A HCV serine protease for the oral treatment of HCV infection. Preclinical data demonstrated that danoprevir binds with high affinity and dissociates slowly from the HCV NS3 protease, allowing high liver drug exposure with only modest plasma drug exposure. A phase Ib, "IFN-free" clinical trial demonstrated that danoprevir, combined with the HCV polymerase inhibitor R7128, was effective in reducing HCV RNA levels in a large proportion of treatment-naive subjects with HCV infection and in approximately half of previously non-responsive subjects with HCV-1 infection, without resistance or safety concerns (Gane *et al*., 2010e). In a phase IIb trial in treatment-naive subjects with HCV-1 infection, danoprevir plus PEG-IFN α-2a and RBV resulted in undetectable levels of HCV RNA in the majority of subjects, showing no evidence of viral resistance. Ritonavir, developed as an HIV protease inhibitor, is a strong inhibitor of the CYP3A enzyme and can improve the pharmacokinetic profile of danoprevir (Deutsch and Papatheodoridis, 2010). Danoprevir/ritonavir was generally well tolerated and demonstrated favorable antiviral activity in combination with PEG-IFN and RBV (Gane *et al*., 2010d). Therefore, at the time of publication, a phase IIb trial to evaluate ritonavir-boosted, low-dose danoprevir in combination with R7128 was planned. Another study showed that HCV protease inhibitor may restore insulin sensitivity in subjects with HCV-1 (Moucari *et al*., 2010). Study results indicated that insulin resistance (IR) may increase the progression of liver fibrosis and induce histological lesions of non-alcoholic steatohepatitis in subjects with chronic hepatitis C. Moreover, IR has shown to be a major predictor of non-response to treatment in subjects with chronic hepatitis C who received PEG-IFN and RBV.

4.8 BMS-650032

BMS-650032 is a potent and selective inhibitor that demonstrated antiviral activity in both single and multiple ascending dose studies in HCV-1a and HCV-1b (McPhee, 2010). Comprehensive in vitro antiviral activity profiles in enzyme- and cell-based replicon assays showed good pharmacokinetic characteristics and strong tolerance. BMS-650032 administered in the range of 10–600 mg BID for 14 d was safe and well tolerated in healthy subjects. Based on calculated PK parameters, BMS-650032 had high oral clearance and preferential hepatic distribution (Levin *et al*., 2010). After a single 600 mg dose of BMS-650032, actively treated subjects experienced a mean decline in HCV RNA by ~2.5 $\log_{10}$ IU/ml at 24 h (McPhee *et al*., 2009). The phase II study

naming a single ascending dose of BMS-650032 in HCV-infected subjects is now complete, and four other phase II studies, including combination therapy with current standard care, are still ongoing.

4.9 ACH-1625

ACH-1625, currently under development by Achillion, is an inhibitor with high potency against the HCV NS3 protease. The anti-HCV potency of this compound was confirmed by targeting viral replicons containing the NS3 protease domain from HCV-1a and HCV-1b clinical isolates. In addition, ACH-1625 showed no anti-viral activity against HIV-1, herpes simplex virus 1 (HSV-1), respiratory syncytial virus (RSV), influenza and bovine virus diarrhoea virus (BVDV); and minimal cytotoxicity in a panel of cell lines and primary cells (50% cytotoxicity concentration (CC_{50})>32 mmol/L). ACH-1625 was specifically targeting the NS3 protease and showed no activity against other cellular proteases (50% inhibition concentration (IC_{50})>10 mmol/L). A combination of ACH-1625 with IFN/RBV and other experimental STAT-C agents yielded additive to synergistic effects. Finally, different mutations emerged from HCV-1a and HCV-1b replicons under ACH-1625 selection. The role of these mutations is currently underway. ACH-1625 shows high hepatoselective distribution in the rat and the dog (Stauber *et al*., 2010).

In clinical trials, ACH-1625 was very well tolerated after administration of single and multiple ascending doses up to 2000 mg in healthy volunteers. Currently, ACH-1625 is being evaluated in chronically infected HCV subjects. The placebo-controlled phase IIa study is aimed at evaluating the safety, tolerability and antiviral activity as a candidate for HCV combination therapy. The clinical data demonstrated reductions in viral RNA between 3–4.25 $\log_{10}$ IU/ml. These mathematical data quantitatively show the percentage of total virus cleared after 5 d of ACH-1625 monotherapy. Specifically, ACH-1625 is being evaluated in conjunction with PEG-IFN α-2a and RBV after 4 and 12 weeks of dosing in subjects infected with chronic HCV-1.

4.10 GS-9256

GS-9256 is a novel HCV NS3 protease inhibitor (in vitro EC_{50} of ~20 nmol/L in HCV-1b replicon assays). GS-9256 was well tolerated, with significant antiviral activity, even at the lowest dose studied. Median HCV RNA declined sharply through Day 2 in a dose-dependent manner. Median changes from baseline in HCV RNA at Day 4 were −1.1, −2.7, −2.6, and −2.9 $\log_{10}$ IU/ml for the 25 mg (capsule), 75 mg (capsule), 75 mg (solution), and 200 mg (solution) BID regimens, respectively. Phase II evaluation of GS-9256 in combination with GS-9190 with or without RBV is currently underway (Lawitz *et al.*, 2010).

Thus far, major shortcomings associated with HCV protease inhibitors are rapidly developing viral resistance, frequent dosing (telaprevir and boceprevir must be TID taken), and anemia/rash (telaprevir). Since the protease inhibitors are designed for combined use with PEG-IFN and RBV, their toxicities is a problem because of their adding to the known toxicities of PEG-IFN and RBV. Phase II studies have made it clear that RBV will be needed to maximize SVR, minimize viral breakthrough and resistance, and avoid relapse (de Bruijne *et al.*, 2009).

5 Polymerase inhibitors

Current HCV therapy has serious adverse effects and leaves a significant proportion of patients infected with HCV-1 with unsatisfactory results. The most promising antiviral target is viral proteins or processes that are not endogenous to host cells. The HCV RNA-dependent RNA polymerase (RdRp) is encoded by the NS5B region and is strictly required for replication of the single-stranded, positive-sense RNA genome. Close structural homologs of this enzyme do not exist within the uninfected host cell. In addition to its active site, at least three known allosteric binding pockets that regulate RNA synthesis are suitable for inhibitor design. Therefore, this well characterized replicative enzyme represents an excellent target for anti-HCV drug development.

The HCV NS5B polymerase contains the classic palm, fingers, and thumb structural subdomains of a polymerase. The function of the palm subdomain is to form the catalytic center of the nucleotidyl transfer reaction. The fingers subdomain interacts with the incoming nucleoside triphosphate, as well as the template base to which it is paired, and the thumb subdomain plays a role in positioning the RNA for

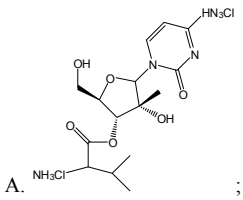
initiation and elongation. The interaction of these subdomains is believed to enhance coordinated movement and assist in modulating initiation, elongation, and termination of RNA synthesis by encouraging high processivity of viral replication (Walker and Hong, 2002).

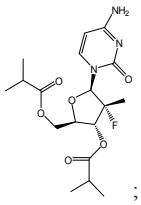
Polymerase inhibitors include nucleoside inhibitors (NIs) and non-nucleoside inhibitors (NNIs) (Table 2). NIs can act as alternative substrates for the viral polymerase, and NNIs act as allosteric inhibitors of the polymerase. In vitro studies showed that the nucleoside type polymerase inhibitors have a higher

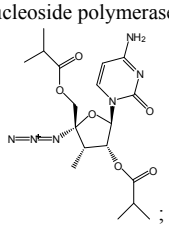
Table 2 Ongoing clinical trials of anti-HCV polymerase inhibitors

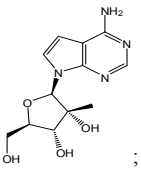
Company	Compound	Class	Clinical phase	Structure[#]
Idenix/Novartis	NM283/NM107[*] (valopicitabine)	NI	II (withdrawn)	A
Roche	R7128	NI	II	B
Roche	R1626/R1479[*] (balapiravir)	NI	II (withdrawn)	C
Merck	MK-0608	NI	unknown	D
Idenix	IDX184	NI	II	E
Anadys	ANA598	NNI	II	Undisclosed
Boehringer Ingelheim	BI 207127	NNI	II	Undisclosed
Bristol-Myers Squibb	BMS 791325	NNI	II	Undisclosed
Vertex	VCH-759	NNI	II	F
Pfizer	PF-00868554 (filibuvir FBV)	NNI	II	G
Gilead	GS-9190	NNI	II	Undisclosed
Pharmasset	PSI-7977	NNI	II	H
Pharmasset	PSI-7851	NNI	II	Diastereoisomer of PSI-7977
ViroPharma/Wyeth	HCV-796	NNI	II (withdrawn)	I
Abbott	ABT-333	NNI	II	Undisclosed
Abbott	ABT-072	NNI	II	Undisclosed
Vertex	VCH-916	NNI	II	J
Boehringer Ingelheim	BILB-1941	NNI	II (withdrawn)	Undisclosed
Pharmasset	PSI-938	NNI	I	Undisclosed
GlaxoSmithKline	GSK625433	NNI	I	K
Merck	MK-3281	NNI	I	L
Inhibitex	INX-189	NNI	I	M
Idenix	IDX375	NNI	I	Undisclosed

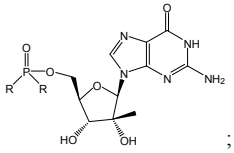
NI: nucleoside polymerase inhibitor; NNI: non-nucleoside polymerase inhibitor. [*] Active moiety. [#] Structure:

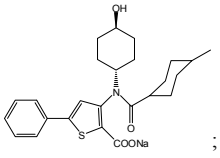

A.

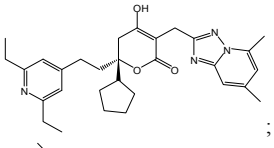
; B.


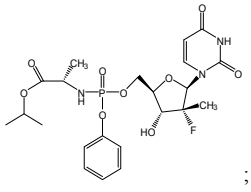
; C.


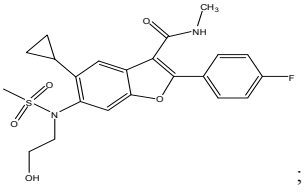
; D.


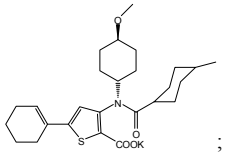
; E.


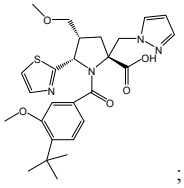
;

F.

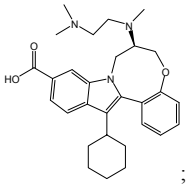
; G.


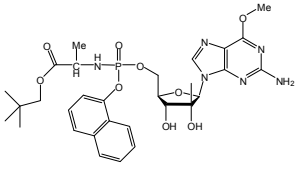
; H.


; I.


;

J.

; K.


; L.


; M.


barrier to viral drug resistance than the non-nucleoside type (McCown *et al*., 2008). Therefore, the combination of a non-nucleoside or protease inhibitor with a nucleoside polymerase inhibitor could have a clear clinical benefit through the delay of resistance emergence. Early clinical data for several nucleosides targeted to HCV RNA polymerase inhibitors indicated marked antiviral effects and a likelihood of relatively slow HCV resistance (Brown, 2009). Optimally effective anti-HCV therapies are likely to be based on multi-class treatment regimens combining polymerase inhibitors and protease inhibitors together with PEG-IFN and RBV or pharmaceutical agents from other mechanistic classes. RBV antagonizes the in vitro anti-HCV activity of the pyrimidine nucleoside analogue 2′-*C*-methylcytidine (2′-*C*-MeCyt), the active component of valopicitabine (formerly NM283). Valopicitabine was the first polymerase inhibitor to enter clinical trials, but this compound is not being further pursued because of the overall risk/benefit profile (Liu-Young and Kozal, 2008). Although valopicitabine demonstrated good anti-HCV activity in combination with PEG-IFN plus RBV in a phase II trial, the FDA still ruled that the drug's risk of gastrointestinal adverse effects appeared to outweigh its benefits. Valopicitabine produced a high rate of gastrointestinal adverse effects such as nausea, vomiting, and diarrhea, which are more common at high dosing levels (e.g., 800 mg/d). For this reason, a low dose of 200 mg was recommended. However, although the lower dose improved the drug's adverse events profile, it was still associated with considerable gastrointestinal toxicity.

5.1 Nucleoside polymerase inhibitors

5.1.1 R7128

R7128 (2′-α-fluoro-2′-β-methyl-3′,5′-diiso-butyryl-deoxycytidine) is the prodrug of PSI-6130 (2′-deoxy-2′-fluoro-2′-*C*-methylcytidine). It is an oral cytidine nucleoside analog with potent and selective inhibition of the HCV NS5B polymerase. It is being developed by Pharmasset and Roche for the treatment of chronic HCV infections and currently in phase IIb trials. PSI-6130 inhibits the replication of HCV through formation of its 5′-triphosphate form, which functions as an alternative substrate for the viral polymerase, competitively inhibiting viral RNA synthesis by preventing further extension after incorporation (Karakama *et al*., 2010). PSI-6130 presents a high barrier to resistance selection in vitro, selects for variants exhibiting only low level resistance, and lacks cross-resistance with R1479 (a nucleoside analog) (Ali *et al*., 2008). Phase I studies completed in 2008 investigated the pharmacokinetics, pharmacodynamics, safety, tolerability, and food effects of R7128 in healthy volunteers and in subjects chronically infected with HCV-1, -2, or -3. Results indicated that all doses of R7128 studied (500 to 9000 mg) were generally safe and well tolerated. No gastrointestinal or other serious adverse events were observed during the study, and no hematologic or other laboratory safety abnormalities of clinical significance were noted. No maximum tolerated dose was identified (Otto *et al*., 2007).

In a multiple ascending dose study conducted in 40 subjects chronically infected with HCV-1 who had previously failed IFN therapy, R7128 demonstrated a potent, dose-dependent antiviral activity. A maximum 4.2 $\log_{10}$ IU/ml (99.9% reduction) HCV RNA decrease from baseline was demonstrated in a subject cohort following 14 d of monotherapy with 1500 mg BID (Reddy *et al.*, 2007).

A four-week study of R7128 was conducted in combination with the current SOC for chronic HCV infection, PEG-IFN plus RBV, in 81 treatment-naive subjects chronically infected with HCV-1. Twenty-five subjects chronically infected with HCV-2 or -3 who did not achieve an SVR with previous IFN-based therapy were also included in the study. Following four weeks of treatment with 500, 1500, or 1000 mg R7128 BID with PEG-IFN plus RBV, or placebo with PEG-IFN plus RBV, subjects achieved a mean 3.8, 5.1, 5.1, and 2.9 $\log_{10}$ IU/ml decrease in HCV RNA, respectively. There were 30% (6 of 20), 85% (17 of 20), 88% (22 of 25), and 18.75% (3 of 16) subjects achieved HCV RNA below the limit of detection (<15 IU/ml), or RVR, respectively. Aside from headache and fatigue in some subjects, no serious adverse events were reported during the treatment period of triple therapy. Thus, R7128 was shown to be generally safe and well tolerated when administered for four weeks in combination with PEG-IFN plus RBV in subjects infected with HCV-1 (Le Pogam *et al.*, 2010). Subjects chronically infected with HCV-2 or -3 who did not achieve an SVR with standard therapy or non-responders achieved a mean 5.0 $\log_{10}$ IU/ml decrease in HCV RNA, and 95% (19 of 20) subjects

achieved RVR. Subjects receiving a placebo with PEG-IFN plus RBV achieved a mean 3.7 $\log_{10}$ IU/ml decrease in HCV RNA, and 60% (3 of 5) achieved RVR (Gane *et al*., 2010e).

In another study, a cohort of 88 subjects chronically infected with HCV-1 received up to 13 d oral combination treatment with R7128 (500 or 1000 mg BID) and danoprevir (100 or 200 mg TID, or 600 or 900 mg BID), or placebo. The median reduction in HCV RNA concentration from baseline to Day 14 ranged from −3.7 to −5.2 $\log_{10}$ IU/ml, as compared with an increase of 0.1 $\log_{10}$ IU/ml in the placebo group. The combination of R7128 and danoprevir was well tolerated with no treatment-related severe adverse events. Pharmacokinetic analysis confirmed that there were no drug-drug interactions between the compounds (Gane *et al*., 2010c). Another combination therapy study including a 13-d oral administration of R7128/danoprevir followed by PEG-IFN plus RBV revealed that all 55 subjects achieved profound reductions in HCV RNA, with no evidence of treatment emergent resistance (Gane *et al*., 2010b).

Ongoing phase IIb trials are aimed at evaluating the dose and duration of R7128 treatment in combination with PEG-IFN plus RBV for the cohort of treatment-naive, HCV-1 or HCV-4 infected subjects. In May 2010, Roche announced that all 408 patients enrolled in the study had completed their scheduled 8 or 12 weeks of the triple combination portion of the assigned treatment. The results indicated that treatment with R7128 administered with SOC was safe and well tolerated, and the on-treatment efficacy data demonstrated that >80% of subjects had undetectable HCV RNA in all cohorts receiving the 12-week triple regimen, compared with <50% for the placebo/SOC cohort. There were no viral rebounds or resistance-related breakthroughs during the time of triple combination therapy. A longer duration trial is currently being undertaken to evaluate the safety and efficacy of R7128 in combination with SOC.

5.1.2 R1626 (balapiravir)

R1626 (balapiravir), the prodrug of the nucleoside analog R1479 (4′-azidocytidine), was developed to increase oral bioavailability and improve antiviral effect. R1479, identified as a selective inhibitor of HCV replication with high antiviral potency across HCV-1a and HCV-1b, showed a time- and dose-dependent reduction of HCV RNA levels and a high barrier to resistance selection. R1626 achieved a more than five-fold increase in oral bioavailability and dose proportionality up to high dose levels (Klumpp *et al*., 2007).

A phase Ib study was designed to evaluate the safety, pharmacokinetics, antiviral activity, and maximum tolerated dose of R1626 in subjects with chronic hepatitis C. Forty-seven treatment-naive subjects infected with HCV-1 were treated with R1626 orally at doses of 500, 1 500, 3 000, 4 500 mg, or placebo BID for 14 d, with 14 d of follow-up. Safety, tolerability, pharmacokinetics, and antiviral activity were assessed. The pharmacokinetics of R1626 was linear over the dose range evaluated. Dose- and time-dependent reductions in HCV RNA were observed. Mean decreases in viral load after 14 d of treatment with doses of 500, 1 500, 3 000, or 4 500 mg were 0.32, 1.20, 2.60, and 3.70 $\log_{10}$ IU/ml, respectively. No resistance to R1626 was observed after 14 d of treatment (Roberts *et al*., 2008). However, there was an increased frequency of adverse events at the highest dose (4 500 mg). The study then evaluated the efficacy and safety of R1626 administered for four weeks in combination with PEG-IFN-γ and RBV in HCV-1-infected treatment-naive subjects. Subjects were randomized to take 1 500 or 3 000 mg BID of R1626 along with PEG-IFN-γ, or 1 500 mg BID of R1626 along with PEG-IFN-γ and RBV. Subjects receiving the SOC treatment of PEG-IFN-γ with RBV were included as control. Following the four-week treatment, HCV RNA was undetectable (<15 IU/ml) in 29%, 69%, and 74% of subjects in the 1 500 BID, 3 000 BID, and 1 500 TID arms, respectively, compared with 5% of subjects receiving SOC. Respective mean reductions in HCV RNA from baseline to Week 4 were 3.6, 4.5, 5.2, and 2.4 $\log_{10}$ IU/ml. A synergistic effect was observed between the three drugs, and there was no evidence of viral resistance development. The incidence of grade 4 neutropenia was 48%, 78%, 39%, and 10% in 1 500 BID, 3 000 BID, 1 500 TID, and SOC, respectively, and was the main reason for dose reductions (Pockros *et al*., 2008b). The highest rates of relapse were observed in the combination arm of 1 500 mg BID R1626 with weekly PEG-IFN-γ (1 500 BID arm; 55%, 6 of 11) and in the 1 500 TID arm (28%, 7 of 25). In five of the seven subjects in the 1 500 TID arm who experienced

relapse, HCV RNA breakthrough occurred during the early stages of treatment. These results further confirm the importance of achieving early and continued suppression of HCV RNA to undetectable levels in order to achieve sustained response (Pockros *et al*., 2008a). Because of fatal drug-induced lymphopenia, the development of R1626 was stopped at the end of 2008.

5.1.3 MK-0608

MK-0608 (2′-*C*-methyl-7-deaza-adenosine), developed by Merck, possesses the most potent antiviral activity (EC_{50} of 0.25 mmol/L). An in vivo test of MK-0608 revealed dose- and time-dependent decreases in plasma viral loads in HCV-infected chimpanzees. In addition, chimpanzees dosed intravenously for 7 d with MK-0608 at 0.2 and 2.0 mg/kg body weight (BW) per day resulted in average reductions in viral load of 1.0 $\log_{10}$ IU/ml and >5 $\log_{10}$ IU/ml, respectively. Two other HCV-infected chimpanzees received daily doses of 1 mg MK-0608 per kg via oral administration. After 37 d of oral dosing, one chimpanzee with a high starting viral load experienced a reduction in viral load of 4.6 $\log_{10}$ IU/ml, and the viral load in the other chimpanzee fell below the limit of quantification (LOQ) of the HCV TaqMan assay (20 IU/ml). Importantly, viral load remained below the LOQ throughout the duration of dosing and for at least 12 d after dosing ended. These results demonstrate a robust antiviral effect on the administration of MK-0608 to HCV-infected chimpanzees (Carroll *et al*., 2009).

5.1.4 IDX184

IDX184 is a prodrug of 2′-methylguanosine monophosphate. Unlike the first generation HCV nucleoside inhibitors, IDX184 is a "liver-target" prodrug, which theoretically will provide increased anti-HCV efficacy and safety. Preliminary studies in monkeys have shown approximately 95% hepatic extraction of orally administered IDX184 with low systemic IDX184 and nucleoside metabolite levels (Cretton-Scott *et al*., 2008). In chimpanzees infected with HCV-1, oral administration of 10 mg/kg BW QD produced a mean viral load reduction of 2.3 $\log_{10}$ IU/ml after 4 d of dosing. IDX184 appeared to be safe and well tolerated in healthy subjects at single doses up to 100 mg (Zhou *et al*., 2009). In treatment-naive HCV-1-infected subjects receiving 25, 50, 75, or 100 mg of IDX184 QD for 3 d and monitored for 14 additional days, −0.47 to −0.74 $\log_{10}$ IU/ml HCV RNA reductions were observed with no serious side effects reported (Lalezari *et al*., 2009). In addition, combining this analogue with an NNI or protease inhibitor was effective in suppressing the emergence of viral resistance (Lallos *et al*., 2009). Studies are now underway to evaluate the inhibitor efficacy when administered in combination with PEG-IFN/RBV in HCV-infected subjects.

5.2 Non-nucleoside polymerase inhibitors

5.2.1 ANA598

ANA598 is a novel, orally available, and highly potent "palm site" non-nucleoside selective inhibitor of HCV-1a and HCV-1b NS5B polymerase (IC_{50}<1 nmol/L) and of HCV replication in cell culture. EC_{50} values for genotypes 1a and 1b HCV replicons are 52 and 3 nmol/L, respectively. ANA598 retains full activity against mutations conferring resistance to protease, nucleoside and complementary non-nucleoside inhibitors (Kirkovsky *et al*., 2007). ANA598 was combined pairwise in vitro with IFN-α, the HCV NS3/4 protease inhibitor telaprevir, the NS5B nucleoside polymerase inhibitor PSI-6130, or the TLR7 agonist ANA773 (currently in clinical development by Anadys). Combinations were evaluated in both wild type (WT) and mutant replicons containing the M414T mutation that confers resistance to palm site non-nucleoside NS5B inhibitors. No cytotoxicity was detected for any of the combinations tested (Thompson *et al*., 2009; 2010a; 2010b; Patel *et al*., 2010). ANA598 was shown to be synergistic and/or additive with IFN-α (WT and M414T mutant replicons), telaprevir, PSI-6130, and ANA773. RBV was not antagonistic with ANA598. No change in susceptibility was observed when ANA598 was tested with replicons containing the primary mutations known to confer resistance to other direct antivirals including A156T (NS3 protease active site), S282T (NS5B active site) and M423T (NS5B non-nucleoside thumb binding site). Genotypic mutations resistant to ANA598 (M414T, M414L, G554D, D559G, and M414T/G554D) were identified in vitro. Reduced potency was observed for ANA598 against all mutations with EC_{50} values of 0.47, 0.30, 0.63,

0.085, and 20 mmol/L, determined for M414T, M414L, G554D, D559G, and M414T/G554D, respectively. Replicons containing mutations that confer resistance to ANA598 remained fully susceptible to IFN-α, telaprevir (NS3 protease inhibitor), and PSI-6130 (nucleoside polymerase inhibitor) (Thompson *et al*., 2008). For each combination evaluated, antiviral interactions were determined to be additive to synergistic. Such combinations may produce a greater viral load reduction and potentially delay the emergence of drug resistance in vivo.

In a phase I study of safety, tolerability, and pharmacokinetics in healthy volunteers, ANA598 showed to be well tolerated at all doses administered. There were no serious adverse events and no withdrawals from the study. ANA598 systemic exposure generally rose with increasing doses. The favorable pharmacokinetic and tolerability profiles of ANA598 support QD or BID administration (Rahimy *et al*., 2008). A phase I study in treating naive HCV-1 subjects demonstrated a rapid and sustained reduction in HCV RNA at all three dose levels tested. The median viral load reduction was 2.4 $\log_{10}$ IU/ml at 200 mg BID, 2.3 $\log_{10}$ IU/ml at 400 mg BID, and 2.9 $\log_{10}$ IU/ml at 800 mg BID on Day 4. In phase IIb trials, ANA598 was well tolerated in HCV subjects for the three tested doses for 3 d, and led to rapid reduction in HCV RNA (median end of treatment (EOT) range 2.3–2.9 $\log_{10}$ IU/ml). This study also evaluated the safety and antiviral activity of ANA598 combined with PEG-IFN and RBV. Twelve-week results for the first cohort (200 mg BID) reported no serious adverse events except for those associated with SOC. The combination of ANA598 with SOC was well tolerated; ANA598 accelerated the rate of achieving undetectable virus in HCV-1 subjects (Lawitz *et al*., 2010a).

5.2.2 BI 207127

BI 207127, developed by Boehringer Ingelheim Pharmaceuticals, is an orally bioavailable, reversible, potent and specific thumb pocket 1 non-nucleoside inhibitor of the HCV RNA-dependent RNA polymerase in vitro. Cell-based HCV sub-genomic replicon EC_{50} values are 23 and 11 nmol/L for HCV-1a and HCV-1b, respectively (Larrey *et al*., 2009b). In a double blind, sequential group comparison of HCV-1 subjects with minimal to mild liver fibrosis, treatments of 100–1200 mg BI 207127 TID were administered over 5 d. All subjects were then tested for plasma HCV RNA virus load (VL) using the Roche COBAS TaqMan assay in the following 10–14 d. As compared to mean 6.4 $\log_{10}$ IU/ml, VL decreased by ≥1 $\log_{10}$ IU/ml in 2/9, 6/9, 8/9, 8/9 and 10/10 subjects treated with 100, 200, 400, 800, and 1200 mg, respectively. No response was seen in subjects treated with placebo, and no breakthrough was observed during treatment. Median VL declines on Day 5 were 0.4, 0.8, 1.3, 3.8, and 3.2 $\log_{10}$ IU/ml, respectively. BI 207127 showed reliable antiviral activity against HCV-1 that correlated with BI 207127 plasma exposure, and reached a maximal effect at 800 mg TID dose (Larrey *et al*., 2009a). Phase II trials incorporating PEG-IFN and RBV combinations in chronic HCV subject are ongoing.

5.2.3 VCH-759

VCH-759, a substituted thiophene-2-carboxylic acid derivative, is a novel, oral, non-nucleoside inhibitor of HCV-1a and HCV-1b NS5B polymerase. This compound inhibits NS5B (IC_{50} HCV-1a 0.41 μmol/L and IC_{50} HCV-1b 0.38 μmol/L) by binding to an allosteric site in the 'thumb' domain situated ~35Å from the active site. VCH-759 is active against the HCV sub-genomic replicon in Huh-7 cells (IC_{50}=0.3 μmol/L for both HCV-1a and HCV-1b). The compound is also selective for the HCV NS5B polymerase relative to human DNA polymerases a, b, and c (IC_{50}>100 μmol/L). VCH-759 shows a good in vitro therapeutic index (TI; CC_{50}/IC_{50}) of >600 and a non-clinical safety profile (Cooper *et al*., 2009b).

A multiple ascending dose study was designed to assess the effect of VCH-759 on viral kinetics, viral resistance, pharmacokinetics, safety, and tolerability after a 10-d monotherapy plus a 14-d follow-up test. Three cohorts of treatment-naive subjects chronically infected with HCV-1 received 400 mg TID, 800 mg TID, or 800 mg BID. The results showed that VCH-759 was rapidly absorbed with peak plasma levels at Day 1 of (1857±773), (3675±2213), and (4627±1688) ng/ml, respectively. The mean maximal decrease in HCV RNA $\log_{10}$ was 1.9, 2.3, and 2.5 for the 400 mg TID, 800 mg BID, and 800 mg TID doses, respectively (Cooper *et al*., 2007). VCH-759 was well tolerated, with the most frequent adverse events appearing to be gastrointestinal disorders. A 28-d

non-clinical toxicology study has been completed and VCH-759 phase IIb clinical trials are underway. HCV variants with mutations conferring resistance to VCH-759 were selected during the course of a 10-d treatment. Results showed that VCH-759 should be used in a combination therapy to maintain viral suppression and prevent emergence of resistance (Nicolas *et al*., 2008).

5.2.4 PF-00868554 (filibuvir)

PF-00868554 (filibuvir), a non-nucleoside inhibitor of the HCV RNA polymerase, exerts its inhibitory effect by binding to the thumb base domain of the protein. It is also a potent and selective inhibitor, with a mean IC_{50} of 0.019 μmol/L against HCV-1 polymerases and a mean EC_{50} of 0.075 μmol/L against the HCV-1b-Con1 replicon. To determine the in vitro antiviral activity of filibuvir against various HCV strains, a panel of chimeric replicons was generated, in which polymerase sequences derived from HCV-1a and HCV-1b clinical isolates were cloned into the HCV-1b-Con1 subgenomic reporter replicon. Results indicated that filibuvir has potent in vitro antiviral activity against a majority (95.8%) of HCV-1a and HCV-1b replicons, with an overall mean EC_{50} of 0.059 μmol/L. Filibuvir showed no cytotoxic effects in several human cell lines up to the highest concentration evaluated (320 μmol/L). Furthermore, the antiviral activity of filibuvir was retained in the presence of human serum proteins. An in vitro resistance study of filibuvir identified M423T as the predominant resistance mutation, resulting in a 761-fold reduction in susceptibility to filibuvir, but no change in susceptibility to IFN-α and a polymerase inhibitor that binds to a different region. Filibuvir also showed good pharmacokinetic properties in preclinical animal species, revealing promising oral bioavailability in both rodent and nonrodent species (Shi *et al*., 2009). Also, filibuvir seemed not to affect any of the major CYP isoforms (IC_{50}>30 μmol/L for 1A2, 2C8, 2D6, 3A4, 2C9, and 2C19) (Li *et al*., 2009).

In monotherapy studies, filibuvir exhibited to be safe and well tolerated with mean reductions in plasma HCV RNA of up to −2.33 $\log_{10}$ IU/ml (Troke *et al*., 2009). In a combination therapy study, the safety and efficacy of filibuvir in combination with PEG-IFN α-2a and RBV was evaluated. The mean reduction ($\log_{10}$ IU/ml) in HCV RNA at Day 4 for placebo (n=8), 200 mg (n=10), 300 mg (n=9), and 500 mg (n=8) BID was −0.58, −2.29, −2.72, and −2.83, respectively, and −2.10, −4.46, −4.67, and −3.62 at Day 28, respectively. The percent of subjects achieving RVR (undetectable HCV RNA by Week 4) for placebo, 200, 300, and 500 mg BID was 0%, 60%, 75%, and 63%, respectively. Of those subjects treated with 200, 300, and 500 mg BID who achieved RVR, 33%, 33%, and 80% achieved undetectable HCV RNA following a two-week treatment, respectively (Jacobson *et al*., 2009). Up to 75% of filibuvir-treated subjects achieved RVR, and the majority remained undetectable on PEG-IFN/RBV therapy, with 63%–88% and 60%–70% of all filibuvir-treated subjects undetectable at Weeks 12 and 48, respectively. However, 20%–50% of those filibuvir-treated subjects undetectable at Week 48 relapsed by Week 60, compared to 0% of placebo-treated subjects. As a result, response rates at Week 60 were similar for the filibuvir- and placebo-treated groups. Filibuvir in combination with PEG-IFN and RBV for four weeks were well tolerated and resulted in higher on-treatment virologic response rates relative to PEG-IFN/RBV alone. However, longer durations of triple therapy will be required to assess the potential for reduced rates of relapse and improvement in SVR rates. A study evaluating 24 weeks of filibuvir/PEG-IFN/RBV is currently underway (Jacobson *et al*., 2010). Another phase IIb study aimed at determining dose selection of filibuvir for combination therapy suggested that filibuvir doses of 300 and 600 mg BID are expected to be efficacious when combined with SOC (Neelakantan *et al*., 2010). Mutations at position 423 caused the predominant amino acid change in subjects receiving filibuvir/PEG-IFN/RBV who did not achieve an RVR, supporting earlier observations that selection of mutations at 423 is the preferred pathway of filibuvir resistance (Mori *et al*., 2010).

5.2.5 GS-9190

GS-9190 is a novel imidazopyridine analogue, non-nucleoside inhibitor of the HCV RNA polymerase. The GS-9190 binding pocket involves a β-hairpin and is in close proximity to the catalytic active site of NS5B, and GS-9190 shows higher inhibitory effect on HCV-1 replicons than on HCV-2 (Shih *et al*., 2007). The EC_{50} values of HCV-1a and HCV-1b replicon replication are 2.5 and 0.6 nmol/L,

respectively. The CC_{50} of GS-9190 in various cell lines is >50 μmol/L, illustrating a high degree of selectivity (SI>20000). GS-9190 has lower potency against an HCV-2a replicon and the JFH1 (HCV virus stain) infectious virus (EC_{50} ~1 μmol/L), and is inactive against a number of related and unrelated viruses. Replicons resistant to various HCV protease and polymerase inhibitors remained fully susceptible to GS-9190. The combination of GS-9190 with either IFN-α or several HCV protease and polymerase inhibitors resulted in an additive antiviral activity (Vliegen *et al*., 2007). Oral bioavailability was greater than 30% in all preclinical species and was not limited by hepatic first pass extraction (Yang *et al*., 2007). Median HCV RNA reduction at 24 h ranged from ~0.7–1.2 $\log_{10}$ IU/ml across all cohorts. Single doses of GS-9190 were well tolerated, without serious or treatment-limiting adverse events reported (Bavisotto *et al*., 2007). Preliminary data from a study with 23 participants who received multiple ascending doses over 8 d suggested that GS-9190 may be associated with Q-T interval prolongation of electrocardiogram. Gilead Sciences, the company developing the drug, has initiated a specific Q-T interval study to look at the potential cardiac risk of GS-9190 among healthy volunteers without hepatitis C. After expert consultation and a separate dose-ranging study in healthy volunteers, Gilead felt that the Q-T interval prolongation at a lower dose of the drug was "clinically manageable" (Susan, 2010). Gilead has begun recruitment for a phase II study of GS-9190 in combination with PEG-IFN and RBV for treatment duration of 24 or 48 weeks (Burton and Everson, 2009). Phase II clinical trials showed that GS-9190 might be associated with Q-T interval elongation.

5.2.6 VCH-222

VCH-222 is a selective inhibitor of the NS5B polymerase of subgenomic HCV, including HCV-1a and HCV-1b, at low-micromolar IC_{50}. VCH-222 was found to be selective against human DNA polymerases a, b, and g (IC_{50}≥56 μmol/L). In the replicon assay, VCH-222 was active against HCV-1a and HCV-1b with EC_{50} values of (23.3±9.0) and (12.0±4.0) nmol/L, respectively; activity against HCV-2a was (4.6±1.4) μmol/L. The compound had an average CC_{50} value of 45 μmol/L when tested on exponentially growing replicon cells with a TI of about 4000 (Bedard *et al*., 2009). VCH-222 has wonderful pharmacokinetic parameters in rats and dogs; it displayed low total body clearance with excellent oral bioavailability (greater than 30%). The exposure of VCH-222 in rat liver was 5-fold higher than in plasma. VCH-222 has a good oral bioavailability and ADME properties in terms of permeability, metabolic behaviors, and distribution in hepatic tissue/cell. VCH-222 is neither a CYP inhibitor/inducer nor a P-glycoprotein inhibitor, reducing the likelihood of drug-drug interactions (Chauret *et al*., 2009). Safety and pharmacokenetics studies in healthy subjects were carried out using 250, 500, 1000, and 1500 mg VCH-222 as treatment. An open cohort of six subjects with HCV-1 infection received 750 mg BID for 3 d. VCH-222 was well tolerated at all doses tested. All adverse events observed were classified as mild to moderate, not serious, without apparent dose relationship. The half-life of VCH-222 was approximately 4 h, and the plasma levels observed at 24 h were above the IC_{90} replicon values in all cohorts. In the subjects treated for 3 d with 750 mg BID, there was a two-fold increase in the maximum observed concentration (c_{max}) and the area under the curve (AUC) observed between Day 1 and Day 3. Preliminary efficacy results on the first four treatment-naive subjects dosed for 3 d revealed a mean reduction of 3.2 $\log_{10}$ IU/ml within 24 h of dosing and of 3.7 $\log_{10}$ IU/ml on Day 4 (Cooper *et al*., 2009a).

5.2.7 NS5A inhibitors

HCV NS5A is a 447 amino-acid, zinc-binding phosphoprotein that plays a critically important but enigmatic role in the virus life cycle (Schmitz and Tan, 2008). Although little is currently known regarding the exact enzymatic activity, the NS5A protein is involved in viral replication and is essential for the production of infectious virus particles (Tellinghuisen and Foss, 2008).

BMS-790052 is the first representative of a group of highly selective HCV NS5A inhibitors with potent activity against all HCV genotypes tested in vitro. The EC_{50} of BMS-790052 ranges from 9 to 50 pmol/L for the HCV replicon genotypes 1a and 1b, and reach up to 146 pmol/L for HCV-3a, making it the most potent inhibitor of HCV replication publicly identified to date. BMS-790052 is also a potent inhibitor of the Japanese fulminant hepatitis-1 infectious

virus (EC_{50}=28 pmol/L). Replicon combination studies also demonstrated additive-synergistic antiviral activity of BMS-790052 with IFN and RBV, as well as inhibitors of the NS3 protease and NS5B polymerase.

In the phase I trial, a randomized, double-blind, placebo-controlled, single ascending dose study, BMS-790052 was administered at six dose levels to healthy, non-HCV-infected subjects over a range of 1–200 mg as an oral solution. The compound was safe and well tolerated, showing no clinically relevant adverse effects when amounts of up to 200 mg were administered. In the phase II trial, a randomized, double-blind, placebo-controlled, single ascending dose study, BMS-790052 was administered to subjects with chronic HCV-1 at oral doses of 1, 10, and 100 mg. BMS-790052 was safe and well tolerated in HCV-infected subjects up to 100 mg. There were no serious adverse events, except for headaches, which were often reported. After single oral doses of 10–100 mg BMS-790052, all subjects had 24 h plasma concentrations above the 10-fold protein binding-adjusted EC_{90} for HCV-1a and HCV-1b, suggesting the possibility of QD administration. A single milligram dose of BMS-790052 produced a mean reduction of 1.8 $\log_{10}$ IU/ml (0.2–3.0 $\log_{10}$ IU/ml) in HCV viral load when measured 24 h after drug administration. Both the 10 and 100 mg doses produced a greater antiviral effect, with mean plasma viral RNA falling by 3.2 and 3.3 $\log_{10}$ IU/ml, respectively, at 24 h post-drug administration. Moreover, the 100 mg dose resulted in a mean maximal HCV RNA decline of 3.6 $\log_{10}$ IU/ml, and a prolonged antiviral response was observed in two subjects infected with HCV-1b (Gao *et al.*, 2010). In a double-blind phase IIb study, 48 treatment-naive HCV-1-infected subjects were randomized to receive placebo, 3, 10, or 60 mg of BMS-790052, once-daily in combination with PEG-IFN α-2a and RBV for 48 weeks. Subject baseline and demographic characteristics were well balanced across treatment arms (n=12 per arm), with mean baseline HCV RNA being 6.5 $\log_{10}$ IU/ml. The proportion of subjects achieving RVR was 42%, 83%, and 75% in the 3, 10, and 60 mg BMS-790052+SOC arms, respectively, compared to 8% for SOC. Confirmed viral breakthrough was not observed in the 10 and 60 mg BMS-790052 arms through Week 12 (Pol *et al*., 2010). BMS-790052 is currently being evaluated in further phase II clinical trials. Because it targets NS5A (different than other anti-HCV potent compounds), identifying BMS-790052 resistance is necessary. As single mutations, Q30E and Y93N in HCV-1a conferred the highest levels of resistance. For HCV-1b, BMS-790052 retained subnanomolar potency against all variants with single amino acid substitutions. Importantly, BMS-790052 resistant variants remained fully sensitive to IFN-α and small-molecule inhibitors of HCV protease and polymerase (Fridell *et al*., 2010).

AZD7295 is a selective inhibitor of HCV NS5A. Preclinical studies demonstrated in vitro antiviral activity of 7 nmol/L and 1.24 μmol/L against HCV-1b and HCV-1a replicons, respectively, with a significant liver concentration. The study assessed the safety, tolerability, pharmacokinetics, and antiviral activity of AZD7295 in HCV-infected subjects. AZD7295 oral solution or placebo was administered for 5 d to treatment-naive and treatment-experienced subjects without cirrhosis. Groups 1 and 2 (n=10, HCV-1a/1b, stratified according to subtype) received 90 mg TID and 233 mg TID, respectively. Group 3 (n=6, HCV-3) received 90 mg TID, and Group 4 (n=5, HCV-1b) received 350 mg BID. HCV RNA declined in a dose-dependent manner in HCV-1b subjects with mean declines from baseline of −1.2 to −2.1 $\log_{10}$ IU/ml by Day 6. HCV RNA levels did not change in HCV-1a or HCV-3 subjects. There were no treatment-related adverse events other than headache and GI effects reported in groups receiving higher doses, which contained higher volumes of recipients. AZD7295 shows a potent antiviral activity in HCV-1b subjects, but no detectable effect on HCV-1a and HCV-3 subjects, which is consistent with plasma levels below in vitro IC_{50} values. A phase II study using a new formulation of AZD7295 in combination with PEG-IFN/RBV is currently being tested in HCV-1b subjects (Gane *et al*., 2010a).

6 Cyclophilin binding molecules

Cyclophilins are high-affinity protein ligands of cyclosporine A in human cells. HCV relies on cyclophilin A as an essential cofactor for cell infection and viral replication. In humans, 19 cyclophilin isoforms with different cellular and tissue distributions have been described (Galat, 2004). Gaither *et al*. (2010) demonstrated that HCV replication relies on

several cellular pathways linked to cyclophilins A, H, 40, and E. Cyclosporine A, the cyclophilin inhibitor, was demonstrated to inhibit HCV replication directly, both in vitro and in vivo (Watashi *et al*., 2003). The finding that cyclosporine A's anti-HCV effect was independent of its immunosuppressive function was promising. In a clinical trial, cyclosporine A was shown to have anti-HCV activity in combination with interferon. Combination of IFN and cyclosporine A was clearly more effective in achieving SVR than IFN monotherapy, especially in subjects with HCV-1, high viral load, or both (Inoue *et al*., 2003). Chatterji *et al*. (2010) demonstrated that full-length NS5A and cyclophilins A form a stable complex. Remarkably, the interaction of cyclophilin A and NS5A is conserved among genotypes, and cyclosporine A is able to prevent the interaction of cyclophilin A and NS5A in a dose-dependent manner (Chatterji *et al*., 2010). More recently, several cyclosporine A derivatives (Table 3) that are devoid of immunosuppressive function have been demonstrated in both preclinical and clinical studies to be efficacious in inhibiting HCV replication (Fischer *et al*., 2010).

6.1 Debio 025 (alisporivir)

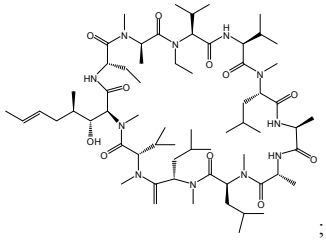
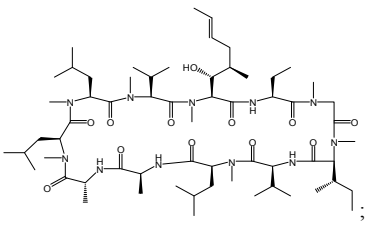
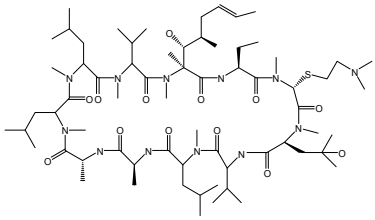
Debio 025 (alisporivir), a non-immunosuppressive cyclosporine A derivative that selectively inhibits cyclophilin, is being developed by Debiopharm S.A. for the potential oral treatment of HCV infection. Debio 025 is at least 10-fold more potent as an anti-HCV agent than cyclosporine (Paeshuyse *et al*., 2006). In virus-infected chimeric mice, Debio 025 was better tolerated than cyclosporine, and the anti-HCV effect of Debio 025 appeared to be synergistic when used in combination with PEG-IFN (Inoue *et al*., 2007). Combining Debio 025 with IFN-α/RBV or STAT-C inhibitors (protease or (non)-nucleoside polymerase HCV inhibitors) resulted in an additive to slightly synergistic antiviral activity in a 3-d test. Debio 025 has the unique ability to rapidly clear hepatoma cells from their HCV replicon when used alone or in combination with IFN-α and STAT-C inhibitors. Moreover, Debio 025 was able to delay the development of escape variants against several STAT-C inhibitors in colony formation assays. Debio 025 proved to be potent against HCV replicons that are resistant to various STAT-C inhibitors for WT HCV. Subgenomic replicons that are resistant to Debio 025 were selected (three independent selections). Mutations were identified in domain II of the NS5A gene. Reintroduction of these mutations in a WT background partially recovered resistance. Debio 025 resistant replicons remained fully susceptible to interferon and several STAT-C inhibitors (Coelmont *et al*., 2009a; 2009b).

During a 15-d phase Ib study in which subjects coinfected with HIV and HCV received 1200 mg BID of Debio 025 or placebo, and Debio 025 resulted in a mean decrease in the viral load of 3.6 $\log_{10}$ IU/ml (Flisiak *et al*., 2008b). In contrast, a phase I trial of Debio 025 suggested that the drug has a limited effect on HIV-1 replication. A phase II clinical trial demonstrated that treatment with ascending doses of Debio 025 (200, 600, and 1000 mg) alone or combined with PEG-IFN α-2a 180 μg/week reduced viral load in subjects with chronic HCV infection. In subjects with HCV-1/HCV-4 (n=12 per arm), the HCV RNA reduction at Day 29 was −4.75 $\log_{10}$ IU/ml in the

Table 3 Ongoing clinical trials of anti-HCV cyclophilin inhibitors

Company	Compound	Class	Clinical phase	Structure*
Debiopharm S.A.	Debio 025 (alisporivir)	CPI	II	A
Novartis	NIM811	CPI	I	B
Scynexis	SCY-635	CPI	I	C

CPI: cyclophilin inhibitor. * Structure:

A.  ; B.  ; C. 

PEG-IFN/Debio 025 1000 mg arm, as compared to −2.49 $\log_{10}$ IU/ml in the PEG-IFN/placebo and −2.20 $\log_{10}$ IU/ml in the Debio 025 1000 mg monotherapy arms. In the groups receiving 200 or 600 mg of Debio 025 in combination with PEG-IFN, HCV RNA reductions were −1.8 and −4.61 $\log_{10}$ IU/ml, respectively. Debio 025 at doses of 600 and 1000 mg daily for 29 d shows a significantly additive anti-HCV effect when co-administered with PEG-IFN α-2a in treatment-naive HCV subjects (Flisiak *et al*., 2008a). Viral kinetics revealed a significant dose-dependent additive antiviral effect of Debio 025, which enhanced overall mean and maximum treatment efficiencies. Viral kinetics does not indicate upcoming resistance against Debio 025 (Herrmann *et al*., 2009). In a double-blind, placebo-controlled study, different dosing regimens of Debio 025 (200, 600, or 1000 mg/d) in monotherapy or with PEG-IFN (180 μg/week) were tested in subjects infected with HCV-1 or HCV-3. HCV RNA clearance was assessed at the end of a 24-week SVR treatment, and/or at later time points. HCV RNA was undetectable after 29 d of treatment in the four subjects (<15 IU/ml). No other anti-HCV treatment was initiated, and all four subjects maintained undetectable HCV RNA until 24 weeks after treatment cessation. SVR was maintained beyond 24 weeks, and HCV RNA remained undetectable for at least one year post-treatment in three subjects (Horban *et al*., 2010). An additional study was carried out in 29 subjects who received Debio 025 plus weekly PEG-IFN α-2a for 29 d, followed by a 21-d drug-free period, at which point they began treatment with a standard regimen of PEG-IFN α-2a plus RBV. The initial effect of a 29-d administration with Debio 025 on HCV RNA levels was maintained by follow-up treatment with SOC. This resulted in a particularly high SVR rate of 67% in subjects infected with HCV-1 or HCV-4. There were no adverse events that could be traced back to the initial treatment with Debio 025 (Flisiak *et al*., 2010). An in vitro study demonstrated that there is a high genetic barrier to resistance for Debio 025. Mutation D320E in NS5A was the only mutation consistently selected in the replicon genome (Coelmont *et al*., 2010).

6.2 NIM811

NIM811 ((melle-4)-cyclosporine) is a cyclosporine derivative developed by Novartis that has higher CYP-binding affinity than cyclosporine. NIM811 is structurally very similar to cyclosporine, with an isobutyl group replaced by a sec-butyl group. However, this small modification essentially blocks the recognition site of CYPA/cyclosporine by calcineurin and thus abolishes the immunosuppressive function associated with cyclosporine. NIM811 induced a concentration-dependent reduction of HCV RNA in replicon cells with IC_{50} of 0.66 μmol/L at 48 h. Furthermore, a greater than 3 $\log_{10}$ IU/ml viral RNA reduction was achieved after treating the cells with as little as 1 μmol/L of NIM811 for 9 d (Ma *et al.*, 2006).

In another study comparing the antiviral activity of cyclosporin A with NIM811 in vitro, treatment with cyclosporin A and NIM811 for 7 d reduced HCV RNA levels by 2–3 $\log_{10}$ IU/ml, and treatment for 3 weeks reduced HCV RNA to an undetectable level. NIM811 exerted higher anti-HCV activity than cyclosporin at lower concentrations (Goto *et al*., 2006). In addition, the combination of NIM811 with IFN-α significantly enhanced anti-HCV activities without causing any increase in cytotoxicity (Ma *et al*., 2006). Antiviral effects of combining NIM811 with other viral-specific inhibitors (including NS3/4A protease and NS5B polymerase) were investigated in vitro using an HCV replicon. All combinations tested led to more pronounced antiviral effects than any single agent alone, with no significant increase in cytotoxicity. Moreover, the combination of NIM811 with an NI (NM107) or an NNI (thiophene-2-carboxylic acid) was synergistic, while the combination with a protease inhibitor (BILN 2061) was additive. Resistant clones were selected in vitro with these inhibitors; interestingly, it was much more difficult to develop resistance against NIM811 than viral specific inhibitors. No cross-resistance was observed. Most notably, NIM811 was highly effective in blocking the emergence of resistance when used in combination with viral protease or polymerase inhibitors. When the combination activities of NIM811 and NM107 were evaluated in vitro, there was a concentration-dependent inhibition of the HCV replicon with NTM811 and NM107, either alone or in combination. The combination of NTM811 and NM107 resulted in synergistic antiviral effect without any significant increase in cytotoxicity, and also increased the barrier to resistance for both agents (Boerner *et al*., 2007). Single oral doses of NIM811 were tested among

healthy volunteers who were enrolled in five sequential dose groups (50, 150, 400, 800, or 1600 mg) and placebo; multiple oral doses of NIM811 (25–600 mg QD or BID) were administered to HCV-1 subjects for 14 d. PK and safety assessments were conducted throughout the study period. NIM811 was well tolerated both in healthy and HCV-infected subjects following single and multiple oral administrations of up to 1600 mg. Blood exposure to NIM811 is dose-dependent. Terminal half-life of NIM811 was around 12−20 h. NIM811 accumulated slightly upon reaching steady state (accumulation factor ~1.2). Food could affect the absorbance of NIM 811 (Ke *et al*., 2009). Phase II trials then investigated the activity of NIM811 combination therapy. Twenty of 21 subjects with relapse after PEG-IFN and RBV received one dose of treatment in a double-blind, placebo controlled trial. Subjects were randomized 1:1 to receive oral 600 mg BID of NIM811 or matching placebo for 14 d in combination with 180 mg PEG-IFN α-2a on study Days 0 and 7. The mean drop in HCV RNA was 2.78 $\log_{10}$ IU/ml in subjects who received the combination of NIM811+PEG-IFN, compared to a 0.58 $\log_{10}$ IU/ml drop in the PEG-IFN arm. No serious adverse events occurred. NIM811, when combined with PEG-IFN α, shows a marked antiviral activity (Lawitz *et al*., 2009).

6.3 SCY-635

SCY-635 is a novel, non-immunosuppressive, cyclosporine-based analog that exhibits potent suppression on HCV replication. SCY-635 inhibits the peptidyl prolyl isomerase activity of cyclophilin A at nanomolar concentrations. SCY-635 is orally bioavailable in multiple species and distributes extensively to hepatocytes. Metabolic studies have indicated that SCY-635 does not induce the major CYP450 enzymes 1A2, 2B6, and 3A4. SCY-635 was shown to produce blood and liver concentrations of the parent drug that exceeded the EC_{50} determined in the bicistronic con1b-derived replicon assay. SCY-635 exhibited synergistic antiviral activity with IFN α-2b and additive antiviral effect with RBV (Houck and Hopkins, 2006; Hopkins *et al*., 2010). A series of two-drug combination studies performed in vitro showed that SCY-635 was able to combine with non-nucleoside polymerase inhibitors, nucleoside polymerase inhibitors, protease inhibitors, RBV, and IFN α-2b, and all produced greater than expected antiviral activity. No combinations exhibited greater than expected cytotoxicity; in fact, less than expected cell cytotoxicity was observed when SCY-635 was combined with boceprevir and telaprevir (Hopkins *et al*., 2009a). During a phase Ib clinical study, HCV-1 subjects were enrolled into one of three ascending cohorts (total daily doses: 300, 600, 900 mg) for 15 d. There were no serious adverse events or no discontinuations, indicating a dose-limiting toxicity. Greater than proportional increases in plasma exposure to SCY-635 were observed and steady state was achieved on Day 3. In the 900-mg treatment cohort, mean trough plasma concentrations remained above the replicon-derived EC_{90} value from Days 3 through 15 with consistent decreases in plasma RNA. Maximum responses were observed on Days 11 and 15. Group mean and median nadir values were 2.20 and 1.82 $\log_{10}$ IU/ml below baseline. One subject achieved undetectable RNA levels at Day 15 (Hopkins *et al*., 2009b).

7 Other small molecular anti-HCV compounds

Other compounds, such as entry inhibitors (ITX4520, ITX5061, Pro 206, JTK-652, Cyanovirin-N), P7 inhibitors (BIT225), NS4A binding inhibitors (ACH-1095, GS9525), and cholesterol- and sphingolipid-lowering compounds (statins), have been designed to target other steps of the HCV replication cycle. All are currently being investigated in clinical trials.

8 Combination therapy

Because HCV has a high replication rate without proof-reading mechanism, the emergence of viral resistance is a notable obstacle for STAT-C. The FDA has restricted the use of monotherapy to 3 d in early studies (Opar, 2010); thus, the combination of molecules with different modes of action will be the focus for future development of anti-HCV therapy. Combination therapy will enable increased antiviral activity while delaying the development of viral resistance.

The INFORM-1 trial is the first attempt to study the combination of a nucleoside polymerase inhibitor (R7128) and a protease inhibitor (danoprevir) in

HCV-infected subjects (Delang *et al*., 2010). Roche has also announced that it will not conduct the previously planned a 28-d INFORM-2 study, designed to evaluate the combination of R7128 with R7227 with and without PEG-IFN and RBV. A small study using ritonavir (100 mg BID) could boost danoprevir (200 mg BID), achieved 100% undetectable HCV RNA after 15 d and was generally well tolerated (Deutsch and Papatheodoridis, 2010). Based on these findings, Roche has decided to conduct longer duration studies, including the INFORM-3 clinical study of R7128 and R7227 with and without PEG-IFN and RBV.

Boehringer Ingelheim reported results from a phase Ib study, named Sound-C1, which showed that the combination of two oral HCV compounds, the protease inhibitor BI 201335 and the polymerase inhibitor BI 207127, in combination with RBV, reduced viral load to the lower limit of quantifiable level in HCV treatment-naive subjects. The regimen did not include interferon through the first 28 d of treatment. This data was presented at the American Association for the Study of Liver Diseases (AASLD) 2010 Liver Meeting in Boston, MA. In this randomized, open-label trial, 32 treatment-naive HCV-1 subjects received a combination of BI 207127 in either 400 mg or 600 mg TID, 120 mg QD of BI 201335, and RBV (in two doses 1000 and 1200 mg daily) for 28 d. All subjects showed a rapid and sharp decline in HCV viral load during the first two days, followed by a slower second phase decline. In the lower and higher dose groups, 73% and 100% of subjects achieved a rapid virological response, respectively.

In another study, a combination of BMS-650032 (protease inhibitor) and BMS-790052 was administered to HCV-1 null-responders to a prior course of therapy. The interim results at 12 weeks (total study duration 24 weeks) produced early antiviral activity, but 6 out of the 11 subjects treated exhibited viral breakthrough, indicating that PEG-IFN and/or RBV will be needed to completely suppress the virus (at least in this study). When the combination was accompanied with PEG-IFN and RBV, 9 out of 10 subjects became HCV RNA undetectable by Week 12 (Lok *et al*., 2010).

A study with many doses and arms showed optimal anti-HCV effect when GS-9256 (HCV protease inhibitor) and tegobuvir (GS-9190, a polymerase inhibitor) were combined with PEG-IFN and RBV. Of the 14 subjects that received quadruple therapy for four weeks, followed by 44 weeks of PEG-IFN plus RBV, all subjects were HCV RNA negative by Day 28. An ongoing four-month study of the quadruple therapy is underway. A phase IIa study has shown that its investigational compounds, GS-9190 and GS-9256, used in conjunction with current SOC therapies, produced substantial suppression of HCV within 28 d of treatment (Susan, 2010).

9 Summary

One of the biggest challenges in HCV treatment is that about half of patients are infected with HCV-1, which is typically considered the most difficult genotype for treatment. In addition, the arduous adverse effects of present SOC have led many patients to curtail their treatment. Furthermore, because the development of HCV resistance is relatively rapid, it is important and necessary to test and develop many new target drugs to enhance current anti-HCV therapy. Fortunately, important progress in the development of potent and selective inhibitors of HCV replication has been made in the last decade. Some newly developed drugs such as telaprevir, which is competing to be the first protease inhibitor, are expected to enter the market in the near future. We are now entering a phase in which there are a large number of antiviral drugs available, which will enable us to choose selective inhibitors to block specific replication steps (or targets) of the HCV life cycle and different HCV genotypes may also be targeted. Ultimately, previous dependence on interferon and RBV, and drugs that have serious adverse events, could potentially be abolished.

References

Ali, S., Leveque, V., Le Pogam, S., Ma, H., Philipp, F., Inocencio, N., Smith, M., Alker, A., Kang, H., Najera, I., *et al*., 2008. Selected replicon variants with low-level in vitro resistance to the hepatitis C virus NS5B polymerase inhibitor PSI-6130 lack cross-resistance with R1479. *Antimicrob. Agents Chemother.*, **52**(12):4356-4369. [doi:10.1128/AAC.00444-08]

Bavisotto, L., Wang, C.C., Jacobson, I.M., Marcellin, P., Zeuzem, S., Lawitz, E.J., Lunde, M., Sereni, P., O′Brien, C., Oldach, D.W., *et al*., 2007. 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*, **46**(4):255A.

Bedard, J., Nicolas, O., Bilimoria, D., L′Heureux, L., Fex, P., David, M., Chan, L., 2009. Identification and characterization of VCH-222, a novel potent and selective non-nucleoside HCV polymerase inhibitor. *J. Hepatol*., **50**(S1):S340. [doi:10.1016/S0168-8278(09)60937-5]

Boerner, J., Ma, S., Compton, T., Lin, K., 2007. NIM811, a cyclophilin inhibitor, and NM107, an HCV polymerase inhibitor, synergistically inhibits HCV replication and suppresses the emergence of resistance in vitro. *Antivir. Res*., **74**(3):A57. [doi:10.1016/j.antiviral.2007.01.081]

Brown, N.A., 2009. Progress towards improving antiviral therapy for hepatitis C with hepatitis C virus polymerase inhibitors. Part I: Nucleoside analogues. *Expert Opin. Inv. Drugs*, **18**(6):709-725. [doi:10.1517/13543780902854194]

Burton, J.R., Everson, G.T., 2009. HCV NS5B polymerase inhibitors. *Clin. Liver Dis*., **13**(3):453-465. [doi:10.1016/j.cld.2009.05.001]

Cai, Z., Zhang, C., Chang, K.S., Jiang, J., Ahn, B.C., Wakita, T., Liang, T.J., Luo, G., 2005. Robust production of infectious hepatitis C virus (HCV) from stably HCV cDNA-transfected human hepatoma cells. *J. Virol.*, **79**(22):13963-13973. [doi:10.1128/JVI.79.22.13963-13973.2005]

Carroll, S.S., Ludmerer, S., Handt, L., Koeplinger, K., Zhang, N.R., Graham, D., Davies, M.E., MacCoss, M., Hazuda, D., Olsen, D.B., 2009. Robust antiviral efficacy upon administration of a nucleoside analog to hepatitis C virus-infected chimpanzees. *Antimicrob. Agents Chemother*., **53**(3):926-934. [doi:10.1128/AAC.01032-08]

Chatterji, U., Lim, P., Bobardt, M.D., Wieland, S., Cordek, D.G., Vuagniaux, G., Chisari, F., Cameron, C.E., Targett-Adams, P., Parkinson, T., *et al*., 2010. HCV resistance to cyclosporin A does not correlate with a resistance of the NS5A-cyclophilin A interaction to cyclophilin inhibitors. *J. Hepatol.*, **53**(1):50-56. [doi:10.1016/j.jhep.2010.01.041]

Chauret, N., Chagnon-Labelle, C., Diallo, M., Laquerre, J., Laterreur, J., May, S., Ste-Marie, L., 2009. Preclinical pharmacokinetic and ADME characterization of VCH-222, a novel non-nucleoside HCV NS5B polymerase inhibitor. *J. Hepatol*., **50**(S1):S341-S342. [doi:10.1016/S0168-8278(09)60941-7]

Chen, K.X., Njoroge, F.G., 2009. A review of HCV protease inhibitors. *Curr. Opin. Investig. Drugs*, **10**(8):821-837.

Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., Houghton, M., 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, **244**(4902):359-362. [doi:10.1126/science.2523562]

Coelmont, L., Gallay, P., Bobardt, M., Kaptein, S., Paeshuyse, J., Vliegen, I., Vuagniaux, G., Neyts, J., 2009a. In vitro anti-hepatitis C virus (HCV) activities and resistance profile of Debio 025, a non-immunosuppressive cyclophilin binding molecule. *Antivir. Res*., **82**(2):A20. [doi:10.1016/j.antiviral.2009.02.024]

Coelmont, L., Gallay, P., Bobardt, M., Kaptein, S., Paeshuyse, J., Vliegen, I., Vuagniaux, G., Neyts, J., 2009b. Particular in vitro anti-HCV activities and resistance profile of the cyclophilin inhibitor Debio 025. *J. Hepatol*., **50**(S1):S36. [doi:10.1016/S0168-8278(09)60090-8]

Coelmont, L., Hanoulle, X., Chatterji, U., Berger, C., Snoeck, J., Bobardt, M., Lim, P., Vliegen, I., Paeshuyse, J., Vuagniaux, G., *et al*., 2010. DEB025 (alisporivir) inhibits hepatitis C virus replication by preventing a cyclophilin A induced *cis-trans* isomerisation in domain II of NS5A. *PLoS ONE*, **5**(10):e13687. [doi:10.1371/journal.pone.0013687]

Cooper, C., Lawitz, E.J., Ghali, P., Rodriguez-Torres, M., Anderson, F.H., Lee, S.S., Proulx, L., 2007. Antiviral activity of the non-nucleoside polymerase inhibitor, VCH-759, in chronic hepatitis C patients: Results from a randomized, double-blind, placebo-controlled, ascending multiple dose study. *Hepatology*, **46**(4):864A.

Cooper, C., Larouche, R., Bourgault, B., Chauret, N., Proulx, L., 2009a. Safety, tolerability and pharmacokinetics of the HCV polymerase inhibitor VCH-222 following single dose administration in healthy volunteers and antiviral activity in HCV-infected individuals. *J. Hepatol*., **50**(S1):S342. [doi:10.1016/S0168-8278(09)60942-9]

Cooper, C., Lawitz, E.J., Ghali, P., Rodriguez-Torres, M., Anderson, F.H., Lee, S.S., Bedard, J., Chauret, N., Thibert, R., Boivin, I., *et al*., 2009b. Evaluation of VCH-759 monotherapy in hepatitis C infection. *J. Hepatol*., **51**(1):39-46. [doi:10.1016/j.jhep.2009.03.015]

Cretton-Scott, E., Perigaud, C., Peyrottes, S., Licklider, L., Camire, M., Larsson, M., La Colla, M., Hildebrand, E., Lallos, L., Bilello, J., *et al*., 2008. In vitro antiviral activity and pharmacology of IDX184, a novel and potent inhibitor of HCV replication. *J. Hepatol*., **48**(S2):S220. [doi:10.1016/S0168-8278(08)60590-5]

de Bruijne, J., Weegink, C.J., Jansen, P.L.M., Reesink, H.W., 2009. New developments in the antiviral treatment of hepatitis C. *Vox Sanguinis*, **97**(1):1-12. [doi:10.1111/j.1423-0410.2009.01187.x]

Delang, L., Coelmont, L., Neyts, J., 2010. Antiviral therapy for hepatitis C virus: beyond the standard of care. *Viruses*, **2**(4):826-866. [doi:10.3390/v2040826]

Deutsch, M., Papatheodoridis, G.V., 2010. Danoprevir, a small-molecule NS3/4A protease inhibitor for the potential oral treatment of HCV infection. *Curr. Opin. Investig. Drugs*, **11**(8):951-963.

Feld, J.J., Hoofnagle, J.H., 2005. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature*, **436**(7053):967-972. [doi:10.1038/nature04082]

Fischer, G., Gallay, P., Hopkins, S., 2010. Cyclophilin inhibitors for the treatment of HCV infection. *Curr. Opin. Investig. Drugs*, **11**(8):911-918.

Flisiak, R., Feinman, S.V., Jablkowski, M., Horban, A., Kryczka, W., Halota, W., Heathcote, J.E., Mazzella, G., Vandelli, C., Liz, J.S., *et al*., 2008a. Efficacy and safety of increasing doses of the cyclophilin inhibitor Debio 025 in combination with pegylated interferon α-2a in treatment naive chronic HCV patients. *J. Hepatol.*, **48**(S2):62. [doi:10.1016/S0168-8278(08)60145-2]

Flisiak, R., Horban, A., Gallay, P., Bobardt, M., Selvarajah, S.,

Wiercinska-Drapalo, A., Siwak, E., Cielniak, I., Higersberger, J., Kierkus, J., *et al*., 2008b. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. *Hepatology*, **47**(3):817-826. [doi:10.1002/hep.22131]

Flisiak, R., Woynarowski, M., Jablkowski, M., Halota, W., Kryczka, W., Horban, A., Czauz-Andrzejuk, A., Bialkowska, J., Pawlowska, M., Zarebska-Michaluk, D., *et al*., 2010. Efficacy of standard of care therapy following experimental Debio 025 treatment in patients with chronic hepatitis C. *J. Hepatol.*, **52**(S1):S291. [doi:10.1016/S0168-8278(10)60750-7]

Foy, E., Li, K., Sumpter, R.Jr., Loo, Y.M., Johnson, C.L., Wang, C., Fish, P.M., Yoneyama, M., Fujita, T., Lemon, S.M., *et al*., 2005. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. *PNAS*, **102**(8):2986-2991. [doi:10.1073/pnas.0408707102]

Franco, S., Parera, M., Aparicio, E., Clotet, B., Martinez, M.A., 2007. Genetic and catalytic efficiency structure of an HCV protease quasispecies. *Hepatology*, **45**(4):899-910. [doi:10.1002/hep.21623]

Franco, S., Clotet, B., Martinez, M.A., 2008. A wide range of NS3/4A protease catalytic efficiencies in HCV-infected individuals. *Virus Res.*, **131**(2):260-270. [doi:10.1016/j.virusres.2007.10.003]

Fridell, R.A., Qiu, D.K., Wang, C.F., Valera, L., Gao, M., 2010. Resistance analysis of the HCV NS5A inhibitor BMS-790052 in an in vitro replicon system. *Antimicrob. Agents Chemother*., **54**(9):3641-3650. [doi:10.1128/AAC.00556-10]

Friebe, P., Lohmann, V., Krieger, N., Bartenschlager, R., 2001. Sequences in the 5′ nontranslated region of hepatitis C virus required for RNA replication. *J. Virol*., **75**(24):12047-12057. [doi:10.1128/JVI.75.24.12047-12057.2001]

Fried, M.W., Shiffman, M.L., Reddy, K.R., Smith, C., Marinos, G., Goncales, F.L.Jr., Haussinger, D., Diago, M., Carosi, G., Dhumeaux, D., *et al*., 2002. Peginterferon α-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med*., **347**(13):975-982. [doi:10.1056/NEJMoa020047]

Gaither, L.A., Borawski, J., Anderson, L.J., Balabanis, K.A., Devay, P., Joberty, G., Rau, C., Schirle, M., Bouwmeester, T., Mickanin, C., *et al*., 2010. Multiple cyclophilins involved in different cellular pathways mediate HCV replication. *Virology*, **397**(1):43-55. [doi:10.1016/j.virol.2009.10.043]

Galat, A., 2004. Function-dependent clustering of orthologues and paralogues of cyclophilins. *Prot. Struct. Funct. Bioinf.*, **56**(4):808-820. [doi:10.1002/prot.20156]

Gane, E., Foster, G.R., Cianciara, J., Stedman, C., Ryder, S., Buti, M., Clark, E., Tait, D., 2010a. 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.*, **52**(S1):S464. [doi:10.1016/S0168-8278(10)61194-4]

Gane, E., Roberts, S., Stedman, C., Angus, P., Ritchie, B., Elston, R., Ipe, D., Morcos, P., Baher, L., Najera, I., *et al*., 2010b. Early on-treatment responses during pegylated IFN plus ribavirin are increased following 13 days of combination nucleoside polymerase (RG7128) and protease (RG7227) inhibitor therapy (INFORM-1). *J. Hepatol*., **52**(S1):S291-S292. [doi:10.1016/S0168-8278(10)60751-9]

Gane, E.J., Roberts, S.K., Stedman, C.A., Angus, P.W., Ritchie, B., Elston, R., Ipe, D., Morcos, P.N., Baher, L., Najera, I., *et al*., 2010c. 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*, **376**(9751):1467-1475. [doi:10.1016/S0140-6736(10)61384-0]

Gane, E., Rouzier, R., Stedman, C., Wiercinska-Drapalo, A., Horban, A., Chang, L., Zhang, Y., Sampeur, P., Najera, I., Shulman, N., *et al*., 2010d. 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.*, **52**(S1):S16-S17. [doi:10.1016/S0168-8278(10)60039-6]

Gane, E.J., Rodriguez-Torres, M., Nelson, D.E., Jacobson, I.M., McHutchison, J.G., Duca, A., Hyland, R., Hill, G., Albanis, E., Symonds, W.T., *et al*., 2010e. Sustained virologic response (SVR) following RG7128 1500 mg BID PEG-IFN/RBV for 28 days in HCV genotype 2/3 prior non-responders. *J. Hepatol*., **52**(S1):S16. [doi:10.1016/S0168-8278(10)60039-6]

Gao, M., Nettles, R.E., Belema, M., Snyder, L.B., Nguyen, V.N., Fridell, R.A., Serrano-Wu, M.H., Langley, D.R., Sun, J.H., O′Boyle, D.R., *et al*., 2010. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature*, **465**(7294):96-100. [doi:10.1038/nature089960]

Goto, K., Watashi, K., Murata, T., Hishiki, T., Hijikata, M., Shimotohno, K., 2006. Evaluation of the anti-hepatitis C virus effects of cyclophilin inhibitors, cyclosporin A, and NIM811. *Biochem. Biophys. Res. Commun.*, **343**(3):879-884. [doi:10.1016/j.bbrc.2006.03.059]

Grakoui, A., McCourt, D.W., Wychowski, C., Feinstone, S.M., Rice, C.M., 1993. A second hepatitis C virus-encoded proteinase. *PNAS*, **90**(22):10583-10587. [doi:10.1073/pnas.90.22.10583]

Hadziyannis, S.J., Sette, H.Jr., Morgan, T.R., Balan, V., Diago, M., Marcellin, P., Ramadori, G., Bodenheimer, H.Jr., Bernstein, D., Rizzetto, M., *et al*., 2004. Peginterferon-α 2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann. Intern. Med*., **140**(5):346-355.

Hayashi, N., Takehara, T., 2006. Antiviral therapy for chronic hepatitis C: past, present, and future. *J. Gastroenterol*., **41**(1):17-27. [doi:10.1007/s00535-005-1740-7]

Herrmann, E., Kafer, A., Flisiak, R., Nicolas-Metral, V., Zeuzem, S., Crabbe, R., 2009. PK-PD modeling of viral ki-

netics during treatment with Debio-025 plus pegylated interferon α-2a in treatment-naive HCV patients. *J. Hepatol*., **50**(S1):S344. [doi:10.1016/S0168-8278(09)60948-X]

Hezode, C., Forestier, N., Dusheiko, G., Ferenci, P., Pol, S., Goeser, T., Bronowicki, J.P., Bourliere, M., Gharakhanian, S., Bengtsson, L., *et al*., 2009. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N. Engl. J. Med*., **360**(18):1839-1850. [doi:10.1056/NEJMoa0807650]

Hopkins, S., Huang, Z.H., Scribner, A., Peel, M., 2009a. Antiviral activity of the non-immunosuppressive cyclophilin inhibitor SCY-635 in combination with investigational and approved anti-HCV agents. *Hepatology*, **50**(4):1042A.

Hopkins, S., Heuman, D., Gavis, E., Lalezari, J., Glutzer, E., DiMassimo, B., Rusnak, P., Wring, S., Smitley, C., Ribeill, Y., 2009b. Safety, plasma pharmacokinetics, and anti-viral activity of SCY-635 in adult patients with chronic hepatitis C virus infection. *J. Hepatol*., **50**(S1):S36. [doi:10.1016/S0168-8278(09)60091-X]

Hopkins, S., Scorneaux, B., Huang, Z.H., Murray, M.G., Wring, S., Smitley, C., Harris, R., Erdmann, F., Fischer, G., Ribeill, Y., 2010. SCY-635, a novel nonimmunosuppressive analog of cyclosporine that exhibits potent inhibition of hepatitis C virus RNA replication in vitro. *Antimicrob. Agents Chemother*., **54**(2):660-672. [doi:10.1128/AAC.00660-09]

Horban, A., Berak, H., Kolakowska-Rzadzka, A., Patel, H., Flisiak, R., Crabbe, R., Liz, J.S., Heathcote, J.E., 2010. Sustained virological response after 4-week treatment with Debio 025 monotherapy or coadministered with PEG-IFN α-2a in HCV genotype 1 and 3 patients. *J. Hepatol*., **52**(S1):S294. [doi:10.1016/S0168-8278(10)60757-X]

Houck, D.R., Hopkins, S., 2006. Preclinical evaluation of SCY-635, a cyclophilin inhibitor with potent anti-HCV activity. *Hepatology*, **44**(S1):534A-535A.

Houghton, M., Abrignani, S., 2005. Prospects for a vaccine against the hepatitis C virus. *Nature*, **436**(7053):961-966. [doi:10.1038/nature04081]

Inoue, K., Sekiyama, K., Yamada, M., Watanabe, T., Yasuda, H., Yoshiba, M., 2003. Combined interferon α-2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. *J. Gastroenterol.*, **38**(6):567-572. [doi:10.1007/s00535-002-1104-5]

Inoue, K., Umehara, T., Ruegg, U.T., Yasui, F., Watanabe, T., Yasuda, H., Dumont, J.M., Scalfaro, P., Yoshiba, M., Kohara, M., 2007. Evaluation of a cyclophilin inhibitor in hepatitis C virus-infected chimeric mice in vivo. *Hepatology*, **45**(4):921-928. [doi:10.1002/hep.21587]

Jacobson, I., Pockros, P., Lalezari, J., Lawitz, E., Rodriguez-Torres, M., DeJesus, E., Haas, F., Martorell, C., Pruitt, R., Durham, K., *et al*., 2009. Antiviral activity of Filibuvir in combination with pegylated interferon α-2a and Ribavirin for 28 days in treatment naive patients chronically infected with HCV genotype 1. *J. Hepatol*., **50**(S1):S382-S383. [doi:10.1016/S0168-8278(09)61054-0]

Jacobson, I., Pockros, P.J., Lalezari, J., Lawitz, E., Rodriguez-Torres, M., DeJesus, E., Haas, F., Martorell, C., Pruitt, R., Purohit, V., *et al*., 2010. Virologic response rates following 4 weeks of Filibuvir in combination with pegylated interferon alfa-2a and Ribavirin in chronically-infected HCV genotype-1 patients. *J. Hepatol*., **52**(S1):S465. [doi:10.1016/S0168-8278(10)61197-X]

Karakama, Y., Sakamoto, N., Itsui, Y., Nakagawa, M., Tasaka-Fujita, M., Nishimura-Sakurai, Y., Kakinuma, S., Oooka, M., Azuma, S., Tsuchiya, K., *et al*., 2010. Inhibition of hepatitis C virus replication by a specific inhibitor of serine-arginine-rich protein kinase. *Antimicrob. Agents Chemother.*, **54**(8):3179-3186. [doi:10.1128/AAC.00113-10]

Ke, J., Lawitz, E., Rozier, R., Marbury, T., Nguyen, N., Serra, D., Dole, K., Praestgaard, J., Huang, M., Evans, T., 2009. Safety, and tolerability of NIM 811, a novel cyclophilin inhibitor for HCV, following single and multiple ascending doses in healthy volunteers and HCV-infected patients. *J. Hepatol.*, **50**(S1):S229. [doi:10.1016/S0168-8278(09)60624-3]

Kirkovsky, L., Zhou, Y., Norris, D., Okamoto, E., Nolan, T.G., Bartkowski, D., Khandurina, J., Sergeeva, M., Murphy, D., Ayida, B., *et al*., 2007. ANA598, a novel non-nucleoside inhibitor of HCV NS5B polymerase, exhibits favorable pharmacokinetic properties in multiple preclinical species. *Hepatology*, **46**(S1):858A.

Klumpp, K., Smith, D., Brandl, M., Alfredson, T., Sarma, K., Smith, M., Najera, I., Jiang, W.R., Le Pogam, S., Leveque, V., *et al*., 2007. Design and characterization of R1626, a prodrug of the HCV replication inhibitor R1479 (4′-azidocytidine) with enhanced oral bioavailability. *Antivir. Res*., **74**(3):A35. [doi:10.1016/j.antiviral.2007.01.029]

Kolykhalov, A.A., Mihalik, K., Feinstone, S.M., Rice, C.M., 2000. Hepatitis C virus-encoded enzymatic activities and conserved RNA elements in the 3′ nontranslated region are essential for virus replication in vivo. *J. Virol*., **74**(4):2046-2051. [doi:10.1128/JVI.74.4.2046-2051.2000]

Kukolj, G., Benhamou, Y., Manns, M.P., Bourliere, M., Pol, S., Schuchmann, M., Cartier, M., Huang, D., Lagace, L., Steinmann, G., *et al*., 2009. BI 201335, a potent HCV NS3 protease inhibitor, in treatment-naive and -experienced chronic HCV genotype-1 infection: genotypic and phenotypic analysis of the NS3 protease domain. *J. Hepatol*., **50**(S1):S347. [doi:10.1016/S0168-8278(09)60956-9]

Kukolj, G., Bousquet, C., Dansereau, N., Do, F., Lagace, L., Llinas-Brunet, M., Marquis, M., Massariol, M.J., Maurice, R., Spickler, C., *et al*., 2010. Characterization of resistant mutants selected in vitro by the HCV NS3/4A protease inhibitor BI 201335. *J. Hepatol*., **52**(S1):S295. [doi:10.1016/S0168-8278(10)60760-X]

Kwo, P.Y., Lawitz, E.J., McCone, J., Schiff, E.R., Vierling, J.M., Pound, D., Davis, M.N., Galati, J.S., Gordon, S.C., Ravendhran, N., *et al*., 2010. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon α-2b and ribavirin in treatment-naive patients with

genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet*, **376**(9742):705-716. [doi:10.1016/S0140-6736(10)60934-8]

Lalezari, J., Asmuth, D., Casiro, A., Vargas, H.E., Patrick, G.D., Liu, W., Pietropaolo, K., Zhou, X.J., Sullivan-Bolyai, J., Mayers, D.L., 2009. Antiviral activity, safety and pharmacokinetics of IDX184, a liver-targeted nucleotide HCV polymerase inhibitor, in patients with chronic hepatitis C. *Hepatology*, **50**(6):11A-12A.

Lallos, L., LaColla, M., Serra, I., Bilello, J.P., Soubasakos, M.A., Li, B., Panzo, R.J., Gillum, J.M., Bonin, A., Seifer, M., *et al*., 2009. Combination of IDX184, a nucleotide prodrug polymerase inhibitor, with other classes of HCV inhibitors is additive to synergistic in the HCV replicon in vitro. *Hepatology*, **50**(4):1045A.

Lam, A.M., Frick, D.N., 2006. Hepatitis C virus subgenomic replicon requires an active NS3 RNA helicase. *J. Virol*., **80**(1):404-411. [doi:10.1128/JVI.80.1.404-411.2006]

Larrey, D., Benhamou, Y., Lohse, A.W., Trepo, C., Moelleken, C., Bronowicki, J.P., Heim, M.H., Arasteh, K., Zarski, J.P., Bourliere, M., *et al*., 2009a. BI 207127 is a potent HCV RNA polymerase inhibitor during 5 days monotherapy in patients with chronic hepatitis C. *Hepatology*, **50**(4):1044A.

Larrey, D., Benhamou, Y., Lohse, A.W., Trepo, C., Moelleken, C., Bronowicki, J.P., Arasteh, K., Bourliere, M., Heim, M., Enriquez, J., *et al*., 2009b. 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*., **50**(S1): S383-S384. [doi:10.1016/S0168-8278(09)61056-4]

Lavanchy, D., 2009. The global burden of hepatitis C. *Liver Int.*, **29**(1):74-81. [doi:10.1111/j.1478-3231.2008.01934.x]

Lawitz, E., Rouzier, R., Nguyen, T., Huang, M., Ke, J., Praestgaard, J., Serra, D., Koziel, M., Evans, T., 2009. Safety and antiviral efficacy of 14 days of the cycophilin inhibitor NIM811 in combination with pegylated interferon α-2a in relapsed genotype 1 HCV infected patients. *J. Hepatol*., **50**(S1):S379. [doi:10.1016/S0168-8278(09)61047-3]

Lawitz, E.J., Marbury, T.C., Vince, B.D., Grunenberg, N., Rodriguez-Torres, M., de Micco, M.P., Tarro, J.N., Shelton, M.J., West, S., Zong, J., *et al*., 2010. Dose-ranging, three-day monotherapy study of the HCV NS3 protease inhibitor GS-9256. *J. Hepatol*., **52**(S1):S466-S467. [doi:10.1016/S0168-8278(10)61199-3]

Lawitz, E., Rodriquez-Torres, M., Rustgi, V.K., Hassanein, T., Rahimy, M.H., Crowley, C.A., Freddo, J.L., Muir, A., McHutchison, J., 2010a. Safety and antiviral activity of ANA598 in combination with pegylated interferon α-2a plus ribavirin in treatment-naive genotype-1 chronic HCV patients. *J. Hepatol*., **52**(S1):S467. [doi:10.1016/S0168-8278(10)61200-7]

Le Pogam, S., Seshaadri, A., Ewing, A., Kang, H.S., Kosaka, A., Yan, J.M., Berrey, M., Symonds, B., de la Rosa, A., Cammack, N., *et al*., 2010. RG7128 alone or in combination with pegylated interferon-α 2a and ribavirin prevents hepatitis C virus (HCV) replication and selection of resistant variants in HCV-infected patients. *J. Infect. Dis*., **202**(10):1510-1519. [doi:10.1086/656774]

Lemke, C.T., Zhao, S.P., Goudreau, N., Hucke, O., Thibeault, D., Llinas-Brunet, M., White, P.W., 2010. Bound structure and biochemical mechanism of action of BI 201335, a potent, non-covalent inhibitor of HCV NS3-NS4A protease. *Antivir. Res*., **86**(1):A20-A21. [doi:10.1016/j.antiviral.2010.02.336]

Levin, J., Eley, T., Pasquinelli, C., Wendelburg, P., Villegas, C., He, B., Liao, S., Jiang, H., Marbury, T., Bertz, R., *et al*., 2010. Safety and Pharmacokinetics of BMS-650032 Following Multiple Ascending Doses for 14 Days in Healthy Subjects. Bristol-Myers Squibb, Princeton, FL.

Li, H., Tatlock, J., Linton, A., Gonzalez, J., Jewell, T., Patel, L., Ludlum, S., Drowns, M., Rahavendran, S.V., Skor, H., *et al*., 2009. Discovery of (*R*)-6-cyclopentyl-6-(2-(2,6-diethylpyridin-4-yl)ethyl)-3-((5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)methyl)-4-hydroxy-5,6-dihydropyran-2-one (PF-00868554) as a potent and orally available hepatitis C virus polymerase inhibitor. *J. Med. Chem*., **52**(5):1255-1258. [doi:10.1021/jm8014537]

Lin, C., Kwong, A.D., Perni, R.B., 2006. Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect. Disord. Drug Targets*, **6**(1):3-16. [doi:10.2174/187152606776056706]

Lin, K., Kwong, A.D., Lin, C., 2004. Combination of a hepatitis C virus NS3-NS4A protease inhibitor and α interferon synergistically inhibits viral RNA replication and facilitates viral RNA clearance in replicon cells. *Antimicrob. Agents Chemother*., **48**(12):4784-4792. [doi:10.1128/AAC.48.12.4784-4792.2004]

Lin, K., Perni, R.B., Kwong, A.D., Lin, C., 2006. VX-950, a novel hepatitis C virus (HCV) NS3-4A protease inhibitor, exhibits potent antiviral activities in HCV replicon cells. *Antimicrob. Agents Chemother*., **50**(5):1813-1822. [doi:10.1128/AAC.50.5.1813-1822.2006]

Lin, T.L., Lenz, O., Fanning, G., Verbinnen, T., Delouvroy, F., Scholliers, A., Vermeiren, K., Rosenquist, A., Edlund, M., Samuelsson, B., *et al*., 2009. In vitro activity and preclinical profile of TMC435350, a potent hepatitis C virus protease inhibitor. *Antimicrob. Agents Chemother*., **53**(4):1377-1385. [doi:10.1128/AAC.01058-08]

Lindenbach, B.D., Evans, M.J., Syder, A.J., Wolk, B., Tellinghuisen, T.L., Liu, C.C., Maruyamam, T., Hynes, R.O., Burton, D.R., McKeating, J.A., *et al*., 2005. Complete replication of hepatitis C virus in cell culture. *Science*, **309**(5734):623-626. [doi:10.1126/science.1114016]

Liu-Young, G., Kozal, M.J., 2008. Hepatitis C protease and polymerase inhibitors in development. *AIDS Patient Care St.*, **22**(6):449-457. [doi:10.1089/apc.2007.0199]

Liverton, N.J., Carroll, S.S., Dimuzio, J., Fandozzi, C., Graham, D.J., Hazuda, D., Holloway, M.K., Ludmerer, S.W., McCauley, J.A., McIntyre, C.J., *et al*., 2010. MK-7009, a potent and selective inhibitor of hepatitis C virus NS3/4A protease. *Antimicrob. Agents Chemothe*r., **54**(1):305-311.

[doi:10.1128/AAC.00677-09]

Lohmann, V., Korner, F., Koch, J., Herian, U., Theilmann, L., Bartenschlager, R., 1999. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science*, **285**(5424):110-113. [doi:10.1126/science.285.5424.110]

Lok, A.S., Gardiner, D.F., Lawitz, E., Martorell, C., Everson, G.T., Ghalib, R.H., Reindollar, R., Rustgi, V.K., Wendelburg, P., Zhu, K., *et al*., 2010. Combination therapy with BMS-790052 and BMS-650032 alone or with pegylated interferon and ribavirin (PEGIFN/RBV) results in undetectable HCV RNA through 12 weeks of therapy in HCV genotype 1 null responders. *Hepatology*, **52**(4): 877A.

Ma, S., Boerner, J.E., TiongYip, C., Weidmann, B., Ryder, N.S., Cooreman, M.P., Lin, K., 2006. NIM811, a cyclophilin inhibitor, exhibits potent in vitro activity against hepatitis C virus alone or in combination with α interferon. *Antimicrob. Agents Chemother*., **50**(9):2976-2982. [doi:10.1128/AAC.00310-06]

Malcolm, B.A., Liu, R., Lahser, F., Agrawal, S., Belanger, B., Butkiewicz, N., Chase, R., Gheyas, F., Hart, A., Hesk, D., *et al*., 2006. SCH 503034, a mechanism-based inhibitor of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral activity of α interferon in replicon cells. *Antimicrob. Agents Chemother*., **50**(3):1013-1020. [doi:10.1128/AAC.50.3.1013-1020.2006]

Manns, M., Reesink, H., Moreno, C., Berg, T., Benhamou, Y., Horsmans, Y., Dusheiko, G., Flisiak, R., Meyvisch, P., Lenz, O., *et al*., 2009. Opera-1 Trial: interim analysis of safety and antiviral activity of TMC435 in treatment-naive genotype 1 HCV patients. *J. Hepatol*., **50**(S1):S7. [doi:10.1016/S0168-8278(09)60013-1]

Manns, M.P., McHutchison, J.G., Gordon, S.C., Rustgi, V.K., Shiffman, M., Reindollar, R., Goodman, Z.D., Koury, K., Ling, M., Albrecht, J.K., 2001. Peginterferon α-2b plus ribavirin compared with interferon α-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*, **358**(9286):958-965. [doi:10.1016/S0140-6736(01)06102-5]

Manns, M.P., Foster, G.R., Rockstroh, J.K., Zeuzem, S., Zoulim, F., Houghton, M., 2007. The way forward in HCV treatment—finding the right path. *Nat. Rev. Drug Discov*., **6**(12):991-1000. [doi:10.1038/nrd2411]

McCauley, J.A., McIntyre, C.J., Rudd, M.T., Nguyen, K.T., Romano, J.J., Butcher, J.W., Gilbert, K.F., Bush, K.J., Holloway, M.K., Swestock, J., *et al*., 2010. Discovery of vaniprevir (MK-7009), a macrocyclic hepatitis C virus NS3/4A protease inhibitor. *J. Med. Chem*., **53**(6):2443-2463. [doi:10.1021/jm9015526]

McCown, M.F., Rajyaguru, S., le Pogam, S., Ali, S., Jiang, W.R., Kang, H., Symons, J., Cammack, N., Najera, I., 2008. The hepatitis C virus replicon presents a higher barrier to resistance to nucleoside analogs than to non-nucleoside polymerase or protease inhibitors. *Antimicrob. Agents Chemother*., **52**(5):1604-1612. [doi:10.1128/AAC.01317-07]

McPhee, F., 2010. Identification and preclinical profile of the novel HCV NS3 protease inhibitor BMS-650032. *J. Hepatol*., **52**(S1):S296. [doi:10.1016/S0168-8278(10)60763-5]

McPhee, F., Hernandez, D., Sheaffer, A., Lee, M., Falk, P., Yu, F., Zhai, G.Z., Chaniewski, S., Pasquinelli, C., 2009. Genotypic and phenotypic analysis of samples from HCV-infected subjects treated with BMS-650032 in a single ascending dose study. *Hepatology*, **50**(S4):1048A. [doi:10.1002/hep.23307]

Mori, J., Hammond, J.L., Srinivasan, S., Jagannatha, S., van der Ryst, E., 2010. Genotypic characterisation of filibuvir (PF-00868554) resistance in patients receiving four weeks co-administration of filibuvir with PEGIFN/RBV (12 week analysis). *J. Hepatol*., **52**(S1):S15. [doi:10.1016/S0168-8278(10)60037-2]

Moucari, R., Forestier, N., Larrey, D., Guyader, D., Couzigou, P., Benhamou, Y., Voitot, H., Vidaud, M., Seiwert, S., Bradford, B., *et al*., 2010. Danoprevir, an HCV NS3/4A protease inhibitor, improves insulin sensitivity in patients with genotype 1 chronic hepatitis C. *Gut*, **59**(12):1694-1698. [doi:10.1136/gut.2010.219089]

Neelakantan, S., Purohit, V.S., O′Gorman, M., Hammond, J., Tensfeldt, T.G., 2010. Exposure-response relationship of Filibuvir in HCV infected patients: application to dose selection for combination therapy. *J. Hepatol*., **52**(S1): S119-S120. [doi:10.1016/S0168-8278(10)60288-7]

Nguyen, M.H., Keeffe, E.B., 2005. Prevalence and treatment of hepatitis C virus genotypes 4, 5, and 6. *Clin. Gastroenterol. Hepatol.*, **3**(10):S97-S101. [doi:10.1016/S1542-3565(05)00711-1]

Nicolas, O., Boivin, I., St-Denis, C., Bedard, J., 2008. Genotypic analysis of HCV NS5B variants selected from patients treated with VCH-759. *J. Hepatol*., **48**(S2):S317. [doi:10.1016/S0168-8278(08)60846-6]

Ohno, T., Mizokami, M., Tibbs, C.J., Ohba, K., Suzuki, K., Wu, R.R., Nouri-Aria, K.T., Williams, R., 1994. New genotype of hepatitis C virus in South Africa. *J. Med. Virol*., **42**(4):409-413. [doi:10.1002/jmv.1890420414]

Opar, A., 2010. Excitement grows for potential revolution in hepatitis C virus treatment. *Nat. Rev. Drug Discov*., **9**(7): 501-503. [doi:10.1038/nrd3214]

Otto, M.J., Robson, R., Rodríguez, C.A., Beard, A., Symonds, W.T., Hill, G., Berrey, M.M., 2007. Pharmacokinetics, Safety, and Tolerability of R7128, a Novel Nucleoside Polymerase Inhibitor for HCV Following Single, Ascending Oral Doses in Healthy Volunteers. Pharmasset Inc., Princeton, NJ.

Paeshuyse, J., Kaul, A., de Clercq, E., Rosenwirth, B., Dumont, J.M., Scalfaro, P., Bartenschlager, R., Neyts, J., 2006. The non-immunosuppressive cyclosporin DEBIO-025 is a potent inhibitor of hepatitis C virus replication in vitro. *Hepatology*, **43**(4):761-770. [doi:10.1002/hep.21102]

Patel, R., Thompson, P., Showalter, R., Appleman, J., 2010. In vitro combination studies of ANA598 with anti-HCV agents demonstrate enhanced anti-viral activity. *Antivir. Res*., **86**(1):A41-A42. [doi:10.1016/j.antiviral.2010.02.397]

Pawlotsky, J.M., 2006. Therapy of hepatitis C: from empiricism to eradication. *Hepatology*, **43**(S1):S207-S220. [doi:10.1002/hep.21064]

Perni, R.B., Almquist, S.J., Byrn, R.A., Chandorkar, G., Chaturvedi, P.R., Courtney, L.F., Decker, C.J., Dinehart, K., Gates, C.A., Harbeson, S.L., *et al*., 2006. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob. Agents Chemother.*, **50**(3):899-909. [doi:10.1128/AAC.50.3.899-909.2006]

Pockros, P., Nelson, D., Godofsky, E., Rodriguez-Torres, M., Everson, G.T., Fried, M.W., Ghalib, R., Harrison, S., Nyberg, L., Shiffman, M.L., *et al*., 2008a. High relapse rate seen at week 72 for patients treated with R1626 combination therapy. *Hepatology*, **48**(4):1349-1350. [doi:10.1002/hep.22593]

Pockros, P.J., Nelson, D., Godofsky, E., Rodriguez-Torres, M., Everson, G.T., Fried, M.W., Ghalib, R., Harrison, S., Nyberg, L., Shiffman, M.L., *et al*., 2008b. R1626 plus peginterferon α-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin. *Hepatology*, **48**(2):385-397. [doi:10.1002/hep.22357]

Pol, S., Everson, G., Ghalib, R., Rustgi, V., Martorell, C., Tatum, H.A., Lim, J., Hezode, C., Diva, U., Yin, P.D., *et al*., 2010. Once-daily NS5A inhibitor (BMS-790052) plus peginterferon α-2a and Ribavirin produces high rates of extended rapid virologic response in treatment-naive HCV-genotype 1 subjects: phase 2a trial. *J. Hepatol.*, **52**(S1):S462. [doi:10.1016/S0168-8278(10)61190-7]

Rahimy, M.H., Crowley, C.A., Freddo, J.L., Sergeeva, M.V., Golec, B., 2008. Results of a phase I safety, tolerability and pharmacokinetic study of ANA598, a non-nucleoside NS5B polymerase inhibitor, in healthy volunteers. *Hepatology*, **48**(4):1026A.

Reddy, R., Rodriguez-Torres, M., Gane, E., Robson, R., Lalezari, J., Everson, G.T., Dejesus, E., McHutchison, J.G., Vargas, H.E., Beard, A., *et al*., 2007. 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*, **46**(4):862A-863A.

Reesink, H.W., Fanning, G.C., Farha, K.A., Weegink, C., van Vliet, A., van′t Klooster, G., Lenz, O., Aharchi, F., Marien, K., van Remoortere, P., *et al*., 2010. Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. *Gastroenterology*, **138**(3):913-921. [doi:10.1053/j.gastro.2009.10.033]

Reiser, M., Timm, J., 2009. Serine protease inhibitors as anti-hepatitis C virus agents. *Expert Rev. Anti.-Infect. Ther.*, **7**(5):537-547. [doi:10.1586/eri.09.30]

Reiser, M., Hinrichsen, H., Benhamou, Y., Reesink, H.W., Wedemeyer, H., Avendano, C., Riba, N., Yong, C.L., Nehmiz, G., Steinmann, G.G., 2005. Antiviral efficacy of NS3-serine protease inhibitor BILN-2061 in patients with chronic genotype 2 and 3 hepatitis C. *Hepatology*, **41**(4):832-835. [doi:10.1002/hep.20612]

Roberts, S.K., Cooksley, G., Dore, G.J., Robson, R., Shaw, D., Berns, H., Hill, G., Klumpp, K., Najera, I., Washington, C., 2008. Robust antiviral activity of R1626, a novel nucleoside analog: a randomized, placebo-controlled study in patients with chronic hepatitis C. *Hepatology*, **48**(2):398-406. [doi:10.1002/hep.22321]

Salmon-Ceron, D., Lewden, C., Morlat, P., Bevilacqua, S., Jougla, E., Bonnet, F., Heripret, L., Costagliola, D., May, T., Chene, G., 2005. Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. *J. Hepatol.*, **42**(6):799-805. [doi:10.1016/j.jhep.2005.01.022]

Sarrazin, C., Rouzier, R., Wagner, F., Forestier, N., Larrey, D., Gupta, S.K., Hussain, M., Shah, A., Cutler, D., Zhang, J., *et al*., 2007. SCH 503034, a novel hepatitis C virus protease inhibitor, plus pegylated interferon α-2b for genotype 1 nonresponders. *Gastroenterology*, **132**(4):1270-1278. [doi:10.1053/j.gastro.2007.01.041]

Schiff, E., Poordad, F.F., Jacobson, I.M., Flamm, S.L., Bacon, B.R., Lawitz, E.J., Gordon, S.R., McHutchison, J.G., Ghalib, R., Poynard, T., *et al*., 2008. Role of interferon response during RE-Treatment of null responders with boceprevir combination therapy: results of phase II trial. *Gastroenterology*, **134**(4):A755. [doi:10.1016/S0016-5085(08)63527-1]

Schmitz, U., Tan, S.L., 2008. NS5A—from obscurity to new target for HCV therapy. *Recent Pat. Anti-Infect. Drug Discov.*, **3**(2):77-92. [doi:10.2174/157489108784746597]

Seth, R.B., Sun, L., Chen, Z.J., 2006. Antiviral innate immunity pathways. *Cell Res.*, **16**(2):141-147. [doi:10.1038/sj.cr.7310019]

Shepard, C.W., Finelli, L., Alter, M.J., 2005. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.*, **5**(9):558-567. [doi:10.1016/S1473-3099(05)70216-4]

Shi, S.T., Herlihy, K.J., Graham, J.P., Nonomiya, J., Rahavendran, S.V., Skor, H., Irvine, R., Binford, S., Tatlock, J., Li, H., *et al*., 2009. Preclinical characterization of PF-00868554, a potent nonnucleoside inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. *Antimicrob. Agents Chemother.*, **53**(6):2544-2552. [doi:10.1128/AAC.01599-08]

Shih, I.H., Vliegen, I., Peng, B., Yang, H., Paeshuyse, J., Purstinger, G., Fenaux, M., Mabery, E., Bahador, G., Lehman, L.S., *et al*., 2007. 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*, **46**(4):859A.

Simmonds, P., 2004. Genetic diversity and evolution of hepatitis C virus—15 years on. *J. Gen. Virol.*, **85**(11):3173-3188. [doi:10.1099/vir.0.80401-0]

Stauber, K., Marlor, C.W., Mushtaq, G., Gadhachanda, V., Pais, G., Hashimoto, A., Phadke, A., Huang, M., Deshpande, M., 2010. Characterization of the hepatoselective distribution of ACH-1625, a potent, clinical stage HCV NS3 protease inhibitor. *J. Hepatol.*, **52**(S1):S299. [doi:10.1016/S0168-8278(10)60772-6]

Susan, H., 2010. Gilead's Investigational Hepatitis C Compounds GS 9190 and GS 9256 in Combination with Standard of Care Therapies Achieve Substantial Viral Suppression in Phase II Study. Available from http://www.gilead.com/pr_1489408 [Accessed on Nov. 20, 2011]

Tang, H., Grise, H., 2009. Cellular and molecular biology of HCV infection and hepatitis. *Clin. Sci.*, **117**(2):49-65. [doi:10.1042/CS20080631]

Tellinghuisen, T.L., Foss, K.L., 2008. Regulation of hepatitis C virion production via phosphorylation of the NS5A protein. *PLoS Pathog.*, **4**(3):e1000032. [doi:10.1371/journal.ppat.1000032]

Thompson, P.A., Patel, R., Showalter, R.E., Li, C., Applemon, J.R., Steffy, K., 2008. In vitro studies demonstrate that combinations of antiviral agents that include HCV polymerase inhibitor ANA598 have the potential to overcome viral resistance. *Hepatology*, **48**(4):1164A.

Thompson, P., Patel, R., Steffy, K., Appleman, J., 2009. Preclinical studies of ANA598 combined with other anti-HCV agents demonstrate potential of combination treatment. *J. Hepatol.*, **50**(S1):S37. [doi:10.1016/S0168-8278(09)60092-1]

Thompson, P., Showalter, R., Patel, R., Appleman, J., 2010a. In vitro combination of ANA598 with other anti-HCV agents can eliminate the emergence of resistant colonies. *Antivir. Res.*, **86**(1):A48. [doi:10.1016/j.antiviral.2010.02.415]

Thompson, P.A., Showalter, R.E., Patel, R.A., Appleman, J.R., 2010b. Enhanced in vitro antiviral activity of ANA598 in combination with other anti-HCV Agents support combination treatment. *J. Hepatol.*, **52**(S1):S262. [doi:10.1016/S0168-8278(10)60675-7]

Tong, X., Arasappan, A., Bennett, F., Chase, R., Feld, B., Guo, Z., Hart, A., Madison, V., Malcolm, B., Pichardo, J., *et al*., 2010. Preclinical characterization of the antiviral activity of SCH 900518 (narlaprevir), a novel mechanism-based inhibitor of hepatitis C virus NS3 protease. *Antimicrob. Agents Chemother.*, **54**(6):2365-2370. [doi:10.1128/AAC.00135-10]

Troke, P., Lewis, M., Simpson, P., van der Ryst, E., Hammond, J., Craig, C., Perros, M., Westby, M., 2009. Genotypic characterisation of HCV NS5B following 8-day monotherapy with the polymerase inhibitor PF-00868554 in HCV-infected subjects. *J. Hepatol.*, **50**(S1):S351. [doi:10.1016/S0168-8278(09)60970-3]

Vliegen, I., Paeshuyse, J., Marbery, E., Peng, B., Shih, H., Lehman, L.S., Dutartre, H., Selisko, B., Canard, B., Bondy, S., *et al*., 2007. GS-9190, a novel substituted imidazopyridine analogue, is a potent inhibitor of hepatitis C virus replication in vitro and remains active against known drug resistant mutants. *Hepatology*, **46**(4):855A.

Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., Murthy, K., Habermann, A., Krausslich, H.G., Mizokami, M., *et al*., 2005. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat. Med.*, **11**(7):791-796. [doi:10.1038/nm1268]

Walker, M.P., Hong, Z., 2002. HCV RNA-dependent RNA polymerase as a target for antiviral development. *Cur. Opin. Pharmacol.*, **2**(5):534-540. [doi:10.1016/S1471-4892(02)00195-9]

Wang, C., Sarnow, P., Siddiqui, A., 1993. Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. *J. Virol.*, **67**(6):3338-3344.

Watashi, K., Hijikata, M., Hosaka, M., Yamaji, M., Shimotohno, K., 2003. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology*, **38**(5):1282-1288. [doi:10.1053/jhep.2003.50449]

Weisberg, I.S., Jacobson, I.M., 2009. Telaprevir: hope on the horizon, getting closer. *Clin. Liver Dis.*, **13**(3):441-452. [doi:10.1016/j.cld.2009.05.009]

White, P.W., Llinas-Brunet, M., Amad, M., Bethell, R.C., Bolger, G., Cordingley, M.G., Duan, J.M., Garneau, M., Lagace, L., Thibeault, D., *et al*., 2010a. Preclinical characterization of BI 201335, a C-terminal carboxylic acid inhibitor of the hepatitis C virus NS3-NS4A protease. *Antimicrob. Agents Chemother.*, **54**(11):4611-4618. [doi:10.1128/AAC.00787-10]

White, P.W., Llinas-Brunet, M., Amad, M., Bolger, G., Cordingley, M.G., Duan, J., Garneau, M., Lagace, L., Thibeault, D., Kukolj, G., 2010b. Preclinical characterization of non-covalent HCV NS3/4A protease inhibitor BI 201335. *J. Hepatol.*, **52**(S1):S302. [doi:10.1016/S0168-8278(10)60779-9]

Wong, D.A., Tong, L.K., Lim, W., 1998. High prevalence of hepatitis C virus genotype 6 among certain risk groups in Hong Kong. *Eur. J. Epidemiol.*, **14**(5):421-426. [doi:10.1023/A:1007400304726]

Yang, C., Wang, Y., Wieman, L., Eisenberg, E., Lee, M., Murray, B., Tong, L., Ray, A., Bondy, S., Tse, W., *et al*., 2007. Preclinical pharmacokinetic characterization of GS-9190, a novel non-nucleoside HCV NS5B polymerase inhibitor. *Hepatology*, **46**(4):860A-861A.

Yi, M., Lemon, S.M., 2003. 3′-nontranslated RNA signals required for replication of hepatitis C virus RNA. *J. Virol.*, **77**(6):3557-3568. [doi:10.1128/JVI.77.6.3557-3568.2003]

Zhong, J., Gastaminza, P., Cheng, G., Kapadia, S., Kato, T., Burton, D.R., Wielandm, S.F., Uprichard, S.L., Wakita, T., Chisari, F.V., 2005. Robust hepatitis C virus infection in vitro. *PNAS*, **102**(26):9294-9299. [doi:10.1073/pnas.0503596102]

Zhou, X.J., Pietropaolo, K., Sullivan-Bolyai, J., Kuca, B., Liu, W., Xu, L., Belanger, B., Khan, S., Mayers, D., 2009. IDX184, a liver-targeted nucleotide HCV polymerase inhibitor: results of a first-in-man safety and pharmacokinetic study. *J. Hepatol.*, **50**(S1):S351. [doi:10.1016/S0168-8278(09)60968-5]