Hepatitis C virus replication and potential targets for direct-acting agents

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Abstract: We finally stand at the brink of novel, oral, direct-acting antivirals for the treatment of hepatitis C virus (HCV) infection. Basic science research has led to a greater understanding of the viral life cycle and identified numerous potential targets for therapy. Early compounds were plagued by inconsistent *in vivo* activity and side effects that led to discontinuation of investigational efforts. However, several agents have now progressed to phase 2 human studies and two protease inhibitors have completed enrolment for their phase 3 clinical trials and look promising. Thus, while it appears that protease inhibitors will likely be the next available drugs for the treatment of HCV infection, the quest for additional therapeutic agents will continue. The future of HCV therapy lies in multidrug cocktails of several agents targeted against a variety of targets. In the near future these agents will be added to the current standard therapy consisting of pegylated interferon and ribavirin; however, the ultimate and probably realistic goal will be to develop multidrug oral regimens to replace the need for interferon.

Keywords: HCV replication, new treatment, polymerase inhibitors, protease inhibitors, therapy

Introduction

Hepatitis C infects 170 million people worldwide and 1.6% of the United States population [Armstrong *et al.* 2006; Davis *et al.* 2003; Alter *et al.* 1999; WHO, 1999]. After acute infection, 55% to 85% of patients develop chronic disease. The natural history of chronic hepatitis C varies significantly because of host, viral and environmental factors. Chronic infection leads to cirrhosis in approximately 20% of patients after 20 years of infection [Freeman *et al.* 2001]. Thereafter, other complications including hepatic decompensation (ascites, encephalopathy, variceal hemorrhage, hepatorenal syndrome, or hepatic synthetic dysfunction) and hepatocellular carcinoma ensue at a rate of about 3% per year [Sangiovanni *et al.* 2006; El-Serag, 2004; Serfaty *et al.* 1998; Fattovich *et al.* 1997]. Without liver transplantation, decompensated cirrhosis leads to death in 50–72% of patients after 5 years [Fattovich *et al.* 2002]. As a result of the high prevalence of hepatitis C virus (HCV) infection and resultant complications, HCV is the leading indication for liver transplantation in the United States and the world as a whole [Wasley and Alter, 2000].

Chronic hepatitis C is the only chronic viral infection that can be cured with antiviral therapy.

Unlike human immunodeficiency virus and hepatitis B, a sustained virologic response (SVR), defined as HCV-RNA undetectable by a sensitive amplification test 6 months after the completion of therapy, is equivalent to a cure in >99% of cases [Fried *et al.* 2002; Manns *et al.* 2001]. Patients with compensated cirrhosis who achieve an SVR essentially eliminate their subsequent risk of decompensation, may achieve histologic regression, and decrease their risk of hepatocellular carcinoma by two thirds [Bruno *et al.* 2007; Di Bisceglie *et al.* 2007; Camma *et al.* 2004].

The current standard of care for the treatment of HCV infection remains the combination of pegylated interferon and ribavirin [Fried *et al.* 2002; Manns *et al.* 2001]. This therapy eradicates HCV in 40–50% of genotype 1 non-cirrhotic patients and 70–80% of genotype 2 and 3 non-cirrhotic patients. The response to treatment is lower in obese, insulin-resistant, or African-American patients and in those with advanced hepatic fibrosis or high viral loads. Of greatest concern are patients with decompensated cirrhosis and immunosuppressed patients, such as liver transplant recipients, who are rarely able to tolerate full doses of therapy.

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Figure 1. A graphic depiction of the viral life cycle with the potential antiviral targets listed. NS, nonstructural proteins; ER, endoplasmic reticulum; IRES, internal ribosome entry side.

Fortunately, we are entering a new era of HCV therapy. A greater understanding of the HCV replication machinery has led to a large list of potential targets for therapy (Figure 1). One such target is the nonstructural 3 (NS3) viral protease, an enzyme that is critical to post-translational processing of the viral polyprotein. Drugs that effectively inhibit this enzyme are in the final stages of clinical trials, and it appears that the combination of a protease inhibitor with pegylated interferon and ribavirin will significantly improve the SVR rate, perhaps even with a shorter duration of treatment. In addition, these medications will allow us to retreat previous relapsers and nonresponders to pegylated interferon and ribavirin with some success. However, while such drugs will be a tremendous addition to our therapeutic armamentarium, it is important to recognize that they will not eradicate infection in all patients and barriers to using interferon or ribavirin in many patients will still be a problem.

This article will review the most promising potential targets for new direct-acting antiviral agents (DAA). Drugs for some of these targets are well along in clinical development while others are only hypothetical and supported by *in vitro* studies (Table 1). It is important for the reader to understand that the high replication and nucleic acid substitution rate of HCV will

likely lead to rapid emergence of viral drug resistance if a single one of these replicative steps is targeted. Thus, the future of HCV therapy lies in the combination of multiple agents against different targets such as receptor binding, cell entry, viral transcription, translation, polyprotein processing, particle assembly and export of virus progeny. These DAAs might also be combined with non-specific antiviral agents such as those that enhance the endogenous immune response to the virus, e.g. interferons or therapeutic vaccines, or neutralize extracellular virus, e.g. hyperimmune globulins. The goal, of course, is to seek combinations that will both increase efficacy and improve tolerability.

Early inhibitors: receptor binding and cell entry

Entry of HCV into the hepatocyte involves a series of sequential interactions with soluble and cell surface host factors, and remains incompletely understood. Plasma-derived HCV is complexed with low-density and very-low-density lipoproteins (LDL and VLDL), which probably facilitates the initial attraction and concentration of virus on the cell surface via interaction with the low-density lipoprotein receptor (LDL-R) [Andre *et al.* 2005]. The highly glycosylated viral envelope proteins E1 and E2

Table 1. Direct-acting agents to treat hepatitis C in phase 2 and 3 clinical trials.

Class	Drug name	Drug Company
Entry and RNA-Binding Inhibitors		
Neutralizing Antibodies	Civacir	NABI Pharmaceuticals
Glycosylation Inhibitors	Celgosivir	Migenix, Inc.
	*UT-231B	Unither Pharmaceuticals
IRES Inhibitors	*Heptazyme	Ribozyme Pharmaceuticals, Inc.
	*ISIS-14803	ISIS Pharmaceuticals
	VGX-410C	VGX Pharmaceuticals
Protease Inhibitors		
NS2 Inhibitors	none	
NS3 Inhibitors	Telaprevir	Vertex Pharmaceuticals
	Boceprevir	Schering-Plough Corporation
	ITMN-191	Intermune, Inc.
	TCM435	Tibotec Pharmaceuticals Limited
	MK-7009	Merck
	BMS-650032	Bristol-Myers Squibb
	BMS-791325	Bristol-Myers Squibb
	*ACH-806	Achillion Pharmaceuticals Inc.
NS4A Inhibitors		
RNA Transcription		
NS4B Inhibitors	none	
Helicase Inhibitors	none	
Cyclophilin A Inhibitors	Debio-025	DebioPharm Group
	NIM-811	Novartis
NS5B Polymerase Inhibitors	R7128	Pharmasset, Inc. and Roche
	*R1626	Roche
	*NM283	Idenix Pharmaceuticals, Inc.
	*HCV-796	ViroPharm, Inc.
	VCH-759	Vertex Pharmaceuticals
	PF-868554	Pfizer
	IDX184	Idenix Pharmaceuticals, Inc.
	GS 9190	Gilead
NS5A Inhibitors	BMS790052	Bristol-Myers Squibb
Other		
TLR-9 Agonists	*CPG 10101	Pfizer
NKT Cell Agonist	KRN7000	Kyowa Hakko Kirin Company, Limited
Other	Nitazoxanide	Romark Laboratories L.C.

Data available at: www.clinicaltrials.gov and www.hcvdrugs.com. NS, nonstructural; IRES, internal ribosome entry site; TLR-9, toll-like receptor-9.

*Future investigation of these compounds has been aborted.

conformationally attach to glycosaminoglycans at the hepatocyte cell surface and to the c-type lectins DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin; CD209) and L-SIGN (DC-SIGNr; liver and lymph node specific; CD209L) on neighboring dendritic cells and liver sinusoidal cells [Cormier *et al.* 2004a]. E2 then sequentially attaches to the tetraspanin CD81 and scavenger receptor class B type 1 (SR-B1) to form a receptor complex that utilizes the tight junction claudin proteins, particularly CLDN1, for internalization [Zeisel *et al.* 2007; Cormier *et al.* 2004b; Pileri *et al.* 1998]. Occludin (OCLN), a tight junction protein, is also essential for cell entry but its exact role remains to be defined [Lanford *et al.* 2009; Ploss *et al.* 2009; Evans *et al.* 2007]. Interestingly, EWI-2wint, a small

protein that is associated with CD81 in several cell lines and efficiently blocks HCV entry, is absent in hepatocytes and this probably explains the selective susceptibility of hepatocytes to HCV infection [Schuster and Baumert, 2009; Rocha-Perugini *et al.* 2008].

The role of the humoral immune response in controlling HCV infection is not clear. Monoclonal anti-CD81 antibodies have been utilized to effectively block HCV infection of mice with humanized livers [Lanford *et al.* 2009]. However, they do not affect HCV infection after it has been established. Monoclonal and polyclonal antibodies with HCV envelope neutralizing capacity have been tested in humans at the time of liver transplant but have been ineffective in preventing reinfection of the donor liver; however, this

remains a potential perioperative strategy during transplantation [Davis *et al.* 2005]. The currently available antibodies' lack of efficacy may be attributed to the heterogeneity of the virus, its association with apolipoproteins, or other factors.

Another approach to blocking cellular infection is to inhibit binding and processing of HCV via the cell surface receptor proteins involved in cell entry. Glycosylation inhibitors may alter the structure of cell surface glycosaminoglycans thereby decreasing or eliminating viral concentration at the cell surface [Pawlotsky *et al.* 2007]. MX-3256 (Celgosivir; Migenix Inc.) is an oral alpha-glucosidase I inhibitor that acts through host-directed glycosylation to prevent proper folding of the HCV envelope. In preclinical studies, celgosivir demonstrated strong synergy with pegylated interferon plus ribavirin, but a phase IIa monotherapy study did not show any reduction in HCV-RNA [Kaita *et al.* 2007; Yoshida *et al.* 2006]. Unfortunately, a 12-week study of another glycosylation inhibitor, UT-231B (Unither Pharmaceuticals), in HCV-infected patients who previously failed interferon-based therapy also failed to reduce virus levels. Despite these early setbacks, this remains an interesting target for future therapies.

In addition, the lectin cyanovirin-N interacts with HCV envelope glycoproteins and blocks the association between E2 and CD81 [Helle *et al.* 2006]. Therefore, targeting the cell surface and/or viral glycans could be a promising approach to anti-viral therapy.

Virus uncoating, HCV-RNA release and attachment to the endoplasmic reticulum

The HCV and receptor complex fuses with the hepatocyte cell membrane and undergoes clathrin-mediated endocytosis. Acidification of the vesicle leads to fusion of the viral and vesicle membranes resulting in uncoating and release of the viral RNA into the cytoplasm. The presence of viral RNA in the cytosol has pathogenic and potential therapeutic implications.

The cell recognizes the presence of single-strand RNA (ssRNA) in the cytosol as abnormal and initiates a pathogen-associated molecular pattern (PAMP)-associated response via toll-like receptors [Sen, 2001]. This process involves activation of retinoic acid inducible gene-1 (RIG-1) and Toll-IL-1 receptor domain containing

adaptor-inducing interferon-beta (TRIF) that under normal circumstances leads to cellular production of type 1 interferons and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis [Saito and Gale, 2008]. However, HCV is capable of down-regulating this step of the innate immune response. The HCV NS3/4 protease cleaves and inactivates the RIG-1 adaptor protein IFN-beta promoter stimulator-1 (IPS-1) and TRIF itself thereby blocking downstream activation of the interferon regulatory genes [Zhu *et al.* 2007; Foy *et al.* 2005; Li *et al.* 2005]. Therefore, NS3/4 inhibition (see later), in addition to its direct effect on viral polyprotein processing, has the potential to restore the RIG-1 and TRIF pathways of innate immunity.

The cytosolic viral ssRNA is also a vulnerable potential target for therapeutic oligonucleotides such as antisense nucleotides (small non-coding strands of RNA that hybridize and inactivate mRNA) or ribozymes (RNA molecules that catalyze cleavage of a target RNA). However, these agents require an absolutely conserved target in an otherwise very heterogeneous virus in order to have their effect. The internal ribosomal entry site (IRES) at the 5' end of the viral RNA is that highly conserved target. IRES is the landing pad that directs the positive strand HCV-RNA to the endoplasmic reticulum (ER) for protein translation. Thus, inhibition of attachment of IRES to both cellular and viral proteins by oligonucleotides could effectively inhibit HCV replication. While several class-specific problems with oligonucleotides such as drug delivery, instability, proinflammatory effects, and other unintended 'off-target' side effects have been partially overcome by modifications of the compounds, all candidate drugs to date have been plagued by safety concerns. Development of Heptazyme (Ribozyme Pharmaceuticals, Inc.), a ribozyme that cleaved IRES, was stopped secondary to toxicity. ISIS-14803 (ISIS Pharmaceuticals), a 20 base pair antisense oligodeoxynucleotide, led to a 1.2 to 1.7 log decline in HCV RNA when given as monotherapy three times a week for 4 weeks in three out of 28 patients; however, asymptomatic liver function test abnormalities also occurred in five treated patients [McHutchison *et al.* 2006]. VGX-410C (VGX Pharmaceuticals) was a small molecule that also targeted HCV IRES binding. It appeared to be safe in phase 2 clinical trials, but was not effective. Despite these early setbacks, this approach

remains intriguing and warrants further exploration.

Polyprotein translation and protein processing

Once the viral RNA attaches to the ER, a single large polyprotein is translated. This polyprotein is then co- and post-translationally processed by host and viral proteases into at least 11 viral proteins. Two host cellular pepsidases are required for cleaving HCV structural proteins. These are not targets for HCV therapy as they are essential for cellular function. NS2 complexes with NS3 and zinc to form a cystine protease. This complex autocatalytically cleaves NS2 from NS3, and is then degraded by the proteasome. To date, no inhibitors of the NS2 protease have entered clinical development.

In contrast, the NS3 protease has been the primary focus of recent drug development. NS3 complexes with NS4A and acts as a serine protease to cleave the polyprotein at the NS3-NS4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B sites. The NS3-NS4A complex's catalytic triad lies adjacent to a shallow substrate binding area that has made design of potent inhibitors challenging. It is this active site that is the target for drugs that are currently in clinical trials. Telaprevir (VX-950; Vertex Pharmaceuticals) and boceprevir (SCH503034; Schering-Plough Corporation) are both in phase 3 clinical trials. While these agents are potent inhibitors of HCV replication, monotherapy leads to rapid selection of drug-resistant strains of the virus, typically within days [Kieffer *et al.* 2007]. Therefore, effective treatment, where each drug is dosed three times per day, requires combination with other agents which means that all current studies include pegylated interferon and ribavirin. This significantly reduces the likelihood of drug resistance, but the requirement for frequent dosing with these first-generation agents may impede compliance and increase the chance of resistance outside the setting of clinical trials.

Telaprevir administered during the first 12 weeks of a 24 week course of pegylated interferon and ribavirin in previously untreated genotype-1-infected patients resulted in an SVR in 61% compared with 41% in those treated with pegylated interferon and ribavirin alone for 48 weeks (standard treatment) [McHutchison *et al.* 2008]. A similar trial in Europe achieved an SVR in 68% with the 24-week triple-drug regimen compared to 48% with standard treatment

[Dusheiko *et al.* 2008]. A lower dose or elimination of ribavirin resulted in a lower chance of response. Thus, it appears that, at least for the time being, both pegylated interferon and ribavirin continue to be essential components of treatment. Telaprevir in combination with pegylated interferon and ribavirin is also effective in retreating genotype 1 patients who had relapsed or failed to respond to a prior course of pegylated interferon and ribavirin. The previously described 24 week regimen achieved an SVR in 69% of prior relapsers and 39% of previous non-responders [Manns *et al.* 2009b]. Extension of the total length of treatment to 48 weeks did not appear to improve the response to retreatment. Efficacy and side effects are both increased with triple drug therapy. Rash is most common, but rarely leads to drug discontinuation. Pruritus, nausea, diarrhea and rectal discomfort are also more common.

Twenty-eight weeks of therapy with Boceprevir, given orally three times a day, in combination with peginterferon and ribavirin, led to an SVR rate of 55% in previously untreated genotype 1-infected patients [Kwo *et al.* 2008]. To address concerns about resistance, a second group of patients was treated with a 4-week lead-in phase of pegylated interferon and ribavirin to reduce HCV-RNA levels before the introduction of boceprevir; however, the SVR rate remained unchanged indicating that this is unnecessary. Unlike telaprevir, boceprevir treated patients may benefit from longer therapy. Patients treated with 4 weeks of pegylated interferon and ribavirin were then treated with either 44 weeks of pegylated interferon and ribavirin (38% SVR), 24 weeks of boceprevir, pegylated interferon and ribavirin (56% SVR), or 44 weeks of triple therapy (75% SVR) [Kwo *et al.* 2009]. Side effects with boceprevir include headache, gastrointestinal complaints and anemia that may limit the ability to maintain the ribavirin dose.

ITMN-191 (Intermune, Inc.), another protease inhibitor, has been used in combination with pegylated interferon and ribavirin for 14 days to achieve an undetectable HCV-RNA in 71% of treated patients [Kamal and Nasser, 2008]. ITMN-191 is active against HCV strains that are resistant to telaprevir and boceprevir. This drug is being studied in combination with pegylated interferon and ribavirin, as well as in combination with a polymerase inhibitor (R7128; Pharmasset, Inc. and Roche) without either

interferon or ribavirin. The latter study is the first proof-of-concept study of an interferon-free regimen in humans. After 14 days of double therapy, virus levels had declined by 4.8 to 5.2 logs and 63%–71% of patients had no detectable virus in serum [Gane *et al.* 2009].

TCM435 (Tibotec Pharmaceuticals Limited and Medivir) is a once daily oral NS3 protease inhibitor. A 4.3 to 5.5 log drop (depending on the dose) in viral load was achieved after 28 days when TCM435 was combined with pegylated interferon and ribavirin in genotype 1 HCV-infected patients who were previous nonresponders or relapsers to interferon based therapy [Marcellin *et al.* 2009]. Three more clinical trials utilizing TCM435 with pegylated interferon and ribavirin have been registered at ClinicalTrials.gov, two in genotype 1 and one in other genotypes.

Although in the preclinical phase of testing, MK-7009 (Merck) is another NS3 protease inhibitor with impressive potency. This drug caused a drop of over 5 log₁₀ in HCV RNA in 5 days in chimpanzees [Lanford *et al.* 2009]. When combined with pegylated interferon and ribavirin 69 to 82% achieved a rapid virologic response compared with 6% who were treated with pegylated interferon and ribavirin [Manns *et al.* 2009a]. Like MK-7009, there are many other protease inhibitors in various stages of clinical trials.

Another potential target at the translation step of replication is NS4A. NS4A complexes with NS3 to stabilize the protease functions and anchor the protein complex to the endoplasmic reticulum. ACH-806 (Achillion Pharmaceuticals Inc.), a selective NS4A binder, *in vitro* caused synergistic inhibition of HCV viral replication when used in combination with NS3 protease inhibitors such as telaprevir [Wyles *et al.* 2008].

The addition of a protease inhibitor to pegylated interferon and ribavirin remains the most promising next step in the near future to improve SVR rates with HCV therapy. These drugs are potent HCV polyprotein processing inhibitors, may restore host innate immunity, may improve the sensitivity to interferon and are orally bioavailable. These drugs are not without their limitations, however. They are genotype and probably subtype specific to various degrees, have their own unique side effects, and will likely remain

susceptible to resistance, particularly if there is not strict adherence to the dosing regimen. Therefore, the future of HCV therapy lies with improved pharmacodynamics and in combinations of agents targeting different sites and mechanisms of the viral life cycle.

HCV-RNA transcription

Viral transcription occurs in a replicase complex built upon a membranous web within the hepatocyte that is comprised of host and viral elements, including the viral polymerase. NS4B is essential to construction of the membranous web and has important RNA-binding activity facilitating attachment of the positive strand viral RNA [Egger *et al.* 2002]. Cyclophilin A recruits NS5B, the RNA-dependent RNA polymerase, to the viral replication complex. After gathering these essential elements at the replication complex, the process of strand replication is directed by the viral polymerase and generates both the negative strand template and positive strand progeny to incorporate into new virus particles. The newly transcribed positive strand is unwound from the template by the viral helicase and this single positive sense strand is now available for translation, transcription or packaging in new virions.

The HCV RNA-dependent RNA polymerase can be inhibited by targeting the RNA-binding site or one of the other four nonnucleoside allosteric sites. Several agents are now in various stages of clinical development. R7128 is an active site NS5B inhibitor that, in combination with pegylated interferon and ribavirin, led to an 85% loss of detectable virus after just 4 weeks (rapid virologic response, RVR) in naïve genotype 1 patients. Only 10% of controls receiving pegylated interferon and ribavirin achieved a RVR [Lalezari *et al.* 2008]. R1626 (Roche), another active site NS5B inhibitor, given orally twice a day in combination with pegylated interferon and ribavirin led to a 74% RVR as compared to a 5% RVR in patients treated with pegylated interferon and ribavirin [Pockros *et al.* 2008]. Unfortunately, R1626 was associated with dose-limiting neutropenia. NM283 (Idenix Pharmaceuticals, Inc.), a third active site inhibitor of NS5B, was aborted secondary to gastrointestinal side effects.

Several new drugs have also been developed against the HCV polymerase's allosteric sites. HCV-796 (ViroPharma, Inc.) had entered

phase 2 clinical trials in combination with pegylated interferon and ribavirin; however, further study of this drug was discontinued secondary to elevated liver function tests in treated patients [Evans *et al.* 2007]. VCH-759 (Vertex Pharmaceuticals), another HCV allosteric inhibitor of the NS5B polymerase, was used in phase 1 clinical trials as an oral agent dosed three times per day [Cooper *et al.* 2009]. Although drug resistance was seen, a 2.5 log₁₀ drop in HCV viral load was documented after 10 days of monotherapy with the highest dose. Pfizer's compound, PF-00868554, in monotherapy at the highest dose resulted in a 1.95 log drop in viral load in HCV-infected patients [Hammond *et al.* 2008]. Therefore it has progressed to phase 2 clinical trials.

Polymerase inhibitors represent the second most attractive target for HCV therapy after protease inhibitors. In addition to the agents described above, numerous others are currently in early development. Perhaps more than with the protease inhibitors, this class of drug has been plagued with unacceptable side effects that have led to discontinuation of investigation of several otherwise promising compounds. The predominant unacceptable side effects have been nausea, vomiting and diarrhea, but like the protease inhibitor side effects, these may be unique to each agent rather than class effects. Although the nucleoside inhibitors may be genotype specific, they may have fewer problems with resistance than drugs against other targets. The non-nucleoside inhibitors have encountered rapid emergence of resistance, which may limit their usefulness unless they are part of a multi-drug cocktail.

There are several potential targets at the step of viral transcription other than direct polymerase inhibitors. The NS4B, critical for both construction of the replicase complex and RNA-binding, is a potential target for therapy that has yet to be exploited [Lanford *et al.* 2009]. Cyclophilin inhibitors, such as Debio-025 (Debiopharm Group) and NIM-811 (Novartis), inhibit RNA polymerase incorporation in the replicase complex. Debio-025 is a once-a-day oral agent that has already been utilized alone and in combination with pegylated interferon and ribavirin in HCV-infected patients [Flisiak *et al.* 2008]. When combined with pegylated interferon and ribavirin, it resulted in a 4.75-log drop in serum HCV RNA levels in 29 days, compared with a 2.49-log

decline in HCV RNA levels with pegylated interferon alone and a 2.2-log decline in HCV RNA levels with DEBIO-025 alone. NS5A is a multi-function protein that is essential to genome replication, particle assembly and the host response to the virus [Best *et al.* 2005]. Its precise role in replication is not entirely understood, but because it is a multifunctional protein it is an extremely attractive target for therapeutic intervention. Two novel NS5A inhibitors have been reported [Lanford *et al.* 2009]. BMS790052 (Bristol-Myers Squibb) resulted in a 3.6 log decline in HCV RNA following a single dose. Like the protease inhibitors, resistance to NS5A inhibitors has already been reported. Finally, the viral helicase is a potential target. Helicase inhibitors must be specific for the viral helicase, avoiding inhibition of host cellular helicases [Dubuisson, 2007; Myong *et al.* 2007]. No HCV helicase inhibitors are currently in clinical trials, but preclinical studies of a herpes simplex virus helicase inhibitor have shown promise [Jankowsky and Fairman, 2007; Kwong *et al.* 2005].

Viral assembly and export

Viral particle formation is initiated by the interaction of the core protein with genomic RNA in the endoplasmic reticulum [Mizuno *et al.* 1995; Tanaka *et al.* 2000]. Viral particle assembly requires N-glycosylation of envelope proteins for proper envelope folding. This process of envelope folding and configuration is essential for assembly of new virions, virus export, antigenicity and receptor binding for reinfection. Therefore, agents that alter envelope glycosylation may interfere with numerous steps in the viral life cycle (see MX-3256 under 'Early inhibitors: receptor binding and cell entry').

Other

Interferons have been the cornerstone of hepatitis C treatment for more than two decades. Although interferons stimulate the host innate immune response and initiate numerous antiviral mechanisms within the cell, the precise effects responsible for its efficacy in HCV infection are not known. However, some specific host innate immune pathways have recently been identified and exploited as potential targets for therapeutics. For example, two toll-like receptor-9 (TLR-9) agonists are in clinical trials. One such compound, CPG 10101 (Pfizer), is a synthetic oligodeoxynucleotide that activates TLR-9 leading to stimulation of B cells and plasmacytoid

dendritic cells that in turn secrete antiviral cytokines [McHutchison *et al.* 2007]. CPG 10101 when given as monotherapy to HCV-infected patients decreased HCV viral loads in a dose dependent manner, with the highest dose achieving a 1.7 log reduction after 4 weeks. One of its major effects is to increase cellular interferon. Whether this endogenous interferon will prove superior to administration of exogenous interferon is not clear.

KRN7000 (Kyowa Hakko Kirin Company, Limited) is another immune activator; specifically, a synthetic analog of α -galactosylceramide, which stimulates natural killer T cells (NKT) in mice and humans. Immune activation of NKT cells leads to cytokine production of interferon- γ and tumor necrosis factor- α (TNF- α). Although a phase one clinical trial has been completed no results have been published (<http://clinicaltrials.gov/ct2/show/NCT00352235>). It remains unclear if further development of drugs that target immune activation will lead to oral medications with minimal side effects, or if we might simply discover other ways of producing cytokines with unacceptable side effects.

NS5A, described above in its role in viral replication, also impairs host immune response to HCV infection in numerous ways. The N-terminal end of NS5A appears to prevent phosphorylation of the Janus Kinases Jak1 and Tyk2 and is essential for interferon signaling. NS5A also induces the pro-inflammatory cytokine interleukin-8 (IL-8), which as been associated with an impaired response to interferon treatment [Mihm *et al.* 2004; Polyak *et al.* 2001]. In addition, NS5A inhibits apoptosis of infected hepatocytes. In other words, NS5A facilitates viral evasion of the host innate immune response. *In vitro* replicon experiments have shown that knockout mutations of NS5A result in improved interferon sensitivity [Bonte *et al.* 2004]. Thus, NS5A inhibitors could enhance the sensitivity of infected cells to interferon and restore the host's innate antiviral response.

Finally, nitazoxanide (Romark Laboratories L.C.) is an antiparasitic agent that was serendipitously discovered to have antiviral activity against hepatitis B and C infection. The proposed mechanism of action is the increase in phosphorylation of the protein kinase PKR that phosphorylates eukaryotic initiation factor 2 α , which leads to the inhibition of HCV-RNA

translation [Darling and Fried, 2009; Rossignol *et al.* 2009; Elazar *et al.* 2008]. The drug has been shown to improve SVR rates in patients infected with genotype 4 HCV, the predominant viral genotype in Egypt where these studies were conducted. Specifically, 79% of patients achieved an SVR when 12 weeks of nitazoxanide was followed by 36 weeks of the drug combined with pegylated interferon and ribavirin. Just 50% of those treated with pegylated interferon and ribavirin alone achieved an SVR [Rossignol *et al.* 2009]. Studies in patients infected with genotypes common in the United States and Europe are underway.

Conclusions

Future therapy for HCV is firmly rooted in the exploitation of our understanding of the viral life cycle. As our understanding of the viral life cycle continues to evolve, novel targets are revealed and refined. Protease inhibitors of the NS3 protease are the farthest along in clinical development and show great promise for the very near future. We are hopeful that at least one will be available for clinical use by 2011. Polymerase inhibitors are not far behind, and combinations of the two together are already beginning. Cell entry and virus assembly are the least understood steps in the viral life cycle and therefore development of drugs targeting those steps is lagging. Through further understanding of the viral life cycle, the future promises highly effective combinations of agents that attack different targets. Since drug resistance will likely be a significant problem, even multiple targets in the same step of replication may be utilized.

Conflict of interest statement

GD has received research grants from Human Genomic Science, Merck, Novartis, Schering-Plough, Tibotec, Vertex.

References

- Alter, M.J., Kruszon-Moran, D., Nainan, O.V., McQuillan, G.M., Gao, F., Moyer, L.A. *et al.* (1999) The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 341: 556–562.
- Andre, P., Perlemuter, G., Budkowska, A., Brechot, C. and Lotteau, V. (2005) Hepatitis C virus particles and lipoprotein metabolism. *Semin Liver Dis* 25: 93–104.
- Armstrong, G.L., Wasley, A., Simard, E.P., McQuillan, G.M., Kuhnert, W.L. and Alter, M.J. (2006) The prevalence of hepatitis C virus infection in

- the United States, 1999 through 2002. *Ann Intern Med* 144: 705–714.
- Best, S.M., Morris, K.L., Shannon, J.G., Robertson, S.J., Mitzel, D.N., Park, G.S. *et al.* (2005) Inhibition of interferon-stimulated JAK-STAT signaling by a tick-borne flavivirus and identification of NS5 as an interferon antagonist. *J Virol* 79: 12828–12839.
- Bonte, D., Francois, C., Castelain, S., Wychowski, C., Dubuisson, J., Meurs, E.F. *et al.* (2004) Positive effect of the hepatitis C virus nonstructural 5A protein on viral multiplication. *Arch Virol* 149: 1353–1371.
- Bruno, S., Stroffolini, T., Colombo, M., Bollani, S., Benvegna, L., Mazzella, G. *et al.* (2007) Sustained virological response to interferon- α is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 45: 579–587.
- Camma, C., Di Bona, D., Schepis, F., Heathcote, E.J., Zeuzem, S., Pockros, P.J. *et al.* (2004) Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. *Hepatology* 39: 333–342.
- Cooper, C., Lawitz, E.J., Ghali, P., Rodriguez-Torres, M., Anderson, F.H., Lee, S.S. *et al.* (2009) Evaluation of VCH-759 monotherapy in hepatitis C infection. *J Hepatol* 51: 39–46.
- Cormier, E.G., Durso, R.J., Tsamis, F., Boussemart, L., Manix, C., Olson, W.C. *et al.* (2004a) L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. *Proc Natl Acad Sci USA* 101: 14067–14072.
- Cormier, E.G., Tsamis, F., Kajumo, F., Durso, R.J., Gardner, J.P. and Dragic, T. (2004b) CD81 is an entry coreceptor for hepatitis C virus. *Proc Natl Acad Sci USA* 101: 7270–7274.
- Darling, J.M. and Fried, M.W. (2009) Nitazoxanide: beyond parasites toward a novel agent for hepatitis C. *Gastroenterology* 136: 760–763.
- Davis, G.L., Albright, J.E., Cook, S.F. and Rosenberg, D.M. (2003) Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl* 9: 331–338.
- Davis, G.L., Nelson, D.R., Terrault, N., Pruett, T.L., Schiano, T.D., Fletcher, C.V. *et al.* (2005) A randomized, open-label study to evaluate the safety and pharmacokinetics of human hepatitis C immune globulin (Civacir) in liver transplant recipients. *Liver Transpl* 11: 941–949.
- Di Bisceglie, A.M., Everson, G.T., Lindsay, K.L., Everhart, J.E., Wright, E.C., Lee, W.M. *et al.* (2007) Prolonged antiviral therapy with peginterferon to prevent complications of advanced liver disease associated with hepatitis C: results of the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) trial. *Hepatology* 46: ALB1.
- Dubuisson, J. (2007) Hepatitis C virus proteins. *World J Gastroenterol* 13: 2406–2415.
- Dusheiko, G.M., Hezode, C., Pol, S., Goeser, T., Bronowicki, J.P., Bourliere, M. *et al.* (2008) Treatment of chronic hepatitis C with telaprevir (TVR) in combination with peginterferon- α -2a with or without ribavirin: further interim analysis results of the PROVE2 study. *J Hepatol* 48: A58.
- Egger, D., Wolk, B., Gosert, R., Bianchi, L., Blum, H.E., Moradpour, D. *et al.* (2002) Expression of hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex. *J Virol* 76: 5974–5984.
- El-Serag, H.B. (2004) Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 127: S27–34.
- Elazar, M., Lui, M., McKenna, S., Liu, P., Gehrig, E.A., Elfert, A. *et al.* (2008) Nitazoxanide (NTZ) is an induced or eIF2 α and PKR phosphorylation. *Hepatology* 48: A1881.
- Evans, M.J., von Hahn, T., Tscherne, D.M., Syder, A.J., Panis, M., Wolk, B. *et al.* (2007) Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 446: 801–805.
- Fattovich, G., Giustina, G., Degos, F., Tremolada, F., Diiodati, G., Almasio, P. *et al.* (1997) Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 112: 463–472.
- Fattovich, G., Pantalena, M., Zagni, I., Realdi, G., Schalm, S.W. and Christensen, E. (2002) Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. *Am J Gastroenterol* 97: 2886–2895.
- Flisiak, R., Feinman, S.V., Jablkowski, M., Horban, A., Kryczka, W., Halota, W. *et al.* (2008) Efficacy and safety of increasing doses of the cyclophilin inhibitor DEBIO 025 in combination with pegylated interferon α -1a in treatment naive chronic HCV patients. *J Hepatol* 48: A143.
- Foy, E., Li, K., Sumpter, R., Loo, Y.M., Johnson, C.L., Wang, C. *et al.* (2005) Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. *Proc Natl Acad Sci USA* 102: 2986–2991.
- Freeman, A.J., Dore, G.J., Law, M.G., Thorpe, M., Von Overbeck, J., Lloyd, A.R. *et al.* (2001) Estimating progression to cirrhosis in chronic hepatitis C virus infection. *Hepatology* 34: 809–816.
- Fried, M.W., Shiffman, M.L., Reddy, K.R., Smith, C., Marinos, G., Goncales, F.L. *et al.* (2002) Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347: 975–982.
- Gane, E., Roberts, S., Stedman, C., Angus, P., Ritchie, B., Elston, R. *et al.* (2009) First-in-man demonstration of potent antiviral activity with a nucleoside polymerase (R7128) and protease (R7227/ITMN-191) inhibitor combination in HCV; safety,

- pharmacokinetics, and virologic results from inform-1. *J Hepatol* 50: A1046.
- Hammond, J., Rosario, M., Wagner, F., Mazur, D., Kantaridis, C., Purohit, V. *et al.* (2008) Antiviral activity of hte HCV polymerase inhibitor PF-00868554 administered as monotherapy in HCV genotype 1 infected subjects. *Hepatology* 48: LB11.
- Helle, F., Wychowski, C., Vu-Dac, N., Gustafson, K.R., Voisset, C. and Dubuisson, J. (2006) Cyanovirin-N inhibits hepatitis C virus entry by binding to envelope protein glycans. *J Biol Chem* 281: 25177–25183.
- Jankowsky, E. and Fairman, M.E. (2007) RNA helicases – one fold for many functions. *Curr Opin Struct Biol* 17: 316–324.
- Kaita, K., Yoshida, E., Kunimoto, D., Anderson, F., Morris, S., Marotta, P. *et al.* (2007) Proof of concept study of celgosivir in combination with peginteron a-2b and ribavirin in chronic hepatitis C genotype-1 non-responder patients. *J Hepatol* 46: A127.
- Kamal, S.M. and Nasser, I.A. (2008) Hepatitis C genotype 4: what we know and what we don't yet know. *Hepatology* 47: 1371–1383.
- Kieffer, T.L., Sarrazin, C., Miller, J.S., Welker, M.W., Forestier, N., Reesink, H.W. *et al.* (2007) Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. *Hepatology* 46: 631–639.
- Kwo, P., Lawitz, K.P., McCone, J., Schiff, E., Vierling, J., Pound, D. *et al.* (2008) Interim results from HCV SPRINT-1: RVR/EVR from phase 2 study of boceprevir plus peginteron (peginterferon alpha-2b)/ribavirin in treatment-naïve subjects with genotype-1 CHC. *J Hepatol* 48: A995.
- Kwo, P., Lawitz, E., McCone, J., Schiff, E., Vierling, J., Pound, D. *et al.* (2009) HCV SPRINT-1 Final results: SVR 24 from a phase 2 study of boceprevir plus peginteron (peginterferon alpha-2b)/ribavirin in treatment naïve subjects with genotype-1 chronic hepatitis C. *J Hepatol* 50: A4.
- Kwong, A.D., Rao, B.G. and Jeang, K.T. (2005) Viral and cellular RNA helicases as antiviral targets. *Nat Rev Drug Discov* 4: 845–853.
- Lalezari, J., Gane, E., Rodriguez-Torres, M., DeJesus, E., Nelson, D., Everson, G.T. *et al.* (2008) Potent antiviral activity of the HCV nucleoside polymerase inhibitor R7128 with PEG-IFN and ribavirin: interim results of R7128 500mg for 28 days. *J Hepatol* 48: A66.
- Lanford, R.E., Evans, M.J., Lohmann, V., Lindenbach, B., Gale, M., Rehermann, B. *et al.* (2009) The accelerating pace of HCV research: a summary of the 15th International Symposium on Hepatitis C Virus And Related Viruses. *Gastroenterology* 136: 9–16.
- Li, K., Foy, E., Ferreon, J.C., Nakamura, M., Ferreon, A.C., Ikeda, M. *et al.* (2005) Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc Natl Acad Sci USA* 102: 2992–2997.
- Manns, M., Gane, E., Rodriguez-Torres, M., Stoeckl, A., Yeh, C.-T., Wiedmann, R. *et al.* (2009a) MK-7009 significantly improves rapid viral response (RVR) in combination with pegylated interferon alfa-2a and ribavirin in patients with chronic hepatitis C (CHC) genotype 1 infection. *J Hepatol* 50: A1056.
- Manns, M., Muir, A., Adda, N., Jacobson, I., Afdhal, N., Heathcote, J. *et al.* (2009b) Telaprevir in hepatitis C genotype-1-infected patients with prior non-response, viral breakthrough or relapse to peginterferon-alpha-a/b and ribavirin therapy: SVR results of the PROVE3 study. *J Hepatol* 50: A1044.
- Manns, M.P., McHutchison, J.G., Gordon, S.C., Rustgi, V.K., Shiffman, M., Reindollar, R. *et al.* (2001) Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 358: 958–965.
- Marcellin, P., Reesink, H., Cramp, M., Flisiak, R., Van Vlierberghe, H., Verloes, R. *et al.* (2009) Antiviral activity and safety of TMC435 combined with peginterferon a2a and ribavirin in patients with genotype-1 hepatitis c infection who failed previous IFN based therapy. *J Hepatol* 50: A1058.
- McHutchison, J.G., Bacon, B.R., Gordon, S.C., Lawitz, E., Shiffman, M., Afdhal, N.H. *et al.* (2007) Phase 1B, randomized, double-blind, dose-escalation trial of CPG 10101 in patients with chronic hepatitis C virus. *Hepatology* 46: 1341–1349.
- McHutchison, J.G., Everson, G.T., Gordon, S.C., Jacobson, I., Kauffman, R. and McNair, L. (2008) PROVE1: Results from a phase 2 study of telaprevir with peginterferon alpha-2a and ribavirin in treatment-naïve subjects with hepatitis C. *J Hepatol* 48: A4.
- McHutchison, J.G., Patel, K., Pockros, P., Nyberg, L., Pianko, S., Yu, R.Z. *et al.* (2006) A phase I trial of an antisense inhibitor of hepatitis C virus (ISIS 14803), administered to chronic hepatitis C patients. *J Hepatol* 44: 88–96.
- Mihm, U., Herrmann, E., Sarrazin, U., von Wagner, M., Kronenberger, B., Zeuzem, S. *et al.* (2004) Association of serum interleukin-8 with virologic response to antiviral therapy in patients with chronic hepatitis C. *J Hepatol* 40: 845–852.
- Mizuno, M., Yamada, G., Tanaka, T., Shimotohno, K., Takatani, M. and Tsuji, T. (1995) Virion-like structures in HeLa G cells transfected with the full-length sequence of the hepatitis C virus genome. *Gastroenterology* 109: 1933–1940.
- Myong, S., Bruno, M.M., Pyle, A.M. and Ha, T. (2007) Spring-loaded mechanism of DNA unwinding by hepatitis C virus NS3 helicase. *Science* 317: 513–516.

- Pawlotsky, J.M., Chevaliez, S. and McHutchison, J.G. (2007) The hepatitis C virus life cycle as a target for new antiviral therapies. *Gastroenterology* 132: 1979–1998.
- Pileri, P., Uematsu, Y., Campagnoli, S., Galli, G., Falugi, F., Petracca, R. *et al.* (1998) Binding of hepatitis C virus to CD81. *Science* 282: 938–941.
- Ploss, A., Evans, M.J., Gaysinskaya, V.A., Panis, M., You, H., de Jong, Y.P. *et al.* (2009) Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 457: 882–886.
- Pockros, P.J., Nelson, D., Godofsky, E., Rodriguez-Torres, M., Everson, G.T., Fried, M.W. *et al.* (2008) R1626 plus peginterferon Alfa-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin. *Hepatology* 48: 385–397.
- Polyak, S.J., Khabar, K.S., Rezeiq, M. and Gretch, D.R. (2001) Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. *J Virol* 75: 6209–6211.
- Rocha-Perugini, V., Montpellier, C., Delgrange, D., Wychowski, C., Helle, F., Pillez, A. *et al.* (2008) The CD81 partner EWI-2wint inhibits hepatitis C virus entry. *PLoS One* 3: e1866.
- Rossignol, J.F., Elfert, A., El-Gohary, Y. and Keeffe, E.B. (2009) Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. *Gastroenterology* 136: 856–862.
- Saito, T. and Gale, M. (2008) Regulation of innate immunity against hepatitis C virus infection. *Hepatology* 48: 115–122.
- Sangiovanni, A., Prati, G.M., Fasani, P., Ronchi, G., Romeo, R., Manini, M. *et al.* (2006) The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. *Hepatology* 43: 1303–1310.
- Schuster, C. and Baumert, T.F. (2009) EWI-2wint – a host cell factor inhibiting hepatitis C virus entry. *J Hepatol* 50: 222–224.
- Sen, G.C. (2001) Viruses and interferons. *Annu Rev Microbiol* 55: 255–281.
- Serfaty, L., Aumaitre, H., Chazouilleres, O., Bonnard, A.M., Rosmorduc, O., Poupon, R.E. *et al.* (1998) Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 27: 1435–1440.
- Tanaka, Y., Shimoike, T., Ishii, K., Suzuki, R., Suzuki, T., Ushijima, H. *et al.* (2000) Selective binding of hepatitis C virus core protein to synthetic oligonucleotides corresponding to the 5' untranslated region of the viral genome. *Virology* 270: 229–236.
- Wasley, A. and Alter, M.J. (2000) Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 20: 1–16.
- WHO. (1999) Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J Viral Hepat* 6: 35–47.
- Wyles, D.L., Kaihara, K.A. and Schooley, R.T. (2008) Synergy of a hepatitis C virus (HCV) NS4A antagonist in combination with HCV protease and polymerase inhibitors. *Antimicrob Agents Chemother* 52: 1862–1864.
- Yoshida, E., Kunimoto, D., Lee, S., Sherman, M., Heathcote, J. and Enns, R. (2006) Results of a phase IIa dose ranging study of orally administered Celgosivir as monotherapy in chronic hepatitis C genotype-1 patients. *Gastroenterology* 130: A784.
- Zeisel, M.B., Koutsoudakis, G., Schnober, E.K., Haberstroh, A., Blum, H.E., Cosset, F.L. *et al.* (2007) Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. *Hepatology* 46: 1722–1731.
- Zhu, H., Dong, H., Eksioglu, E., Hemming, A., Cao, M., Crawford, J.M. *et al.* (2007) Hepatitis C virus triggers apoptosis of a newly developed hepatoma cell line through antiviral defense system. *Gastroenterology* 133: 1649–1659.

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