Antiviral Research 100 (2013) 555–561

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

Host-targeting agents in the treatment of hepatitis C: A beginning and an end?

James M. Baugh [1], Jose A. Garcia-Rivera [1], Philippe A. Gallay *

Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA 92037, USA

ARTICLE INFO

Article history:
Received 13 June 2013
Revised 8 September 2013
Accepted 23 September 2013
Available online 30 September 2013

Keywords:
Host-targeting antivirals
Alisporivir
HCV
Cyclophilin A

ABSTRACT

The development of two distinct classes of hepatitis C antiviral agents, direct-acting antivirals (DAAs) and host-targeting antivirals (HTAs), have distinctly impacted the hepatitis C virus (HCV) field by generating higher sustained virological response (SVR) rates within infected patients, via reductions in both adverse side effects and duration of treatment when compared to the old standard of care. Today DAAs are actively incorporated into the standard of care and continue to receive the most advanced clinical trial analysis. With a multitude of innovative and potent second-generation DAA compounds currently being tested in clinical trials, it is clear that the future of DAAs looks very bright. In comparison to the other class of compounds, HTAs have been slightly less impactful, despite the fact that primary treatment regimens for HCV began with the use of an HTA – interferon alpha (IFNα). The compound was advantageous in that it provided a broad-reaching antiviral response; however deleterious side effects and viral/patient resistance has since made the compound outdated. HTA research has since moved onward to target a number of cellular host factors that are required for HCV viral entry and replication such as scavenger receptor-BI (SR-BI), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoA reductase), cyclophilin A (CypA), fatty acid synthase (FASN) and miRNA-122. The rationale behind pursuing these HTAs is based upon the extremely low mutational rate that occurs within eukaryotic cells, thereby creating a high genetic barrier to drug resistance for anti-HCV compounds, as well as pan-genotypic coverage to all HCV genotypes and serotypes. As the end appears near for HCV, it becomes important to ask if the development of novel HTAs should also be analyzed in combination with other DAAs, in order to address potential hard-to-treat HCV patient populations. Since the treatment regimens for HCV began with the use of a global HTA, could one end the field as well?

Contents

* Corresponding author. Address: Department of Immunology and Microbial Science, IMM-9, The Scripps Research Institute, 10550N Torrey Pines Rd, La Jolla, CA 92037, USA. Tel.: +1 858 784 8180.
E-mail address: gallay@scripps.edu (P.A. Gallay).
[1] These authors contributed equally to this work.

0166-3542/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2013.09.020

1. Introduction

Hepatitis C virus (HCV) is a growing global pandemic with an estimated 2–3% of the world's population thought to be infected with the virus, superseding HIV as the leading cause of death due to an infectious agent in the United States (Lavanchy, 2009). HCV comprises seven genotypes, more than 50 subtypes, and billions of quasi species (Grimm, 2012). It is spread via contaminated blood with 50–80% of infected patients eventually developing chronic hepatitis C, which in turn can lead to liver cirrhosis. After the onset of cirrhosis, hepatocellular carcinoma occurrence becomes the next step in the progression of the disease with annual rates between 1–8% (Ghany et al., 2009; Lauer and Walker, 2001; Poynard et al., 2003). At the moment, no vaccine is available and up until recently the traditional standard of care (SOC) was merely comprised of the combination of injected pegylated-interferon alpha (pegIFN) and oral ribavirin (RBV) administered for up to 48 weeks, which in itself is associated with serious and potentially life-threatening side effects (Ghany et al., 2009). Although this regimen was capable of achieving up to 80% SVR_{12} (undetectable HCV-RNA in the blood 12 weeks after the completion of a treatment regimen) rates in nongenotype 1 patients, the percentage of genotype 1 patients who reach SVR_{12} on this treatment was drastically reduced to 40–50%. Given that genotype 1 infections account for approximately 60% of global infections, more effective therapies were developed (Ghany et al., 2009; Magiorkinis et al., 2009).

A better understanding of virus replication coupled with the need to achieve higher SVR rates, particularly in genotype 1 patients, brought about the development of the first set of DAAs. In 2011, the U.S. Food and Drug Administration (FDA) granted approval for the use of two small molecule inhibitors telaprevir (Incivek/Incivo) and boceprevir (Victrelis), whose target is the HCV-encoded protease NS3. Phase III clinical trials achieved SVR rates of up to 75% in treatment-naïve genotype-1-infected patients when combined with pegIFN/RBV. However, major limitations still exist within these DAA regimens, namely: (i) additional side effects; (ii) increased risk of developing drug-resistant variants; and (iii) limited to only genotype 1 patients. Buhler and Bartenschlager, (2012) recently proposed a set of criteria that ideal future therapies of chronic hepatitis C should (i) have to be free of pegIFN in order to reduce side effects; (ii) impose a high barrier of drug resistance; (iii) require only short treatment durations; and (iv) provide more than 90% SVR_{12} rates.

2. HCV Host-targeting antivirals

A number of host-targeting antivirals (HTAs) have been developed and tested to combat HCV. Many are broad target HTAs that work by creating an activated anti-viral state within the host by triggering arms of innate immune response, examples of which include IFNα, interferon lambda (IFNλ), or Toll Like Receptor (TLR) agonists. The targeted group of HTAs are more precise in that they act upon key host enzymes or cellular factors that are required for the HCV lifecycle, such as cyclophilin A (CypA), fatty acid synthase (FASN), and miRNA-122. Generally speaking, the primary advantage of targeting host factors is the extremely low rate of mutation within the host that arises during times of viral or chemotoxic stress, thereby affording a higher barrier to drug resistance while also limiting the potential of viral breakthrough. Since each HTA acts upon a unique step of the virus life cycle, it is also reasonable to believe that these compounds should act synergistically with one another, or even approved DAAs, further expanding the anti-HCV arsenal to address the most difficult to treat cases. Finally, since a number of viruses hijack similar cellular machine it also seems reasonable to develop HTAs that could potentially be used to combat new and emerging infectious diseases.

Of all of the general HTAs that are targeted to activate the immune response, the one that is the best studied, tested, and understood is IFNα. Treatment of HCV patients with IFNα has been ongoing for the last 25 years (Heim, 2013). The presence of circulating IFNα within a patient leads to receptor binding via the cell, followed by a signaling cascade that promotes the induction of hundreds of antiviral proteins, so-called interferon-stimulated genes (ISGs) (Stetson and Medzhitov, 2006). In an infected cell these ISGs can shut down the host transcription and translation machinery in a myriad of ways, ranging from simple post-translational modifications to proteolytic cleavage of host regulatory elements, collectively serving to create an antiviral state within the cell (Heim, 2013). IFNα has cured many cases of HCV, over a long period of time, during which no other viable alternatives were even available. However the treatment itself has less favorable cure rates in the predominant HCV genotype 1, often leads to the generation of viral escape mutants, and is commonly associated with dramatic side effects (Ghany et al., 2009). Recent cell culture work has gone onto further highlight that HCV is quite capable of creating escape mutants in a few select regions of the viral genome, over a range of IFNα concentrations (Perales et al., 2013). Ongoing clinical trials are predominantly IFNα-free and have shown to afford very promising results with DAA combinations alone (Gilead Sciences, 2013. Gilead Reports Interim Data from Phase 2 LONESTAR Study. Press Release, May 2, 2013).

In addition to IFNα, the Type III interferon family member IFNλ1 (IFNlambda) has also been tested, in its pegylated form, on HCV patients with either genotype 1, 2, 3, or 4 and showed promising efficacy in combatting the virus (Muir et al., 2010; Zeuzem et al., 2011). The advantages and rationale behind testing IFNlambda is that: (i) it possesses a receptor specificity distinct from IFNα, (ii) while maintaining antiviral inducing effects comparable to IFNα, and (iii) the receptors are selectively expressed only on specific cell types, which should translate to fewer patient side effects (Donnelly and Kotenko, 2010). Recent work in a cell culture model system has shown that HCV is capable of generating escape mutants to IFN lambda treatment alone (Friborg et al., 2013b), however IFNlambda has also been shown to act synergistically with DAAs (Friborg et al., 2013a). Taken together, it appears that a general antiviral tool such as IFNlambda should continue in development, especially since it could potentially be a frontline broad range antiviral for new and emerging infectious diseases. Continuing on with the theme of broad-range antiviral inducers, work with TLR agonist also shows a marked enhancement of the antiviral response accompanied by HCV elimination. Specifically two separate clinical trials looked at the treatment of HCV patients with either a Toll-like receptor (TLR) 7 agonist, or a TLR 9 agonist, wherein both demonstrated robust activation of the immune response (Boonstra et al., 2012; Rodriguez-Torres et al., 2010). Importantly, the TLR9 agonist IMO-2125, was shown to lower viral loads in the null responder patient population, (Rodriguez-Torres et al., 2010) exemplifying the importance of developing a variety of drug target options.

2.1. Phosphatidylinositol 4-kinase III alpha

The advent of cell culture models of subgenomic and then fully infectious HCV enabled the expansion of targeted HTA drugs. Specifically, the identification of many essential host proteins required for the HCV lifecycle were discovered by exhaustive siRNA screens directed against the host (Randall et al., 2007). One of the first host targets that were shown to be essential to HCV replication was phosphatidylinositol 4-kinase III alpha (PI4KIIIα) (Berger et al., 2009). It has recently been shown that PI4KIIIα can modulate the

phosphorylation status of NS5A, which in turn modulates the morphology of replication sites, presumably leading to an enhancement in HCV replication (Reiss et al., 2013). Many groups have come out with compounds that target PI4KIIIα and block its downstream phosphorylation activity. However, when Boehringer Ingelheim created conditional PI4KIIIα knockout mice they found that targeting this kinase was lethal, with a complete degeneration of mucosal epithelium in the intestinal track, clearly demonstrating the requirement by the host for this enzyme as well (Vaillancourt et al., 2012). This highlights the key point in specific targeting of host proteins, particularly enzymes, you must know exactly how the drug is working and what are the possible side effects to the host system if you block the activity of said target. Now we will look at more feasible targets.

2.2. Scavenger Receptor B1

HCV uses a multitude of cell surface receptors to enter a hepatic cell (Scheel and Rice, 2013). Recently, a number of groups have developed compounds to block one of these receptors, Scavenger Receptor B1 (SR-B1). The concept of blocking a virus prior to entry into the host is not new; however, the stringent requirement of SR-B1 for HCV entry has afforded some potent efficacy in a cell culture system (Syder et al., 2011). There are two different approaches taken in blocking SR-B1: one group of SR-B1 targeted compounds is antibody based, while the other is a small molecule antagonist, ITX 5061 (Scheel and Rice, 2013). Interestingly, monoclonal antibodies directed against SR-B1 have been tested in a humanized mouse model and were seen to block both HCV infection and dissemination (Lacek et al., 2012). ITX 5061 is a compound that is already in the clinical stage as it was shown to increase high-density lipoproteins (HDLs) in both humans and mice by interfering with SR-B1, the main HDL receptor in the liver (Masson et al., 2009). Importantly, ITX 5061 was shown to act synergistically with protease and polymerase DAAs in a fully infectious HCV cell culture system (Zhu et al., 2012). ITX 5061 is currently being tested in mono-infected HCV patients in a proof-of-concept Phase 1 clinical trial, carried out by the AIDS Clinical Trial Group (ACTG). All of this aside, an important caveat to this group of receptor-targeted compounds is the fact that CD-81-independent cell-to-cell transmission has been previously shown by others (Jones et al., 2010; Witteveldt et al., 2009). Therefore it is imperative, before moving too much further, to demonstrate that HCV cannot undergo cell–cell transmission in the absence of SR-B1, or any other receptor that is targeted.

2.3. FASN inhibitors

The FASN gene encodes for a polypeptide composed of seven functional domains in addition to an acyl-carrier protein, which together, catalyze the *de novo* synthesis of fatty acids (FA) (Kridel et al., 2007). FAs have been previously shown to have a role in the regulation of HCV replication with FASN's primary products, being 16-carbon FA palmitate, 14-carbon myristate and 18-carbon stearate FAs (Huang et al., 2007; Kapadia and Chisari, 2005). Yang et al. (2008) were the first to successfully demonstrate FASN's role in HCV replication, by showing it to be upregulated in the presence of both the genomic JFH-1 virus (genotype 2a) and subgenomic Con1b, although FASN upregulation was substantially weaker in the subgenomic replicon compared to that of whole virus. In another study done by Huang et al. (2013), FASN was shown to interact with the N-terminal domain of NS5B. It was also found to be associated with detergent-resistant lipid rafts and colocalized with NS5B in active HCV replication complexes. Moreover, FASN was shown to directly increase HCV NS5B RNA-dependent RNA polymerase (RdRp) activity *in vitro*, suggesting that this interaction plays an important role in modulating HCV replication.

3-V Biosciences recently presented positive preclinical data with their novel FASN inhibitor, TVB-2640. It was demonstrated that TVB-2640 safely causes FASN inhibition, is rapidly absorbed, is highly bioavailable (~60%) and is well tolerated in both rats and dogs (Evanchik et al., 2012). With FASN inhibitors representing an entirely new category of HTA therapy, combined with a novel mechanism of action and potent antiviral activity, it will be interesting to see data from Phase I/II clinical trials poised to start in 2013. In the case of FASN inhibitors such as 3-V Bioscience's TVB-2640, the exact mechanism of action (MOA) of the compound is not completely understood with regard to its role in impairing HCV replication. What is known is that FASN is upregulated in the presence of HCV infection, it interacts with the HCV NS5B polymerase and increases HCV's NS5B polymerase (Huang et al., 2013; Yang et al., 2008). FASN inhibitors are thought to exert their anti-HCV activity by binding to FASN and thus impeding its ability to interact with NS5B. Again a cautious approach must be taken with this group of compounds, as they are targeting a host enzyme that carries out a host need, it is unclear if there is a redundant compensatory activity in the host.

2.4. miRNA-122

A discovery in 2005 demonstrated the requirement of the liver-specific microRNA, miR-122, to HCV replication (Jopling et al., 2005). MicroRNAs (miRNAs) are small, endogenous non-coding RNAs, which regulate post-transcriptional gene expression by binding to partially complementary sites within target messenger RNAs (mRNAs), resulting in translational repression either by transcript cleavage and degradation or suppression of robust translation (Ambros, 2004; Fabian and Sonenberg, 2012; Janssen et al., 2013). While the typical function of most miRNAs is to suppress gene expression, the opposite holds true in the relationship between HCV and miR-122. In this scenario, binding of miR-122 to two sites at the 5′ end of the HCV positive-strand RNA genome does not result in the degradation of the RNA strand, rather it stabilizes the transcript by acting as a cap – which in turn promotes viral replication and stability (Garcia-Sastre and Evans, 2013). As the HCV genome does not contain the traditional cap structure at its 5′ end and undergoes IRES mediated translation via the direct recruitment of ribosomal components, it therefore requires shielding the 5′ end from cytosolic RNA exonuclease digestion (Garneau et al., 2007; Shimakami et al., 2012). In the case of HCV, miR-122 acts to shield viral RNA from Xrn1 degradation and increased RNA stability (Li et al., 2013). This mechanism is also important for transcript fitness and virus survival as the cap also protects aberrant transcripts from triggering RIG-I, an intracellular RNA-triggered innate immune sensor. It is important to note that the miRNA-122-binding sites are conserved among all HCV genotypes and subtypes, thus allowing miRNA-122 to represent a very attractive host target for antiviral therapy (Li et al., 2011).

Miravirsen is the latest HTA from Santaris Pharma to enter into Phase IIa clinical trials. It is a 15-nucleotide locked nucleic acid-modified antisense oligonucleotide complementary sequence to the 5′ region of mature miR-122 (Janssen et al., 2013). Initial studies conducted in chimpanzees with chronic genotype 1 HCV infection provided evidence of miravirsen's antiviral potency, by demonstrating long-lasting viral suppression without evidence of resistant mutations (Lanford et al., 2010). Importantly, when Phase I clinical trials were conducted in healthy volunteers there were no observable adverse effects (Hildebrandt-Eriksen et al., 2009). Phase IIa studies conducted with miravirsen demonstrated prolonged dose-dependent reductions in HCV RNA levels, without evidence of viral breakthrough mutations. In this study, 36 treatment-naïve chronic HCV genotype 1 patients were randomly assigned to receive five weekly subcutaneous injections of miravirsen at doses

of 3, 5, or 7 mg/kg of body weight or placebo over a 29-day period. Five patients who received the highest dose (a weekly dose of 7 mg/kg) of miravirsen alone achieved undetectable HCV RNA levels, strongly demonstrating the potent efficacy of this drug. Hopes that miravirsen could be utilized as a monotherapy were dashed however, as four of these five patients experienced viral rebound within 14 weeks (Janssen et al., 2013). These results suggest that the given time frame and/or dosage were insufficient to achieve SVR in these patients. Santaris is currently testing the effect of a 12-week regimen in genotype 1 prior null-responder patients (ClinicalTrials.gov number, NCT01727934) (Janssen et al., 2013; Lieberman and Sarnow, 2013). Hopefully this work will further elucidate the true potential of this category of antiviral compounds that can likely be applied to a variety of viral targets.

2.5. Cyclophilin A

Cyclosporine A (CsA), an immunosuppressant drug used in organ transplantation to prevent rejection, was the first cyclophilin inhibitor (CypI) shown to have anti-HCV activity *in vitro* (Goto et al., 2006; Ishii et al., 2006; Nakagawa et al., 2004; Watashi et al., 2003). CsA exerts its immunosuppressive activity by binding to its intracellular partner, CypA, forming a ternary complex with calcineurin that triggers a reduction in the stimulation of the growth and differentiation of T cells (Borel, 2002). The clinical effectiveness of CsA was first demonstrated when it was shown to be more effective in combination with pegIFN than pegIFN alone (Inoue et al., 2003; Inoue and Yoshiba, 2005). Subsequent *in vitro* studies done in hepatoma cell lines attributed this effect to CsA's ability to prevent HCV RNA replication and protein synthesis (Goto et al., 2006; Ishii et al., 2006; Nakagawa et al., 2004; Watashi et al., 2003). This discovery led to the development of a second generation of CypIs, which lack the ability to bind calcineurin and in turn are devoid of the immunosuppressive properties of CsA.

Nonimmunosuppressive CypIs derived from CsA include NIM-811, SCY-635, EDP-546 and alisporivir (ALV) (previously known as Debio-025) (Hopkins et al., 2010; Ma et al., 2006; Owens et al., 2013). Cyclophilin Inhibitor EDP-546 is a Potential Cornerstone Drug for Use in Combination with NS5A and Protease Inhibitors Due to its High Barrier to HCV Resistance; (Paeshuyse et al., 2006).

A number of CypIs are currently under development and at various stages of preclinical and clinical phases. Among these, SCY-635, currently in Phase II clinical trials has demonstrated clinical efficacy in genotype 1 patients by reducing mean viral load of 2.2 $\log_{10}$ after 15 days of treatment with 300 mg TID (three times a day) of SCY-635 alone (Hopkins et al., 2012; Hopkins et al., 2010). Other CsA-derived CypIs such as EDP-546 (Jiang et al., 2012; Owens et al., 2013). Cyclophilin Inhibitor EDP-546 is a Potential Cornerstone Drug for Use in Combination with NS5A and Protease Inhibitors Due to its High Barrier to HCV Resistance, together with another class of CypI, sanglifehrins, a group of naturally occurring cyclophilin-binding polyketides, which are structurally distinct from cyclosporines, such as BC556, are currently being tested in preclinical studies and have shown promising *in vitro* anti-HCV activities (Ahmed-Belkacem et al., 2012; Gregory et al., 2011; Moss et al., 2012).

ALV was the first oral nonimmunosuppressive CypI to enter into clinical trials, and to date, is the most advanced with the largest clinical database of HCV genotype 1, 2, 3 and 4 patients. ALV was initially developed for HIV-1 and was discovered in a Phase I clinical trial with 23 HIV-1 patients, including 19 co-infected with HCV, to be incredibly effective against HCV, while having only minimal effects against HIV. ALV decreased HCV RNA by 3.63 $\log_{10}$ in patients treated with 1200 mg ALV BID (twice daily) for 15 days vs. 0.73 $\log_{10}$ in the placebo group (Flisiak et al., 2008; Lin and Gallay, 2013). This study demonstrated for the first time the clinical efficacy of CypIs in HCV-infected patients, paving the way for HTA treatments.

Subsequent Phase IIa clinical trials with ALV in combination with pegIFN and RBV demonstrated that very impressive mean viral load reductions could be achieved in treatment-naïve HCV-infected patients after only four weeks, with 4.6 $\log_{10}$ and 5.9 $\log_{10}$ viral load reductions attained in genotypes 1/4 and genotypes 2/3, respectively (Flisiak et al., 2009; Gallay and Lin, 2013; Lin and Gallay, 2013). Based on these results, the Phase IIb study, known as ESSENTIAL, focused on treatment-naïve genotype 1 HCV patients who were treated with ALV or placebo in combination with pegIFN and RBV for 24–48 weeks (Flisiak et al., 2011). A loading dose of 600 mg BID ALV was given during the first week of treatment with 600 mg QD for the remaining weeks. 76% of patients on ALV triple therapy successfully attained SVR compared to 55% on placebo triple therapy, with the rate of viral breakthrough being much lower in the ALV-treated group, thereby confirming its high barrier to resistance. In another Phase II study known as FUNDAMENTAL, pegIFN null-responder genotype 1 HCV-infected patients were treated for 24 weeks with either ALV 400 mg BID with pegIFN and RBV or pegIFN and RBV alone. Remarkably, 75.4% of patients on ALV triple therapy attained SVR_{12} compared to 8.9% in the control group (Alberti et al., 2012).

The VITAL-1 study, an open label Phase IIb clinical trial, was set up to evaluate the safety and efficacy of ALV administered alone, with pegIFN, or with pegIFN and RBV in 340 treatment-naïve genotype 2/3 HCV patients. Within this study one third of the patients were infected with genotype 2 and the remainder with genotype 3. Patients were randomly assigned to 1 of 5 treatment arms: 1000 mg QD ALV monotherapy, 600 mg QD ALV plus RBV, 800 mg QD ALV plus RBV, 600 mg QD ALV plus pegIFN, or standard therapy using pegIFN/RBV. All patients were started with a loading dose of 600 mg BID ALV for 4 weeks before beginning their assigned regimen. Those who achieved RVR stayed on the assigned regimen through week 24. Those who did not switched to 600 mg ALV plus pegIFN/RBV and continued for the same duration. At the conclusion of this study, it was determined that ALV improved SVR_{24} rates to 80–85% compared to 58% for pegIFN and RBV treatment. Overall, ALV was well tolerated within patients with very low relapse rates occurring within patients treated with ALV/RBV (between 4% and 9%). Also it is worth noting that genotype 3 patients across all the pegIFN-free arms responded the same or better as genotype 2 patients to ALV, emphasizing the major advantage ALV may have in treating genotype 3 infected patients (Pawlotsky et al., 2012).

Phase III clinical trials began in early 2011 to study the combination of ALV with pegIFN and RBV in treatment-naïve genotype 1 HCV patients. Unfortunately, this study was put on partial clinical hold after six cases of acute pancreatitis were reported, including one death in early 2012, in patients receiving ALV with pegIFN and RBV. Acute pancreatitis is a known adverse event with pegIFN/RBV therapy and no cases of pancreatitis were reported with ALV, pegIFN-free therapies in the VITAL-1 study (Lin and Gallay, 2013).

The exact MOA of CypIs is also not yet fully understood, but a number of critical discoveries are helping to unravel their role in the inhibition of HCV replication: (i) CypA governs HCV replication via its peptidyl-prolyl isomerase hydrophobic pocket (Chatterji et al., 2009; Liu et al., 2009); (ii) CypIs inhibit peptidyl-prolyl isomerase activity by binding to the enzymatic pocket of CypA (Ke et al., 1991; Zydowsky et al., 1992); and (iii) the HCV NS5A protein is the viral ligand for CypA (Chatterji et al., 2010; Coelmont et al., 2010; Fernandes et al., 2010; Hanoulle et al., 2009; Waller et al., 2010). It is believed that the main antiviral mechanism of action of CypIs is the disruption of CypA-NS5A interactions that regulate multiple

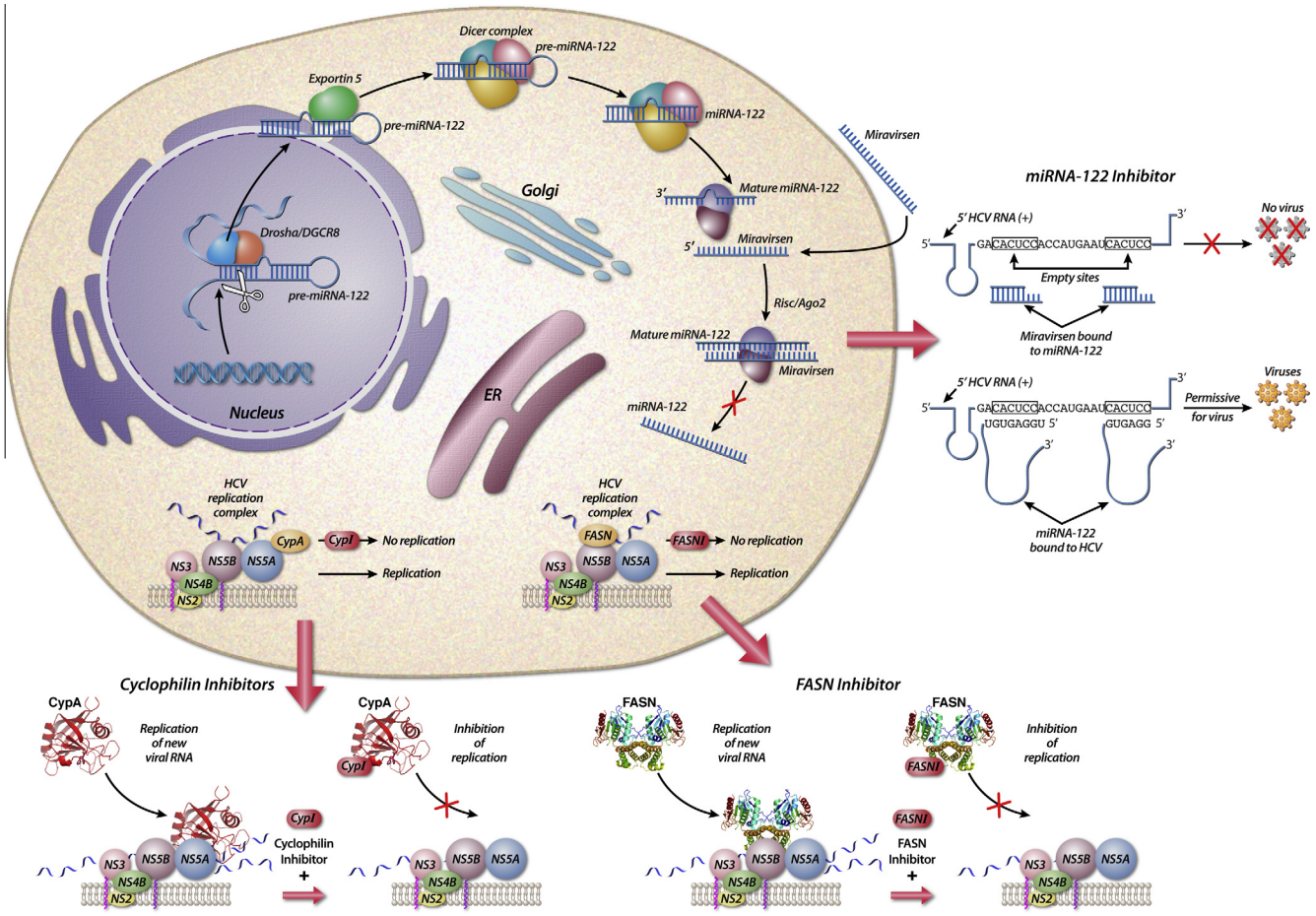

Fig. 1. Mechanism of action of host-targeting antivirals against hepatitis C. A number of host pathways are exploited by HCV during its life-cycle. Adapted from Baugh and Gallay (2012) and Santaris (2010).

Table 1
Some of the host-targeting antivirals currently under clinical development for the treatment of hepatitis C.

Compound	Sponsor	Host target	Structure	HCV EC50	Tested genotype coverage	Status	References
NIM811	Novartis	Cyclophilin A		120 nM	N/A	Terminated	Lawitz et al. (2011)
Alisporivir	Novartis	Cyclophilin A		45 nM	GT1/2/3/4	Phase III	Pawlotsky et al. (2012)
SCY-635	Scynexis	Cyclophilin A		100 nM	GT1	Phase IIa	Hopkins et al. (2012)
EDP-546	Enanta	Cyclophilin A	Unpublished	67 nM	N/A	Preclinical	Jiang et al (2012)
NVPO18	Neurovive	Cyclophilin A		38 nM	N/A	Preclinical	Moss et al. (2012)
Miravirsen	Santaris Pharma	miR-122	Unpublished	671 nM	GT1	Phase II	Janssen et al. (2013)
TVB-2640	3-V Biosciences	FASN	Unpublished	<100 nM	GT1/2	Phase I	Kemble et al. (2012)

phases of HCV replication (Gallay and Lin, 2013; Owens et al., 2013. Cyclophilin Inhibitor EDP-546 is a Potential Cornerstone Drug for Use in Combination with NS5A and Protease Inhibitors Due to its High Barrier to HCV Resistance) (See Fig. 1 and Table 1).

3. Host targeting antivirals a beginning and an end

PegIFN- and RBV-free treatment regimens for HCV infection are currently being tested in the clinic and will likely be within the grasp of patients in the coming years. Thus, as we bid farewell to the first generation of HTAs we must look toward the future generation of HTA products that are in the pipeline for a number of companies. DAAs are undeniably effective against HCV and as patient-based treatment progresses we will likely see subsets of individuals that prove to be difficult to treat. It is these populations of patients that HTAs should be directly tested, in conjunction with DAAs. Given the distinct advantages that HTAs possess over DAAs – high barrier to resistance, broad pan-genotypic coverage, and potential synergistic activity with DAAs – it is easy to envision in the

near future that this class of drugs, in combination with next generation DAAs, will afford a novel approach to combat the most difficult of HCV cases. Moreover it is reasonable to see this class of compounds be tested against a variety of other pathogens.

Acknowledgements

We acknowledge financial support from the U.S. Public Health Service grant no. AI087746 (P.A.G.). This is publication no. 24052 from the Department of Immunology & Microbial Science, The Scripps Research Institute, La Jolla, CA.

References

Ahmed-Belkacem, A., Colliandre, L., Ahnou, N., Lerat, N., Bessin, Y., Barthe, P., Bourget, W., Douguet, D., Guichou, J.F., Pawlotsky, J.M., 2012. New cyclophilin inhibitors unrelated to cyclosporine potently inhibit HCV replication and revert HCV-induced mitochondrial dysfunction. Hepatology 56, 1067A–1068A.

Alberti, A., Chuang, W.L., Flisiak, R., Mazzella, G., Horban, A., Goeser, T., Calistru, P., Buti, M., Davis, G., Gong, Y., Avila, C., Kao, J.H., 2012. Alisporivir (Alv) plus peg-interferon/ribavirin (Pr) in Hcv G1 treatment-experienced patients achieves primary endpoint with superior efficacy at treatment week 12 compared to retreatment with Pr. Journal of Hepatology 56, S553–S554.

Ambros, V., 2004. The functions of animal microRNAs. Nature 431, 350–355.

Baugh, J., Gallay, P., 2012. Cyclophilin involvement in the replication of hepatitis C virus and other viruses. Biological Chemistry 393, 579–587.

Berger, K.L., Cooper, J.D., Heaton, N.S., Yoon, R., Oakland, T.E., Jordan, T.X., Mateu, G., Grakoui, A., Randall, G., 2009. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. Proceedings of the National Academy of Sciences of the United States of America 106, 7577–7582.

Boonstra, A., Liu, B.S., Groothuismink, Z.M.A., Bergmann, J.F., de Bruijne, J., Hotho, D.M., Hansen, B.E., van Vliet, A.A., de Rooij, J.V., Fletcher, S.P., Bauman, L.A., Rahimy, M., Appleman, J.R., Freddo, J.L., Reesink, H.W., de Knegt, R.J., Janssen, H.L.A., 2012. Potent immune activation in chronic hepatitis C patients upon administration of an oral inducer of endogenous interferons that acts via Toll-like receptor 7. Antiviral Therapy 17, 657–667.

Borel, J.F., 2002. History of the discovery of cyclosporin and of its early pharmacological development. Wiener klinische Wochenschrift 114, 433–437.

Buhler, S., Bartenschlager, R., 2012. New targets for antiviral therapy of chronic hepatitis C. Liver international: Official Journal of the International Association for the Study of the Liver 32 (Suppl. 1), 9–16.

Chatterji, U., Bobardt, M., Selvarajah, S., Yang, F., Tang, H., Sakamoto, N., Vuagniaux, G., Parkinson, T., Gallay, P., 2009. The isomerase active site of cyclophilin A is critical for hepatitis C virus replication. The Journal of Biological Chemistry 284, 16998–17005.

Chatterji, U., Lim, P., Bobardt, M.D., Wieland, S., Cordek, D.G., Vuagniaux, G., Chisari, F., Cameron, C.E., Targett-Adams, P., Parkinson, T., Gallay, P.A., 2010. HCV resistance to cyclosporin A does not correlate with a resistance of the NS5A-cyclophilin A interaction to cyclophilin inhibitors. Journal of Hepatology 53, 50–56.

Coelmont, L., Hanoulle, X., Chatterji, U., Berger, C., Snoeck, J., Bobardt, M., Lim, P., Vliegen, I., Paeshuyse, J., Vuagniaux, G., Vandamme, A.M., Bartenschlager, R., Gallay, P., Lippens, G., Neyts, J., 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, e13687.

Donnelly, R.P., Kotenko, S.V., 2010. Interferon-lambda: a new addition to an old family. Journal of Interferon and Cytokine Research 30, 555–564.

Evanchik, M., Cai, H.Y., Feng, Q.S., Hu, L., Johnson, R., Kemble, G., Kosaka, Y., Lai, J., Oslob, J.D., Sivaraja, M., Tep, S., Yan, H.B., Zaharia, C.A., McDowell, R., 2012. TVB-2640, a novel anti-HCV agent, safely causes sustained host-target inhibition in vivo. Hepatology 56, 1066A–1067A.

Fabian, M.R., Sonenberg, N., 2012. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nature Structural and Molecular Biology 19, 586–593.

Fernandes, F., Ansari, I.U., Striker, R., 2010. Cyclosporine inhibits a direct interaction between cyclophilins and hepatitis C NS5A. Plos One 5, e9815.

Flisiak, R., Feinman, S.V., Jablkowski, M., Horban, A., Kryczka, W., Pawlowska, M., Heathcote, J.E., Mazzella, G., Vandelli, C., Nicolas-Metral, V., Grosgurin, P., Liz, J.S., Scalfaro, P., Porchet, H., Crabbe, R., 2009. The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naive hepatitis C patients. Hepatology 49, 1460–1468.

Flisiak, R., Horban, A., Gallay, P., Bobardt, M., Selvarajah, S., Wiercinska-Drapalo, A., Siwak, E., Cielniak, I., Higersberger, J., Kierkus, J., Aeschlimann, C., Grosgurin, P., Nicolas-Metral, V., Dumont, J.M., Porchet, H., Crabbe, R., Scalfaro, P., 2008. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology 47, 817–826.

Flisiak, R., Pawlotsky, J.M., Crabbe, R., Calistru, P.I., Kryczka, W., Haussinger, D., Mazzella, G., Romero-Gomez, M., Purcea, D., Vuagniaux, G., Bao, W., Avila, C., Zeuzem, 2011. Once Daily Alisporivir (Deb025) Plus Pegifnalfa2a/Ribavirin Results in Superior Sustained Virologic Response (Svr24) in Chronic Hepatitis C Genotype 1 Treatment Naive Patients. Journal of Hepatology 54, S2-S2.

Friborg, J., Levine, S., Chen, C.Q., Sheaffer, A.K., Chaniewski, S., Voss, S., Lemm, J.A., McPhee, F., 2013a. Combinations of Lambda Interferon with Direct-Acting Antiviral Agents Are Highly Efficient in Suppressing Hepatitis C Virus Replication. Antimicrobial Agents and Chemotheraphy 57, 1312–1322.

Friborg, J., Lin, B., Chen, C., McPhee, F., 2013b. Isolation and characterization of interferon lambda-resistant hepatitis C virus replicon cell lines. Virology 444, 384–393.

Gallay, P.A., Lin, K., 2013. Profile of alisporivir and its potential in the treatment of hepatitis C. Drug Design, Development and Therapy 7, 105–115.

Garcia-Sastre, A., Evans, M.J., 2013. MiR-122 is more than a shield for the hepatitis C virus genome. Proceedings of the National Academy of Sciences of the United States of America 110, 1571–1572.

Garneau, N.L., Wilusz, J., Wilusz, C.J., 2007. The highways and byways of mRNA decay. Nature Reviews. Molecular Cell Biology 8, 113–126.

Ghany, M.G., Strader, D.B., Thomas, D.L., Seeff, L.B., 2009. American Association for the Study of Liver, D., Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49, 1335–1374.

Gilead Sciences. Gilead Reports Interim Data from Phase 2 LONESTAR Study. Press Release, May 2, 2013.

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. Biochemical and Biophysics Research Communication 343, 879–884.

Gregory, M.A., Bobardt, M., Obeid, S., Chatterji, U., Coates, N.J., Foster, T., Gallay, P., Leyssen, P., Moss, S.J., Neyts, J., Nur-e-Alam, M., Paeshuyse, J., Piraee, M., Suthar, D., Warneck, T., Zhang, M.Q., Wilkinson, B., 2011. Preclinical characterization of naturally occurring polyketide cyclophilin inhibitors from the sanglifehrin family. Antimicrobial Agents and Chemotherapy 55, 1975–1981.

Grimm, D., 2012. All for one, one for all: new combinatorial RNAi therapies combat hepatitis C virus evolution. Molecular Therapy: The Journal of the American Society of Gene Therapy 20, 1661–1663.

Hanoulle, X., Badillo, A., Wieruszeski, J.M., Verdegem, D., Landrieu, I., Bartenschlager, R., Penin, F., Lippens, G., 2009. Hepatitis C virus NS5A protein is a substrate for the peptidyl-prolyl cis/trans isomerase activity of cyclophilins A and B. The Journal of Biological Chemistry 284, 13589–13601.

Heim, M.H., 2013. 25 years of interferon-based treatment of chronic hepatitis C: an epoch coming to an end. Nature Reviews Immunology 13, 535–542.

Hildebrandt-Eriksen, E.S., Bagger, Y.Z., Knudsen, T.B., Petri, A., Persson, R., Boergesen, H.M., McHulchison, J.G., Levin, A.A., 2009. A Unique Therapy for Hcv Inhibits Microrna-122 in Humans and Results in Hcv Rna Suppression in Chronically Infected Chimpanzees: Results from Primate and First-in-Human Studies. Hepatology 50, 12A-12A.

Hopkins, S., DiMassimo, B., Rusnak, P., Heuman, D., Lalezari, J., Sluder, A., Scorneaux, B., Mosier, S., Kowalczyk, P., Ribeill, Y., Baugh, J., Gallay, P., 2012. The cyclophilin inhibitor SCY-635 suppresses viral replication and induces endogenous interferons in patients with chronic HCV genotype 1 infection. Journal of Hepatology 57, 47–54.

Hopkins, S., Scorneaux, B., Huang, Z., 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, 660–672.

Huang, H., Sun, F., Owen, D.M., Li, W., Chen, Y., Gale Jr., M., Ye, J., 2007. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. Proceedings of the National Academy of Sciences of the United States of America 104, 5848–5853.

Huang, J.T., Tseng, C.P., Liao, M.H., Lu, S.C., Yeh, W.Z., Sakamoto, N., Chen, C.M., Cheng, J.C., 2013. Hepatitis C Virus Replication Is Modulated by the Interaction of Nonstructural Protein NS5B and Fatty Acid Synthase. Journal of Virology 87, 4994–5004.

Inoue, K., Sekiyama, K., Yamada, M., Watanabe, T., Yasuda, H., Yoshiba, M., 2003. Combined interferon alpha2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. Journal of Gastroenterology 38, 567–572.

Inoue, K., Yoshiba, M., 2005. Interferon combined with cyclosporine treatment as an effective countermeasure against hepatitis C virus recurrence in liver transplant patients with end-stage hepatitis C virus related disease. Transplantation Proceedings 37, 1233–1234.

Ishii, N., Watashi, K., Hishiki, T., Goto, K., Inoue, D., Hijikata, M., Wakita, T., Kato, N., Shimotohno, K., 2006. Diverse effects of cyclosporine on hepatitis C virus strain replication. Journal of Virology 80, 4510–4520.

Janssen, H.L., Reesink, H.W., Lawitz, E.J., Zeuzem, S., Rodriguez-Torres, M., Patel, K., van der Meer, A.J., Patick, A.K., Chen, A., Zhou, Y., Persson, R., King, B.D., Kauppinen, S., Levin, A.A., Hodges, M.R., 2013. Treatment of HCV infection by targeting microRNA. The New England Journal of Medicine 368, 1685–1694.

Jiang, L.J., Liu, S., Phan, T.U., Polemeropoulos, A.J., Rhodin, M.H., Owens, C.M., Luo, X., Hoang, K., Long, J., Gao, X., Wang, G.Q., Or, Y.S., 2012. EDP-546, a potent and novel cyclophilin inhibitor with favorable preclinical pharmacokinetic and safety profiles. Hepatology 56, 1076A-1076A.

Jones, C.T., Catanese, M.T., Law, L.M.J., Khetani, S.R., Syder, A.J., Ploss, A., Oh, T.S., Schoggins, J.W., MacDonald, M.R., Bhatia, S.N., Rice, C.M., 2010. Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. Nature Biotechnology 28, 167-U116.

Jopling, C.L., Yi, M., Lancaster, A.M., Lemon, S.M., Sarnow, P., 2005. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 309, 1577–1581.

Kapadia, S.B., Chisari, F.V., 2005. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proceedings of the National Academy of Sciences of the United States of America 102, 2561–2566.

Ke, H.M., Zydowsky, L.D., Liu, J., Walsh, C.T., 1991. Crystal structure of recombinant human T-cell cyclophilin A at 2.5 A resolution. Proceedings of the National Academy of Sciences of the United States of America 88, 9483–9487.

Kridel, S.J., Lowther, W.T., Pemble, C.W.t., 2007. Fatty acid synthase inhibitors: new directions for oncology. Expert Opinion on Investigational Drugs 16, 1817–1829.

Lacek, K., Vercauteren, K., Grzyb, K., Naddeo, M., Verhoye, L., Slowikowski, M.P., Fafi-Kremer, S., Patel, A.H., Baumert, T.F., Folgori, A., Leroux-Roels, G., Cortese, R., Meuleman, P., Nicosia, A., 2012. Novel human SR-BI antibodies prevent infection and dissemination of HCV in vitro and in humanized mice. Journal of Hepatology 57, 17–23.

Lanford, R.E., Hildebrandt-Eriksen, E.S., Petri, A., Persson, R., Lindow, M., Munk, M.E., Kauppinen, S., Orum, H., 2010. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 327, 198–201.

Lauer, G.M., Walker, B.D., 2001. Hepatitis C virus infection. The New England Journal of Medicine 345, 41–52.

Lavanchy, D., 2009. The global burden of hepatitis C. Liver International: Official Journal of the International Association for the Study of the Liver 29 (Suppl 1), 74–81.

Lawitz, E., Godofsky, E., Rouzier, R., Marbury, T., Nguyen, T., Ke, J., Huang, M., Praestgaard, J., Serra, D., Evans, T.G., 2011. Safety, pharmacokinetics, and antiviral activity of the cyclophilin inhibitor NIM811 alone or in combination with pegylated interferon in HCV-infected patients receiving 14 days of therapy. Antiviral Res. 89 (3), 238–245.

Li, Y., Masaki, T., Yamane, D., McGivern, D.R., Lemon, S.M., 2013. Competing and noncompeting activities of miR-122 and the 5' exonuclease Xrn1 in regulation of hepatitis C virus replication. Proceedings of the National Academy of Sciences of the United States of America 110, 1881–1886.

Li, Y.P., Gottwein, J.M., Scheel, T.K., Jensen, T.B., Bukh, J., 2011. MicroRNA-122 antagonism against hepatitis C virus genotypes 1–6 and reduced efficacy by host RNA insertion or mutations in the HCV 5' UTR. Proceedings of the National Academy of Sciences of the United States of America 108, 4991–4996.

Lieberman, J., Sarnow, P., 2013. Micromanaging hepatitis C virus. The New England Journal of Medicine 368, 1741–1743.

Lin, K., Gallay, P., 2013. Curing a viral infection by targeting the host: the example of cyclophilin inhibitors. Antiviral research.

Liu, Z., Yang, F., Robotham, J.M., Tang, H., 2009. Critical role of cyclophilin A and its prolyl-peptidyl isomerase activity in the structure and function of the hepatitis C virus replication complex. Journal of Virology 83, 6554–6565.

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 alpha interferon. Antimicrobial Agents and Chemotherpy 50, 2976–2982.

Magiorkinis, G., Magiorkinis, E., Paraskevis, D., Ho, S.Y., Shapiro, B., Pybus, O.G., Allain, J.P., Hatzakis, A., 2009. The global spread of hepatitis C virus 1a and 1b: a phylodynamic and phylogeographic analysis. PLoS Medicine 6, e1000198.

Masson, D., Koseki, M., Ishibashi, M., Larson, C.J., Miller, S.G., King, B.D., Tall, A.R., 2009. Increased HDL cholesterol and ApoA-I in humans and mice treated with a novel SR-BI inhibitor. Arteriosclerosis, Thrombosis, and Vascular Biology 29, 2054–U2170.

Moss, S.J., Garcia-Rivera, J.A., Leyssen, P., Coates, N., Foster, T., Kendrew, S., Nur-E-Alam, M., Stanley, A.E., Suthar, D., Warneck, T., Neyts, J., Gallay, P., Wilkinson, B., Gregory, M.A., 2012. Bc556, a potent, pan-genotypic, high barrier to resistance, second generation cyclophilin inhibitor for treatment of chronic Hcv infection. Journal of Hepatology 56, S328–S328.

Muir, A.J., Shiffinan, M.L., Zaman, A., Yoffe, B., de la Torre, A., Flamm, S., Gordon, S.C., Marotta, P., Vierling, J.M., Lopez-Talavera, J.C., Byrnes-Blake, K., Fontana, D., Freeman, J., Gray, T., Hausman, D., Hunder, N.N., Lawitz, E., 2010. Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. Hepatology 52, 822–832.

Nakagawa, M., Sakamoto, N., Enomoto, N., Tanabe, Y., Kanazawa, N., Koyama, T., Kurosaki, M., Maekawa, S., Yamashiro, T., Chen, C.H., Itsui, Y., Kakinuma, S., Watanabe, M., 2004. Specific inhibition of hepatitis C virus replication by cyclosporin A. Biochemical and Biophysical Research Communications 313, 42–47.

Owens CM, R.M., Polemeropoulos A, Long J, Wang G, Jiang L, Or YS 2013. Cyclophilin Inhibitor EDP-546 is a Potential Cornerstone Drug for Use in Combination with NS5A and Protease Inhibitors Due to its High Barrier to HCV Resistance. Cyclophilin Inhibitor EDP-546 is a Potential Cornerstone Drug for Use in Combination with NS5A and Protease Inhibitors Due to its High Barrier to HCV Resistance, 48th Annual Meeting of the European Association for the Study of the Liver (EASL 2013), Amsterdam. Abstract 1213.

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, 761–770.

Pawlotsky, J.M., Sarin, S.K., Foster, G.R., Peng, C.Y., Rasenack, J., Flisiak, R., Piratvisuth, T., Wedemeyer, H., Chuang, W.L., Zhang, W., Naoumov, N.V., 2012. Alisporivir plus Ribavirin achieves high rates of sustained HCV clearance (SVR24) as interferon (IFN)-free or IFN-add-on regimen in treatment-naive patients with HCV GT2 or GT3: final results from VITAL-1 study. Hepatology 56, 309A–310A.

Perales, C., Beach, N.M., Gallego, I., Soria, M.E., Quer, J., Esteban, J.I., Rice, C., Domingo, E., Sheldon, J., 2013. Response of hepatitis C virus to long-term passage in the presence of alpha interferon: multiple mutations and a common phenotype. Journal of virology 87, 7593–7607.

Poynard, T., Yuen, M.F., Ratziu, V., Lai, C.L., 2003. Viral hepatitis C. Lancet 362, 2095–2100.

Randall, G., Panis, M., Cooper, J.D., Tellinghuisen, T.L., Sukhodolets, K.E., Pfeffer, S., Landthaler, M., Landgraf, P., Kan, S., Lindenbach, B.D., Chien, M., Weir, D.B., Russo, J.J., Ju, J., Brownstein, M.J., Sheridan, R., Sander, C., Zavolan, M., Tuschl, T., Rice, C.M., 2007. Cellular cofactors affecting hepatitis C virus infection and replication. Proceedings of the National Academy of Sciences of the United States of America 104, 12884–12889.

Reiss, S., Harak, C., Romero-Brey, I., Radujkovic, D., Klein, R., Ruggieri, A., Rebhan, I., Bartenschlager, R., Lohmann, V., 2013. The Lipid Kinase Phosphatidylinositol-4 Kinase III Alpha Regulates the Phosphorylation Status of Hepatitis C Virus NS5A. Plos Pathog 9.

Rodriguez-Torres, M., Ghalib, R.H., Gordon, S.C., Lawitz, E., Patel, K., Pruitt, R., Sheikh, A.M., Arbeit, R.D., Bexon, A.S., Kandimalla, E.R., Precopio, M., Sullivan, T., Muir, A.J., McHutchison, J.G., 2010. Imo-2125, a Tlr9 Agonist, Induces Immune Responses Which Correlate with Reductions in Viral Load in Null Responder Hcv Patients. Hepatology 52, 336A-336A.

Scheel, T.K.H., Rice, C.M., 2013. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. Nat Med 19, 837–849.

Shimakami, T., Yamane, D., Jangra, R.K., Kempf, B.J., Spaniel, C., Barton, D.J., Lemon, S.M., 2012. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. Proceedings of the National Academy of Sciences of the United States of America 109, 941–946.

Stetson, D.B., Medzhitov, R., 2006. Type I interferons in host defense. Immunity 25, 373–381.

Syder, A.J., Lee, H., Zeisel, M.B., Grove, J., Soulier, E., Macdonald, J., Chow, S., Chang, J., Baumert, T.F., McKeating, J.A., McKelvy, J., Wong-Staal, F., 2011. Small molecule scavenger receptor BI antagonists are potent HCV entry inhibitors. Journal of Hepatology 54, 48–55.

Vaillancourt, F.H., Brault, M., Pilote, L., Uyttersprot, N., Gaillard, E.T., Stoltz, J.H., Knight, B.L., Pantages, L., McFarland, M., Breitfelder, S., Chiu, T.T., Mahrouche, L., Faucher, A.M., Cartier, M., Cordingley, M.G., Bethell, R.C., Jiang, H.P., White, P.W., Kukolj, G., 2012. Evaluation of Phosphatidylinositol-4-Kinase III alpha as a Hepatitis C Virus Drug Target. Journal of virology 86, 11595–11607.

Waller, H., Chatterji, U., Gallay, P., Parkinson, T., Targett-Adams, P., 2010. The use of AlphaLISA technology to detect interaction between hepatitis C virus-encoded NS5A and cyclophilin A. J Virol Methods 165, 202–210.

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, 1282–1288.

Witteveldt, J., Evans, M.J., Bitzegeio, J., Koutsoudakis, G., Owsianka, A.M., Angus, A.G.N., Keck, Z.Y., Foung, S.K.H., Pietschmann, T., Rice, C.M., Patel, A.H., 2009. CD81 is dispensable for hepatitis C virus cell-to-cell transmission in hepatoma cells. J Gen Virol 90, 48–58.

Yang, W., Hood, B.L., Chadwick, S.L., Liu, S., Watkins, S.C., Luo, G., Conrads, T.P., Wang, T., 2008. Fatty acid synthase is up-regulated during hepatitis C virus infection and regulates hepatitis C virus entry and production. Hepatology 48, 1396–1403.

Zeuzem, S., Arora, S., Bacon, B., Box, T., Charlton, M., Diago, M., Dieterich, D., Mur, R.E., Everson, G., Fallon, M., Ferenci, P., Flisiak, R., George, J., Ghalib, R., Gitlin, N., Gladysz, A., Gordon, S., Greenbloom, S., Hassanein, T., Jacobson, I., Jeffers, L., Kowdley, K., Lawitz, E., Lee, S., Leggett, B., Lueth, S., Nelson, D., Pockros, P., Rodriguez-Torres, M., Rustgi, V., Serfaty, L., Sherman, M., Shiffman, M., Sola, R., Sulkowski, M., Vargas, H., Vierling, J., Yoffe, B., Ishak, L., Fontana, D., Xu, D., Lester, J., Gray, T., Horga, A., Hillson, J., Ramos, E., Lopez-Talavera, J.C., Muir, A., Grp, E.S., 2011. PEGYLATED INTERFERON-LAMBDA (PEGIFN-lambda) SHOWS SUPERIOR VIRAL RESPONSE WITH IMPROVED SAFETY AND TOLERABILITY VERSUS PEGIFN alpha-2A IN HCV PATIENTS (G1/2/3/4): EMERGE PHASE IIB THROUGH WEEK 12. Journal of Hepatology 54, S538–S539.

Zhu, H.H., Wong-Staal, F., Lee, H., Syder, A., McKelvy, J., Schooley, R.T., Wyles, D.L., 2012. Evaluation of ITX 5061, a Scavenger Receptor B1 Antagonist: Resistance Selection and Activity in Combination With Other Hepatitis C Virus Antivirals. Journal of Infectious Diseases 205, 656–662.

Zydowsky, L.D., Etzkorn, F.A., Chang, H.Y., Ferguson, S.B., Stolz, L.A., Ho, S.I., Walsh, C.T., 1992. Active site mutants of human cyclophilin A separate peptidyl-prolyl isomerase activity from cyclosporin A binding and calcineurin inhibition. Protein Science 1, 1092–1099.