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Current and emerging antiviral treatments for hepatitis C infection

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Newly licensed direct acting antivirals for hepatitis C virus HCV are able to cure up to 75% of patients chronically infected with genotype-1 infection, which is the predominant HCV strain in Europe and North America. Emerging antiviral therapies promise further increases in virological response, as well as improved tolerability, reduced duration of therapy, and will potentially eliminate the need for interferon use. This review highlights the main therapeutic agents used in current standard of care, including telaprevir and boceprevir. It goes on to evaluate the mechanisms of emerging drugs, their stage of development and response rates seen in research to date. Finally, it projects into the not too distant future to consider treatment strategies involving combinations of agents and interferon-free therapies, and in which patients they might prove most successful.

Introduction

Approximately 170 million people are infected with hepatitis C virus (HCV) and it is the leading cause of liver transplantation and second most common cause of liver cancer globally [1–3]. Despite receiving less attention than other blood borne viruses, HCV recently surpassed HIV in terms of attributable deaths in the United States [4]. The sequelae of chronic hepatitis C (CHC) infection are preventable by viral eradication. Peginterferon-alpha (PEG-IFN) and ribavirin (RBV) have been standard of care therapy for the past decade. Unfortunately, PEG-IFN and RBV will cure only at best 50% of patients infected with genotype-1 HCV, the predominant HCV strain in Europe and North America. Use is also limited by significant toxicities, including psychiatric morbidity, influenza-like symptoms and cytopenias, mandating careful patient selection and monitoring [5–8]. Newly licensed directly acting agents (DAA) for HCV, telaprevir and boceprevir, both used in combination with PEG-IFN and RBV, dramatically improve effectiveness and can cure up to 75% of patients chronically infected with genotype-1 HCV. Emerging antiviral therapies promise further improvements in virological response, improved tolerability, reduced duration of therapy, and may potentially eliminate the need for IFN use.

This review highlights the main therapeutic agents used in current standard of care and their limitations, and includes the two newly available DAAs, telaprevir and boceprevir. It evaluates the mechanisms of emerging drugs with the greatest promise, including the next wave of HCV protease inhibitors, as well as the HCV polymerase inhibitors, NS5A inhibitors and cyclophilin inhibitors, outlining their stage of development and response rates seen in clinical trials to date. Finally, it projects into the not too distant future to consider individualized treatment strategies involving combinations of agents and interferon-free therapies, and for which patients they might prove most appropriate.

The current backbone of HCV therapy: peginterferon-alpha and ribavirin

PEG-IFN and RBV were the standard of care treatment for chronic HCV infection until 2011 and remain so for non-

Table 1

Currently licensed therapies for chronic HCV

Drug	Mechanism	Sustained virological response Treatment naïve	Re-treatment	Main limitations
Pegylated interferon alpha-2a/b	Non-specific antiviral agent	45% genotype-1 and -4 65–80% genotype-2 and 3 (EASL 2011 [21])	Relapsers: 20–29% genotype-1 (Bacon *et al*. 2011 [39], Zeuzem *et al*. 2011 [42]) 40% genotypes-2 and -3 Ghany *et al*. 2009 [84]	Injectable agent Psychiatric side effects 24–48 weeks for standard therapy
Ribavirin	Non-specific antiviral agent		Non-responders: 5–7% genotype-1 (Bacon *et al*. 2011 [39], Zeuzem *et al*. 2011 [42]) 15–20% genotypes-2 and -3 (Ghany *et al*. 2009 [84])	Anaemia
Boceprevir	NS3 protease inhibitor	67–68% genotype-1 with PEG/RBV *vs*. 40% PEG/RBV alone (non-Black patients) (Poordad *et al*. 2011 [35])	Null responders: 23–30% Partial responders: 40–52% Relapsers: 69–75% (Bacon *et al*. 2011 [39])	Anaemia, dysgeusia Only active in HCV genotype-1 Requires PEG/RBV backbone
Telaprevir	NS3 protease inhibitor	69–75% genotype-1 with PEG/RBV *vs*. 44% PEG/RBV alone (non-Black patients) (Jacobson *et al*. 2011 [43])	Null-responders: 29–33% Partial responders: 54–59% Relapsers: 83–88% (Zeuzem *et al*. 2011 [42]	Rash, anaemia, gastrointestinal side effects Only active in HCV genotype-1 Requires PEG/RBV backbone

PEG, Pegylated interferon alpha-2a/b; RBV, ribravirin.

genotype-1 HCV. In treatment naïve individuals it results in a sustained viral response (SVR) around 45% in HCV genotype-1 infection, compared with 75% in genotypes-2 and –3 infection (Table 1) [9]. With the introduction of telaprevir and boceprevir therapy, SVR rates up to 75% can also be achieved in genotype-1, but PEG-INF/RBV continue to play an important role to prevent the emergence of resistance-associated HCV variants. Treatment response rates in acute HCV infection are very high, with SVR rates 70–90% using PEG-IFN monotherapy. Unfortunately acute HCV infection is normally asymptomatic, and the diagnosis is not made until chronicity is established. In the minority of patients diagnosed acutely, IFN-based therapy is often contra-indicated by comorbidities. Treatment of acute HCV inection has recently been reviewed in detail elsewhere [10, 11].

IFN has both direct and indirect antiviral effects. Direct effects are mediated through the induction of interferon stimulated genes which code for effector proteins and cytokines that inhibit virus replication and generate an antiviral state. Indirect effects are mediated through up-regulation of major histocompatibility complex class 1 genes in antigen presenting cells, which leads to cytotoxic T-cell clearance of HCV infected cells [7]. Polyethylene glycol (PEG) polymer chains covalently attached to IFN reduce renal and hepatic clearance, allow for weekly administration and superior SVR over standard interferon [12, 13].

The mechanism of action of ribavirin in HCV treatment is not completely understood. There are multiple hypotheses. Viral kinetic studies suggest that HCV mutagenesis leading to error catastrophe and consequent lowering of HCV fitness is an important mechanism of action [8, 14]. RBV is also a guanosine analogue, and may act as a chain terminator through incorporation of ribavirin into the HCV genome during viral replication [8]. Ribavirin may also modulate host T-cell immunity as viral infection.

Side effects of PEG-IFN/RBV are common and are a major limitation of current therapy. Important PEG-IFN side effects include influenza-type symptoms and fatigue, psychiatric morbidity and bone marrow suppression [6, 7]. Psychiatric side effects, including depression or aggression, mandate careful patient selection and monitoring, and may preclude some patients from accessing current PEG-IFN based therapies. Ribavirin causes haemolysis and anaemia [8], and on-treatment cytopaenias due to both agents are more common in the setting of cirrhosis. Developing HCV treatment regimens with less toxicity is clinically important, and should expand the number of people appropriate for treatment and successfully completing therapy.

Factors predicting IFN-based treatment response

Host genetics are highly predictive of PEG-IFN/RBV treatment outcome. Genome-wide studies have confirmed an association between a polymorphism in the region of the IL-28B gene and response to HCV treatment [15, 16]. Individuals who carry a favourable IL-28B genotype have a two to three times increase in response to PEG-IFN/RBV [17, 18]. Other important predictors of PEG-IFN response include

pre-treatment viral load, liver fibrosis state and insulin resistance [19].

On-treatment virological response is the most accurate predictor of SVR, and time to viral clearance has been adopted as a guide to treatment duration (response-guided therapy, RGT) or futility. A rapid virological response (RVR, undetectable HCV RNA at week 4) is 86–100% predictive of SVR [20], regardless of HCV genotype, and is achieved in approximately 10–27% of genotype-1 and 64–76% of genotype-2/-3 infections [21]. Patients with a low baseline viral load who achieve an RVR can be considered for short duration therapy. Genotype-1 patients with complete early virological response (EVR, undetectable HCV RVA at week 12) have a 68–84% rate of SVR [20]. Patients with a slow but persistent virological decline may be considered for extended therapy with PEG-IFN/RBV. Week 12 and 24 HCV RNA measurements are important for predicting treatment failure. Treatment should be abandoned if HCV RNA has declined by $<2\ \log_{10}$ IU ml^{-1} at week 12, or is still detectable at week 24, given an expected SVR of 1–3% [22, 23].

The success rate of re-treatment with PEG-IFN/RBV after failing initial therapy has been disappointing, estimated at a pooled SVR of 16% in a meta-analysis [24]. Prior 'relapsers' (individuals who achieved an undetectable HCV RNA at the end of treatment, but did not achieve an SVR) with genotype-1 infection have a 15–25% SVR rate with re-treatment. Previous 'null-responders' (defined as $<2\ \log_{10}$ IU ml^{-1} decline in HCV RNA viral load after 12 weeks of therapy) with genotype-1 infection have a 4–14% SVR on re-treatment [25, 26].

The presence of HIV co-infection marginally reduces the effectiveness of PEG-IFN/RBV treatment: the APRICOT study reported an SVR of 29% among genotype-1 infection and 62% among genotype-2/-3 infection [27]. In contrast, active injecting drug use does not affect treatment response. In a systematic review of chronic HCV treatment with PEG-IFN/RBV, median SVR among people who inject drugs was 54% (range 18%-94%), compared with 54%–63% among non-injectors [28]. Acute HCV treatment is also effective among people who inject drugs [29].

The moderate effectiveness of PEG-IFN/RBV for chronic HCV treatment, and the poorer SVR in populations with advanced liver disease, who previously failed therapy and HIV co-infection, demonstrates the need for improvements in treatment efficacy.

New and emerging direct acting antiviral therapy

Improved understanding of HCV replication has allowed for the development of a plethora of new therapeutic agents that target enzymes directly (Figure 1). HCV is a flavivirus with an RNA genome encoding a polyprote in [30]. After HCV enters hepatocytes, translation takes place to produce the structural polyprotein which must then be cleaved into functional proteins [31]. Several

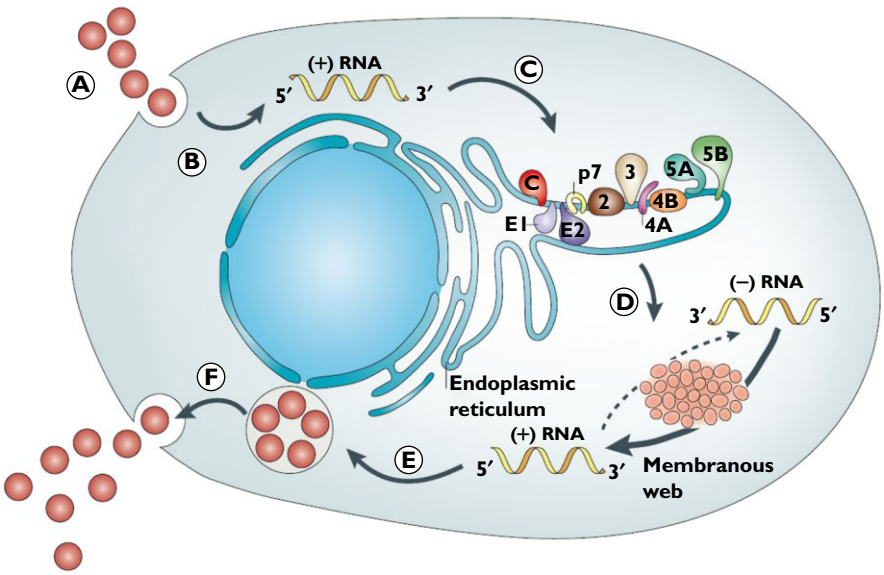

Figure 1

HCV viral life cycle and therapeutic targets for new drug classes (A) HCV virus binding and entry via receptor-mediated endocytosis (targeted by entry inhibitors); (B) RNA release into the cytoplasm; (C) Translation into a polypeptide on the ribosome, and processing into viral proteins that form structural components of the virus (targeted by protease inhibitors); (D) RNA replication in the endoplasmic reticulum (targeted by protease, polymerase, NS5A and cyclophilin inhibitors, and antagomirs); (E) RNA packaging and assembly (targeted by NS5A inhibitors); and (F) virion maturation and release (targeted by glycosylation inhibitors). (Figure reproduced with permission from Nature Publishing Group, Moradpour *et al*. 2007 [31], copyright)

non-structural proteins (NS2-NS5) mediate these intracellular functions and have proven promising therapeutic targets for DAAs [32, 33]. Host target inhibitors include the cyclophilin inihibitors, a host enzyme intimately involved in HCV replication, and the antagomir targeting miR-122. Novel immunomodulators, new interferons or ribavirin analgoues are not HCV specific but generate an anti-viral state. A recent systematic review found more than 50 molecules currently in development to treat chronic HCV, divided across various therapeutic classes [34]. In light of the rapidly evolving literature, we have selected the more promising agents from each major class as illustrations of drugs in development (Table 2). It is worth noting that most of these drugs are in the early stages of development, have been studied in small cohorts, and have been presented in conference form only. Therefore their results need to be considered with some caution.

Current HCV protease inhibitors

Two HCV protease inhibitors (PIs), boceprevir and telaprevir, are now licensed for chronic HCV treatment. The HCV non-structural (NS)-3/4A HCV protease is responsible for cleaving the HCV viral polyprotein into mature proteins. Both drugs bind reversibly to the NS3 active site, blocking polyprotein cleavage and preventing HCV replication [35]. In addition to this direct antiviral action, inhibition of NS3 protease may also act to restore the hepatocyte interferon-signalling pathways [36]. Both drugs were designed using genotype-1 HCV-specific *in vitro* systems, and have limited activity against other HCV genotypes. When used as monotherapy, virological resistance develops rapidly to boceprevir and telaprevir [37, 38], necessitating combination with PEG-IFN/RBV.

The pivotal trials of boceprevir or telaprevir triple therapy demonstrated significantly increased rates of SVR compared with PEG-IFN/RBV in both treatment-naïve and treatment experienced HCV genotype-1 infected patients.

Boceprevir Boceprevir treatment consists of 800 mg (4×200 mg capsules) administered orally, every 8 h, and is introduced after a 4 week lead-in of standard PEG-IFN/RBV therapy in patients with genotype-1 HCV infection. Treatment naïve patients receive boceprevir for a further 24 weeks (if HCV RNA was undetectable from week 8) or 44 weeks (if HCV RNA was detectable at week 8), accompanied by PEG-IFN/RBV [35]. Treatment experienced patients receive the same dose for 44 weeks after the 4-week PEG-IFN/RBV lead-in [39]. The rationale for a lead-in period was to lower HCV RNA levels prior to boceprevir treatment, thereby reducing the risk of viral breakthrough or resistance [40]. The lead-in allows assessment of IFN responsiveness – if HCV RNA decreases <1 $\log_{10}$ IU ml^{-1} by week 4, predicted SVR with PEG-IFN/RBV alone is 5% *vs*. 29–39% with the addition of boceprevir.

Among treatment naïve patients, SVR improved when boceprevir was added to PEG-IFN/RBV from 40% to 67–68% in non-Black patients, and from 23% to 42–53% in Black patients (Table 1) and 44% of patients were eligibile for short duration therapy using RGT. Anaemia occurred nearly twice as often in boceprevir patients as in controls (49% *vs*. 29%), but there was no significant difference in the frequency of treatment discontinuation due to adverse events [35]. Erythropoietin use to treat anaemia was more frequent among patients on boceprevir (43% *vs*. 24%). Dysgeusia also occurred more than twice as often in boceprevir patients than controls (37% *vs*. 18%). Among patients who had previously failed PEG-IFN/RBV, boceprevir improved SVR from 29% to 75% among previous relapsers, and improved SVR from 7% to 52% among partial responders [39]. Prior null responders had an SVR rate of 38% with boceprevir therapy [41].

The registration studies for both boceprevir [35, 39] and telaprevir [42, 43] were well designed and powered randomized control trials. Their main limitations stem from generalizability from the trial to clinical settings. Firstly, boceprevir's 4 week lead-in period in their study design offers advantages in predicting overall outcome based on week 4 RVR. However the lead-in contributes to treatment complexity in practice. Secondly, the high treatment completion rates and corresponding high SVR rates for both PIs may also have been augmented by erythropoietin administration used to manage anaemia during the trials in >40% of participants which may not be available outside of a research setting. Finally, recent data from a French observational cohort on the use of HCV PIs in clinical practice found high rates of serious adverse events (38–49%) compared with the boceprevir and telaprevir clinical trials (9–14%) [44]. However, there are no data available yet on whether the SVR observed in trials will differ in practice.

Telaprevir The telaprevir treatment paradigm involves 750 mg (2×375 mg capsules) administered orally for 12 weeks, concurrently with PEG-IFN for 24 weeks (with RVR and EVR) or 48 weeks (for patients who do not achieve EVR) in treatment naïve patients. In the registration trial, telaprevir treatment for 8 or 12 weeks in addition to PEG-IFN/RBV for 24–48 weeks improved SVR from 44% to 69–75% in previously untreated patients [43]. Rash and anaemia were higher in the groups that received telaprevir, and discontinuation of treatment was more frequent (7–11% telaprevir group *vs*. 3% control group). Rashes were primarily eczematous and reversed on discontinuation of telaprevir. However one rare case of Stevens-Johnson syndrome and DRESS syndrome have been reported. Erythropoietin use was not permitted. Gastrointestinal side effects (nausea, diarrhoea, anorectal pain and haemorrhoids) were also more common with telaprevir compared wiht controls (40–43% *vs*. 31%).

Telaprevir also improves SVR rates in HCV genotype-1 patients who have previously failed PEG-IFN/RBV. Telaprevir used for 12 weeks with 48 weeks of PEG-IFN/RBV, with or without a 4 week lead-in phase, improved SVR from 24%

Table 2

Class leading agents in development for chronic HCV treatment

Class	Mechanism	Drug	Trial phase	Patients	End point, virological response	Reference
NS5A inhibitor	NS5A inhibitor	Daclatasvir (BMS-790052)	II	Genoytpe-1, naïve	+PEG-IFN/RBV: SVR12 83–92%	Pol *et al*. 2011 [59]
			II	Genoytpe-1, null responders	+protease inhibitor: SVR 36% +protease inhibitor + PEG-IFN/RBV: SVR 90%	Lok *et al*. 2012 [58]
Polymerase inhibitors	NS5B inhibitor	GS-7977	II	Genotype-2 and -3, naïve	+PEG-IFN/RBV: SVR 100% +RBV: SVR 100%	Gane *et al*. 2011 [51]
			II	Genotype-1, naïve	+PEG-IFN/RBV: ETR 100%, SVR12 88–91%, SVR24 93%	Lawitz *et al*. 2011 [53]
	NS5B inhibitor	Mericitabine, (RG7128)	II	Genotype-1, naïve	+PEG-IFN/RBV: SVR12 76%	Pockros *et al*. 2011 [54]
			II	Genoytpe-1, naïve	+protease inhibitor: Day14 viral load decline 5.1 $\log_{10}$ IU ml^{-1}	Gane *et al*. 2010 [55]
			II	Genoytpe-1, nullnresponders	+protease inhibitor: Day14 viral load decline 4.9 $\log_{10}$ IU ml^{-1}	Gane *et al*. 2010 [55]
	Non-nucleoside	Tegobuvir (GS-9190)	II	Genoytpe-1, naïve	+PEG-IFN/RBV: EVR 67% +protease inhibitor + PEG-IFN/RBV: EVR 92%	Lawitz *et al*. 2011 [85] Foster *et al*. 2011 [86]
NS3/4a Protease inhibitors	NS3/4A inhibitor	TMC435	II	Genotype-1, naïve	+PEG-IFN/RBV: SVR 89–97%	Fried 2011 [48]
				Genotype-1, experienced	+PEG-IFN/RBV: Week 24 treatment response 84–91%	Zeuzem *et al*. 2011 [87]
		Asunaprevir (BMS-650032)	II	Genoytpe-1, naïve	+PEG-IFN/RBV: EVR 83–100%	Bronowicki *et al*. 2011 [88]
			II	Genoytpe-1, experienced	+polymerase inhibitor: SVR 36% +polymerase inhibitor + PEG-IFN/RBV: SVR 90%	Lok *et al*. 2012 [58]
		Danoprevir (RG7227)	II	Genoytpe-1, naïve	+PEG-IFN/RBV: SVR 60–71% +polymerase inhibitor: Day14 viral load –5.1 $\log_{10}$ IU ml^{-1}	Larrey *et al*. 2011 [89] Gane *et al*. 2010 [55]
				Genoytpe-1, experienced	+polymerase inhibitor: Day14 viral load –4.9 $\log_{10}$ IU ml^{-1}	Gane *et al*. 2010 [55]
Quadruple therapy	Interferon +ribavirin +NS5A +NS3 inhibitor	PEG-IFN/RBV +daclatasvir +asunaprevir	II	Genotype-1, experienced, non-responders	SVR 90%	Lok *et al*. 2012 [58]
Host-targeted agents	Cylcophilin inhibitor	Alisporivir (Debio025)	II	Genotype-1, naïve	+PEG-IFN/RBV: SVR 76% compared to 55% standard therapy	Flisiak *et al*. 2011 [65]
Antagomirs	Micro-RNA (miR-122) antagomir	Miravirsen	II	Genotype-1, naïve	+PEG-IFN/RBV: 10 weeks HCV RNA decline 2.7 $\log_{10}$ IU ml^{-1}	Janssen *et al*. 2011 [68]
Ribavirin analogues	Ribavirin analogues	Taribavirin (Viramidine)	III	All genotypes	+PEG-IFN: SVR 38% (inferior to RBV)	Benhamou *et al*. 2009 [77] Marcellin *et al*. 2010 [78]
Interferons	PEG-interferon lambda	PEG-IFN-lamba	II	Genotypes-1–4	+RBV: EVR 40–71%	Zeuzem *et al*. 2011 [75]
Immunomodulators	Nitazoxadide	Nitazoxadide	II	Genotypes-1, -2 and -4, naïve	+PEG-IFN: SVR 61% +PEG-IFN/RBV: SVR 79%	Rossignol *et al*. 2010 [69]
	Toll-like Receptor-7 agonist	ANA733	II	Genotype-1, naïve	Monotherapy: 10 days viral load decline 1.3 $\log_{10}$ IU ml^{-1}	Bergmann *et al*. 2011 [71]
	Toll-like Receptor-9 agonist	IMO-2125	II	Genotype-1, null responder	Increased serum IFN-alpha, IP-10, cytokines	Rodriguez-Torres *et al*. 2010 [72]
	Therapeutic vaccination	GI-5005	2	Genotype-1, naïve	+PEG-IFN/RBV: SVR 58%	McHutchison *et al*. 2010 [73] Jacobson *et al*. 2010 [74]

PEG-IFN, pegylated interferon alpha-2a/b; RBV, ribavirin; Gt, HCV genotype; EVR, early virological response 12 weeks on-therapy SVR12, sustained virological response 12 weeks post-therapy; SVR, sustained virological response 24 weeks post-therapy.

to 83–88% among prior relapsers, from 15% to 54–59% in partial responders, and from 5% to 29–33% among null responders [42].

Patients with cirrhosis had much improved SVR when treated with either telaprevir (62% vs 33%) or boceprevir (50% *vs*. 39%, non-Black cohort) combination therapy but treatment toxicities were more challenging with higher discontinuation rates (15% *vs*. 11% among non-cirrhotics) [45]. In a sub-analysis of the telaprevir registration study, rash, pruritus and anaemia were more frequent in patients with cirrhosis (43%, 55% and 44%, respectively) than in those who received PEG-IFN/RBV (27%, 35% and 27%, respectively).

Protease inhibitor drug interations Drug interactions between HCV PIs and other medications introduce extra treatment complexity. HCV PIs seem to exert strong, reversible inhibition of CYP34A. However other data indicate that another non-CYP34A pathway is involved in boceprevir metabolism and excretion [46]. PI concentrations can vary with co-administration of other cytochrome P450 metabolized drugs including HIV combination antiretroviral therapy, with implications most important for HIV/HCV co-infected patients [47].

Novel HCV protease inhibitors

Beyond telaprevir and boceprevir, a number of new NS3A PIs are being developed in phase II/III trials. The next PI to market will likely be TMC-435, which is dosed once daily, offering a benefit over current generation NS3/4A PIs. A phase II trial of treatment naïve, genotype-1 individuals used TMC-435 with PEG-IFN/RBV for 24 of 48 weeks total therapy, guided by HCV RNA at weeks 4 to 20. 68–76% of patients achieved RVR, of whom 88–95% achieved SVR [48]. 79–86% of patients were eligible for short duration (24 weeks) therapy. The control group in this study also had a high SVR response, so the overall virological response in this cohort may have been over-estimated. It had a favourable side effect profile, with similar rates of rash and anaemia compared with the control group.

MK-5712 is a potent second generation NS3 PI in early stage development. It requires once daily dosing, and has efficacy against HCV genotypes 1–6 *in vitro* [49]. MK-5172 also has activity against a number of variants that are resistant to other protease inhibitors in development. There are several other PIs in development (Table 2). These new PIs will likely replace the first generation PIs due to their improved side effect profile and simplified use, regardless of any additional improvement in SVR.

HCV NS5B polymerase inhibitors

NS5B polymerase inhibitors can be classified as nucleoside inhibitors (NI) or non-nucleoside inhibitors (NNIs). NIs are potent and are active against all HCV genotypes, as the HCV catalytic site is conserved across genotypes. They have a good resistance profile and NI-resistant HCV variants have displayed very poor fitness to date [50]. The most promising NI at present is GS-7977 which has entered phase III development for genotype-1 HCV in combination with PEG-IFN/RBV [51–53]. GS-7977 has also entered phase III development as IFN-free treatment for genotype-2/-3 HCV (see below). Mericitabine is a second NI in advanced clinical development. In one study, HCV treatment naive patients infected with HCV genotypes-1/-4 received response guided mericitabine plus PEG-IFN/RBV or PEG-IFN/RBV alone for at least 24 weeks. Virological response 12 weeks post-therapy (SVR12) was 76% in the intervention group, compared with 56% in the standard therapy group [54]. Its antiviral potency (91% RVR) has been confirmed in other phase II studies [55].

NNIs bind to allosteric sites around the active site of the NS5B enzyme, induce conformational changes and down-regulate the polymerase's activity. There are multiple NNIs that have entered clinical development, including tegobuvir in phase II development, and others in earlier stages including filibuvir [56] and silibinin [57]. The class-wide limitations of NNIs to date include their relatively weak potency and rapid emergence of resistance. They may have a role in combination DAA regimens.

HCV NS5A inhibitors

NS5A replication complex inhibitors are potent, pan-genotypic antivirals. Daclatsavir is a potent NS5A inhibitor with efficacy in HCV genotype-1 treatment naïve and experienced patients [58, 59]. In a phase II study of treatment naïve patients, daclatasvir given in combination with PEG-IFN/RBV *vs*. standard therapy had an SVR12 of 83–92% *vs*. 25%, respectively [59]. Natural polymorphisms at the HCV NS5A gene conferring daclatasvir resistance have been identified from gene bank studies and have been shown to be clinically relevant *in vivo* [60]. Further study is underway to determine how these primary resistance mutations might affect the NS5A class.

HCV resistance associated with DAA therapy

The high replication rate and error-prone HCV polymerase give rise to naturally occurring resistance-associated variants (RAVs) [50]. In the setting of PI monotherapy, RAVs are selected within days, leading to virological breakthrough [61]. Single nucleotide substitutions have been identified that are associated with resistance to all PIs in development, and the R155/A156 substitutions are cross-resistant for all PIs. Combination with PEG-IFN/RBV can prevent mutants from emerging [62, 63] Hence current PI trials underway in phase III are using triple therapy with PEG-IFN/ RBV.

Host target inhibitors

Cyclophilin inhibitors

Cyclophilin A is required for HCV replication and this has led to the development of several cyclophilin inhibitors

[54]. Alisporivir (Debio025) is a representative, non-immunosuppressive ciclosporin analogue that inhibits HCV assembly and replication by binding to the host protein, cyclophilin A [64]. In a phase II trial of HCV genotype-1 treatment naïve patients, those receiving alisporivir and PEG-IFN/RBV had superior SVR (76%) compared with PEG-IFN/RBV alone (55%) [65]. Alisporivir is now in phase III development for HCV genotype-1. Alisporivir also has activity against HCV genotypes-2/-3 and IFN-free regimens are currently being evaluated. Resistance to cyclophilin inhibitors has been described, although is thought to be rare *in vivo* [66].

Antagomirs

The liver-expressed microRNA-122 (miR-122) is essential for HCV accumulation in hepatocytes [67]. In animal models, an oligonucleotide complementary to miR-122 (a so called 'antagomir') led to prolonged suppression of HCV viraemia. In an early phase II trial, miravirsen (an miR-122 antagomir) was given in weekly subcutaneous weight-based injections to individuals with HCV genotype-1 for 1 month, followed by standard therapy [68]. A continuous and prolonged anti-viral activity was observed beyond the end of active therapy with minimal side effects.

Immunomodulators

Immunomodulatory agents improve innate immune responses indirectly promoting HCV elimination. Nitazoxanide is an anti-parasitic agent that improves SVR in combination with standard therapy, and is thought to act by improving interferon signalling [69]. In a phase II study of treatment naïve, predominantly HCV genotype-4 patients, nitazoxanide was used as a 4 week lead-in, then continued with PEG-IFN for 36 weeks, with or without ribavirin. The SVR was 79% *vs.* 61%, with or without ribavirin, respectively. but side effects were more frequent among those receiving nitazoxanide [69].

Anti-HCV activity can be induced by toll-like receptor (TLR)-7 and TLR-9 stimulation by mediating endogenous interferon and cytokine release [70]. A small molecule inducer of the TLR-7 pathway is in phase II development indicating anti-viral activity of -1.3 $\log_{10}$ IU ml^{-1} from baseline, compared with -0.3 $\log_{10}$ IU ml^{-1} in the placebo group [71]. A TLR-9 agonist has also completed phase-1 development [72]. Therapeutic vaccination targets are also in early development. One vaccine agent (GI-5005) expresses a protein encompassing HCV NS3 and core protein sequences, and demonstrated antiviral activity in phase II studies of genotype-1, treatment naïve patients, improving SVR from 48% to 58% when added to PEG-IFN/RBV [73, 74].

Other therapeutic approaches

There are a number of novel indirectly acting antivirals in various stages of development. Their main advantage is that by acting non-specifically, they do not engender viral resistance, but they add to treatment complexity if used additively.

Interferon analogues

Alternative interferon agents have been studied to improve tolerability and efficacy. PEG-IFN-lambda is active against all HCV genotypes and binds a more hepatocyte-specific receptor, thus reducing haematological side effects. A phase II study demonstrated fewer dose interferon reductions, less marrow toxicity and flu-like symptoms, and generally improved rapid virological response (between 40–71% dependent on HCV genotype) [75]. Albinterferon is a fortnightly preparation of PEG-IFN-alpha bound to albumin to prolong its half-life, with consequently reduce side effects. Although the SVR was 51% in non-responders with genotypes 1–3, development has been discontinued [76].

Ribavirin analogues

Ribavirin analogues have been studied in place of ribavirin primarily as a means of reducing ribavirin-associated anaemia [77]. Taribavirin is a prodrug, which preferentially targets the liver, and accumulates less in red blood cells. A phase III study of PEG-IFN with taribavirin demonstrated significantly less anaemia compared with PEG-IFN/RBV, but inferior SVR in the taribavirin group (38% *vs.* 52%) [78].

The promise of new combinations: with and without interferon

The challenge of new therapeutic agents and combinations is to improve virological response, shorten the length of therapy and offer treatment free of interferon. New treatments will need to be evaluated by several markers: first, in terms of their improvements in efficacy beyond the new standard of PI/PEG-IFN/RBV response; second, their reductions in side effects; third, improved treatment simplicity and pill burden; and finally cost for the individual and society. There is the real potential to have interferon free regimes available within several years with the consequent reduction in side effects and availability of treatment for IFN-intolerant individuals (Table 3). However, there may remain a selective role for IFN since quadruple therapy with two DAAs plus PEG-IFN/RBV has been shown to improve outcomes for patients with multiple poor treatment response predictors (such as previous treatment non-response, cirrhosis, genotype-1 infection and high HCV viral load). In these difficult to treat individuals, the increase in efficacy may outweigh increases in complexity and toxicity. Another area whether combination therapy is likely to improve in the next few years will be incremental changes in the current triple therapy PI/PEG-IFN/RBV as newer once daily protease inhibitors replace boceprevir and telaprevir.

Table 3
Interferon free combination strategies in development

Mechanism	Drugs	Trial phase	Patients	Virological response, end point	References
Nucleoside polymerase +NS3 protease inhibitor	Daclatasvir +asunaprevir	II	Genotype-1, null responders, USA	RVR 64%, SVR 36% (n = 11)	Lok *et al.* 2012 [58]
		II	Genotype-1b, null reponders, Japan	SVR24 100% (n = 10)	Chayama *et al.* 2012 [79]
Nucleoside polymerase inhibitor + RBV	GS-7977 +RBV	II	Genotype-2 and -3, naïve	RVR 100%, SVR 100% (n = 10)	Gane *et al.* 2011 [51]
		II	Genotype-1, null responders	SVR4 10% (n = 10)	Gane *et al.* 2012 [52]
Nucleoside polymerase +NS3 protease inhibitor +Ribavirin	BI201335 +BI207127 +RBV	II	Genotype -1, naïve	RVR 100% (n = 17)	Zeuzem *et al.* 2011 [90]
Nucleoside polymerase +NS3 protease inhibitor	Tegobuvir +GS-9256	II	Genotype-1, naïve	EVR 80% (n = 15) +RBV: EVR 100%) (n = 13)	Foster *et al.* 2011 [86]
Nucleoside polymerase +NS3 protease inhibitor	Mericitabine +danoprevir	II	Genotype-1, experienced, non-responders	Day14 viral load -4.9 $\log_{10}$ IU ml^{-1}	Gane *et al.* 2010 [55]
		II	Genotype-1, naïve	Day14 viral load -5.1 $\log_{10}$ IU ml^{-1}	Gane *et al.* 2010 [55]

PEG-IFN, pegylated interferon alpha-2a/b; RBV, ribavirin; RVR, rapid virological response with HCV RNA undetectable at week 4; EVR, early virological response with HCV RNA undetectable at week 12.

Interferon-free therapy

Several studies have explored IFN-free therapy combinations in different groups (Table 3). Two agents, GS-7977 and daclatasvir, are likely to be included in the first IFN-free regimens. A study of an NS5B polymerase (GS-7977) with ribavirin among treatment naïve, genotype-2/-3 patients treated 40 participants for 12 weeks, randomly assigned PEG-IFN for different durations or not at all [51]. All participants achieved an RVR and SVR, regardless of PEG-IFN administration. Viral kinetics were no different in the IFN-free arm, and there were no cases of viral breakthrough, suggesting that the combination has a high barrier to resistance. GS-7977 monotherapy for 12 weeks among 10 genotype-2/-3 patients resulted in an end-of-treatment response for all patients. However four relapsed within 4 weeks of stopping therapy, consistent with a need for RBV in IFN-free regimens [51]. Among harder to treat, genotype-1 null responders, GS-7977 plus RBV for 12 weeks demonstrated a 100% end-of-treatment response (n = 10). However nine out of 10 relapsed within 4 weeks of stopping treatment [52]. Previous null responders may require longer DAA therapy, the addition of other DAA agents, or might require PEG-IFN.

Another phase II trial of treatment experienced, genotype-1, non-responders without cirrhosis studied daclatasvir, an NS5A polymerase inhibitor, and asunaprevir, an NS3 protease inhibitor, randomized to receive PEG-IFN/RBV *vs*. no PEG-IFN/RBV [58]. Amongst the IFN-free group (n = 11), 4/11 of their non-responder population achieved an SVR, 1/11 relapsed after an undetectable HCV RNA at the end of treatment, while 6/11 had viral breakthrough on therapy. When examining genotype-1 subtype, both genotype-1b infected patients achieved an SVR (n = 2), while only 2/9 genotype-1a patients achieved an SVR. Resistance mutations to both NS5A and NS3 agents occurred in patients with virological failure. It is unclear whether those mutations will affect subsequent therapy. The same IFN-free drug combination was used for 24 weeks in a small Japanese cohort (n = 10) of HCV genotype-1b, IL28B favourable, treatment experienced null responders and was demonstrated to have a 100% SVR [79]. These early studies suggest that differences in genotype-1 subtype will have implications for IFN-free treatment success.

Quadruple therapy

In a phase II study of PEG-IFN and RBV in combination with daclatasvir and asunaprevir, very high SVR rates were achieved in a null responder population [58]. Ten treatment experienced, genotype-1, non-responders without cirrhosis had undetectable HCV RNA on treatment. SVR response measured at 12, 24 and 48 weeks was 10/10, 9/10 and 9/10 respectively. These results suggest that quadruple therapy may achieve very high SVR rates in patients who respond poorly to IFN, a dramatic improvement on standard therapy which would have predicted less than 10% SVR.

Conclusions

2011 was a watershed year for HCV treatments, heralding the licensing of the first DAA for HCV, as well as proof-of-concept that HCV could be cured without IFN therapy [1]. Challenges still remain to develop effective, durable, IFN-free regimens without promoting HCV resistance, and in

turn improve treatment side effects allowing those with significant psychological co-morbidities to access treatment for the first time. Few people who inject drugs, the group most at risk of HCV infection, receive HCV treatment currently [80, 81]. Improved regimens of shorter duration with fewer side effects may increase the number of patients on treatment overall, and people who inject drugs in particular. Current HCV practice is evolving rapidly and newer agents and classes of drug will add to treatment complexity in the short term. In addition to the individual benefits from curing HCV, increasing treatment uptake among people who inject drugs has the potential to reduce HCV prevalence in this high risk population [82, 83]. The rapid improvements, successes to date and the number of HCV agents in development should give hope to millions of patients living with HCV infection.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work, JD and EA had no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, MH received research support from Roche, DL received honoraria from Abbott and Merck, and AT received research/grant support from Merck, Roche Pharmaceuticals, Gilead Sciences and speaker's fees from Merck, Roche Pharmaceuticals, Bristol-Myers Squibb, JD, EA, DL and MH had no other relationships or activities that could appear to have influenced the submitted work, and AT had a consulting/advisory capacity with Merck, Roche Pharmaceuticals, Janssen-Cilag (Johnson and Johnson) and is a co-inventor of a patent related to the *IL28B*-HCV discovery.

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