

Review article: novel therapeutic options for chronic hepatitis C

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SUMMARY

Background

The efficacy of treatment against hepatitis C virus has improved, but it is still far from ideal. Thus, new antihepatitis C virus therapies are required.

Aim

To evaluate the data on antihepatitis C virus approaches beyond the current standard combination of pegylated interferon-alpha and ribavirin.

Method

We reviewed the available literature regarding novel antihepatitis C virus options, given alone or in combination with existing agents.

Results

New interferons and ribavirin alternatives have been tried aiming to improve the efficacy and the safety/tolerability profile of standard agents. The hepatitis C virus polymerase and NS3/4A protease have been rather popular targets for new antihepatitis C virus agents. The combination of such inhibitors with pegylated interferon-alpha and ribavirin seems to act synergistically and to prevent viral resistance, compared to monotherapies. Several novel immunomodulators are currently evaluated and may be useful in combination therapies. Alternative strategies (inhibition of hepatitis C virus protein translation, assembly/release or binding) or agents with different modes of action (statins, S-adenosylmethionine and herbs) need further evaluation.

Conclusions

Many novel promising antihepatitis C virus agents are being developed, offering hope for future therapies that may target multiple points of the viral life cycle and/or host immune response. Newer approaches should ideally provide safe, effective and more tolerable therapy to all chronic hepatitis C virus patients.

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INTRODUCTION

The management of chronic hepatitis C virus (HCV) infection is extremely important, as approximately 500 000 patients die every year because of complications of HCV-related end-stage liver disease.¹ The efficacy of therapy has improved over the last decade, but still more than 40% of chronic hepatitis C (CHC) patients do not eventually respond to the combination of pegylated interferon-alpha (PEG-IFN α) and ribavirin (RBV), which is the current standard of care.² In particular, sustained virological response (SVR), which is the desirable therapeutic end-point, is achieved in 45–55% of naïve patients infected with genotype 1 or 4 and 80% of those infected with genotypes 2 or 3.^{2, 3} As patients with genotype 1 or 4 represent the majority of CHC cases in most parts of the world, it is evident that current treatment is far from ideal. Moreover, in the increasing numbers of nonresponders to a previous standard course, the current combination of PEG-IFN α and RBV offers rather poor SVR rates and is therefore not currently recommended. Finally, the current combination therapy is associated with frequent and potentially severe side effects and cannot be given to all patients, particularly to those with advanced liver disease who mostly require therapeutic

intervention. From all these, it is evident that novel and more efficacious anti-HCV therapies are urgently needed.

The availability of new culture systems has created opportunities not only for the better understanding of HCV infection biology, but also for the identification of new methods that could lead to the discovery of novel anti-viral therapies.^{4, 5} In particular, the development of HCV subgenomic replicons, which are able to replicate autonomously in cell culture, and a system derived from a genotype 2a genome isolated from a Japanese patient with fulminant hepatitis, which produces infectious virus particles, have reinforced this field.^{4, 5} On the basis of new insights into virology of HCV (attachment, entry into cells, replication and release; Figure 1) and host cellular immune response, the effort to find new agents has focused on targeting specific steps of the viral life cycle, including inhibitors of HCV enzymes, nucleoside analogues targeting viral RNA and immunomodulators.⁶ Although many new anti-viral compounds have been synthesized and several of them shown promising anti-viral efficacy in preclinical and early clinical trials, their safety and clinical efficacy have to be proved in the long term.⁷

This review focuses on the therapeutic approaches, beyond the standard combination of PEG-IFN α and

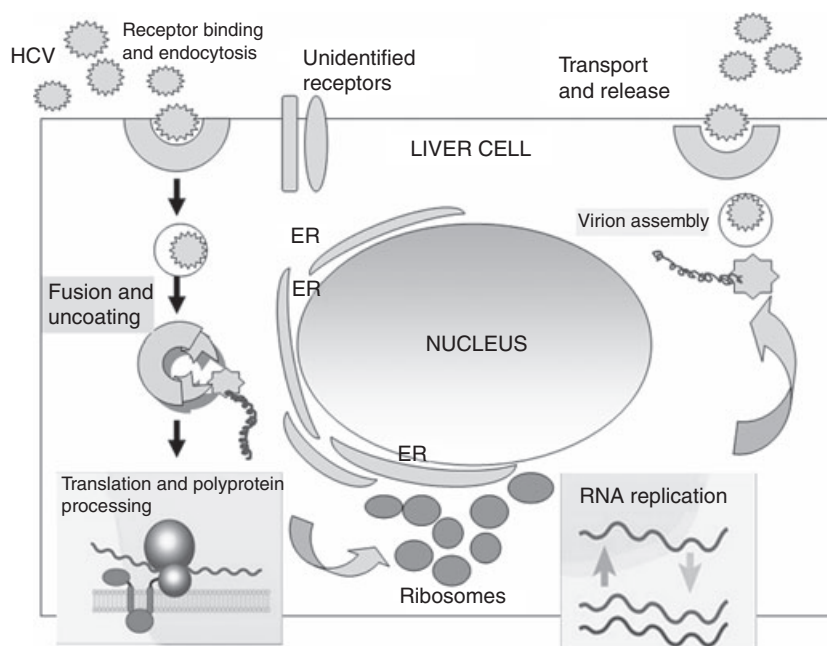


Figure 1. Schematic picture of the replicative cycle of hepatitis C virus in hepatocytes. Each step represents a potential site of therapeutic intervention.

RBV, which are currently being evaluated for the treatment against HCV (Table 1).

HCV VIRION AND ITS LIFE CYCLE

Hepatitis C virus is a small enveloped virus with a 9.6 kb positive-sense single-stranded RNA genome that encodes a unique large polyprotein, which is processed by cellular and virally encoded proteases to produce at least 10 mature structural and nonstructural (NS) proteins (Figure 2).⁸ Amongst the structural proteins, the two envelope glycoproteins, E1 and E2, are essential components of the HCV virion envelope and are necessary for viral entry and fusion with cellular membrane.⁹ The latter is an important step for the release of HCV nucleocapsid in the cell cytoplasm and initiation of HCV polyprotein translation in a membranous web made of the NS proteins and host cell proteins called 'replication complex', located in close contact with perinuclear membranes.⁸ HCV 5'-nontranslated region contains a highly structured element, called internal ribosome entry site (IRES), which is essential for the initiation of HCV polyprotein translation.¹⁰ Amongst the NS proteins, the NS3 serine-like protease and the RNA-dependent RNA polymerase (RdRp), which is encoded by the NS5 region, are essential for viral maturation and replication, and therefore represent ideal targets for the development of small molecule anti-HCV compounds.^{11, 12} Genome encapsidation occurs in the endoplasmic reticulum and nucleocapsids are enveloped and matured into the Golgi apparatus before they are released in the pericellular space by exocytosis (Figure 1).¹³

NEW THERAPEUTICS APPROACHES

New interferons

Consensus interferon. Consensus interferon (CIFN) is a recombinant, non-naturally, 166-amino acid IFN, containing the most frequently observed amino acids from various natural IFNs in each corresponding position.^{14, 15} Some studies suggested that CIFN in combination with RBV may not be superior to the combination of PEG-IFN α -2b plus RBV in naïve CHC patients with genotype 2/3¹⁶ or genotype 1.^{17, 18} CIFN-based regimens have also been tried in nonresponders and relapsers CHC patients. CIFN (18 μ g for 8 weeks and 9 μ g for another 40 weeks) achieved low (22%) SVR rates in nonresponders to PEG-IFN α -based

therapy.¹⁹ Moreover, in a phase III, open-label multi-centre US-based study (DIRECT trial), CIFN (15 μ g) plus RBV for 48 weeks achieved SVR rates of only 10% in 343 genotype 1 nonresponders to PEG-IFN α -based therapy.²⁰

A major drawback of CIFN is the need for daily dosage because of its short half-life. It should be also noted that most of the data on CIFN-based therapies have not been published as full papers to date.^{16-18, 20-23}

Albuferon. Albuferon (alb-IFN) is an 86 kDa novel recombinant protein consisting of IFN α genetically fused to human albumin extending its serum half-life.²⁴ Recent studies have shown that its prolonged half-life (6 days) supports dosing at 2- to 4-week intervals,²⁵ regardless of other factors, such as age, gender, race or stage of liver fibrosis. An ongoing phase IIb trial in 458 IFN-naïve CHC patients with genotype 1 showed that the efficacy of alb-IFN (900–1200 μ g every 2 or 4 weeks) in combination with RBV for 48 weeks exhibits efficacy and safety at least comparable to PEG-IFN α -2a plus RBV with similar frequency in adverse events, significantly less frequent development of antibodies against IFN α (3% vs. 19%, $P < 0.0001$), and better quality of life because of its improved dosing schedule.²⁶ In this study, the efficacy of alb-IFN and RBV appeared to be similar with PEG-IFN α -2a and RBV (SVR rates: 51–59% vs. 58%) and greater in heavier (≥ 75 kg) patients (SVR: 61–74% vs. 53%).²⁶ A phase III trial of alb-IFN in naïve CHC patients started in late 2006.

The efficacy of alb-IFN in difficult-to-treat patients has been evaluated in 115 nonresponders to previous IFN α -based therapies.²⁷ This study showed that alb-IFN (900–1800 μ g every 2 or 4 weeks) in combination with weight-based RBV (1000–1200 mg daily) for 48 weeks was effective [the overall end-of-treatment response (ETR) in the alb-IFN cohorts was on average 31% (range: 25–44) and the SVR rate 20% (range: 9–30)] and safe at doses up to 1800 μ g every 2 weeks. The HCV-RNA undetectability rates at week 24 were comparable across the 900–1800 μ g cohorts, whereas they ranged from 15% to 27% in genotype 1 patients with the highest rates achieved in the 1500 and 1800 μ g cohorts. Most patients with undetectable HCV-RNA at week 24 maintained the virological response until the end-of-treatment (week 48), while the virological response at week 12 and week 24

Table 1. Therapeutic options currently under evaluation for the treatment against HCV

Anti-HCV agent	Phase of development	Comments
New IFN		
Consensus IFN, albuferon, omega IFN, PEG-IFN β , R7025, Locteron	II-III	Albuferon: better dosing schedule (every 2-4 weeks) Omega IFN: continuous infusion
RIB analogues – IMPDH inhibitors		
Viramidine or taribavirin	III	Less frequently anemia but lower response rates than RBV*
VX-497 or Merimepodip	II	Preliminary data in combination with PEG-IFN α + RBV
Amantadine	IV	Combination with PEG-IFN α + RBV may improve SVR in NRs
Inhibition of RNA replication		
NS5B Polymerase inhibitors	Preclinical-III	
Nucleoside inhibitors (NM283, R1626, R7128, A837093)		NM283: stopped because of low efficacy and poor tolerability R1626: no evidence for resistance after 4 weeks
Non-nucleoside inhibitors (R803, HCV-371, HCV-086, HCV-796, Bilb1941, AG021541, PSI6130, CSK625433, GS9190, VCH-759, ANA598)		R803, HCV-371, HCV-086: stopped due to insufficient anti-viral activity, HCV-796: stopped due to elevations of liver enzymes
Prevention of functional replication complexes (ACH806)	I	ACH806: stopped due to renal dysfunction
Inhibition of protein translation	Preclinical-II	
Antisense oligonucleoside (ISIS 14803, AVI-4065)		ISIS 14803: stopped because of aminotransferase flares and poor anti-viral efficacy
RNA interference (SirmaAV34, BLT-HCV)		BLT-HCV: multi-targeting approach to prevent resistance
Ribozymes (Heptazyme)		Heptazyme: stopped because of toxicity in animal models
Post-translational modification		
NS3/4A serine proteinase inhibitors (BILN2061, VX950, SCH503034, ITMN191, TMC435350)	I-III	BILN2061: stopped due to cardiotoxicity Addition of VX-950 seems to improve the efficacy of PEG-IFN α or PEG-IFN α /RBV
NS5A (A831) and helicase inhibitors (QU663)	Preclinical-I	
Inhibitors of viral assembly/release (Celgosivir)	II	
Prevention of binding		
Polyclonal or monoclonal antibodies, GNS-037	Preclinical-II	Most studies in transplant CHC patients
Other immunomodulators		
Toll-like receptors agonists (resiquimod, ANA245, ANA975, SM360320, CPG10101)	I	Need further evaluation CPG10101 was suspended
Histamine dihydrochloride	II	Studies with PEG-IFN α /RBV were stopped
Thymalfasin	II-III	Stopped because of insufficient efficacy
Interleukin (IL-10, IL-12, IL-29)	II-III	IL-12: poor anti-viral efficacy and severe adverse events, PEG-IL-29: active in liver but not circulating white cells
Vaccines (recombinant E1, TG4040, IC-41, T cell, dendritic cell)	Preclinical-II	Induction of cytotoxic and T-helper cells response
Ciclosporin A analogues (DEBIO025, NIM811, SCY635)	I-II	DEBIO-025: 10-fold greater anti-HCV activity than ciclosporin
GI-5005		Monotherapy gave promising results. Trial of GI-5005 plus PEG-IFN α /RBV in progress

Table 1. Continued		
Anti-HCV agent	Phase of development	Comments
Others	Preclinical-II	
Agents implicated in lipid biosynthesis (NA255, Bezafibrate, statins) S-Adenosylmethionine, Betaine		Statins: inhibition of geranylgeranylation of host cell proteins <i>In vitro</i> : increase of IFN α anti-viral effect, reduce oxidative stress
Hepatoprotectants and other herbs (Silymarin, MK-001, Glycyrrhizin)	-	Controversial efficacy
Nitazoxanide	-	Nitazoxanide/PEG-IFN α -2a/RBV: higher end-of-therapy response rate compared to standard therapy
Antiapoptotic agents (IDN-6556, PF-03491390)	-	Pan-caspase inhibitors: promising results

IMPDH, inosine monophosphate dehydrogenase; NR, nonresponder; SVR, sustained virological response; CHC, chronic hepatitis C; RBV, ribavirin; IFN, interferon; RIB, ribavirin; HCV, hepatitis C virus.

* Perhaps higher responses at viramidine doses >18 mg/kg daily.

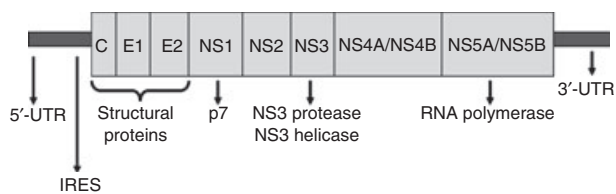


Figure 2. Structure of the 9.6 kb positive-sense single-stranded RNA genome of hepatitis C virus that encodes the structural and nonstructural proteins of the virus.

predicted 48-week responses in the 900–1200 μg cohorts.²⁷ Interestingly, in the 1800 μg group, six (50%) of the 12 CHC genotype 1 patients ‘null-responders’ to prior PEG-IFN α /RBV combination, achieved early virological response (undetectable HCV-RNA at week 12).

Omega interferon. Omega IFN is a new type-1 IFN, homologous to IFN α in 60% and homologous to IFN β in 30%, that has been designed for continuous delivery by an implantable device. In 102 IFN-naïve CHC patients with genotype 1, 48 weeks of treatment with omega IFN 25 μg daily in combination with RIB achieved undetectable HCV-RNA in 36% of cases at 12 weeks after the end-of-treatment, compared to only 6% of patients with omega IFN monotherapy. Omega IFN was well tolerated in both

groups with discontinuation because of adverse events in only two patients.²⁸

Other interferons. R7025 is a novel human pegylated IFN α -2a molecule generated using DNA shuffling technology. R7025 has the same (40 kDa) PEG molecule used in PEG-IFN α -2a, but it has 50-fold higher *in vitro* anti-viral activity. Single ascending doses of R7025 in 72 healthy volunteers gave promising results with mild flu-like symptoms and reversible drop in neutrophils and without serious adverse events.²⁹ Locteron is another controlled release recombinant IFN α -2a. In a phase Ib trial, combination of Locteron (at doses 160, 320, 480 and 640 μg every 2 weeks) with RBV was given in 32 naïve CHC genotype 1 patients for 12 weeks. At the end-of-treatment, the mean HCV-RNA reduction was 1.8, 4.5, 4.2 and 4.7 log₁₀ IU/mL respectively, while arthralgia, weakness, myalgia and headache were the most common adverse events.³⁰

Ribavirin analogues – inosine monophosphate dehydrogenase inhibitors

Ribavirin acts via inhibition of inosine monophosphate dehydrogenase (IMPDH), a cellular enzyme implicated in the production of guanine nucleotides.³¹ RBV treatment alone does not produce a significant anti-viral effect, but its addition to IFN α therapy significantly increases the rate of complete viral eradication,

compared to IFN α monotherapies, possibly because of the production of viruses, which are more sensitive to IFN α .³¹ However, its tolerability is frequently limited because of RBV induced haemolytic anaemia.³¹ Thus, RBV analogues lacking a haemolytic effect are needed, but their development is difficult, as the exact anti-viral mechanisms of RBV remain unknown.

Viramidine or taribavirin. Taribavirin hydrochloride or viramidine is a RBV prodrug that is metabolized preferentially in the liver by adenosine deaminase and thus it does not accumulate in erythrocytes.³² In a phase II trial, viramidine (400–800 mg daily) combined with PEG-IFN α -2a, achieved a relatively lower SVR rate, but significantly less frequently haemolytic anaemia, compared with RBV.³³ In this study, the daily dose of 600 mg of viramidine achieved the highest SVR rate.

A recent phase III trial revealed that viramidine combined with PEG IFN α -2b, compared with PEG IFN α -2b plus RBV combination, exhibited significantly lower anaemia rates (5% vs. 24%, $P < 0.001$), but again lower virological response rates (SVR: 38% vs. 52%).³⁴ Viramidine at doses >18 mg/kg yielded response rates similar to the current standard combination therapy without increasing the rates of anaemia or other side effects.^{35, 36} In addition, 970 patients with slow virological response (particularly those with HCV-RNA drop <2 log within the first 4 weeks of treatment) had higher SVR when treated with viramidine, compared with RBV, attributable to improved tolerability of viramidine over RBV (63% vs. 36% respectively).³⁷ Interestingly, pre-treatment with viramidine monotherapy (perhaps with RBV as well) for 4 weeks prior to PEG-IFN α -2b therapy resulted in a steeper decline of HCV-RNA levels after PEG-IFN α -2b introduction, compared with viramidine and PEG-IFN α -2b simultaneous therapy, but this difference was not significant. Thus, further studies are needed to assess the impact of pre-dosing of viramidine (or RBV) on viral response, as well as the development of weight-based dosing of viramidine.^{38, 39} For the time being, viramidine seems to be a potential substitute of RBV only for cases who develop severe anaemia under RBV but not for all CHC patients.

VX-497 or Merimepodip. VX-497 (Merimepodip) is a potent, specific and orally taken inhibitor of IMPDH. Although it has been found that VX-497 monotherapy

may increase viral replication because of T-cells inactivation⁴⁰ and IFN α plus VX-497 combination did not have stronger anti-viral activity than IFN α monotherapy in naïve CHC patients,⁴¹ the addition of VX-497 to PEG-IFN α and RBV was reported to improve significantly the virological response at week 24 in non-responders CHC patients.⁴²

Amantadine

Amantadine is a tricyclic amine, which has been used to treat and limit influenza A infection. Amantadine monotherapy has little activity against HCV, but its combination with PEG-IFN α and RBV has been shown to increase anti-viral response in patients with CHC. The results of a phase IV trial aiming to compare the efficacy of the triple PEG-IFN α -2a/RBV/amantadine (400 mg/day) combination for 48 weeks against the standard combination of PEG-IFN α -2a/RBV in 703 naïve genotype 1 CHC patients were recently presented. SVR rates were similar between the two groups (49% vs. 53%), while the drop-out rate was significantly higher in the amantadine group (32% vs. 23%, $P = 0.01$).⁴³ Although, amantadine seems to have no impact on virological response in naïve CHC patients, a recent meta-analysis of 31 randomized-controlled trials suggested that the triple therapy (PEG-IFN α /RBV/amantadine) may significantly improve the SVR rates (23%) in nonresponders to previous standard therapy.⁴⁴

Inhibition of RNA replication

NS5B polymerase inhibitors. The NS5B RdRp is an attractive target for anti-viral therapy.^{6, 12} Several research groups have discovered nucleoside analogues, which are converted to nucleotides and terminate the RNA chain.¹¹ Laboratory efforts have also uncovered a variety of structurally unrelated non-nucleoside inhibitors (NNIs) of RNA polymerase, which target the allosteric sites of the RdRp.^{11, 12} Interestingly, the different classes of NS5B polymerase inhibitors elicit diverse patterns of resistance, whereas several different binding sites for NNIs exist on the HCV polymerase. Thus, although these agents target the same viral enzyme, they offer the potential to be used in combination. Further studies are needed to clarify their exact anti-viral effect in the clinical setting after mid- and long term administration, but generally monotherapies must be avoided in favour of combination strategies because

of the susceptibility of these agents for development of viral resistance.¹¹

Nucleoside inhibitors: NM283 or Valopicitabine is the oral prodrug of the nucleoside analogue of 2'-C-methyl-cytidine. Pharmacokinetic studies have supported its co-administration with PEG-IFN α .⁴⁵ NM283 has less potent anti-viral efficacy, compared with BILN 2061 or VX-950, but it has demonstrated anti-viral activity at tolerated doses. In an ongoing phase IIb trial including 173 naïve genotype 1 CHC patients,⁴⁶ combination of NM283 (200–800 mg daily) and PEG-IFN α -2a achieved significant dose-dependent HCV-RNA reductions with high undetectability rates of HCV-RNA at weeks 24 and 36 by the sensitive Taq-Man assay (range: 49–68% of patients at both time points). Gastrointestinal side effects were common and occasionally severe at the NM283 daily dose of 800 mg,⁴⁶ leading to a protocol amendment with reduction in the 800 mg dose after 14–22 weeks of therapy.

In another phase IIb trial,⁴⁷ 178 genotype 1 non-responders to PEG-IFN α and RBV, received NM283 (400–800 mg daily) plus PEG-IFN α -2a. At 48 weeks, HCV-RNA undetectability by the Amplicor and Taq-Man assay was achieved in 40% and 28% of patients treated with NM283 plus PEG-IFN α -2a compared to 27% and 24% of those treated with PEG-IFN α -2a plus RBV re-treatment. HCV-RNA levels reduction was six-fold greater in the NM283/PEG-IFN α -2a than in the PEG-IFN α -2a/RBV group ($P = 0.06$). Again, the high NM283 dose (800 mg daily) was reduced to 400 mg daily after 40 weeks of therapy because of gastrointestinal side effects.⁴⁷ Recently, however, further development of the agent was stopped before the onset of phase III trials because of low efficacy and poor tolerability.

R1626, a prodrug of R1479 (4'-azido-cytidine), is a potent, specific inhibitor of HCV polymerase-mediated RNA synthesis. *In vitro* experimental studies have shown that R1479 has additive anti-viral effect combined with other HCV replication inhibitors, such as IFN α , RBV, NM107 and BILN2061, without any antagonistic effects⁴⁸ and without any effect on stability of human erythrocytes membrane when combined with RBV.⁴⁹ R1626 gave promising results in initial clinical trials. In particular, in a phase Ib trial including 47 naïve genotype 1 CHC patients, R1626 monotherapy at doses of 1500–4500 mg twice daily achieved 1.2–3.7

log₁₀ IU/mL reductions in HCV-RNA levels at 14 days.⁵⁰ The drug was generally well tolerated, but with increasing rates of adverse events at the highest dose (e.g. significant drop in haemoglobin at doses of 6000–9000 mg daily). In a phase II study, the combination of R1626 (1500–3000 mg twice daily) plus PEG-IFN α -2a with or without RBV was compared to standard therapy in 104 genotype 1 naïve CHC patients. At 4 weeks, HCV-RNA was undetectable in 81% of patients treated with triple therapy (mean reduction of 5.2 log₁₀ IU/mL), compared to only 5% of patients treated with standard therapy (mean reduction of 2.4 log₁₀ IU/mL).⁵¹ There was no evidence of resistance to R1626 during this 4-week period.⁵² Gastrointestinal adverse events were commonly observed with higher doses of R1626, while neutropaenia was not associated with increased incidence of infection. Further studies of different dosages of R1626 in combination with PEG-IFN α -2a and RBV are in progress.

R7128 is a new oral nucleoside HCV-RNA polymerase inhibitor. The anti-viral efficacy and tolerability of ascending dose of R7128 (750–3000 mg/day) were evaluated in 40 genotype 1 CHC patients. At 2 weeks, a significant dose-dependent reduction in serum HCV-RNA was observed (mean: 0.3–2.1 log₁₀ IU/mL, range: 0.9–2.7). No evidence of viral rebound was detected and no serious adverse events were recorded (headache, dry mouth, nausea and upper respiratory infection were the most frequent adverse events).⁵³

A-837093 is a new potent HCV polymerase inhibitor, with a strong anti-viral efficacy in the chimpanzee model, which may be useful in combination with other small-molecules (such as NS3/4A protease inhibitors) to treat HCV infection effectively. Its oral bioavailability was very good in animals.⁵⁴

Non-nucleoside inhibitors: JTK-109 and JTK-003 were the first NNIs of NS5B polymerase that entered clinical trials, but further clinical development is still awaited. Subsequently, several other HCV polymerase NNIs have been developed and evaluated in clinical trials, such as R803, HCV-371, HCV-086 and HCV-796. Further clinical investigation, however, has been stopped because of insufficient anti-viral activity of the first three and elevations of liver enzymes of the latter.

HCV-796 is an oral NNI of HCV-RNA polymerase, which belongs to the benzofuran family and has demonstrated potent anti-viral activity in both *in vitro* and

in vivo studies. In a clinical dose-escalation study including 102 treatment-naïve CHC patients (72% with genotype 1), HCV-796 administered orally at doses of 50–1500 mg twice daily for 14 days demonstrated an initial rapid anti-viral activity without significant side effects apart from mild-to-moderate headache.⁵⁵ In particular, the highest dose of HCV-796 achieved 1.4 log₁₀ IU/mL mean reduction in HCV-RNA levels at day 4.⁵⁵ However, selection of viral variants (Cys316Tyr) was observed with subsequent increase in viraemia, which returned to nearly baseline levels (the mean reduction of HCV-RNA at day 14 was only 0.7 log₁₀ IU/mL).⁵⁶ In a phase II clinical trial, the combination of HCV-796 and PEG-IFN α -2b was evaluated in patients with CHC. At 14 days, the combination therapy achieved greater reduction in HCV-RNA levels, compared with PEG-IFN α -2b monotherapy (3.5 vs. 1.6 log₁₀ IU/mL).⁵⁷ However, the combination of HCV-796 with PEG-IFN α -2b/RBV was halted because of significant elevation of liver enzymes.

Bilb1941⁵⁸ is another specific NNI of HCV polymerase, which, at an oral dose of 10–450 mg thrice daily, was found to have potent anti-viral activity at 5 days in 96 males, genotype 1, CHC patients. Although serious adverse events were not recorded, Bilb1941 was associated with frequent gastrointestinal intolerance (particularly at higher doses) and exacerbations of aminotransferases levels.⁵⁸

AG-021541 is a representative compound from a novel series of HCV polymerase inhibitors characterized by a dihydropyrene core. AG-021541 has exhibited satisfactory *in vitro* anti-viral activity against HCV, but the development of resistance because of mutations in the inhibitor-binding region needs further evaluation.⁵⁹

Thiophene derivatives and benzothiadiazines are other two classes of allosteric inhibitors of the HCV polymerase, but clinical trials are missing. The latter class of inhibitors showed anti-viral synergy with IFN in the HCV replicon, but a mutant replicon resistant to benzothiadiazines has been identified (M414T within NS5B).⁶⁰

PSI-6130⁶¹ and GSK625433⁶² are also potent and selective HCV NS5B inhibitors. PSI-6130 seems to be compatible with IFN, RBV, NNIs and BILN-2061.⁶³ GSK625433 belongs to a novel series of acyl-pyrrolidine, which binds to the palm region of HCV polymerase and its combination with other polymerase/protease inhibitors seems to be promising. VCH-759, a new oral HCV-RNA polymerase inhibitor given at

ascending doses (1200–2400 mg/day) for 10 days in 32 naïve genotype 1 CHC patients, achieved a significant (≥ 2 log₁₀ IU/mL) decline in HCV-RNA at doses of 1600–2400 mg/day (e.g. 22% of patients under VCH-759 2400 mg/day had ≥ 3 log₁₀ IU/mL reduction in viral load) with only mild gastrointestinal adverse events.⁶⁴ GS-9190, a novel HCV NS5B inhibitor, was also recently evaluated in 31 genotype 1 CHC patients. In this study, a single dose of GS-9190 (40–480 mg) showed a potent dose-dependent reduction in serum HCV-RNA levels (range: 0.19–2.5 log₁₀ IU/mL) without serious adverse events.⁶⁵ Recent data indicated that GS9190 remains active against known resistant HCV drug-resistant mutants.⁶⁶ Finally, the pharmacokinetic properties of ANA598, a potent NNI of NS5B polymerase, were evaluated in a recent preclinical trial.⁶⁷ ANA598 had high oral bioavailability and was suggested to achieve higher concentration in the liver compared with other compounds of the same class.⁶⁷

Prevention of functional replication complexes. ACH-806 is a new potent inhibitor of HCV replication,⁶⁸ which has been shown to act synergistically in combination with different classes of HCV inhibitors,⁶⁹ such as VX-950 and NM283. Its exact mechanism of anti-viral action remains unclear, but it seems to prevent the development of functional replication complexes via inhibition of NS3–NS4A interaction.⁶⁸ However, further evaluation was stopped because of possible development of renal dysfunction.⁷⁰

Inhibition of protein translation

Antisense oligonucleotides. Antisense oligonucleotides are short synthetic nucleic acids (usually with <25 nucleotides) that bind an RNA target forming RNA–RNA (antisense RNA) or RNA–DNA (antisense DNA) hybrids resulting in inhibition of RNA translation of viral proteins and/or replication.⁷¹ Several oligonucleotides targeting the 5′-UTR, the most conserved region of the HCV genome, have been reported to inhibit HCV gene expression *in vitro*. ISIS 14803 is a 20-base antisense oligonucleotide, which is complementary to the HCV translation initiation region within the IRES. ISIS 14803 gave promising results in early phase II clinical trials, but subsequent aminotransferases flares and poor anti-viral efficacy led to discontinuation of further studies.^{15, 72} AVI-4065 has been shown to inhibit HCV protein

translation *in vitro* and in animal models, whereas a phase II study is under progress.⁷⁰

RNA interference. RNA interference is a method of specific degradation of messenger RNA leading to RNA silencing. Small interfering RNA or short hairpin RNA are used, but both approaches currently are not orally bioavailable and require parenteral administration.⁷³ SirnaAV34 is planned for further evaluation following preclinical animal studies. BLT-HCV (Benitec), the first clinical candidate to treat HCV infection through RNA interference, consists of three components targeting different HCV sequences, underlining the importance of a multi-targeting approach to prevent resistance development.⁷⁴⁻⁷⁶

Ribozymes. Ribozymes are synthetic nuclease-resistant catalytic RNA molecules acting by cleavage of specific HCV-RNA sequences.⁷⁷ *In vitro* studies have demonstrated the potent anti-viral activity of various ribozymes to inhibit HCV polyprotein translation.⁷⁰ Heptazyme is a ribozyme against the HCV IRES, which had progressed to early phase clinical studies in patients with CHC. It showed moderate anti-viral efficacy, but further development was stopped because of toxicity in animal models.

Post-translational modification

NS3/4A protease inhibitors. NS3 serine protease, with the cofactor NS4A, forms a heterodimeric protease, which acts as a serine protease and plays an essential role for the generation of components of the viral RNA replication complex.⁷⁸ In addition, it has been implicated in the initiation of the cellular anti-viral response. Although it was difficult to design potent and high-affinity inhibitors because of the characteristics of the protease-binding pocket which is wide and shallow, the NS3-4A protease has emerged as one of the most popular targets of many novel small-molecule inhibitors.^{6, 79} There is, however, great concern regarding the development of viral resistance to this group of anti-HCV agents.

BILN 2061 or Ciluprevir, a noncovalent inhibitor of NS3-4A protease, was the first compound of this class tested in clinical trials. Although the compound demonstrated dramatic reductions in serum HCV-RNA levels in genotype 1 patients within 48 h, further development was halted because of cardiotoxicity.⁸⁰

VX-950 or Telaprevir is another peptidomimetic inhibitor of the viral NS3-4A serine protease, which, in contrast to BILN 2061, forms a covalent but reversible complex with the target enzyme through the inclusion of an α -ketoamide in the active site of the enzyme.⁸¹ Recent studies have shown that VX-950 is capable of reducing serum levels of neopterin (a marker of inflammatory activity),⁸² but its *in vitro* activity against genotype non-1 HCV genotypes has not been elucidated yet.^{70, 83, 84} In a 14-day dose-defining study including eight healthy volunteers and 36 CHC genotype 1 patients, the VX-950 dose of 750 mg every 8 h achieved the greatest effect with 4.4 log₁₀ IU/mL median HCV-RNA reduction.⁸⁵ However, viral breakthroughs were observed during the second week of treatment because of selection of telaprevir-resistant variants associated with substitution of alanine to serine at position 156, whereas replacement of the same residue with threonine or valine conferred cross-resistance to both VX-950 and BILN 2061. After treatment discontinuation, the sensitive wild-type virus slowly replaced the resistant variants. The most commonly reported drug-related adverse events were headache and diarrhoea.⁸⁵

In a subsequent 14-day randomized trial, the efficacy of VX-950 and PEG-IFN α -2a combination was compared to VX-950 or PEG-IFN α -2a monotherapy in 20 naive CHC genotype 1 patients, while all patients received standard PEG-IFN α -2a and RBV combination for 24 or 48 weeks within 5 days after completing the 14-day dosing period. The VX-950/PEG-IFN α -2a combination was well tolerated and achieved greater median decline in viral load, compared with VX-950 or PEG-IFN α -2a monotherapy (5.5 vs. 4.0 or 1.0 log₁₀ IU/mL respectively).⁸⁶ Additionally, four of eight patients treated with the VX-950/PEG-IFN α -2a combination had undetectable HCV-RNA at day 14, while no patient had an increase in HCV-RNA levels during treatment.⁸⁶ Interestingly, SVR was achieved in nine of 15 patients from the VX-950 monotherapy and VX-950/PEG-IFN α -2a combination groups, who continued with PEG-IFN α -2a/RBV therapy for 24 or 48 weeks, suggesting that VX-950-based regimens may increase SVR rates.⁸⁷ However, three of the five patients who relapsed after the end of the standard therapy had developed known VX-950 resistance mutations within the NS3 protease gene (V36/A156, V36/R155 and T54).⁸⁸ Nevertheless, combination therapy seems to be a superior therapeutic option, without the disadvantage of VX-950 monotherapy, which frequently leads to viral rebound because of presence of uncovered viral

variants with low-level (V36M/A, T54A or R155K/T) or high-level (A156V/T and 36/155) of resistance to telaprevir after wild-type virus clearance. These variants have decreased replication capacity and are fully sensitive to PEG-IFN α /RBV therapy.^{89, 90}

In another study, VX-950 (750 mg thrice daily) and PEG-IFN α -2a/RBV for 4 weeks followed by standard PEG-IFN α -2a/RBV combination for 44 weeks were given in 12 naïve genotype 1 patients.⁹¹ The triple therapy was well tolerated and achieved undetectable HCV-RNA (<10 IU/mL) within 4 weeks in all patients without any breakthrough. Serum HCV-RNA remained undetectable in all but one patient after 12 weeks of follow-on therapy with PEG-IFN α -2a/RBV,⁹² while SVR was observed in eight (67%) patients.⁹² In a phase II trial (PROVE-1), 250 naïve genotype 1 patients received VX-950 and PEG-IFN α -2a/RBV for 12 weeks followed by 0, 12 or 36 weeks of PEG-IFN α -2a/RBV or standard PEG-IFN α -2a/RBV combination for 48 weeks. At 12 weeks, HCV-RNA remained undetectable (<10 IU/mL) in 88% and 52% of patients treated with triple and double therapy respectively ($P = 0.0001$). The rate of adverse events was similar but discontinuations were more common in the triple combination arm (11% vs. 3%). SVR rate was higher after the 12-week triple followed by 12-week standard double combination than after the 12-week triple combination only (61% or 35%), while end-of-therapy response rate was higher after the 12-week triple followed by 36-week standard double combination than after the 48-week standard double combination (65% vs. 45%).⁹³ In another trial (PROVE 2), 323 naïve genotype 1 patients received VX-950 and PEG-IFN α -2a \pm RBV for 12 weeks or VX-950 and PEG-IFN α -2a/RBV for 12 weeks followed by 12 weeks of PEG-IFN α -2a/RBV or standard PEG-IFN α -2a/RBV therapy for 48 weeks.⁹⁴ Triple combination achieved greater 4- and 12-week responses than any double combination, while VX-950/PEG-IFN α -2a achieved greater on-therapy responses than PEG-IFN α -2a/RBV. SVR rates were higher in the two triple combination arms (59–65%) than in the VX-950/PEG-IFN α -2a arm (29%).⁹⁴ Viral breakthroughs usually because of selection of VX-950-resistant variants developed less frequently in the triple than in the VX-950/PEG-IFN α -2a combination arm (at 12 weeks: 2% vs. 24%).^{94, 95} Rash, pruritus, nausea and diarrhoea were the most common adverse events in the VX950 arms of the latter two trials.^{93, 94}

SCH 503034 is an oral well-tolerated protease inhibitor of NS3/4A protease.⁹⁶ In a phase IIa,

placebo-controlled trial, SCH 503034 monotherapy (at a dose ranging from 100 mg twice daily to 400 mg thrice daily for 14 days), showed potent anti-viral efficacy (2.1 log₁₀ IU/mL mean reduction of viral load at a dose of 400 mg thrice daily) in 61 genotype 1 non-responders to previous PEG-IFN α -based therapy.⁹⁷ The most common side effect of SCH 503034 was headache, but the overall side effects did not significantly differ from those observed in patients taking placebo.⁹⁷ Similar to the other compounds of the same class, combination of SCH 503034 with IFN α was considered as a strategy for protection against the development of resistant variants leading to enhanced anti-viral activity.⁹⁸ In a recent 14-day study, SCH 503034 (400 mg thrice daily) and PEG-IFN α -2b combination achieved greater reduction in viral load, compared with PEG-IFN α -2b or SCH 503034 monotherapy (2.9 log₁₀ IU/mL for SCH 503034 plus PEG-IFN α -2b vs. 1.1 log₁₀ for PEG-IFN α -2b alone vs. 0.5–2.5 log₁₀ for SCH 503034 alone) in genotype 1 nonresponders to previous PEG-IFN α -2b and RBV therapy.⁹⁹ Further phase II trials assessing 24 and 48 weeks of combination treatment are under progress.

ITMN-191 and TMC435350 are new potent oral inhibitors of the NS3/4A protease. Pharmacokinetic properties of ITMN-191 have been evaluated in animal models (rats and cynomolgus monkeys).¹⁰⁰ ITMN-191 has a favourable cross-resistance profile with VX-950 and other related linear tetrapeptides,¹⁰¹ while recent data support its use against multiple HCV genotypes.¹⁰² In addition, recent *in vitro* studies suggested synergistic anti-viral activity of ITMN-191 and PEG-IFN α -2a, but further clinical studies are required.¹⁰³ TMC435350 has been evaluated in HCV-negative volunteers at single and multiple ascending doses with good safety profile. This compound will be further investigated at once daily administration in CHC patients.¹⁰⁴

NS5A and helicase inhibitors. New potent anti-viral agents, such as NS5A (A-831) and helicase (QU663) inhibitors have been evaluated *in vitro* with encouraging results, but further clinical evaluation is needed.^{70, 105}

Inhibitors of viral assembly and release

Inhibitors of cellular glycosidases are potential candidates of anti-viral therapy, inhibiting viral assembly and release. Celgosivir or MX-3253 is a novel potent

inhibitor of the host enzyme α -glucosidase I, which is involved in HCV assembly and release.¹⁵ In an open-label phase II trial, 43 naïve or IFN α -intolerant genotype 1 CHC patients were randomized to receive celgosivir 200 mg daily, 400 mg daily or 200 mg twice daily for 12 weeks. During the study period, celgosivir was well tolerated with only mild gastrointestinal side effects and asymptomatic dose-related elevation of creatine phosphokinase, while 5% of patients had peak on-treatment viral load reductions of $\geq 1 \log_{10}$ IU/mL.¹⁰⁶ Although celgosivir monotherapy had moderate anti-viral activity, further studies of combination with PEG-IFN α plus RBV are ongoing.

Prevention of binding

Similar to many other viruses, HCV entry into the cells is based on different steps, i.e. binding, internalization and cell penetration. The E1 and E2 are type I transmembrane highly glycosylated proteins of the HCV envelope, and E2 is considered to play an important role in viral attachment interacting with one or more components of the cell membrane, such as low-density lipoprotein receptor, glycosaminoglycans and CD81.⁷⁰ Thus, inhibition of HCV entry can be based on the development of specific inhibitor molecules, which act at the receptor(s)-binding site(s) or specific antibodies that neutralize infectious particles.⁷⁰ Recently, the anti-viral activity of specific E2-derived peptides, such as GNS-037, have been evaluated *in vitro* with encouraging results.¹⁰⁷ Polyclonal immune globulins and monoclonal antibodies, such as HCV-AB 68 and HCV-AB 65 have been evaluated in early clinical trials including CHC patients who underwent liver transplantation.⁷⁰ Only HCV-AB 68 has been evaluated in the nontransplant setting. In particular, HCV-AB 68 given intravenously in 40 CHC patients was well tolerated and resulted in transient reduction of viraemia following single or multiple doses up to 120 mg without serious adverse events.¹⁰⁸

Immunomodulators

Toll-like receptor agonists. Toll-like receptors (TLR) are molecules on the cells surface that recognize the presence of invading pathogens, such as bacteria and viruses.¹⁵ TLRs are expressed by immune cells and their activation leads to acute inflammatory response by inducing the expression of proinflammatory cytokines, such as IFN. There are 10 human TLRs, each

recognizing molecular signatures associated with specific class of microbial species.

ANA245 or isatoribine, a selective agonist of TLR7, was found to reduce viraemia in 12 naïve CHC patients after 7 days of intravenous administration¹⁰⁹ with some patients achieving >90% reduction in serum HCV-RNA levels. The drug had relatively mild side effects. Following these encouraging results, ANA975, an oral pro-drug of ANA245 was evaluated. ANA975 was found to have comparable anti-viral efficacy with ANA245 and to be well tolerated after a single dose in 36 healthy volunteers,¹¹⁰ but it was suspended following intense immune stimulation in animal models. The safety, pharmacokinetics and pharmacodynamic properties of resiquimod, a new oral TLR7–8 agonist, were analysed in two recent randomized phase II trials. Resiquimod was given at 0.01 and 0.02 mg/kg twice per week for 1 month. Although the high dose had greater anti-viral efficacy than the low dose, it was associated with intense adverse events because of induction of IFN α .¹¹¹ SM360320 is another TLR7 agonist, which has been found to reduce *in vitro* HCV-RNA levels by induction of type I IFN.⁷⁹

CPG 10101 (Actilon) is a synthetic TLR-9 agonist. In a phase I study, 74 genotype 1 patients, who had relapsed after PEG-IFN α and RBV therapy, were randomized to receive GPG10101 subcutaneously, alone or in combination with PEG-IFN α and/or RBV. Triple CPG 10101/PEG-IFN α /RIB combination, compared with PEG-IFN α /RBV, was well tolerated and achieved greater reduction in serum HCV-RNA levels at week 12 (50% vs. 13%, $P < 0.05$),¹¹² whereas end-of-therapy response was achieved in 45% of patients who continued the triple therapy for 48 weeks.¹¹³ However, further development of CPG 10101 was stopped because of lack of efficacy.

Histamine dihydrochloride. Histamine dihydrochloride (HID) is considered to have both immunomodulatory and antioxidant properties.¹¹⁴ Initial studies supported the co-administration of HID with IFN α . In a phase II trial including 129 naïve CHC patients, this combination given for 48 weeks achieved SVR rates ranging between 30% and 40%,¹¹⁵ which seem to be somewhat inferior to those reported with the combination of IFN α and RBV.¹¹⁶ The triple therapy (HID/IFN α /RBV) was also tried in 18 CHC patients without SVR after IFN α monotherapy, in who 50% virological response was achieved at the end of 48 weeks of therapy, but

SVR rates were not reported.¹¹⁷ The trials with HD in combination with PEG-IFN α and RBV were recently discontinued.

Thymalfasin. Thymalfasin or Zadaxin is a synthetic analogue of the natural immunomodulatory peptide thymosin- α 1, which promotes T lymphocyte and natural killer cell activation.¹⁵ Zadaxin has been evaluated in 25 nonresponders to IFN α and RBV combination. The triple combination of Thymalfasin (1.6 mg twice per week), PEG-IFN α -2a and RBV given for 48 weeks achieved end-of-therapy responses in 12 (48%) of 25 patients,¹¹⁸ but further investigation of Zadaxin was halted because the combination of Zadaxin and PEG-IFN α showed no benefit over PEG-IFN α alone.¹⁵

Interleukins. Interleukin (IL)-10 given in nonresponders to IFN α -based therapies has been found to improve serum aminotransferases levels and liver histology.¹¹⁹ IL-12 is unlikely to become an alternative to conventional IFN α -based therapy because of its poor anti-viral efficacy and substantial severe adverse events.¹²⁰ IL-29 binding to a heterodimeric IL-28R/IL-10R receptor activates, similar to IFN α , the JAK/STAT pathway. Recently, the activity of a pegylated form of IL-29 (PEG-IL-29) in different doses (0.03–3 mg/kg) intravenously was evaluated in animals. This study showed that PEG-IL-29 is pharmacologically active inducing the expression of known IFN serum biomarkers (e.g. neopterin and β -2 microglobulin) and genes (e.g. MxA and PkR) in liver biopsy specimens, to an extent similar to IFN α . Interestingly, in contrast to IFN α , PEG-IL-29 is active in liver, but not in circulating white cells.¹²¹

Therapeutic vaccines. Although no prophylactic vaccine is available for prevention of HCV infection, several therapeutic vaccines have been developed to induce HCV-specific immune responses. A recombinant E1 vaccine against the E1 envelope glycoprotein has been found to stimulate both humoral and cellular immune responses. This vaccine has yielded encouraging results in preliminary clinical trials. In particular, E1 vaccine was associated with improvement in serum ALT levels and liver histology, but with no effect on HCV-RNA levels in 35 genotype 1 nonresponders to previous standard therapy.¹²²

IC-41 is another vaccine containing several epitopes of the HCV genome and polyarginine as an adjuvant.

In a phase II trial, IC-41 was able to induce significant immune responses and transient reduction of HCV-RNA levels in 60 nonresponders.¹²³

T-cell vaccine based on the HLA and HCV genotype cross-reactivity^{124, 125} and dendritic cell-based vaccines are novel and very promising approaches for therapeutic immunization in patients with CHC. In particular, dendritic cell vaccination is able to elicit potent activation of antigen-specific cellular immunity against HCV proteins, which is a more physiological process of capturing, internalization and presentation of HCV antigens. In recent studies, immunization of mice with beads coated with NS5 protein and anti-DEC205-endocytosis receptor of dendritic cell was able to induce a significant cellular immune response.^{126, 127}

Ciclosporin A and its analogues. Cyclophilins (CyP), a family of peptidyl-propyl *cis/trans* isomerases (PPIase), seem to have a significant role in HCV replication. Based on findings in laboratory animal models (chimpanzees) and small study in CHC patients, the immunosuppressant drug ciclosporin may suppress HCV-RNA levels via inhibition of PPIase.¹²⁸ Interestingly, the anti-viral effect of ciclosporin was related to its blood concentration being greater in combination with IFN α .¹²⁹ DEBIO-025 is a novel non-immunosuppressive form of ciclosporin. *In vitro* studies have shown that DEBIO-025 has a 10-fold greater anti-viral activity on HCV replication, compared with ciclosporin, independently of HCV genotype.^{130, 131} DEBIO-025 is also attractive for HCV patients co-infected with human immunodeficiency virus as well as for combination approaches with NS5B and/or NS3-4A inhibitors.¹⁵

NIM811 and SCY-635¹³² are new ciclosporin analogues, which exhibit stronger suppression of HCV-RNA *in vitro*, compared with ciclosporin itself. Again, their anti-viral activity was greater in combination with IFN α .⁷⁹ Based on *in vitro* studies, NIM811 exerts its anti-viral activity through a CyP-B-dependent mechanism (CyP-B is an important host factor for HCV replication)¹³³ and it can be combined with other anti-viral agents.^{134, 135} Thus, although further clinical studies are needed, ciclosporin and its analogues might provide a new strategy for anti-HCV treatment, particularly in combination with standard or other new anti-HCV treatments (polymerase or protease inhibitors).^{15, 79}

GI-5005. GI-5005 is a whole, heat-inactivated recombinant yeast genetically modified to express HCV-specific (HCV NS3 and core) protein targets, which acts via immune elimination of infected hepatocytes. In a recent 1B trial,¹³⁶ 71 CHC patients received 0.05–40 yeast units/week subcutaneously for 5 weeks followed by monthly dosing for seven additional weeks. There was no serious adverse event, while ALT normalization was observed in a dose-dependent manner and mild HCV-RNA reduction (0.75–1.4 log₁₀ IU/mL) was detected in six patients. A multicentre phase II trial comparing GI-5005 plus PEG-IFN α /RBV vs. PEG-IFN α /RBV is in progress.

Other agents

Agents implicated in lipid biosynthesis. NA-255 is a novel serine palmitoyltransferase inhibitor, which exhibits HCV anti-viral activity via disrupting of HCV assembly and/or NS5B and lipid rafts interaction.¹³⁷ Bezafibrate, a lipid lowering agent acting via peroxisome proliferator-activated receptor- α , was found to reduce serum gamma-glutamyl-transpeptidase and ALT levels in 34 CHC patients, nonresponders to previous PEG-IFN α /RBV therapy.¹³⁸ Statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] have also been shown inhibitory effects on HCV replication.¹³⁹ In a genome-length HCV-RNA replication system (OR6), atorvastatin, fluvastatin, simvastatin and lovastatin, but not pravastatin, were found to have anti-HCV activity and to have a synergistic effect with PEG-IFN α . However, the anti-HCV activity of atorvastatin given at standard doses, and perhaps of all HMG-CoA reductase inhibitors, was not confirmed *in vivo* in a pilot clinical trial including 10 CHC patients.¹⁴⁰ Thus, further clinical studies are required for these agents. As the anti-viral activity of statins is possibly mediated via inhibition of geranylgeranylation of host cell proteins, which is required for HCV assembly rather than the reduction of cholesterol synthesis itself, the selective inhibition of geranylgeranylated proteins, such as FBL2, might be a safer approach for HCV-RNA inhibition in the future.⁷⁹

S-Adenosyl-methionine and oxidative stress. S-Adenosyl-methionine (SAME) and betaine (thimethylglycine) were shown to increase the anti-viral effect of IFN *in vitro*. They may act via methylation of impor-

tant enzymes implicated in the anti-viral function of IFN, such as STAT1, and may improve IFN signalling and thus virological responses.¹⁴¹ Interestingly, SAME may act also synergistically with IFN via reduction of oxidative stress in hepatocytes induced by HCV genes expression.¹⁴² Phase II studies of SAME in combination with PEG-IFN α -2a and RIB are in progress.

Herbs and other hepatoprotectants. Hepatoprotectants, a large family of agents with antihepatotoxic activity, are given in various acute and chronic liver diseases. They are made mostly from traditional remedies, but their mode of action is more or less unknown. Several herbs, such as silymarin and glycyrrhizin, multivitamins, methionine, oleanolic acid and thioctic acid are considered the most frequently prescribed hepatoprotectants.¹⁴³

Although herbs are widely used for the treatment of CHC in Asia, their efficacy and safety remain controversial. Silymarin, the seed extract of milk thistle (*Silybum marianum*), is an ancient herbal remedy used to treat various liver and gall-bladder diseases. In a recent study, MK-001, a standardized extract of Silymarin was evaluated *in vitro*. MK-001 was found to have anti-inflammatory properties with inhibition of tumour necrosis factor- α expression in human peripheral blood mononuclear cells and nuclear factor $\kappa\beta$ in human hepatoma Huh7 cells, while its anti-viral effects were partly related to induction of Stat1 phosphorylation.¹⁴⁴

Glycyrrhizin, a natural compound extracted from the roots of *Glycyrrhiza glabra*, has been evaluated in CHC nonresponders with or with contraindications to IFN α . Glycyrrhizin, given by six infusions weekly for 4 weeks, was able to induce only biochemical responses in 72 (60%) of 121 patients, whereas at the end-of-treatment (22 weeks) there were no significant changes in necroinflammation.¹⁴⁵ Glycyrrhizin was also evaluated in CHC patients, nonresponders to previous PEG-IFN α /RBV therapy. Glycyrrhizin (200 mg) given three or five times a week intravenously for 52 weeks was associated with reduction of aminotransferases and 45% improvement of ≥ 1 point in the necroinflammation score.¹⁴⁶

Nitazoxanide. Nitazoxanide (NTZ), an antiprotozoan agent, has also anti-viral efficacy blocking the viral protein synthesis via inhibition of eukaryotic initiation factor 2. NTZ monotherapy for 12 weeks followed by

combination of NTZ/PEG-IFN α -2a (with or without RBV) for 36 weeks was compared to standard therapy (PEG-IFN α -2a/RBV) in 120 CHC genotype 4 patients.¹⁴⁷ Triple compared to standard double therapy was reported to achieve higher ETR and SVR rates (in naïve patients: 79% vs. 43%, $P = 0.006$) without significant difference in adverse events.¹⁴⁷

Antiapoptotic agents. As hepatocyte apoptosis plays an important role in development of liver injury in CHC, antiapoptotic agents have been investigated in such patients. In a recent double-blind, placebo-controlled trial, IDN-6556, a potent reversible pan-caspase inhibitor, was evaluated in 105 patients with chronic liver diseases (80 with CHC). IDN-6556 (5–40 mg/day) for 14 days was well tolerated and achieved significant ALT reduction, compared to placebo, but no effect on viraemia.¹⁴⁸ PF-03491390, another oral pan-caspase inhibitor, was also tried in CHC (5–50 mg/day for 12 weeks) achieving greater reduction in serum markers of inflammation and fibrosis, compared with placebo.¹⁴⁹

CONCLUSIONS

Several novel agents with encouraging preliminary results have already reached or will soon enter into advanced clinical development programmes to demonstrate definitely their usefulness in the treatment of CHC. Aside from the safety and efficacy, the success of these new agents will be influenced by their ability to inhibit all viral variants and prevent the emergence of escape mutants. To date, the NS5B RdRp and the NS3/4A protease have received more attention as anti-HCV targets. However, it is evident that monotherapy with any anti-viral will be unlikely to eradicate HCV and combinations of several agents with different modes of action against viral and possibly host targets will be needed to prevent the emergence

of drug-resistant viral variants. Thus, additional research is required to determine proper dosing and treatment strategies to overcome drug resistance. On the basis of recent clinical trials, the combination of HCV inhibitors with PEG-IFN α with or without RIB synergistically inhibits viral replication and facilitates viral clearance. Further studies will determine whether the superior responses with these new combinations may offer greater SVR rates allowing shorter treatment durations and avoiding viral resistance. However, the great advance in the anti-HCV treatment will come from effective strategies that will not include poorly tolerated and/or frequently contraindicated agents, like IFN α and/or RBV.

The advances and future improvement in *in vitro* culture systems should lead to a better understanding of the host–viral interaction and will boost the search for new HCV therapeutics, whereas more sensitive HCV-RNA assays will be available to differentiate better responders from relapsers or nonresponders. However, the aim should always be the transformation of very promising substances in culture systems into effective and safe drugs in clinical trials. Given that many agents are being developed, it is hoped that the future standard of care will target multiple points of the viral life cycle and host immune response. Ideally, the newer compounds should provide a more effective and more tolerable therapy to all chronic HCV patients.

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