The way forward in HCV treatment — finding the right path

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Abstract | Infection with the hepatitis C virus (HCV) represents an important health-care problem worldwide. The prevalence of HCV-related disease is increasing, and no vaccine is yet available. Since the identification of HCV as the causative agent of non-A, non-B hepatitis, treatment has progressed rapidly, but morbidity and mortality rates are still predicted to rise. Novel, more efficacious and tolerable therapies are urgently needed, and a greater understanding of the viral life cycle has led to an increase in the number of possible targets for antiviral intervention. Here we review the specific challenges posed by HCV, and recent developments in the design of vaccines and novel antiviral agents.

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doi:10.1038/nrd2411

Hepatitis C virus (HCV) infection represents an important global health-care burden, which is likely to increase over the coming years. There are approximately 3–4 million new cases of HCV infection each year, and current estimates suggest that a minimum of 3% of the world's population (approximately 170 million people) are chronically infected, and are at risk of developing liver cirrhosis and/or hepatocellular carcinoma[1]. Today, in developed countries, most cases are acquired through the sharing of infected needles whilst injecting drugs or, to a much lesser extent, via sexual and perinatal transmission[2]. However, in a significant number of patients the route of infection remains unknown. Before the routine screening of blood for HCV, many patients were infected by blood transfusions or treatment with infected blood products. At present, most new cases of HCV infection occur in the developing world[3], and it is believed that immigration will impact on HCV prevalence and subsequent disease burden in the developed world. In the developed world, infection with HCV is responsible for 50–76% of all cases of liver cancer and accounts for two-thirds of all liver transplants[4].

Since the discovery of the virus in 1989 (REF. 5), the development of HCV therapy has progressed significantly (FIG. 1). With the introduction of IFN monotherapy, and the current recommendation of pegIFN-α and ribavirin, the proportion of patients achieving sustained antiviral response (SVR) has increased significantly[6–15]. The mechanism of action of IFN-α and ribavirin is still incompletely understood. IFN has a direct antiviral effect and acts on the immune system of the host, and ribavirin alone does not inhibit HCV replication significantly but augments the antiviral action of IFN. Importantly, ribavirin prevents relapse after the end of antiviral treatment. Despite this, the morbidity and mortality rates associated with HCV are predicted to rise in the coming years, and more efficacious and tolerable therapies are urgently required, particularly for the increasing proportion of patients who are refractory to treatment with IFN-α and ribavirin. Numerous studies have estimated the extent to which the burden of the disease will increase, but these projections may prove to be an underestimate. Consequently, HCV-related annual mortality is set to increase in most Western countries over the next two decades. In France, for example, the likely future mortality of HCV has been examined using the back-calculation method; this predicted a rise from 3,000 in 1998 to 4,500 in 2022 (REF. 16). This is unlikely to change unless at least 50% of the HCV-infected population is treated effectively. For this, HCV carriers have to be readily identified. Projections in the United States suggest that if half of all patients infected with HCV are identified, even with the most aggressive treatment at optimal doses and durations, the best possible outcome is a 24% reduction in the incidence of decompensated cirrhosis after 20 years[17]. By 2020, the proportion of all US HCV cases with liver cirrhosis is estimated to increase from 16% to 32%, and decompensation will increase by 106% over current levels[17] resulting in an increased need for liver transplantation.

This Review is based on a satellite meeting held at the 12th International Symposium on Viral Hepatitis and Liver Disease in Paris, 2006, and is updated to include the current knowledge and recent developments in the field of HCV therapy at the time of publication. Given the shortcomings

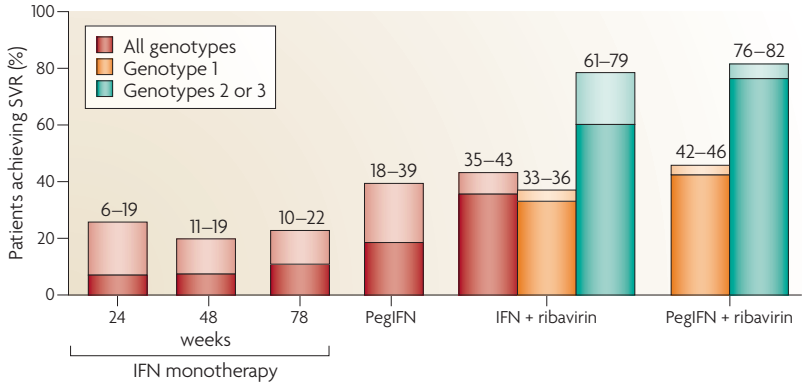

Figure 1 | **Evolution of HCV therapy and response rates.** The proportion of patients achieving a sustained virological response (SVR) has increased with advances in the treatment of hepatitis C virus (HCV) infection, from interferon (IFN) monotherapy to the current standard of care. The numbers above the columns, and the paler shaded area of the columns, represent the ranges of SVR reported in the literature for each treatment or patient population[6–14]. PegIFN, pegylated-interferon.

of current HCV treatment, we examine emerging new therapies for HCV, the impact of viral resistance, and key lessons from HIV management, in particular the potential of combination treatment strategies.

Obstacles in current HCV management

Recent studies have highlighted the barriers and challenges that exist in ensuring patients newly diagnosed with HCV receive appropriate treatment[18,19]. In a US study of patients infected with HCV in primary care, obstacles to receiving appropriate treatment included negative views of patients regarding treatment, inadequate patient follow-up, a tendency for providers not to consider treatment of past drug abusers, and delays in obtaining specialist input[18]. An observational study in the UK found that among all patients diagnosed with HCV over a 2-year period, only about half of all patients were appropriately referred for further management and only 10% began treatment[19]. Conversely, in France, the Ministry of Health has implemented a nationwide viral hepatitis prevention and control programme aimed at increasing both detection of seropositive individuals and provision of antiviral treatment[20]. By 2002, it was estimated that 60% of new HCV patients had been diagnosed through improved HCV screening programmes, and the number of patient referrals to hepatology reference centres had more than doubled from 2,063 in 2000 to 4,259 in 2002. Despite this success, the programme recommended that additional efforts and new strategies were needed to improve treatment compliance and for treating non-responders[20]. Nationwide screening for HCV began in 2002 in Japan, and as a consequence, a reduction in hepatocellular carcinoma and in the number of candidates requiring liver transplantation is anticipated[21].

Sustained antiviral response
HCV RNA below the limit of detection at week 24 following treatment completion.

Limitations of current treatment options

Long-term studies have shown that SVR indicates clearance of virus and cure of the disease[22,23]. However, the response to therapy is dependent on several factors, including viral genotype (FIG. 1) and patient characteristics. There are six different genotypes of HCV, with numerous subtypes. Genotype 1 is the most prevalent and most difficult to treat viral strain in Europe and North America, and represents the greatest unmet treatment need[24]. Genotypes 2 and 3 appear to be more prevalent in the Far East. Of the other genotypes, genotype 4 is common in Africa and the Middle East, whereas genotypes 5 and 6 are predominant in South Africa and South-East Asia, respectively[3].

Certain patient populations are difficult to treat; these include non-responders to prior treatment with IFN-based therapies, patients with severe liver fibrosis or cirrhosis, those of African–American ethnicity, individuals co-infected with HIV, and patients with comorbidities, such as alcohol consumption, fatty liver or insulin resistance[25–32]. For example, response rates in African–American patients with genotype 1 HCV have been shown to be as low as 6–26%, and 50% in those with genotypes 2 or 3 (REFS 29,33). This is compared with overall cure rates of 40–50% for genotype 1 and more than 75% in patient groups with genotypes 2 and 3 (REFS 8,11–13).

There are no approved treatment options available for patients who have failed to respond to previous treatments. Studies suggest that in non-responders to IFN monotherapy, re-treatment with pegIFN and ribavirin can achieve SVR rates of 25–40%; and in non-responders to IFN and ribavirin, re-treatment can achieve SVR rates of up to 10%[28,34]. It has also been shown that extending the treatment duration in slow responders infected with HCV genotype 1 might increase the rate of SVR to the current standard of care for this patient population[105,106]. Trials investigating re-treatment of non-responders with current standard of care are ongoing, but the results available so far are not promising.

Studies in patients co-infected with HIV have shown SVR rates of 17–62% (17–29% for genotypes 1 or 4 and 44–62% for genotypes 2, 3 and 5)[31,32]. These responses may, in part, be explained by viral kinetics — the response to therapy generally being delayed in patients with co-infection[31,32]. For example, Torriani *et al.* state that although patients who are mono-infected with HCV genotype 2 or 3 require 24 weeks of pegIFN-α plus ribavirin therapy, those co-infected with HCV and HIV probably need 48 weeks of treatment[31]. This could be due to the higher viral load in co-infected patients, as well as host immune deficiency. It should be noted that in initial trials for HIV–HCV co-infected patients, lower ribavirin dosages were used than dosages commonly recommended for treatment of HCV infection. Subsequent studies were able to demonstrate significantly higher SVR rates in HIV–HCV co-infected patients with genotype 1 infection if standard weight-adapted ribavirin dosing was used[35].

In addition to inadequate response rates, current therapies are associated with numerous side effects, including flu-like illness, fever, fatigue, haematological disease, anaemia, leucopaenia, thrombocytopaenia, alopecia and depression. Treatment-associated side effects are an important consideration in the management

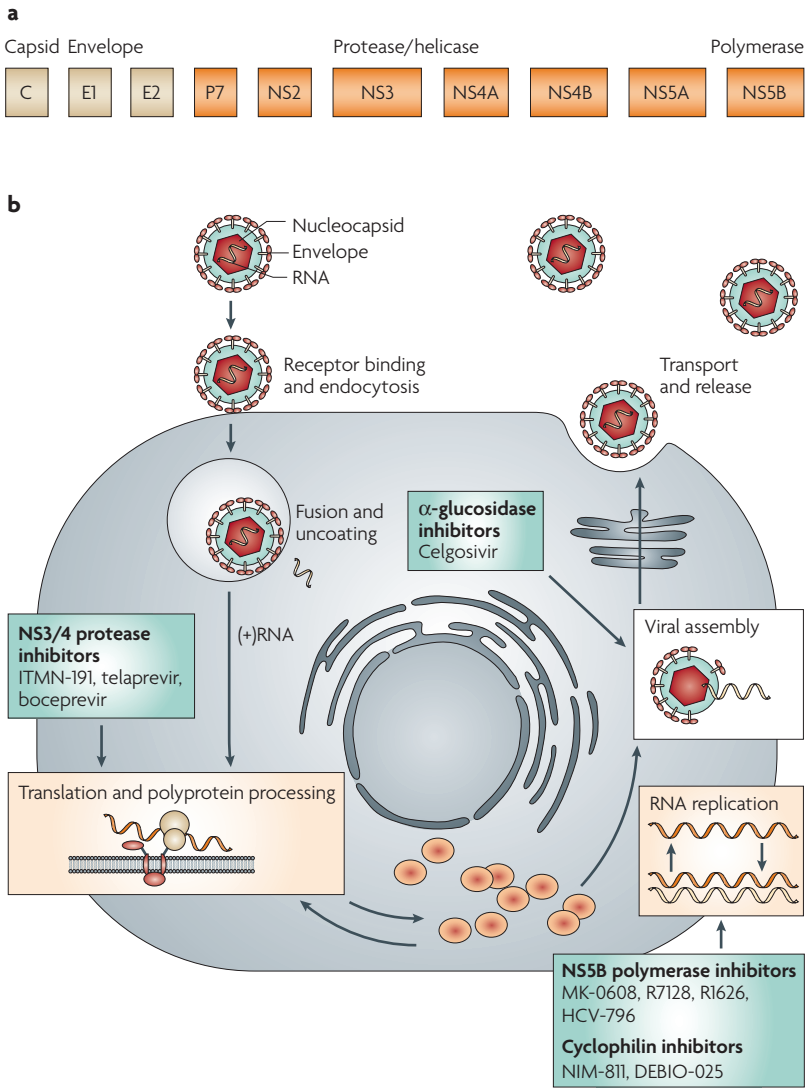

Figure 2 | **Potential targets for antiviral intervention in the HCV life cycle and their location in the HCV genome.** Hepatitis C virus (HCV) is a single-stranded RNA virus belonging to the *Flaviviridae* family[75]. **a** | Genomic organization of proteins encoded by HCV, comprising the structural proteins core (C), envelope 1 (E1), envelope 2 (E2), and P7 (presumed to be an ion channel) and the non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B), which are mainly enzymes essential to the viral life cycle. **b** | The nucleocapsid of the HCV genome is surrounded by an envelope that facilitates attachment and penetration into host cells. Upon enty into the host cell by endocytosis, the virus undergoes a fusion and uncoating step. Its RNA genome is translated into a polyprotein of approximately 3,000 amino acids[5], that is processed by cellular and viral proteases (including NS3) to yield four structural and six non-structural proteins[50]. The non-structural protein NS5B, a RNA-dependent RNA polymerase, catalyses the replication of the viral genome; negative-strand RNA intermediates are formed, which, in turn, serve as templates for the synthesis of new positive-strand RNAs. These are either encapsulated to form new viruses or used as mRNA for viral protein synthesis. The newly formed viral particles are released by exocytosis[97]. Each HCV structure represents a potential antiviral target for drug and vaccine development[46,98]. For example, protease inhibitors target the NS3/4 protease, which is essential for viral polyprotein processing; polymerase inhibitors target the NS5B RNA-dependent RNA polymerase, which is essential for viral RNA replication; cyclophilin inhibitors block cyclophilin-induced stimulation of RNA-binding activity of NS5B; and α-glucosidase inhibitors block the action of a host enzyme required for viral assembly, release and infectivity. Examples of drugs that are or have been in clinical development are included. FIG. 1b modified with permission from *Nature* REF. 99 © (2005) Macmillan Publishers Ltd.

of patients with HCV. A review of current treatments indicates that side effects may reduce adherence to therapy, resulting in 10–20% of premature treatment discontinuations[36]. Consequently, improvements in tolerability and the addition of supportive strategies, such as patient-focused treatment education, may drive overall success rates.

As the ultimate goal of HCV therapy is the complete elimination of the virus in all patients, new strategies for treatment are needed. Prophylactic and therapeutic vaccines are in development, and new approaches include the development of innovative new agents targeting different stages of the viral life cycle, as well as improvements to current strategies. Furthermore, a combination of complementary approaches and individualization based on genotype, viral load and early virological response will improve outcomes.

HCV vaccine development

As yet, no prophylactic vaccine is available for HCV, but extensive studies of a recombinant vaccine in chimpanzees showed encouraging results. Based on the viral envelope proteins E1/E2 (see FIG. 2a), it protected more than 80% of the animals from developing chronic infection following the experimental challenge with either homologous or heterologous HCV-1a viral strains[107]. A T-cell vaccine eliciting broad cellular responses to HCV-1b non-structural proteins 3, 4 and 5, was also shown to exhibit prophylactic activity in chimpanzees after heterologous HCV-1a challenge[108].

Several approaches are also being taken to develop therapeutic vaccines. For example, a clinical-grade HCV E1 protein produced and purified from mammalian cells (InnoVac-C) has been evaluated in clinical trials[37,38]. In a Phase IIa study involving 35 patients with chronic HCV infection, cellular immune responses were boosted with a recombinant E1 vaccine, including a significant T-cell response. However, these cellular immune responses were not accompanied by any significant reductions in serum HCV RNA[37]. Another peptide-based therapeutic HCV vaccine, IC-41, also induced significant T-cell responses, but HCV decay was not more than 1 $\log_{10}$ in individual patients[39]. The only parameter that was shown to correlate with RNA response to IC-41 was an increase in HCV-specific IFN-γ secreting $CD8^+$ cytotoxic T cells above a critical threshold. A clinical trial was initiated with the aim to increase T cell responses, and an optimized schedule increased responder rates, caused a fivefold stronger $CD8^+$ response (sustained for at least 20 weeks), and a broader induction of cytotoxic T-cell responses[40]. The optimized regimen is currently being tested in a clinical trial of treatment-naive HCV patients. Such immune boosting in HCV carriers is likely to be most effective when used as an adjunct therapy with standard-of-care antiviral drugs. Other approaches to therapeutic HCV vaccines include the use of the recombinant core protein adjuvanted with Iscomatrix. This combination elicited an unusually strong T-helper and cytotoxic T-cell response to HCV in rhesus macaques[109], and a clinical trial in HCV patients who previously failed IFN therapy is underway.

Innovative agents in clinical development

For the development of new, specific anti-HCV drugs, an understanding of the HCV life cycle (FIG. 2b), in particular the genomic organization and polyprotein processing, is essential. It has resulted in the development of several agents that target specific stages of the life cycle, the so-called specifically targeted antiviral therapy for HCV (STAT-C) drugs. Potential processes for viral inhibition include virus entry into the host cell, proteolytic processing, RNA replication, and the assembly and release of the new virions. Among the most promising new agents in development are the protease and polymerase inhibitors, as discussed below. RNA-targeted therapies, such as antisense oligonucleotides[41], ribozymes[42] and small interfering RNA (siRNA)-targeting structures[43], have shown substantial success at inhibiting the HCV life cycle *in vitro*, but not *in vivo*. The structural viral envelope proteins E1 and E2, as well as their assembly, represent other potential antiviral targets[44,110]. Analogous to a recently developed HIV cell fusion inhibitor, detailed understanding of HCV cell fusion and cell entry could permit the development of specific HCV entry inhibitors.

Protease inhibitors. The non-structural protein NS3 possesses a protease domain that is responsible for polyprotein processing and is a potential target for antiviral intervention. Despite the catalytic site being a shallow and largely hydrophobic groove, making it difficult to target, several compound inhibitors of the NS3 protease have been successfully designed and are currently in preclinical and clinical development (for example, telaprevir (VX-950), boceprevir (SCH503034) BI12202, MK-7009, TMC435350 and ITMN-191). The proof-of-principle for this class of compounds was provided by BILN 2061, an NS3 protease inhibitor that provides at least a 2–3 $\log_{10}$ decrease in HCV load within 48 hours[45]. However, the clinical development of BILN 2061 was stopped owing to significant side effects.

Protease inhibitors have been associated with substantial reductions in serum HCV RNA in clinical studies when given alone or in combination with pegIFN-α[46–49] (see also clinical trials section below). NS3 possesses a helicase domain that has multiple functions, including RNA-stimulated nucleoside 5′-triphosphate hydrolase (NTPase) activity, RNA binding and unwinding of RNA regions with extensive secondary structure. Other potential targets include the NTP binding site and the binding site for single-stranded RNA[50].

Polymerase inhibitors. The protein NS5B is cleaved from the HCV polyprotein by the NS3 serine protease, and functions as a RNA-dependent RNA polymerase. It is the key enzyme for synthesis of a complementary minus-strand RNA, using the genome as a template, and the subsequent synthesis of genomic plus-strand RNA from this minus-strand RNA template. Several compound inhibitors of the NS5B polymerase are, or have been, in clinical development. Two separate classes of compounds have shown inhibitory effects on the NS5B through two distinct mechanisms: first, nucleoside polymerase inhibitors, which directly inhibit the active site causing chain termination (for example, valopicitabine (NM-283), MK-0608, R1626, PSI-6130 and its prodrug R7128), and second, non-nucleoside polymerase inhibitors, which cause allosteric inhibition resulting in a conformational change of the protein (for example, BILB 1941 and HCV-796)[50]. Preclinical studies have shown that agents targeting the HCV RNA polymerase are associated with significant reductions in serum HCV RNA[51] and clinical studies have demonstrated the promising antiviral effects of NS5B inhibitors when used either as monotherapy or in combination with pegIFN-α (REFS 52–54). However, due to safety concerns and unfavourable risk-benefit profiles, the development of several polymerase inhibitors, including HCV-796, BILB 1941 and valopicitabine, is on hold.

Immune modulators. Other mechanisms that are under investigation include immune modulators targeting the cellular immune response, which plays a major role in HCV infection. Examples include agents that generate and/or promote an effective immune response by inducing or modulating cytokine responses, such as the toll-like receptor (TLR) agonists (for example, CPG 10101 and ANA 975), which have shown antiviral efficacy in initial clinical studies[55]. CPG 10101 (Coley Pharmaceuticals) is a synthetic oligodeoxynucleotide TLR9 agonist that also induces T-helper type 1 cytokine responses, resulting in high levels of type 1 IFN, natural killer (NK) cell stimulation and other viral-specific immunomodulatory responses. In a Phase 1b clinical trial, patients with HCV genotype 1 who received at least 1 mg CPG 10101 twice a week for 4 weeks experienced increases in IFN-α and other markers of immune response along with a mean 1 $\log_{10}$ decline in HCV RNA levels[55,111]. However, improved SVR results have not been reported so far. The clinical development of the TLR7 and TLR9 agonists is currently on hold — Coley Pharmaceuticals has stopped further development of CPG 10101 for viral hepatitis and are concentrating their efforts towards the more promising use of CPG 10101 as an anticancer drug. The development of the TLR7 agonist ANA 975 (Anadys Pharmaceuticals) was stopped owing to preclinical safety issues, as it was found to induce a general inflammatory response in animals.

Further novel investigational agents. The effectiveness of inhibitors of cyclophilin B (for example, NIM-811 and DEBIO-025), a host factor involved in viral replication, is being evaluated in patients with HCV. NIM-811, a cyclosporin A analogue, suppresses HCV genome replication in a cell culture system and may provide a novel strategy for anti-HCV treatment[56,57]. DEBIO-025 has demonstrated strong antiviral activity *in vitro* against HCV genotype 1 and HIV-1. In a Phase Ib study of HCV–HIV co-infected patients, those receiving treatment with DEBIO-025 achieved a mean HCV viral load reduction of 3.6 $\log_{10}$ after 15 days compared with 0.7 $\log_{10}$ for patients receiving placebo[58].

Recently, it has also been reported that NS4A, a cofactor for the NS3 protease, is a valid therapeutic target for chronic HCV infection. ACH-806 (GS-9132) binds to

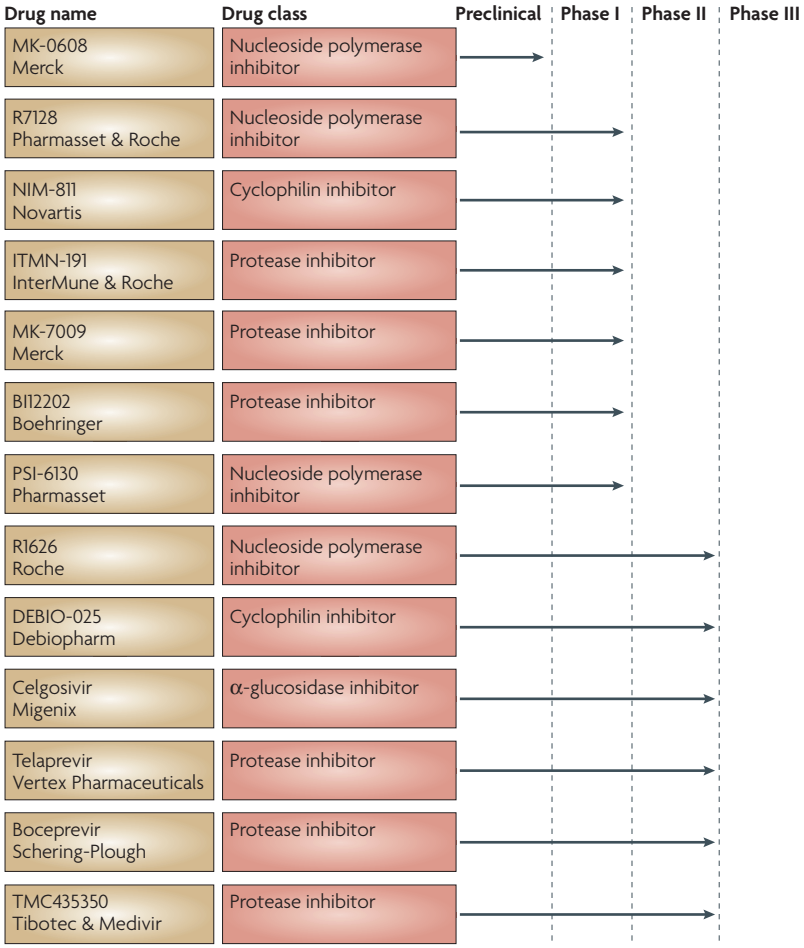

Figure 3 | **New antivirals for the treatment of HCV and present stage of development.**

HCV NS4A, inhibiting the correct proteolytic processing of the HCV polyprotein and thereby the formation of a functional replication complex, consequently decreasing viral RNA synthesis. Results of a randomized, double-blind, placebo-controlled, dose-escalation study demonstrated clinical proof-of-concept, although reversible nephrotoxicity precludes further development of ACH-806 (REF. 59). Furthermore, glucosidase inhibitors have been in development for many years albeit with slow progress.

Improvements to current therapies. Longer-acting IFNs and IFN-inducing molecules are in development. One example is albinterferon-α2b (albIFN-α2b), a fusion protein comprising albumin and IFN-α2b, which has been shown to have antiviral activity in a clinical trial setting, with a less frequent dosing regimen than current pegIFNs[60]. A recent Phase IIb, active-controlled study evaluated the efficacy and safety of three therapeutic dosage regimens of albIFN-α2b (900 μg or 1200 μg every 2 weeks or 1200 μg every 4 weeks) compared with pegIFN-α2a (180 μg once a week) in treatment-naive patients with genotype 1 chronic HCV infection. All treatments were in combination with ribavirin 1,000–1,200 mg per day (based on body weight). SVR rates for the albIFN-α2b arms were 58.5% for 900 μg every 2 weeks, 55.5% for 1,200 μg every 2 weeks and 50.9% for 1200 μg every 4 weeks, compared with 57.9% for the weekly pegIFN-α2a arm[61]. In addition, patients who received albIFN-α2b 900 μg every 2 weeks reported less impairment of quality of life (measured using the SF-36 Health Survey[62]) than those who received weekly pegIFN-α2a. These data suggest that albIFN-α2b given every 2 weeks may offer comparable efficacy to pegIFN-α2a, with an improved dosing schedule and the potential for less impairment of quality of life.

Other strategies to improve IFN efficacy include gene shuffle (this compound was developed by Maxygen and was in development together with Roche), IFN variants[112] and the development of long-lasting IFNs, like albIFN-α2b (Human Genome Sciences and Novartis Pharma), locteron (OctoPlus) and omega IFN with a subcutaneous delivery device (Intarcia Therapeutics) lasting 12 weeks. Furthermore, ribavirin derivatives have been developed to improve efficacy and tolerability — these include levovirin and viramidine (taribavirin). However, combination of levovirin and pegIFN-α2a fails to generate virological responses comparable with ribavirin–pegIFN-α2a combination therapy in patients with chronic HCV[63]. Fixed-dose viramidine was shown to be less efficacious than ribavirin in two Phase III clinical studies, although anaemia rates were significantly lower in patients treated with viramidine compared with those treated with ribavirin[64,65]. Weight-based dosing of viramidine is currently being evaluated in a Phase IIb study of treatment-naive patients with HCV genotype 1.

Clinical trials of NS3 and NS5B inhibitors

The two novel innovative agents furthest in clinical development (late Phase II) (FIG. 3) are the protease inhibitors telaprevir (VX-950) and boceprevir (SCH503034). Valopicitabine (NM-283) was the first polymerase inhibitor to reach Phase IIb clinical testing, but was recently placed on clinical hold in the United States following a review by the Food and Drug Administration (FDA)[66]. These three agents have been shown to have significant antiviral activity in patients with HCV genotype 1, including treatment-naive patients and those not responding to other therapies[54,67–70].

A Phase II clinical study in treatment-naive patients with genotype 1 evaluated valopicitabine 200–800 mg once a day with pegIFN-α2a for 12 weeks compared with pegIFN-α2a alone for the first 4 weeks, followed by pegIFN-α2a and valopicitabine (400–800 mg) from week 5 onwards[71]. At week 4, all combination therapy groups demonstrated greater reductions in HCV RNA than the pegIFN-α2a monotherapy group, and end-of-treatment data indicated that valopicitabine maintained antiviral activity for up to 48 weeks. In a Phase IIb study in non-responders to pegIFN-α2a and ribavirin, SVR data demonstrated comparable results for valopicitabine plus pegIFN-α2a versus re-treatment with pegIFN-α and ribavirin; SVR was not achieved by any patient in the valopicitabine plus pegIFN-α2a arm and one patient (3%)

Table 1 | **Resistant mutants associated with virus inhibitors**

Inhibitor	Mutant	References
Protease		
ITMN-191	D168A	85
Boceprevir	A156T, T54A, V170/A	82
Telaprevir	A156V/T, T54A, R155K/T, V36A/M	81,92,101
BILN 2061	A156V/T, D168V/A	81,84,101
Nucleoside polymerase		
Valopicitabine	S282T	83
R1479	S96T, N142T	87
Non-nucleoside polymerase		
HCV-796	C316Y	100
A-782759	M414T	102
Thiophene-2-carboxylic acid	L419M, M423T	103
Benzimidazoles	P495	104

in the pegIFN-α2a and ribavirin arm[72]. The clinical hold imposed by the FDA was based on the agency's overall assessment of the risk–benefit profile observed to date. R1626, a prodrug of R1479, is a polymerase inhibitor currently in Phase II development, which has shown a maximum mean (median) HCV RNA reduction of 3.7 (4.1) $\log_{10}$ in treatment-naive patients at a dose of 4,500 mg twice daily for 14 days[73].

Phase Ib studies have evaluated telaprevir as monotherapy[69] and in combination with pegIFN-α2a and ribavirin in treatment-naive patients with HCV genotype 1 (REF. 46). Telaprevir was well tolerated as monotherapy (750 mg every 8 hours) for 14 days and in combination with pegIFN-α2a and ribavirin, and patients receiving telaprevir plus pegIFN-α2a and ribavirin demonstrated the largest reduction in plasma HCV RNA levels[46]. Telaprevir is currently being evaluated in three Phase II studies. An interim analysis of one of these studies, PROVE 1, showed that 70% of patients who received telaprevir (750 mg every 8 hours) plus pegIFN-α2a and ribavirin had HCV RNA below 10 IU per ml after 12 weeks of treatment compared with 39% of patients who were treated with pegIFN-α2a and ribavirin alone (intention-to-treat analysis)[74]. According to the study protocol, patients in one of the study treatment arms (telaprevir plus pegIFN-α2a plus ribavirin) were eligible to stop all treatment at week 12 if they met certain on-treatment criteria, including a rapid virological response (RVR, defined as less than 10 IU per ml HCV RNA at week 4) and maintenance of this response at week 10. Nine out of 17 patients achieved week-4 RVR and discontinued therapy at 12 weeks; six of these patients continued to have undetectable HCV RNA 20 weeks post-treatment. Of the remaining eight patients in this study arm, four discontinued owing to adverse events before week 12 and four did not achieve RVR. The first SVR data of the PROVE 1 study[114], as well as first results from PROVE 2, another Phase II trial of telaprevir with treatment naive patients, have just been reported[115].

A dose-ranging study of boceprevir (100–400 mg twice a day) in patients with HCV genotype 1 that had previously failed pegIFN-α2a therapy indicates that this protease inhibitor has dose-related antiviral activity as monotherapy[70]. A Phase Ib 14-day study of boceprevir (200 or 400 mg three times daily) administered in combination with pegIFN-α2a (1.5 μg per kg weekly) demonstrated a dose–response relationship in non-responder patients with HCV genotype 1. Mean maximum $\log_{10}$ reductions in HCV RNA were 2.45 and 2.88 for 200 and 400 mg boceprevir plus pegIFN-α2a, respectively[49], and the combination of agents provided greater antiviral activity than either drug as monotherapy. Boceprevir 800 mg three times a day is currently being evaluated in combination with pegIFN-α2a and ribavirin in a Phase II trial of non-responders. A further Phase II trial of boceprevir 800 mg three times a day in combination with pegIFN-α2a and ribavirin has also been initiated in treatment-naive patients. Recent preliminary results from this so-called SPRINT (Serine Protease Inhibitor Therapy) study are comparable to the two telapravir Phase II studies in treatment naive patients[113–115].

Many of the studies with novel agents conducted so far have focused on the response in patients infected with genotype 1. Studies of the agents in patients infected with other genotypes and in non-responder populations with refractory disease are also required as clinical programmes progress. For example, clinical studies with the now discontinued protease inhibitor BILN-2061 highlighted that antiviral activity may be less pronounced in patients infected with genotypes 2 or 3 compared with those infected with genotype 1 (REFS 48,75).

Resistance to new HCV antivirals

Response to therapy is dependent on several factors including treatment-related factors, host characteristics (including the ability of host cells to respond to IFN, induce antiviral defences and clear infected cells), viral-related factors and disease-related factors[76,78,79]. In addition, the genetic heterogeneity or quasispecies nature of HCV has important therapeutic implications, as the generation and selection of resistant variants can allow the virus to escape the antiviral pressure exerted by treatment[77]. Indeed, mutations in both the polymerase and protease enzymes have already been identified (TABLE 1). In addition, the overall prevalence of individual mutations changes over time, indicating that the relative fitness (that is, the ability to replicate) of a resistant variant will have a role in viral dynamics during treatment.

As previously discussed, many emerging HCV treatments are targeted against specific HCV enzymes; among the most promising are the NS3 serine protease inhibitors and the NS5B RNA-dependent RNA polymerase inhibitors. As the active site for protease inhibitors is a long shallow groove, a single-point mutation in this enzyme might be sufficient to hinder the binding of these antivirals, with different mutations conferring low-level or high-level resistance (FIG. 4). For example, sequencing studies using samples from patients treated with telaprevir have identified several mutations that confer low-level and high-level resistance[80]. Resistant isolates are

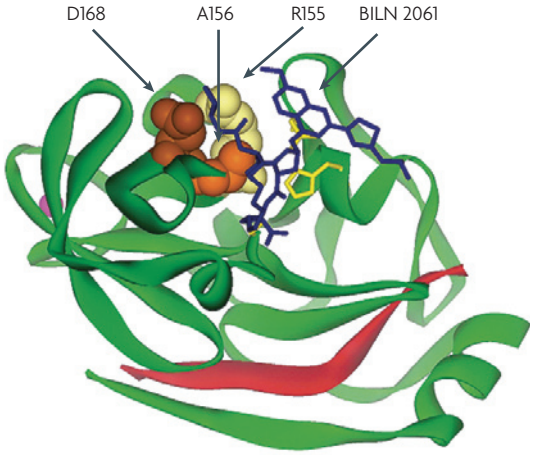

Figure 4 | **HCV mutations conferring resistance against protease inhibitors.** NS3 serine protease (green) and central domain of NS4A (red) showing sites of resistance mutations (D168, A156, R155). The residues that constitute the enzyme catalytic triad (H57, D81 and S139) are shown as yellow (stick representation), and the structural zinc atom is indicated in purple. The protease inhibitor BILN 2061 (discontinued) is modelled in the active site. Figure reproduced with permission from *Nature* REF. 83 © (2005) Macmillan Publishers Ltd.

selected rapidly and therefore combination therapy with pegIFN-α2a or other antiviral agents will be required to limit the development of resistance to telaprevir. As far as we know, telaprevir-resistant mutants are sensitive to IFN-α. The T54A mutation will confer resistance to both telaprevir and boceprevir, whereas the A156S mutation leads to resistance to telaprevir, but not boceprevir[81,82]. There have been several other reports of the selection of HCV-resistant mutants against various protease inhibitors using the *in vitro* replicon system[70,83–86].

The active site of the NS5B RNA-dependent RNA polymerase is a highly conserved region in all HCV genotypes and any amino-acid mutations in this region may inhibit the ability of the virus to replicate (FIG. 5). This suggests that resistance to nucleoside polymerase inhibitors by mutation in the enzyme may not readily develop. Although selection of replicons resistant to 2′-*C*-methyl-nucleosides has shown that HCV is rapidly able to discriminate between antiviral agents and natural nucleosides[81], *in vitro* studies have shown that replicons carrying these mutations showed decreased replication fitness[83,87–89]. There are several binding sites for non-nucleoside analogues within the NS5B polymerase (FIG. 5). Several mutations have been identified as determinants for resistance to non-nucleosides. For example, it has been demonstrated *in vitro* that replacement of P495 with alanine or leucine strongly reduces affinity for non-nucleoside inhibitors[83,89]. Such a mutation decreases the efficiency of viral replication, but viral fitness can be restored by mutations elsewhere in the NS5B coding region[83].

In vitro data suggest a low probability of cross-resistance between some of the different nucleoside polymerase inhibitors or between nucleoside and non-nucleoside inhibitors (see also TABLE 1). For example, production of mutant viral strains by an amino-acid substitution at S96T alone or in combination with N142T confers resistance to R1479 (for which R1626 is the pro-drug), but not valopicitabine[87,90], and the S282T substitution confers resistance to valopicitabine but not to R1479. Furthermore, molecular biology suggests no cross-resistance between protease and polymerase inhibitors[83]. There was also no cross-resistance observed between the cyclophilin B inhibitor NIM-811 and NM-107, the active moiety of valopicitabine[91]. These data suggest that NIM-811, an agent that targets host–viral interactions, provides another option for combination therapy with other antiviral agents, which would reduce the emergence of resistance[82,87].

From the results of *in vitro* studies we can anticipate drug resistance *in vivo* and consider options to reduce it, such as the use of agents with a low probability of cross-resistance in combination. For example, telaprevir monotherapy in treatment-naive patients with HCV genotype 1 produced subsets of patients that had a plateau in HCV RNA decline or breakthrough response during 14 days of dosing[92]. Sequencing assays of the viral RNA in these patients detected that these responses correlated with the selection of viruses containing one or two mutations in the NS3 protease region. *In vitro* analysis demonstrated that specific mutations correlated with the level of resistance; viruses with mutations at A156V/T conferred a high level of resistance to telaprevir, whereas T54A conferred a low level of resistance. In the absence of drug-selective pressure, high-level resistant variants rapidly became undetectable and replaced with wild-type variants[92]. Moreover, administration of telaprevir in combination with pegIFN-α2a alone or with ribavirin appeared to prevent the selection of inhibitor-resistant variants and, hence, viral rebound[46,69].

Lessons from HIV combination therapy?

The HIV epidemic had a major impact on drug development, and antiretrovirals now encompass a number of drug classes of which many have already been developed beyond first and second generation. Drug combinations have significantly changed the face of HIV management, enabling significant viral load suppression, thus preventing or delaying the development of drug-resistant mutations and thereby prolonging patient benefit by slowing disease progression.

HIV and HCV have important differences: HIV is a retrovirus that integrates into the host DNA and establishes persistent infection, whereas HCV does not integrate into the host DNA, and about 15–50% of exposed individuals clear the infection spontaneously. The viruses also differ with regard to response to therapies: HIV therapy can only suppress virus replication below the limit of detection, whereas viral clearance with HCV therapy can be achieved in a high proportion of patients. Despite these obvious differences, there are many similarities between the two diseases, including high levels of viral replication, viral heterogeneity, the importance of patient management, the use of combination therapy, the challenge of resistance to treatment and the lack of an effective vaccine[93,94].

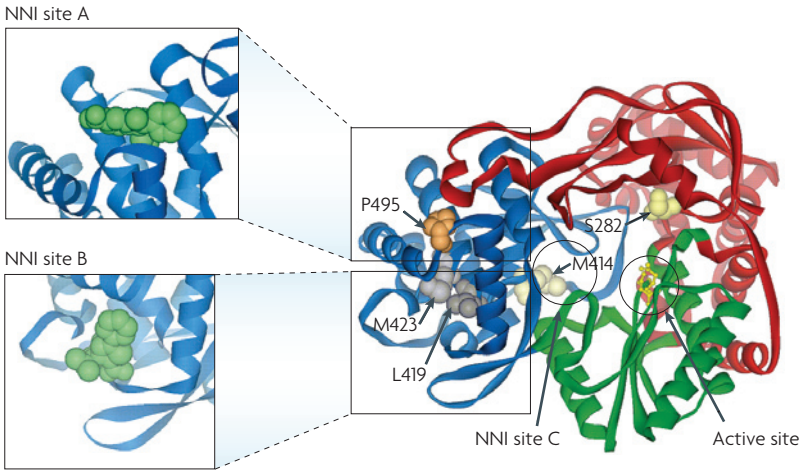

Figure 5 | **HCV mutations conferring resistance against polymerase inhibitors.** NS5B RNA-dependent RNA polymerase (thumb, palm and finger domains are blue, green and red, respectively) showing sites of resistance mutations to nucleoside and non-nucleoside polymerase inhibitors. Figure reproduced with permission from *Nature* REF. 83 © (2005) Macmillan Publishers Ltd.

The lessons learned from the treatment of HIV may influence the approach to the future treatment of chronic HCV infections. Combinations of drugs with different mechanisms of action should allow clinicians to improve efficacy and reduce viral resistance. Analogous to HIV therapy, the success of future HCV antiviral agents will be influenced by their resistance profiles; that is, their ability to inhibit viral variants and prevent the emergence of resistance mutants. Agents with complementary, but different modes of action have the potential to be used in combination and have exhibited limited cross-resistance[82,83,87,89,95]. Thus, combination therapy using multiple small molecules designed to inhibit different virus-specific targets and producing diverse resistance patterns may improve response rates; for example, protease inhibitors with polymerase inhibitors or nucleoside inhibitors with non-nucleoside inhibitors[96]. Development of new combination strategies and the use of short-term therapy will potentially allow improved treatment success while minimizing the potential for developing resistance to any single agent. Ideal antiviral regimens should be based on potency as well as tolerability and convenience, thereby promoting adherence and minimizing the risk of treatment failure.

Outlook

There is an urgent need for a prophylactic vaccine, and for improved strategies for HCV management that achieve the ultimate goal of HCV therapy: a complete cure for all infected patients. In addition to efficacy of treatment, the duration of therapy, viral kinetics, side effects and treatment of patient populations with refractory disease are all factors that need to be addressed. Here, the lessons learned from the development of treatment regimens for HIV could prove valuable. In particular, further improvements in patient outcomes might be gained from the addition of one or more of the new small-molecule antivirals to existing regimens to improve SVR rates and/or reduce treatment duration. As well as potentially improving success rates, the advancement of combination therapies will be vital in the prevention and management of resistance to any single agent. Furthermore, as many patients cannot tolerate IFN-α or ribavirin, there also needs to be a shift toward treatment regimens that are associated with less serious side effects, which might be achieved by the use of all-oral combination therapy regimens.

Demonstrating these possibilities for one or more of the new anti-HCV STAT-C drugs in treatment-naive patients, patients who have relapsed from previous treatment, and non-responders to current treatment regimes is the next step in anti-HCV drug development. The full release of clinical trial information on novel drugs that have been or are being evaluated, whether successful or not, would also considerably enhance efforts to develop more effective therapies that could achieve the ultimate goal of curing all patients infected with HCV.

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Acknowledgements
The authors would like to thank R. Douglas and L. O'Mahony for help in preparing the manuscript; this support was funded by Novartis. M.P.M., J.K.R., S.Z. acknowledge the support of Hep-Net, a network sponsored by BMBF, and M.P.M., S.Z., F.Z. are supported by VIRGIL, an EU6 Framework Project.

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
HCV | hepatocellular carcinoma

FURTHER INFORMATION
ClinicalTrials.gov: http://clinicaltrials.gov/
Hepatitis C Virus Database: http://www.hcvdb.org/index.asp?bhcp=1
Network of competence for hepatitis: http://www.kompetenznetz-hepatitis.de/

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CORRIGENDUM

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Michael P. Manns, Graham R. Foster, Jürgen K. Rockstroh, Stefan Zeuzem, Fabien Zoulim and Michael Houghton

Nature Reviews Drug Discovery **6**, 991–1000 (December 2007) | doi:10.1038/nrd2411

For Figure 3 (page 995), compound TMC435350 is in Phase I and not in Phase II.

Also, the acknowledgements should read:
The authors would like to thank R. Douglas and L. O'Mahony for help in preparing the manuscript; this support was funded by Novartis. M.P.M., J.K.R., S.Z. acknowledge the support of Hep-Net, a network sponsored by BMBF, and M.P.M., S.Z., F.Z. are supported by VIRGIL, an EU6 Framework Project.