Novel therapies for hepatitis C — one pill fits all?

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Abstract | Almost 25 years after the hepatitis C virus (HCV) was identified, and following intense research and development efforts, a large number of direct-acting antiviral drugs are now beginning to reach patient care. Accordingly, the way in which care is delivered is evolving at a breath-taking pace. Here, we review the current and upcoming treatment options for HCV, describe the key challenges facing clinicians and drug developers and discuss how the landscape in the HCV arena will change over the coming years.

More than 30 years ago, a transmissible form of hepatitis that was neither hepatitis A nor B was discovered[1] (FIG. 1 (TIMELINE)). It was shown that some forms of this non-A, non-B hepatitis were responsive to treatment with interferon (IFN)-α[2]. In 1989, a research group at Chiron (later acquired by Novartis) led by Michael Houghton cloned a cDNA encoding a viral plus-strand RNA genome that was named hepatitis C virus (HCV). This discovery paved the way for the development of direct-acting antiviral (DAA) agents for HCV[3]. Today, we are witnessing an enormous acceleration of the speed at which treatment options for HCV have evolved. The hope is that we will soon be able to offer curative treatment to the vast majority of those 160 million individuals worldwide that are chronically infected with HCV and therefore at risk of developing cirrhosis and liver cancer.

In 2003, the first-in-human data on a DAA for HCV, the NS3/4A protease inhibitor BILN 2061, was published[4]. Administered as short-term treatment, it provided proof of concept that a 4 log decrease in viral load was possible within 48 hours. However, there was rapid relapse once the drug was stopped[5,6], and owing to safety issues this HCV protease inhibitor was not developed further. In 2011, the first DAAs — the NS3/4A HCV protease inhibitors boceprevir (Victrelis; Merck) and telaprevir (Incivek; Vertex) — were approved by the US Food and Drug Administration (FDA), each in combination with pegylated IFN (PEG-IFN) plus ribavirin. This new therapeutic regimen has markedly improved treatment results for HCV genotype 1 infection[7–10] (see BOX 1 for an overview of the HCV genotypes). Numerous even more potent and efficacious agents are anticipated to reach the market soon, possibly starting in late 2013. This acceleration in drug development for HCV comes after decades of slow but steady progress in the study of HCV itself and the disease caused by it. Compared to other major blood-borne viral pathogens such as the hepatitis B virus (HBV) and HIV, for which DAAs have been available for decades, HCV has resisted drug development efforts for a long time even in the face of intense research efforts. In this article we discuss the molecular targets of antiviral therapy for HCV infection and the key compounds that are being developed against these targets. Finally, we discuss how, over the coming years, these compounds are anticipated to change the way care is delivered to individuals chronically infected with HCV.

HCV diagnostics and therapeutics

The first clinical benefit that was derived from the molecular identification of HCV was the development of tests for serum anti-HCV antibodies and for viral RNA (FIG. 1 (TIMELINE)). These diagnostic tools allowed the identification of infected individuals and the screening of blood products. Indeed, in the early 1990s large-scale screening programmes were instituted in many industrialized countries, resulting in a dramatic reduction of HCV transmission[11]. Nonetheless, cases of acute HCV infection continue to occur even in countries where blood products are largely safe. These are mostly due to needle-sharing among those who inject substances of abuse as well as healthcare-associated or sexual transmission events[12].

Over the course of the 1990s it was demonstrated that the HCV genome could cause disease when injected as RNA into the liver of a chimpanzee[13]. Several proteins encoded in the viral genome were found to have enzymatic activity, thus presenting potential antiviral targets[14]. However, early attempts to initiate viral replication *in vitro* or in small animal models failed, restricting functional studies to the human or chimpanzee system. This remained a major roadblock to DAA development until 1999 when the HCV replicon system (see below) became available[15].

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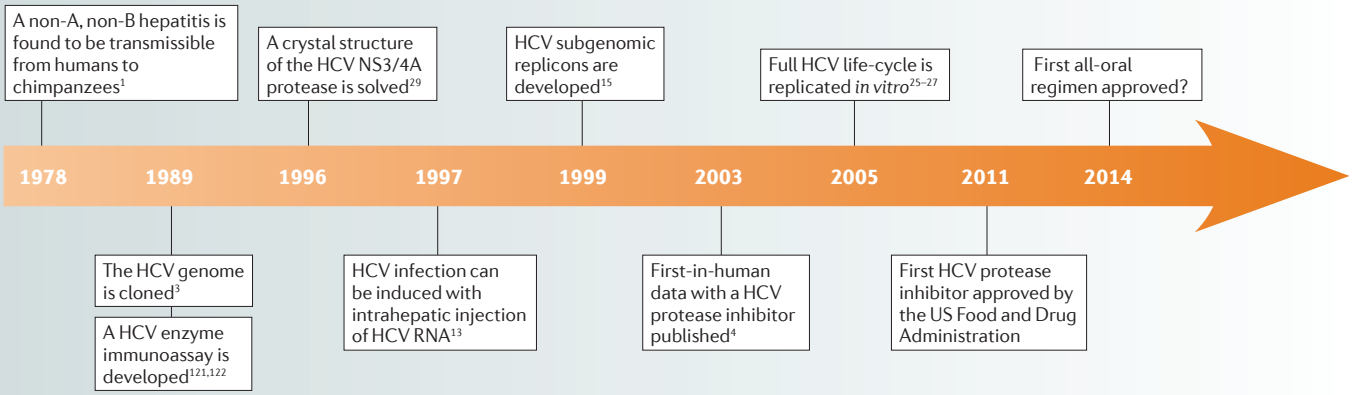
Timeline | **Key discoveries in the basic science of HCV**



HCV, hepatitis C virus.

In contrast to HBV and HIV, the HCV genome is not maintained in the host cell nucleus in any durable form, but only exists as cytosolic RNA. Hence, clearance of infection from a cell without destruction of the cell itself should be possible[16]. In line with this, HCV is the only virus causing a chronic infection that can currently be cured through drug therapy — at least in the majority of cases[17–19]. Conversely, in HBV and HIV, long-term suppression through immune control or antiviral drugs is the ultimate goal of current treatments, but a lifelong potential for reactivation remains. A patient that is negative for HCV RNA 24 weeks after conclusion of therapy is referred to as having achieved a sustained virological response (SVR) (see BOX 2 for a list of the important terms used to describe treatment outcomes); this is thought to be equivalent to cure[18,19]. However, recent data from IFN-free trials seem to suggest that relapses after more than 24 weeks post-treatment may occur[20]. Nonetheless, SVR24 is associated with crucial clinical end points such as survival and protection from developing hepatocellular carcinoma[21] and therefore currently represents the goal of treatment. Recently, the FDA and the European Medicines Agency have accepted SVR12 (BOX 2) as an end point for clinical trials evaluating novel HCV therapies as SVR12 is highly predictive of SVR24. Several other responses at earlier time points (BOX 2) allow the prediction of the likelihood of eventually achieving SVR24 and thus guide the clinician's treatment decisions.

Early trials of IFN monotherapy achieved SVR rates in <10% of cases[22] (FIG. 2). The addition of ribavirin to IFN and the replacement of standard IFN with PEG-IFN (which allowed once-weekly injection and improved pharmacokinetics) increased SVR rates. The combination of PEG-IFN plus ribavirin was the standard of care for HCV genotype 1 infection for 10 years until 2011, when boceprevir and telaprevir were approved[23,24]. For the other HCV genotypes, the PEG-IFN plus ribavirin treatment regimen — at the present time — is still the standard of care.

The development of DAAs for HCV was facilitated in 1999 by a breakthrough discovery by Ralf Bartenschlager's group: they showed that HCV subgenomes, comprising the non-structural proteins NS3-NS5B linked to a selectable marker, could replicate with adequate efficiency *in vitro*[15]. Such subgenomic replicons became the working horse of HCV drug development. Since 2005, a full-length isolate of HCV that not only replicates RNA but also produces infectious viral particles *in vitro* has been available[25–27]. Thus, not unlike the introduction of anti-retroviral drugs into the treatment of HIV infection 25 years ago, the current and upcoming HCV DAAs tell a success story of modern science. Molecular virology has deciphered the viral replication cycle, identified druggable targets and generated tools for compound screening (FIG. 1 (TIMELINE)). Equally important, structural biology has provided high-resolution structures of key viral drug targets such as NS3 (REFS 28,29), NS5A[30] and NS5B[31–33] that allowed modelling of target–inhibitor interactions and thus directed compound refinement.

Molecular targets of anti-HCV drugs

HCV is a small enveloped positive-strand RNA virus that predominantly or exclusively infects hepatocytes. HCV was the first member of the genus hepacivirus within the family of *Flaviviridae* that also contains the classical flaviviruses such as dengue virus, yellow fever virus and tick-born encephalitis virus[34].

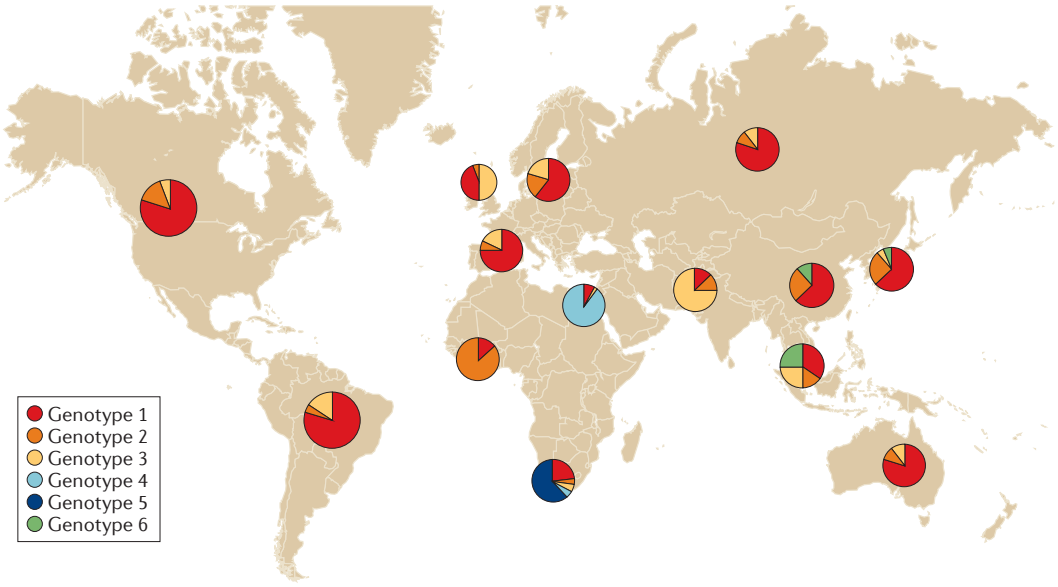
In the blood of the infected host HCV virions are closely associated with host lipoprotein and have therefore been referred to as 'lipo-viro particles'[35] (or lipoviral particles). These interact with at least four essential receptors and co-receptors on the hepatocyte surface before being taken up by clathrin-mediated endocytosis into an endosome[36] (FIG. 3a). Endosomal acidification then triggers the fusion of the viral envelope with the endosomal membrane. This results in the release of the nucleocapsid, which contains the viral genome, into the cytosol. An internal ribosome entry site in the 5′-end

Box 1 | **HCV and host diversity**

Genetic variation in both the hepatitis C virus (HCV) and its human host affect the natural history of the infection and the degree of response by patients to treatment.

As a small RNA virus with an error-prone RNA polymerase HCV exhibits enormous genetic variability. Viral isolates have been grouped into six major genotypes that differ in 30–35% of nucleotides across the genome[93,94]. Genotypes are further divided into subtypes that differ by 20–25% of nucleotides across the genome[93,94]. Even within an infected host HCV exists as a heterogeneous population of related variants often referred to as a "quasispecies swarm"[95]. The predominance of HCV genotypes varies among geographical regions with genotype 1 dominating in Europe and the Americas followed by genotypes 2 and 3 (see the figure; data from the World Health Organization). HCV genotypes are clinically relevant, as genotypes 1 and 4 are less responsive to interferon-based therapy than genotypes 2 and 3. Moreover, subgenotype 1a is associated with a more rapid emergence of resistant-associated variants under treatment with HCV NS3/4A protease inhibitors compared with subgenotype 1b (see main text for more details). Finally, patients infected with genotype 3 are more likely to develop hepatic steatosis, progress more rapidly to hepatic fibrosis and are at a higher risk of developing hepatocellular carcinomas[96,97].

On the host side the rs12979860 single nucleotide polymorphism (SNP), which is commonly referred to as the interleukin-28B (*IL28B*) variant, stands out[98]. The *IL28B* variant (which was recently shown to be located in an intron of the interferon-λ4 (*IFNL4*) gene[98]) was identified through three independent genome-wide association studies showing that its C allele is strongly associated with a better response to interferon-based treatment and higher sustained virological response (SVR) rates (BOX 2) compared with the T allele[99,130,131]. Moreover, it was reported that higher SVR rates in individuals of Caucasian descent compared with individuals of African descent are largely explained by a higher frequency of the C allele[99,130,131]. The *IL28B* genotype remains a predictor of treatment outcome in currently approved triple therapies (that is boceprevir or telaprevir with pegylated interferon-α plus ribavirin). Other SNPs with potential relevance to the natural course of HCV infection or the response to treatment have been reported in the genes encoding cyclophilin A, inosine triphosphatase, the natural killer cell receptor KIR2DL3, patatin-like phospholipase domain-containing protein 3 (PNPLA3) and genes involved in vitamin D metabolism[100–106].



of the genome interacts with cellular ribosomes and a single viral polyprotein is synthesized (FIG. 3b). This is subsequently cleaved by host and viral proteases into 10 viral gene products[34].

The amino terminal part of the polyprotein contains the structural proteins and the small ion channel protein p7. The structural elements include the core protein (the building block of the viral nucleocapsid) and the envelope proteins E1 and E2. Several of the non-structural proteins that make up the carboxyl terminal part of the polyprotein are enzymes and are therefore attractive antiviral drug targets. These include two viral autoproteases (NS2/3 and NS3/4A), the NS3 RNA helicase/NTPase and the NS5B RNA-dependent RNA polymerase.

The targets that have been the most fruitful so far are NS3/4A and NS5B; about two-thirds of the anti-HCV agents currently in Phase II and Phase III trials are directed against these (TABLE 1). In addition, drugs targeting NS5A, an RNA-binding protein of incompletely understood function, are now in late-stage clinical development. The NS4B protein has been attributed with both a GTPase activity and an RNA-binding capability. The non-structural proteins together with a large number of host-encoded factors make up the viral replication and assembly machinery. Genome replication occurs in specialized membrane compartments that are induced in the presence of NS4B and NS5A[37]. Assembly and export of new virions seems to occur in close proximity to lipid

Box 2 | **HCV treatment terminology**

Below is a list of the important terminology used to describe treatment of patients chronically infected with hepatitis C virus (HCV) and the response to the treatment.

Lead-in phase. The phase in which a limited range of compounds is used to treat patients before giving the actual full combination of compounds. For example, in boceprevir-based triple therapy (comprising boceprevir, pegylated interferon-α (PEG-IFNα) and ribavirin) there is generally a 4-week lead-in treatment phase with PEG-IFNα and ribavirin before boceprevir is introduced.

Rapid virological response (RVR). When a patient is HCV RNA negative after 4 weeks of treatment.

Extended rapid virological response: telaprevir (eRVR (TPV)). When a patient is HCV RNA negative between 4 weeks and 12 weeks of treatment with telaprevir.

Extended rapid virological response: boceprevir (eRVR (BOC)). When a patient is HCV RNA negative between 8 weeks and 24 weeks of treatment with boceprevir.

Early virological response (EVR). When a patient has ≥2 log decline in HCV RNA at week 12 of the treatment regimen.

Partial early virological response (pEVR). When a patient has a ≥2 log decline in HCV RNA but is not HCV RNA negative at week 12 of the treatment regimen.

Complete early virological response (cEVR). When a patient is HCV RNA negative at week 12 of the treatment regimen.

Null response. When a patient has a <2 log decline of HCV RNA at week 12 of the treatment regimen.

Partial response. When a patient has a ≥2 log decline in HCV RNA at week 12 of the treatment regimen, but has detectable HCV RNA at week 24.

Breakthrough. When a patient becomes HCV RNA positive under treatment after being undetectable at a previous time point or has a >1 log increase in HCV RNA over nadir.

End of treatment response (EOT). When a patient is HCV RNA negative at the end of treatment.

Relapse. When a patient is HCV RNA negative at the end of treatment but HCV RNA is detected during the next 24 weeks after treatment has ended.

Sustained virological response week 12 (SVR12). When a patient is HCV RNA negative 12 weeks after treatment has ended. SVR12 is accepted by the US Food and Drug Administration as an end point in clinical trials of HCV therapies.

Sustained virological response (SVR24). When a patient is HCV RNA negative 24 weeks after treatment has ended. SVR24 is generally considered as the patient achieving cure.

droplets and is intricately linked to cellular very low-density lipoprotein production[35]. Generally, all stages of the viral replication cycle (entry, replication and assembly and release) depend on support from essential host factors.

Drugs and combinations under development

There is fierce ongoing competition to bring drugs to the market that will replace PEG-IFN, ribavirin and/or the first-generation protease inhibitors (FIG. 4). As of May 2013, over 1,600 studies evaluating drugs for the treatment of hepatitis C are listed on the ClinicalTrials.gov website. These trials are investigating a multitude of compounds (see TABLE 1 for a selection) in different patient populations or subpopulations. There is widespread agreement that no single agent will achieve cure of chronic HCV infection in the foreseeable future. Thus, the rapidly shifting landscape is made even more complex by the many combinations that are being evaluated.

The combinations being evaluated are chosen by their clinical promise as much as by strategic considerations on the part of the pharmaceutical companies developing these drugs and drug combinations. A particular challenge to the development of combination therapies is the fact that pharmaceutical companies are likely to prioritize a combination of their own molecules rather than collaborating with competing companies and combining the best-in-class molecules for the benefit of patients. As more drugs become available, clinicians face the daunting task of finding the most promising regimen for each individual patient under real-life conditions.

In the following sections we discuss the different classes of anti-HCV drugs under development, their general advantages and limitations, as well as a selection of current key compounds. Many novel DAAs are being developed both as combinations with PEG-IFN plus ribavirin and as part of all-oral regimens.

New IFNs and other modulators of the host antiviral response. In the HCV field all-oral or IFN-free therapy is generally seen as a major goal for the near-term or mid-term future. This is because of the notorious side effects and contraindications of IFN in addition to the need for parenteral application. Nonetheless, it is likely that optimal regimens, at least in some patient groups, will have to include IFN for some years to come. Moreover, given their broad antiviral effects, IFN products developed for the treatment of HCV infection may find additional applications in other viral diseases such as hepatitis B or hepatitis B/D. Thus, several companies continue to develop new IFN products despite the high promise of the numerous new and potent DAAs in the pipeline.

The most advanced among the IFNs currently being developed is PEG-IFNλ. It triggers the same intracellular signalling pathway and IFN-induced genes as IFNα, but through a different cell surface receptor that has a more restricted expression pattern across human tissues[38]. Data from the Phase IIb EMERGE study indicated that PEG-IFNλ has comparable efficacy with PEG-IFNα and with fewer serious adverse events, although hyperbilirubinaemia was more common in those receiving the higher dose PEG-IFNλ[39,40]. Other new IFNs at earlier stages of development aim to improve on the pharmacokinetics of IFN, with formulations that have a more prolonged steady-state release or can be taken orally.

Finally, some direct agonists of Toll-like receptor 7 (TLR7) and TLR9 are in early clinical testing for HCV and other infectious and non-infectious diseases. TLR7 and TLR9 (REF. 41) are key transducers of innate antiviral responses, making them theoretically promising targets; however, their future role in treating HCV is hard to predict at this time.

HCV protease inhibitors. Numerous inhibitors of the NS3/4A protease are in clinical development, aiming to replace boceprevir and telaprevir (BOX 3). Despite the availability of high-quality structural data[42], no clinical developments targeting the other essential HCV-encoded

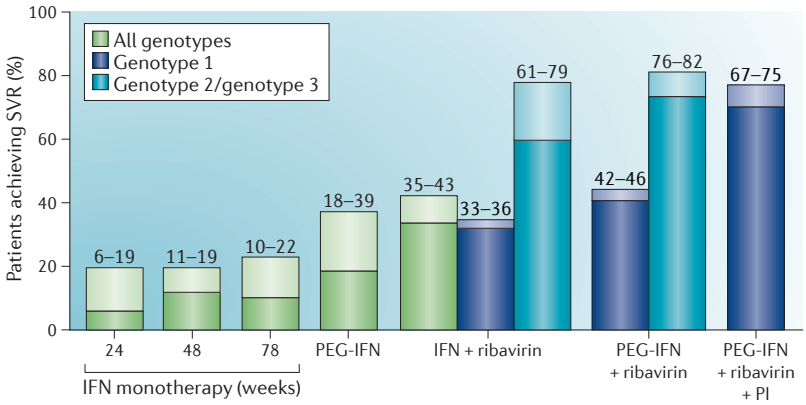


Figure 2 | **Evolution of treatment of HCV.** The percentage of treatment-naive patients chronically infected with hepatitis C virus (HCV) achieving sustained virological response (SVR; see BOX 2 for definition) with evolving treatment standards. Shaded areas represent the range of SVR rates reported in the relevant trials[7,9,23,24,123–129]. IFN, interferon-α; PEG-IFN, pegylated-IFN; PI, HCV NS3/4A protease inhibitor.

protease (NS2/3) have been reported so far. HCV NS3/4A protease inhibitors can be divided into linear compounds (such as boceprevir and telaprevir) and macrocyclic compounds. Protease inhibitor monotherapy generally results in dramatic initial drops in viral load followed by the rapid emergence of resistance-associated variants (RAVs). Thus, protease inhibitors often have high antiviral potency, but specific viral mutations that confer resistance have been reported for all compounds in development and there is widespread cross-resistance within both linear and macrocyclic groups[43].

Substitutions of an arginine residue at position 155 (for example, R155K) of HCV NS3/4A often confer resistance against most or all protease inhibitors at a moderate fitness cost to the virus (that is, in the absence of a drug RAVs replicate with an efficiency that is only moderately reduced compared with the wild-type virus). Drug resistance to protease inhibitors is a particular problem in HCV subgenotype 1a infection, as the genetic barrier (that is, the number of nucleotide exchanges needed to produce a typical RAV) is lower compared with subgenotype 1b[44]. Finally, boceprevir and telaprevir are highly active against genotype 1 only. Although telaprevir has clinically significant activity against genotype 2 and boceprevir seems to have some efficacy against genotype 3, their use is prescribed off-label.

Upcoming 'second-wave' protease inhibitors have one or more incremental advantages over the first-wave compounds (boceprevir and telaprevir). These include higher potency, higher genetic barriers to resistance and more favourable pharmacokinetic profiles, including once-daily dosing[45]. The term 'second-generation' protease inhibitor is mostly used to refer to compounds that are active against RAVs associated with first-wave protease inhibitors and all HCV genotypes.

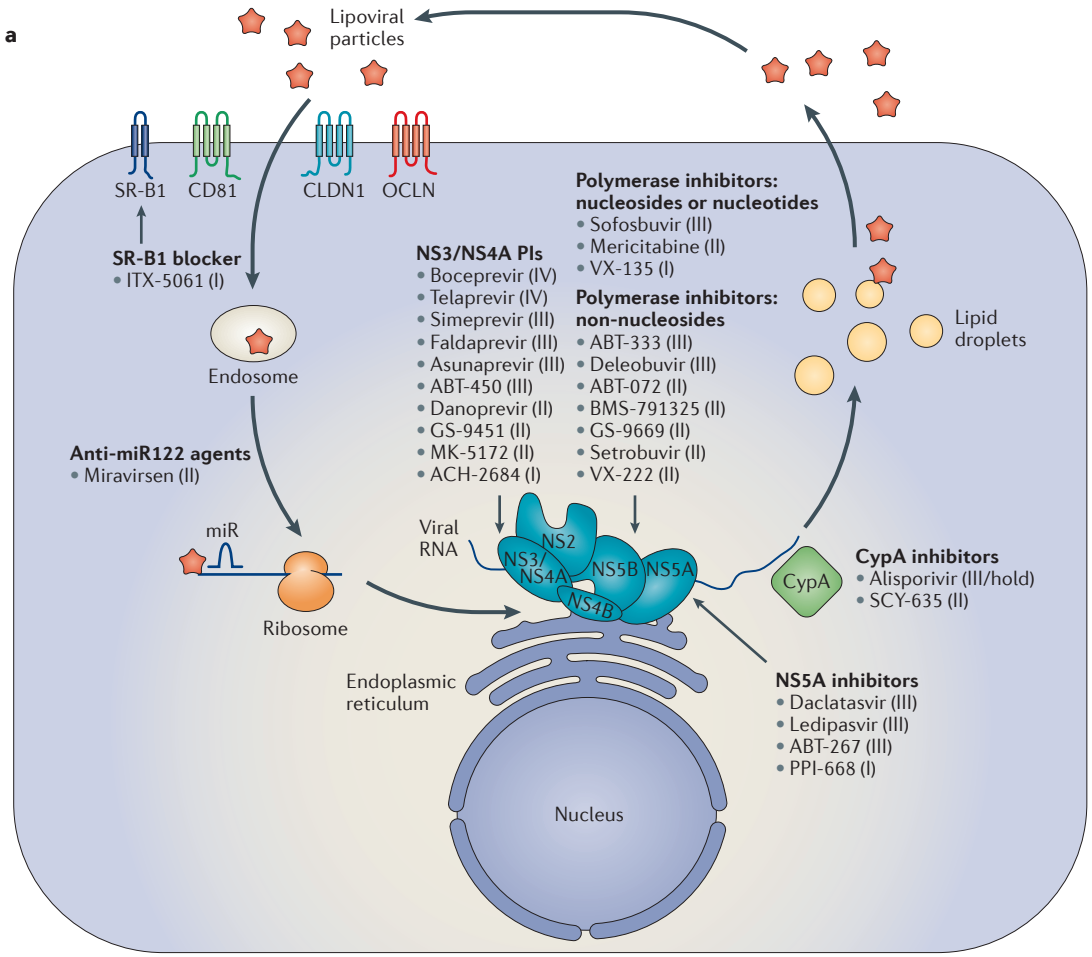
Simeprevir is a second-wave, macrocyclic, once-daily protease inhibitor that has activity against several genotypes; although it is currently being developed primarily for genotype 1 infection. Recently reported data from the Phase III QUEST-1 and QUEST-2 trials using the combination of simeprevir, PEG-IFN plus ribavirin in treatment-naive patients showed SVR12 rates of 80–81%[46,47]. Moreover, high SVR12 rates were also achieved in those with fibrosis: 83–85% (Metavir stage F0–2) and 66–70% (Metavir stage F3–4). Another second-wave protease inhibitor in Phase III trials is faldaprevir (previously known as BI 201335). The Phase II SILEN-C1 and SILEN-C2 studies evaluating the combination of faldaprevir, PEG-IFN plus ribavirin reported SVR24 rates of 72–84% in treatment-naive patients infected with genotype 1, 32–50% in previous partial responders and 21–35% in previous null responders[48,49]. Other second-wave protease inhibitors currently in Phase II or Phase III trials include ABT-450, ACH-1625, asunaprevir, danoprevir and GS-9451 (TABLE 1).

Two drugs currently in earlier clinical development, ACH-2684 and MK-5172, are thought to be true second-generation protease inhibitors, as they have activity against all genotypes and known protease inhibitor-associated RAVs. Interim data presented at the 2013 annual meeting of the European Association for the Study of the Liver (EASL) showed SVR24 rates of 86–92% in treatment-naive non-cirrhotic patients infected with HCV genotype 1 receiving the combination of MK-5172, PEG-IFN plus ribavirin compared with 54% in a control group treated with boceprevir-based triple therapy[50].

It seems likely that one or more second-wave protease inhibitors will reach the market by 2014 and be used in the context of either IFN-containing or all-oral combinations (FIG. 4). There may soon be more HCV protease inhibitors on the market than are needed; however, the best of them may be good candidates for future all-oral combinations.

Nucleoside and nucleotide NS5B polymerase inhibitors. Nucleoside and nucleotide inhibitors of the NS5B RNA-dependent RNA polymerase bind to the enzyme active site and lead to premature chain termination of the nascent viral genome. The NS5B active site is well-conserved across HCV genotypes as amino acid substitutions in this location are generally poorly tolerated and result in a marked loss in viral fitness[51]. Therefore, nucleoside and nucleotide polymerase inhibitors tend to have good activity against a broad range of genotypes and have a high genetic barrier to resistance. That is, the fitness cost of the HCV-resistant clone to nucleoside and nucleotide polymerase inhibitors is high. Indeed, although some resistant mutations, most notably the S282T substitution, have been observed *in vitro* they have not been observed *in vivo*, and no RAV-associated breakthrough has been reported so far.

However, a particularly high number of compounds of this class have been terminated during clinical development. The first HCV nucleoside NS5B polymerase inhibitor that reached the clinic, valopicitabine, was terminated owing to its poor benefit–risk profile, in which gastrointestinal toxicity was the main reason for discontinuation. More recently, the nucleotide NS5B polymerase inhibitor BMS-986094 (formerly known as INX-189) was discontinued, again for toxicity reasons[52].



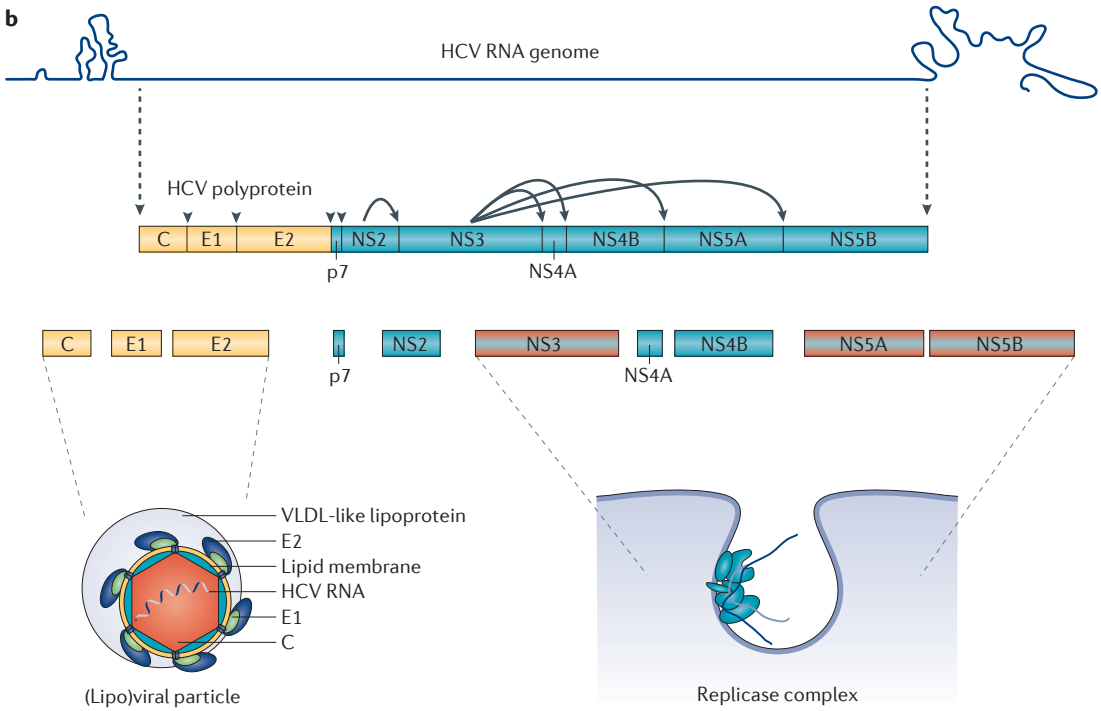


Figure 3 | **Molecular biology and drug targets in HCV. a** | Schematic representation of the hepatitis C virus (HCV) replication cycle. Lipoviral particles from the blood interact with at least four essential cell surface receptors on the hepatocyte, resulting in endocytic uptake of the lipoviral particle into an endosome. Here, acidification triggers fusion of the viral and endosomal membranes and release of the genome-containing viral nucleocapsid into the cytosol. Viral and host factors form the replicase complex in a specialized membrane compartment referred to as the membranous web. Progeny genomes are then packaged into new lipoviral particles. The packaging and export from the cell occurs in close conjunction with lipid droplets and the very low-density lipoprotein (VLDL) synthesis pathway. Eventually, progeny lipoviral particles are released into the bloodstream. Potentially druggable host and viral factors involved in the HCV replication cycle are depicted in green (host) and blue (viral). A selection of approved compounds and compounds in development are shown. Roman numerals in brackets indicate the current clinical phase of development. **b** | The viral genome is translated by cellular ribosomes giving rise to a single viral polyprotein that is then cleaved by cellular proteases (arrowheads) and viral NS2/3 and NS3/4A proteases (curved arrows) into 10 gene products. The structural proteins (core (C), E1 and E2) form part of the lipoviral particle. Both p7 and NS2 play an import yet not fully understood role in the formation of infectious particles; the non-structural proteins NS3, NS4A, NS4B, NS5A and NS5B, together with a number of host factors, form the replicase complex. Viral proteins that are the targets of direct-acting antivirals in advanced clinical development are shaded in red. CLDN1, claudin 1; CypA, cyclophilin A; miR, microRNA; OCLN, occludin; SR-B1, scavenger receptor class B member 1.

Three nucleoside or nucleotide NS5B polymerase inhibitors currently remain in clinical development: mericitabine, sofosbuvir and VX-135 (formerly known as ALS-2200) (TABLE 1). Among these, sofosbuvir and VX-135 seem to have the highest antiviral activity. Sofosbuvir, a nucleotide, has been submitted to the FDA for approval and is therefore the most advanced in this class.

Data from the Phase III FISSION and NEUTRINO studies of sofosbuvir were published recently[53]. In the single-group, open-label NEUTRINO study, 327 patients infected with genotypes 1, 4, 5 or 6 were treated with the combination of PEG-IFN, ribavirin plus sofosbuvir for 24 weeks and an overall SVR12 rate of 90% was achieved. In more detail, SVR12 rates were: 92% for subgenotype 1a ($n = 225$); 82% for subgenotype 1b ($n = 66$); 96% for genotype 4 ($n = 28$); 100% for genotype 5 ($n = 1$); and 100% for genotype 6 ($n = 60$). Notably, sofosbuvir appears to be safe and well tolerated[54]. Regarding the new drug application submitted to the FDA, two combinations are most likely to be approved: sofosbuvir with PEG-IFN plus ribavirin for genotype 1, and sofosbuvir with ribavirin for genotypes 2 and 3. Combinations of sofosbuvir with other potent DAAs, such as the NS5A inhibitor daclatasvir[55], which may become available shortly after the time of sofosbuvir's approval, will be interesting options to evaluate.

Overall, their high potency and high barrier to resistance make nucleoside and nucleotide polymerase inhibitors a highly promising class that may become part of first-choice IFN-containing and all-oral regimens, a so-called backbone of future anti-HCV therapies. However, the fate of this class of drug is tied to a comparatively small number of compounds.

Non-nucleoside NS5B polymerase inhibitors. Non-nucleoside polymerase inhibitors bind the NS5B protein outside the active site, thereby causing a conformational change that inhibits polymerase activity. A limitation of this mechanism of action is that these binding sites are much less conserved among the genotypes compared with the active site. This results in a lower potential for cross-genotypic activity and more potential for viral escape through the emergence of RAVs compared to nucleoside and nucleotide NS5B polymerase inhibitors[43]. Moreover, their potency in terms of viral load reduction in a monotherapy setting is lower compared to protease inhibitors or highly potent nucleoside and nucleotide polymerase inhibitors. There are also considerable safety issues.

Nonetheless, six compounds — ABT-072, ABT-333, deleobuvir (BI 207127), BMS-791325, setrobuvir and VX-222 — are currently in Phase II trials (TABLE 1) and it is likely that several will move forward as part of three-drug or four-drug combinations for genotype 1 (or possibly subgenotype 1b only) infection that may or may not include IFN. For instance, a combination of ABT-072 or ABT-333 plus PEG-IFN and ribavirin given over 12 weeks achieved SVR24 rates of 86% (ABT-072) and 63% (ABT-333) in those receiving the highest dose tested[56]. This is in contrast to SVR24 rates of 9–18% in the control group receiving PEG-IFN plus ribavirin[56]. However, some of the HCV protease inhibitors used to form these combinations, such as ABT-450 or danoprevir, need boosting with the cytochrome P450 3A4 inhibitor ritonavir. That is, ABT-450 and danoprevir need to be co-administered with low-dose ritonavir to improve their pharmacokinetic profile, thereby turning such quadruple therapies into a combination of five different drugs.

NS5A inhibitors. Drugs in clinical development targeting the NS5A RNA-binding protein include ABT-267, ACH-3102, daclatasvir, ledipasvir, MK-8742 and PPI-668 (TABLE 1). NS5A is essential for viral replication; however, its precise function and, therefore, the mechanism of action of NS5A inhibitors is unclear. Nonetheless, the above compounds induce a rapid decline in viral load and the emergence of RAVs with amino acid exchanges located in the NS5A protein confirmed that this is their molecular target[57,58].

Phase II data on the combination of PEG-IFN, ribavirin plus daclatasvir indicated that SVR24 rates >80% were achieved with this combination in treatment-naive patients infected with subgenotype 1b[59]. SVR24 rates are lower in previous non-responders to dual PEG-IFN plus ribavirin therapy and in patients infected with subgenotype 1a[59]. As with NS3/4A protease inhibitors, the lower SVR24 rates seen with daclatasvir are due to a lower genetic barrier to resistance for subgenotype 1a compared with subgenotype 1b[60].

In a combination of four drugs comprising PEG-IFN, ribavirin, daclatasvir and the second-wave protease inhibitor asunaprevir, SVR24 rates of 90–95% were achieved in non-cirrhotic patients infected with genotype 1 and who were prior null-responders to dual therapy of PEG-IFN plus ribavirin (QUAD study)[61]. In two parallel arms evaluating a combination of daclatasvir and asunaprevir only (that is, without PEG-IFN or ribavirin) in patients infected with subgenotype 1b, SVR12 rates of 64–91% were achieved (DUAL

Table 1 | **Selection of approved drugs and drugs in clinical development for chronic HCV infection by class**

Drug name (aliases)	Phase	Developer	Combinations tested (selection)	
			IFN-containing	IFN-free
NS3/4A protease inhibitors (first generation)				
Boceprevir	Approved	Merck	• PEG + RBV (approved)	–
Telaprevir	Approved	Janssen/Vertex	• PEG + RBV (approved)	–
ABT-450*	III	Abbott/AbbVie	• PEG, RBV + ABT-267	• RBV + ABT-072 • ABT-267 + ABT-333 • RBV, ABT-267 + ABT-333
Asunaprevir (BMS-650032)	III	Bristol-Myers Squibb	• Lambda + RBV • PEG, RBV + daclatasvir	• Daclatasvir • Daclatasvir + BMS-791325
Faldaprevir (BI 201335)	III	Boehringer Ingelheim	• PEG + RBV	• Deleobuvir • RBV + deleobuvir
Simeprevir (TMC435)	III	Janssen/Medivir	• PEG + RBV	• RBV + daclatasvir
Vaniprevir (MK-7009)	III	Merck	• PEG + RBV	–
Sovaprevir (ACH-1625)	II	Achillion	• PEG + RBV	–
Danoprevir* (RG-7227)	II	InterMune/Roche	• PEG + RBV	• Mericitabine • RBV + mericitabine
GS-9451	II	Gilead	• PEG, RBV + ledipasvir	• RBV, ledipasvir + tegobuvir
NS3/4A protease inhibitors (second generation)				
MK-5172	II	Merck	• PEG + RBV	• RBV • RBV + MK-8742
ACH-2684	I	Achillion	–	–
Nucleoside polymerase inhibitors				
BMS-986094 (INX-189)	Discontinued	Bristol-Myers Squibb	• N/A	• N/A
Sofosbuvir (GS-7977, PSI-7977)	III	Gilead	• PEG + RBV	• RBV • RBV + ledipasvir • RBV + daclatasvir
IDX-184	Discontinued	Idenix	• N/A	• N/A
Mericitabine (RO5024048, RG7128)	II	Roche	• PEG + RBV • PEG, RBV + boceprevir • PEG, RBV + telaprevir	• RBV + danoprevir
VX-135 (ALS-2200)	II	Alios/Vertex	–	• RBV
Non-nucleoside polymerase inhibitors				
ABT-333	III	Abbott/AbbVie	• PEG + RBV	• ABT-267 + ABT-450 • RBV, ABT-267 + ABT-450 • ABT-072 + ABT-450 • RBV + ABT-450
Deleobuvir (BI 207127)	III	Boehringer Ingelheim	–	• RBV + faldaprevir
ABT-072	II	Abbott/AbbVie	–	• ABT-333 + ABT-450 • RBV + ABT-450
BMS-791325	II	Bristol-Myers Squibb	• PEG + RBV	• Asunaprevir + daclatasvir
Filibuvir	Discontinued	Pfizer	• N/A	• N/A
Setrobuvir (ANA-598, RG-7790)	II	Roche	• PEG + RBV	–
Tegobuvir (GS-9190)	Discontinued	Gilead	• N/A	• N/A
GS-9669	II	Gilead	–	• Ledipasvir + sofosbuvir
VX-222	II	Vertex	• PEG, RBV + telaprevir	• RBV + telaprevir

Table 1 (cont.) | **Selection of approved drugs and drugs in clinical development for chronic HCV infection by class**

Drug name (aliases)	Phase	Developer	Combinations tested (selection)	
			IFN-containing	IFN-free
NS5A inhibitors				
Daclatasvir (BMS-790052)	III	Bristol-Myers Squibb	• PEG + RBV • PEG, RBV + asunaprevir • Lambda + RBV	• Asunaprevir • Asunaprevir + BMS-791325 • RBV + simeprevir • RBV + sofosbuvir
Ledipasvir (GS-5885)	III	Gilead	• PEG, RBV + GS9451	• RBV + sofosbuvir
ABT-267	III	Abbott/AbbVie	• PEG, RBV + ABT-450	• ABT-450 • ABT-333 + ABT-450 • RBV, ABT-333 + ABT-450
MK-8742	II	Merck	–	• RBV + MK-5172
ACH-3102	II	Achillion	–	• RBV
PPI-668	II	Presidio	–	• RBV, faldaprevir + deleobuvir
Host-targeting agents				
Alisporivir (Debio-025, DEB025)	III (on hold)	Novartis	• PEG + RBV	• RBV
SCY-635	II	Scynexis	• PEG + RBV	–
Miravirsen	II	Santaris	–	–
ITX-5061	I	iTherx	–	–
Interferons				
IFNλ	III	Bristol-Myers Squibb	• RBV • RBV + daclatasvir • RBV + asunaprevir	• N/A
IFNα XL	II	Flamel	• RBV	• N/A
Locteron (BLX-883)	II	Biolex	• RBV	• N/A
IFNθ (ITCA 638)	II	Intarcia	• RBV	• N/A
Oral IFN	II	Amarillo	• PEG + RBV	• N/A
TLR7 modulators				
ANA-773	I	Anadys	• RBV	–
GS-9620	I	Gilead	–	–
TLR9 modulators				
IMO-2125	I	Idera	• RBV	–

IFN, interferon; N/A, not applicable; PEG, pegylated interferon; RBV, ribavirin; TLR, Toll-like receptor. *Efficacy of drug is boosted with ritonavir.

study)[132]. For other NS5A inhibitors, data presented at the 2013 EASL meeting indicated that SVR12 rates of up to 98% were achieved using a four-drug combination comprising ledipasvir, PEG-IFN, ribavirin and the protease inhibitor GS-9451 for just 12 weeks[62]. However, the patient population used in this study had a favourable profile in that they were infected with HCV genotype 1, treatment-naive and had the *IL28B* CC genotype[62].

An inhibitor of NS4B, another HCV-encoded RNA-binding protein, has been reported but is still in preclinical development[63]. Currently, NS5A inhibitors look like promising combination partners both for the PEG-IFN plus ribavirin regimen and all-oral regimens although they may be less useful in subgenotype 1a compared with other genotypes.

Do we still need host-targeting agents?

Agents that target host-encoded factors that are essential for HCV replication (host-targeting agents) may offer the advantage of inherent pan-genotypic activity and a high barrier to resistance as host factors are genetically stable in any given host[64]. Potent antiviral activity has been described for the immunosuppressive agent cyclosporine A as well as non-immunosuppressive derivatives of it[65]. Cyclosporin A derivatives perturb the

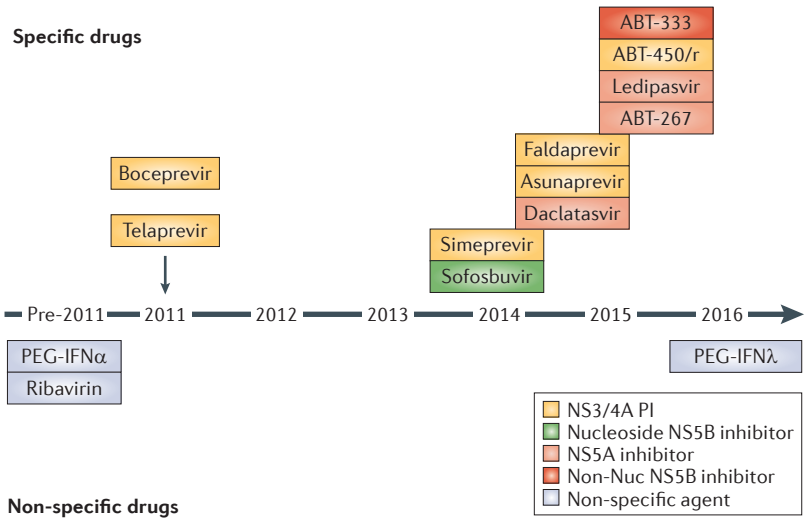


Figure 4 | **Selection of anti-HCV drugs in advanced development.** A schematic of drugs approved for treating hepatitis C virus (HCV) infection as well as drugs in advanced development with tentative future launch dates. PEG-IFN, peglyated-interferon; r, ritonavir.

interaction between the virus and the cellular peptidyl *cis*-trans isomerase cyclophilin A, although their precise mechanisms of action are not fully understood[66,67]. The most advanced non-immunosuppressive cyclosporine A derivative and the most advanced host-targeting agent so far is alisporivir[68] (TABLE 1).

In treatment-naive patients infected with genotypes 2 and 3 high SVR24 rates were achieved with alisporivir alone (72%) or with a combination of alisporivir plus ribavirin (92%)[69], making alisporivir the first drug to show a potential for curing chronic HCV infection by monotherapy. Indeed, the first patient clearing HCV infection by an all-oral monotherapy regimen was a Canadian patient from an early alisporivir trial. This patient received 29 days of alisporivir, then declined the subsequent course of PEG-IFN plus ribavirin that was offered to trial participants, and on follow-up was found to have achieved SVR[70]. However, earlier in 2012 the alisporivir development programme was put on hold by the FDA owing to several cases of acute pancreatitis, including one fatality, in Phase III studies of alisporivir in combination with PEG-IFN plus ribavirin.

Another cyclophilin inhibitor, SCY-635, is currently in Phase II trials. Also in Phase II trials is miravirsen, a locked nucleic acid oligonucleotide that targets the liver-specific microRNA-122 (miR-122)[71]. miR-122 binds to the HCV genome and is essential for replication. Miravirsen is therefore the first miR-targeting drug being developed in humans[72]. In a monotherapy proof-of-concept study there was a 3 log decline in viral load in patients receiving the higher dose of miravirsen[73]. Moreover, resistance and breakthrough were not seen, nor was toxicity[73]. Other host-targeting agents, such as ITX-5061 that perturbs the interaction between HCV and one of its cell surface receptors[74], are in even earlier stages of clinical development.

While the theoretical concept of targeting host factors to treat a highly variable virus such as HCV is appealing, given the recent set-back for the development of alisporivir and the large number of well-advanced and highly potent DAAs, it is currently doubtful whether host-targeting agents will become part of the standard of care for HCV in the foreseeable future. Apart from pancreatitis, mild hyperbilirubinaemia and hypertriglyceridaemia were also observed in trials with alisporivir. Yet, the severe safety signals — most importantly pancreatitis — were only seen when alisporivir was combined with IFN. No such side effects were seen in the all-oral regimen of alisporivir in more than 2,000 patients. Much will depend on whether and when the FDA will allow the clinical development programme for alisporivir to restart. This, however, will depend on the elucidation of the causal relationship between alisporivir and pancreatitis and whether this is related to the alisporivir-associated hypertriglyceridaemia.

Is the end of IFN at hand?

In 2012, important proof-of-concept data showing that chronic HCV infection can indeed be cured by an IFN-free all-oral regimen with a DAA was published[75]. In patients infected with genotype 1 and who were previous null-responders it seemed that a combination of the second-wave protease inhibitor asunaprevir and the NS5A inhibitor daclatasvir could achieve SVR24 rates of ~90% in patients infected with subgenotype 1b. However, viral breakthrough occurred in most of the patients infected with subgenotype 1a[75,76]. Recently, it was reported that the combination comprising the ritonavir-boosted protease inhibitor ABT-450 and the non-nucleoside polymerase inhibitor ABT-333 plus ribavirin achieved SVR12 rates of >90% in non-cirrhotic, treatment-naive patients infected with genotype 1 (REF. 77). However, the same combination only achieved SVR12 rates of 47% in previous partial responders and previous null responders[77].

It is likely that the SVR12 rates of 47% can still be improved using more complex combinations or more potent DAAs. Most notably, the higher SVR rates achieved with several all-oral combinations in subgenotype 1b compared with subgenotype 1a supports the concept that a 'backbone' drug with a high barrier to resistance will be needed to achieve high cure rates with IFN-free regimens for both subgenotypes 1a and 1b. Conversely, a very high rate of early viral breakthrough was observed when two DAAs with a low barrier to resistance were combined[78].

Final data from the AVIATOR study of the combination comprising ritonavir-boosted ABT-450, ABT-267 and/or ABT-333 with or without ribavirin in treatment-naive patients showed SVR24 rates of 83–96% in genotype 1. In the same study SVR12 data in previous null responders were 89–95%. The highest rates were achieved with a combination of three DAAs, one of them ritonavir boosted, plus ribavirin[79,80]. Treatment failures occurred almost exclusively in patients infected with subgenotype 1a. Moreover, it should be noted that this is neither a triple nor a quadruple but a quintuple drug regimen.

Box 3 | **Lessons learned from first-generation HCV protease inhibitors**

Boceprevir (Victrelis; Merck) and telaprevir (Incivek; Vertex) are first-generation peptidomimetic, covalent yet reversible inhibitors of the hepatitis C virus (HCV) NS3/4A protease. Both protease inhibitors — the first direct-acting antivirals (DAAs) approved for HCV — share a similar mechanism of action as well as most of their clinically relevant strengths and weaknesses. Superior sustained virological response (SVR) rates (BOX 2) were achieved in large Phase III studies in patients chronically infected with HCV genotype 1. SVR rates of 67–75% (treatment-naive) and 59–64% (treatment-experienced) were achieved with the triple regimens (pegylated interferon-α (PEG-IFN) and ribavirin plus either telaprevir or boceprevir) compared with standard of care (PEG-IFN and ribavirin only)[7–10]. Lower rates were seen among previous null responders[7–10]. Trial data with first-wave HCV protease inhibitors has recently been reviewed[107], and shows that SVR rates are strongly dependent on the patient's previous response to dual therapy and on their fibrosis stage. Notably, SVR rates were considerably lower in patients with previous null response to dual therapy and lowest for previous null responders with liver cirrhosis[10]. This has led to the recognition that there are patient groups that are more difficult to treat; that is, they are unlikely to respond to currently available regimens. Difficult-to-treat patients are generally considered to be those that have several unfavourable factors such as being infected with HCV subgenotype 1a, a high viral load and poor previous response to IFN-based therapy. Other factors include the patient having advanced liver disease and the interleukin-28B (*IL28B* or *IFNL4*) genotype TT. Among these factors advanced liver disease is of most concern, as apart from low SVR rates it puts patients at risk for severe or even life-threatening adverse events under treatment[108].

After ~18 months of post-marketing experience it has become clear that the introduction of triple therapy has made the clinical management of chronic HCV genotype 1 infection more successful; however, management of side effects has also become more complex. Although the rash associated with telaprevir and the dysgeusia (altered taste) associated with boceprevir, which received much attention before approval, turned out to be generally well manageable on an outpatient basis, marked anaemia often requires patients to receive blood transfusions or to be hospitalized. Moreover, safety in patients with advanced liver disease is a major concern. In one study (the French early access programme)[108], the real-life experience of patients with early-stage compensated cirrhosis (Child–Pugh class A) receiving triple therapy was measured. A high rate of severe adverse events (30–51%), some of them fatal (4 deaths among 307 individuals receiving treatment) was found[108]. This population was underrepresented in the Phase III studies of telaprevir and boceprevir and yet it is clear that these patients are in more urgent need of treatment in order to avert hepatic decompensation. In more recent studies in this patient population, mixed results have been reported with some[109] showing similar findings to the French early access programme[108]. By contrast, others indicated rates of severe adverse events in patients with advanced liver disease that were lower than the French study[108] and similar to what had been observed in Phase III studies[110–113]. Moreover, patients with more decompensated liver disease seem to be at higher risk of adverse events and death[109]. Overall, SVR rates achievable with currently approved triple therapy in patients with compensated cirrhosis range from 28% to 55%. Some success has even been reported for patients with decompensated liver disease awaiting transplantation[114]. Nonetheless, more effective and safer treatment options, especially in HCV-infected patients with advanced liver disease, are clearly a major unmet need in the field.

The extent to which viral resistance will play a role in the management of chronic HCV infection is currently a major unknown. HCV is a highly variable RNA virus with an error-prone RNA polymerase. Accordingly, monotherapy with first-wave protease inhibitors results in the rapid emergence of several largely identical resistance-associated variants (RAVs) that pre-exist in the HCV quasispecies swarm[115,116]. These RAVs are then selected during triple therapy[7]. However, the extent to which a patient's level of preformed RAVs in the quasispecies swarm before treatment affects the resulting SVR rate after treatment is also unclear. With triple therapy, HCV subgenotype 1a consistently shows inferior SVR rates compared to subgenotype 1b. This is probably due to subgenotype 1a having lower genetic barrier to resistance; that is, subgenotype 1a isolates need fewer nucleotide exchanges to create important RAVs. After termination of exposure to protease inhibitors, the frequency of RAVs in the quasispecies swarm slowly decreases[115]. Whether previous exposure to protease inhibitors and the level of pretreatment RAVs in the patient will affect the success of subsequent protease inhibitor-containing regimens (given extensive cross-resistance between all first-wave HCV protease inhibitors) is as yet unknown.

Other complications relating to the currently approved triple regimens is that both boceprevir and telaprevir are inhibitors of cytochrome P450 3A (CYP3A). Consequently, considerable drug–drug interactions with numerous commonly used medications with boceprevir and telaprevir have added a new layer of complexity to the treatment of chronic HCV genotype 1 infection. There are ongoing efforts to gather information on drug–drug interactions with HCV protease inhibitors and to make this information widely available to the medical community through platforms such as the Hepatitis Drug Interactions website from the University of Liverpool, UK. Such measures are crucially important to the success and broad adoption of triple therapy. Finally, other shortcomings of current triple regimens include the restriction to treating HCV genotype 1 infection and the unfavourable pharmacokinetics of boceprevir and telaprevir that necessitate a three times a day dosing schedule.

Thus, even though the introduction of the first DAAs into clinical practice was clearly a milestone, there is considerable need for improvement. Gradual improvements are being made using the currently licensed drugs. For instance, recent data indicated that telaprevir can be administered twice instead of three times a day without diminishing SVR rates[117] and that only 12 weeks of telaprevir-based triple therapy without a subsequent dual (PEG-IFN and ribavirin) phase may suffice in non-cirrhotic individuals with *IL28B* CC genotype who achieve rapid virological response[118]. However, for major leaps forward in the care of chronic HCV infection clinicians may have to wait for novel DAAs that will be approved in the near future.

Box 4 | **Global eradication of HCV — a realistic goal?**

An estimated 160 million people worldwide are infected with hepatitis C virus (HCV) with widely varying prevalence (below 1% up to 15%) among countries. Upcoming combination regimens hold the promise of being able to cure almost any individual patient regardless of the HCV genotype that they are infected with (BOX 1).

One may wonder whether global eradication of HCV might or even should be a realistic goal. The absence of significant non-human reservoirs and the availability of a protective vaccine or at least highly effective treatment are regarded as some of the key preconditions for the eradication of an infectious disease[119]. Pathogens such as smallpox, measles and rubella fulfil these criteria, and indeed smallpox was declared eradicated in 1980 following a major global public health effort. Despite this success, only two other diseases have been formally declared targets for eradication by the World Health Organization (WHO): dracunculiasis (guinea-worm disease) in 1986 (World Health Assembly Resolution: WHA39.21; see Further information) and poliomyelitis in 1988 (World Health Assembly Resolution: WHA41.28; see Further information). As yet neither goal has been achieved, reflecting the enormity of the challenge to achieve complete global eradication even if the above-mentioned preconditions are met. Nevertheless, there are worthwhile goals below the high bar of global eradication; for instance, the WHO aims to eliminate measles and rubella in at least five of the six WHO regions by 2020 (for more details see the Measles & Rubella Initiative website).

HCV largely fulfils the requirement of limited host transmission. That is, transmission events between humans and chimpanzees, the only other species known to be susceptible to HCV, are highly unlikely. There is no protective vaccine for HCV, but one may argue that, with a strictly blood-borne route of transmission, HCV is far less contagious compared to other pathogens targeted for global eradication and it should be possible to effectively prevent new infections even without a vaccine. A bigger obstacle than the absence of a vaccine may be that the vast majority of the HCV-infected population worldwide are unaware of their condition. Indeed, from a global perspective, the greatest two challenges in hepatitis C may be to improve detection rates in order to be able to offer treatment and provide affordable treatment options for poor countries rather than adjusting nuances in antiviral regimens.

Thus, owing to the high percentage of asymptomatic and undiagnosed cases, the high cost of treatment and the existence of more pressing public health priorities, such as malaria, diarrhoeal disease or tuberculosis, global eradication programmes for HCV are unlikely at this time. However, with the help of astute preventive measures, broad testing of at-risk populations and the upcoming new treatment options we have a good chance of seeing HCV prevalence fall rapidly in coming generations once we have passed the anticipated climax of HCV-related morbidity and mortality, such as hepatocelluar carcinoma, that may be expected around 2025 (REF. 120).

A small study in treatment-naive patients infected with genotype 1 ($n = 44$) or genotypes 2 and 3 ($n = 44$) evaluated the combination of sofosbuvir and daclatasvir with or without ribavirin[55]. This regimen, containing sofosbuvir as a likely backbone drug, achieved SVR12 rates of 88–100% independent of viral or *IL28B* genotype[55]. The same combination has also been used in a group of 21 non-cirrhotic, non-responders to telaprevir- or boceprevir-based triple therapy, achieving SVR12 rates of 100%[81]. Moreover, there was a recent case report of a patient with a severe cholestatic HCV reinfection following liver transplantation who achieved rapid recompensation of liver function followed by SVR with the daclatasvir and sofosbuvir combination (without ribavirin)[82].

Along similar lines, a recently presented interim analysis of the COSMOS study of the combination comprising simeprevir plus sofosbuvir with or without ribavirin given for just 12 weeks in previous null responders infected with genotype 1 showed SVR8 rates of 96% (with ribavirin) and 93% (without ribavirin)[83].

In the Phase III FISSION trial, 499 patients infected with genotype 2 or 3 were randomly assigned to receive sofosbuvir plus ribavirin for 12 weeks or PEG-IFN plus ribavirin for 24 weeks[53]. SVR12 was 67% in both groups and non-inferiority was therefore shown. Notably, the all-oral combination was more successful in genotype 2 (97%) compared with genotype 3 (56%). Even lower SVR12 rates of 34% were seen in patients infected with genotype 3 and with cirrhosis[84]. In two more Phase III studies (FUSION and POSITRON) patients infected with genotypes 2 or 3 who were ineligible for PEG-IFN or who were previous non-responders were treated with sofosbuvir plus ribavirin for 12–16 weeks[85]. SVR12 rates of 50–78% were achieved, and again patients infected with genotype 2 responded more favourably. These data support the FDA filing for sofosbuvir in combination with ribavirin in patients infected with genotype 2 or 3.

In the larger SOUND-C2 study, 362 treatment-naive patients infected with genotype 1, including 10% with early-stage cirrhosis (Child–Pugh class A), were treated with the protease inhibitor faldaprevir (BI 201335) and the non-nucleoside polymerase inhibitor deleobuvir with or without ribavirin. SVR12 rates were 52–69% (with ribavirin) and 39% (without ribavirin)[86,87]. Factors associated with high SVR12 rates included female sex, normal serum liver enzymes, HCV subgenotype 1b, and *IL28B* genotype CC. This was the first study showing the impact of the *IL28B* polymorphism and thus the patient's own innate immune system on treatment response for IFN-free all-oral regimen. Interestingly, cirrhotics achieved rates comparable with non-cirrhotics in this study.

Interim data from an IFN-free and ribavirin-free combination of DAAs (the protease inhibitor asunaprevir, the non-nucleoside polymerase inhibitor BMS-791325 and the NS5A inhibitor daclatasvir) were also reported recently[88]. This regimen, given for 12 weeks, achieved an SVR12 rate of 94% in a subgroup of 16 treatment-naive non-cirrhotic patients with HCV genotype 1 (REF. 88).

Considering the large body of data that is available today, it is reasonable to expect within the next 5 years that all-oral IFN-free regimens will become the first-line treatment of choice in most patients chronically infected with HCV. Such regimens will have to contain one or more drugs with a high barrier to resistance, and currently the nucleoside and nucleotide inhibitors, most notably sofosbuvir, are the most likely candidates to be included in such regimens. However, this does not mean that IFN will cease to be used altogether. It has recently been shown that in patients infected with genotype 1, with a low viral load, who achieve a rapid virological response and are considered 'easy to treat', the addition of a first-wave protease inhibitor does not improve SVR24 rates irrespective of *IL28B* genotype or virus subtype[89]. Even in the long term, IFN has some advantages such as the absence of viral resistance and drug–drug interactions, its comparatively low cost, and the vast experience in its use. In the future, IFNs might also find a niche in patients who have been exposed to and failed several oral DAAs and harbour a quasispecies swarm that is enriched with multiple RAVs. Another niche might be patients

taking essential concomitant medications that heavily interact with HCV DAAs, thus precluding their use, and areas of the world where cost of medication will remain a major limiting factor.

Will we have just one anti-HCV pill?

With the advent of more DAAs the treatment of chronic HCV infection is bound to become more effective but also much more complex. It is unlikely that a 'one size fits all' approach will be developed given a broad choice of different drugs and a large number of patient characteristics that have an impact on response to treatment. Clearly, there will continue to be easy and difficult to treat patients and the latter will probably require broader combinations of DAAs and/or longer duration of treatment.

So far, DAAs, in particular first-wave HCV protease inhibitors, have shown that the easy-to-treat HCV patients have become even more easy to treat. It is unclear whether these agents will significantly improve the results for patients with advanced liver disease or even decompensated cirrhosis, post-liver transplant HCV infection, or co-infections with HIV, HBV or HDV. Importantly, it will be the role of clinicians to define the combination of agents with the highest potency and most favourable side-effect profile for their patients. Once these promising DAAs are on the market, the time will come for investigator-initiated trials that will define the best combinations of the best drugs available. This may require off-label combinations of approved drugs that have not been combined in the pre-approval phase for marketing reasons.

First-line protocols may differ owing to cost considerations; for example, while protease inhibitor-based triple therapy is generally deemed cost-effective[90], in resource-poor settings generic versions of PEG-IFN maybe an attractive alternative to highly priced patent-protected DAA combinations. Finally, to what extent pretreatment testing for viral variants and/or host polymorphisms will be useful to guide treatment choices is an important and largely unanswered question. Finding the optimal regimen for each individual patient in a given health-care environment will be a key challenge for clinicians in the coming years.

Perspectives and conclusions

Judging from the data available today it seems feasible that many treatment regimens will contain a nucleoside or nucleotide NS5B polymerase inhibitor together with other DAAs, PEG-IFN and/or ribavirin. Moreover, such regimens will achieve SVR rates >80% across genotypes within 24 weeks of therapy or less. The shortest possible duration for DAA combinations seems to be ~8 weeks for the easy-to-treat patient populations (that is, treatment-naive, *IL28B* CC, HCV subgenotype 1b). This presumably will come in the form of a single-pill fixed-dose combination. These future therapies will be administered by hepatologists, infectious disease specialists, internists or even general practitioners in an outpatient clinic or private practice setting, depending on the country and the health-care system in place. On the other hand, difficult-to-treat patients, such as those with decompensated cirrhosis, may remain a challenge for tertiary referral centres using a tailor-made cocktail of DAAs with or without IFN.

Overall, the future is bright for patients chronically infected with HCV. A major challenge in the Western world will be to identify most or all patients with HCV-associated liver disease that would benefit from therapy. So far, only a minority of HCV-infected individuals are being diagnosed and of those, only a portion receives therapy. In 2009, it was calculated that a means to diagnose 50% more HCV cases than today would have a greater overall benefit in terms of preventing HCV-related deaths than improving SVR rates by 30%[91].

In the developing world, challenges will differ: making HCV therapies economically affordable in such areas of the world will become a major challenge. Furthermore, non-genotype 1 infections are the dominant cause of HCV infection in areas such as the Indian subcontinent (BOX 1). In addition, co-morbidities or environmental factors influencing the natural course of HCV infection differ in different parts of the world. All these challenges have to be overcome before HCV-related morbidity and mortality will significantly decline worldwide.

Another as yet unanswered challenge will be to decrease the public health burden of liver transplantation. The first step will be to prevent reinfection of the graft with HCV through the use of novel DAA-based therapies. The second step will be to successfully treat recurrent chronic HCV infection in the immunosuppressed transplant patient in the light of daunting drug–drug interactions such as those between immunosuppresants and first-wave protease inhibitors and thus avoid the need for re-transplantation.

Chronic HCV infection is not only the most common indication for liver transplantation, but also the indication with the most unfavourable outcomes in the Western world. Hopefully, after the advent of the all-oral DAA-based regimen this will change. HBV may have paved the way in this direction. In the 1980s and early 1990s HBV had been a contraindication for liver transplantation because reinfection was almost universal for patients with replicating HBV infection. Once HBV reinfection had occurred, 50% of patients died within 3 years. Now, HBV reinfection can be prevented by oral HBV polymerase inhibitors (nucleosidic as well as nucleotidic) pre-transplantation and by a combination of oral HBV polymerase inhibitors plus the costly hepatitis B hyperimmunoglobulins (HBIg) post-transplantation. Moreover, HBV-related liver disease has become one of the indications with the best outcome for liver transplantation[92]. Latest results and developments indicate that the costly HBIg has only to be given for a limited time period post-transplantation, reducing the cost burden for preventing HBV reinfection. Indeed, the best way to ameliorate the donor shortage for liver transplants may be to make HCV-related end-stage liver disease — currently still the number one indication for liver transplantation in most countries of the world — disappear from the transplantation waiting lists. This may become an achievable short-term goal.

Whether the ultimate goal of global HCV eradication is deemed realistic or not (BOX 4), major progress against this important disease on a worldwide scale will only be made if the multiple novel HCV drugs will become available and affordable in all parts of the world and will be effective in all types of HCV-associated diseases. Even if this becomes a reality, it will remain a major challenge to identify all HCV infected patients in the first place. Nevertheless, hepatitis C is an inspiring example of what molecular medicine can achieve. Soon after cDNA screening resulted in the identification of the virus, availability of anti-HCV antibody tests based on recombinant HCV proteins led to a dramatic decrease in new HCV infections by screening blood units. Specific drugs were developed as a direct consequence of studying and understanding the HCV life cycle. The replicon system allowed *in vitro* drug testing against multiple antiviral targets. Clinicians and, more importantly, patients are about to reap the rewards of the efforts made by countless researchers for more than two decades.

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

FURTHER INFORMATION
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