Review

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Host-targeting agents for prevention and treatment of chronic hepatitis C – Perspectives and challenges

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Summary

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease and hepatocellular carcinoma worldwide. Furthermore, HCV-induced liver disease is a major indication of liver transplantation. In the past years, direct-acting antivirals (DAAs) targeting HCV enzymes have been developed. DAAs increase the virologic response to anti-HCV therapy but may lead to selection of drug-resistant variants and treatment failure. To date, strategies to prevent HCV infection are still lacking and antiviral therapy in immunocompromised patients, patients with advanced liver disease and HIV/HCV-co-infection remains limited. Alternative or complementary approaches addressing the limitations of current antiviral therapies are to boost the host's innate immunity or interfere with host factors required for pathogenesis. Host-targeting agents (HTAs) provide an interesting perspective for novel antiviral strategies against viral hepatitis since they have (i) a high genetic barrier to resistance, (ii) a pan-genotypic antiviral activity, and (iii) complementary mechanisms of action to DAAs and might therefore act in a synergistic manner with current standard of care or DAAs in clinical development. This review highlights HTAs against HCV infection that have potential as novel antivirals and are in preclinical or clinical development.

Introduction

With approximately 130 million chronically infected individuals worldwide, hepatitis C virus (HCV) infection is a major cause of chronic liver disease including liver cirrhosis, liver failure and hepatocellular carcinoma (HCC) [1–3]. HCV-induced liver cirrhosis and HCC are major indications for liver transplantation (LT) [4]. Thus, HCV-induced liver disease is a major challenge for public health [5].

HCV is a single-stranded RNA virus of positive polarity belonging to the *Flaviviridae* family and the *hepacivirus* genus (reviewed in [6]). While six major genotypes and several different subtypes have been described worldwide, the virus also circulates as a quasispecies within a given infected individual. This high variability represents a challenge for preventive and therapeutic antiviral strategies as the virus may rapidly evade the host immune responses and antivirals [7,8]. The current standard of care (SOC) of chronic HCV infection consists of pegylated interferon-α (PegIFN-α) and ribavirin (RBV). Moreover, since 2011, the new SOC for HCV genotype 1 infected patients is a triple combination of PegIFN-α/RBV and a HCV protease inhibitor (telaprevir or boceprevir). Although the addition of these direct-acting antivirals (DAAs) improves outcome, an important limitation of these DAAs that may contribute to therapy failure is their low genetic barrier for resistance resulting in drug-escape mutants during long-term treatment due to their general mechanism of action [9] and without imposing a large viral fitness cost. DAAs are not approved for LT [10] and IFN-α-based antiviral therapies have limited efficacy and tolerability in LT recipients. In addition to licensed DAAs, other DAAs are at various stages of clinical development in combination with PegIFN-α or in IFN-free regimens, including second-generation protease inhibitors, polymerase and non-structural protein 5A (NS5A) inhibitors. Although a rapid decline in HCV RNA levels and/or eradication of HCV in IFN-free regimens have been demonstrated in clinical trials, viral breakthroughs due to the selection of HCV resistant variants as well as differences in virological outcomes for different genotypes and subtypes have been reported. Furthermore, many of these drugs were associated with side effects and raised issues related to drug–drug interactions [11]. Finally, it is not yet clear whether DAA-based therapies will be effective in difficult-to-treat patients, such as null responders to prior PegIFN-α/RBV therapy, patients with advanced liver disease, LT recipients, HIV/HCV-co-infected individuals, hemodialysis patients, or immunosuppressed patients [10].

Another challenge in the management of chronically infected patients is the absence of strategies for prevention of liver graft infection. Development of preventive strategies based on anti-HCV envelope antibodies has been challenged by the high variability of HCV, resulting in rapid viral escape [12–15]. Proof-of-concept of broadly cross-neutralizing antibodies in

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Abbreviations: DAA, direct-acting antiviral; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTA, host-targeting agent; IFN, interferon; miR, microRNA; RBV, ribavirin.

Review

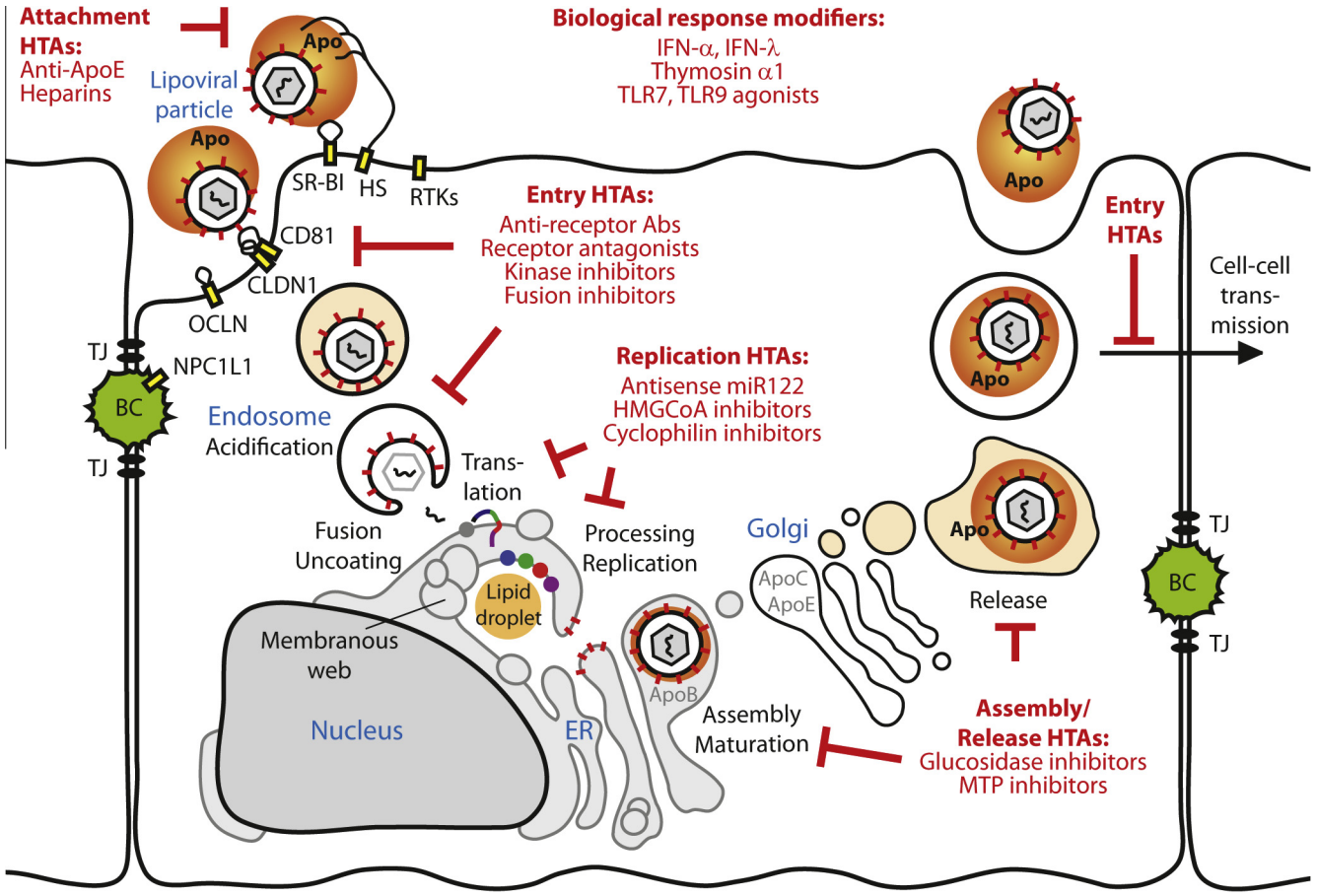

Fig. 1. Host factors required for the hepatitis C virus life cycle as antiviral targets. Outline of the hepatitis C virus (HCV) life cycle in polarized hepatocytes. Host-targeting agents (HTAs) and biological response modifiers (BRMs) are indicated in the figure according to their presumable point of interference with the viral life cycle. ER, endoplasmic reticulum; HS, heparan sulfate proteoglycans; RTKs, receptor tyrosine kinases; SR-BI, scavenger receptor BI; CD81, cluster of differentiation 81; CLDN1, claudin-1; OCLN, occludin; NPC1L1, Niemann-Pick C1-like 1 cholesterol absorption receptor; apo, apolipoprotein; BC, bile canaliculus; TJ, tight junction; Ab, antibody; miR, microRNA; HMGCoA, 3-hydroxy-3-methyglutaryl CoA reductase; MTP, microsomal triglyceride transfer protein; TLR, Toll-like receptor; IFN, interferon.

humans remains to be demonstrated. Thus, there is an unmet medical need for efficient and safe antiviral strategies for difficult-to-treat patients and for prevention of HCV graft infection during LT.

Recent proof-of-concept studies in preclinical models and clinical trials have highlighted that host-targeting agents (HTAs) provide a novel and promising strategy to address current unmet medical needs and limitations of SOC. Two main concepts for HTAs are explored: the first strategy aims at interfering with host factors required for pathogenesis, i.e., to target host factors indispensible for the viral life cycle. These include host cell entry, replication and assembly factors. The second strategy is to target the host by boosting the host's innate immunity, e.g., through the administration of IFN-λ [16] or Toll-like receptor (TLR) agonists [17–19].

HTAs offer a promising perspective due to the following features distinguishing them from DAAs: compared to the viral variability, genetic variability of the host is low. Thus, HTAs impose a very high genetic barrier to resistance [14,15,20–23]. As HTAs are essential for the viral life cycle, HTAs are characterized by a broad pan-genotypic activity while first generation DAAs targeting HCV are characterized by a very narrow antiviral activity limited to genotype 1. Indeed, HTAs have been shown to inhibit infection by HCV of all major genotypes, highly variable quasispecies isolated from individual patients and highly infectious escape variants that are resistant to host neutralizing antibodies [14,20,21,24–27]. Finally, by acting through a complementary mechanism of action, HTAs may synergistically act with current anti-HCV SOC [28,29]. It is expected that this synergy will increase the genetic barrier for resistance, shorten treatment schedules and ameliorate adverse effects by reducing the doses of the individual compounds.

This review will highlight recent progress in the development of HTAs targeting HCV infection that have the potential to clear chronic HCV infection or prevent HCV infection of the liver graft.

Host-targeting agents against hepatitis C virus infection

The HCV life cycle may be divided into three main steps: viral entry into the target cell, viral replication as well as assembly and release of new infectious virions (Fig. 1). Each step of the HCV life cycle is dependent on host cell factors [30], thereby offering numerous targets for HTAs (Figs. 1–3 and Table 1).

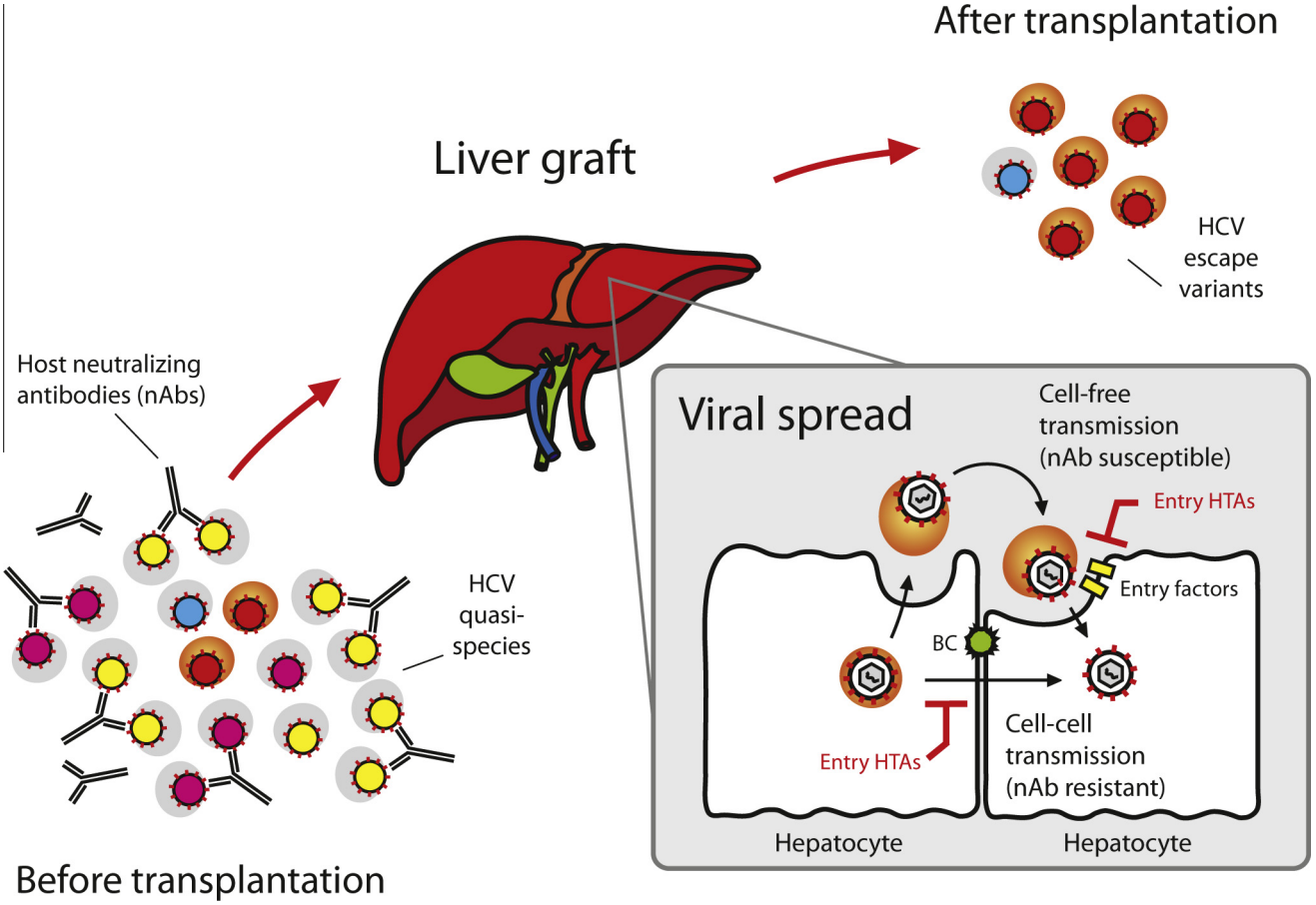

Fig. 2. Host-targeting entry inhibitors for prevention of HCV liver graft infection. During liver transplantation, highly infectious variants of the HCV quasispecies escaping from the host neutralizing antibodies (nAbs) infect the liver graft. This "bottleneck" effect is related to the implantation of a new graft and the lack of selective pressure due to the strong immunosuppression (inset). The inset shows the mechanism of re-infection of naïve hepatocytes and viral spread in the liver graft. HCV variants may spread (i) by cell-free transmission and (ii) by cell–cell transmission. As a consequence, highly infectious HCV variants escaping the host neutralizing immune response are selected during re-infection of the new liver graft through a "bottleneck" effect [14,15]. HCV entry factors are required for both ways of transmission and are targets of HTAs. Entry HTAs targeting HCV entry factors inhibit HCV entry and spread of all major genotype as well as of HCV escape variants that re-infect the liver graft [14,20,21,26,119].

Entry inhibitors

Viral entry is the first step of HCV-host cell interactions and involves the HCV envelope glycoproteins E1 and E2 as well as several host factors. It is believed that cell-free HCV entry is a highly coordinated multistep process (Fig. 1). Highly sulfated heparan sulfate proteoglycans [31] represent first attachment sites, allowing viral concentration on the basolateral hepatocyte membrane. The virus then interacts with several entry factors including scavenger receptor BI (SR-BI) [32], CD81 [33], claudin-1 (CLDN1) [34] and occludin (OCLN) [35]. The formation of CD81-CLDN1 complexes is essential for HCV infection [36,37]. In addition, host cell kinases play an important role in regulating the HCV entry process [21,38,39]. Among them, two cell surface receptor tyrosine kinases (RTKs) have been identified as HCV entry factors: epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2). EGFR and EphA2 promote CD81-CLDN1 co-receptor interaction that is required for HCV entry [21]. The Niemann-Pick C1-Like1 (NPC1L1) cholesterol absorption receptor has recently been proposed as another host entry co-factor [40]. Given its physiological role, NPC1L1 may promote HCV entry either directly by interacting with the HCV lipoviral particle cholesterol or act as indirect entry factor by modulating cholesterol homeostasis and membrane composition required for HCV entry. HCV is internalized via clathrin- and dynamin-dependent endocytosis and is subsequently delivered to the early endosome [41–44]. CD81 and CLDN1 associate during internalization [44,45], but it remains unclear whether other HCV host factors internalize together with HCV. Although required for CD81-CLDN1 interaction, EGFR does not seem to be essential for CD81 internalization [44]. The fusion of the viral and the endosomal membrane is pH-dependent and involves both viral and host proteins [41,46–48]. Among host entry factors, CD81 and CLDN1 play a role in the HCV envelope glycoprotein-dependent cell–cell fusion process [34,49], which is regulated by RTK function [21].

An alternative route of viral entry is direct cell–cell transmission, which also requires numerous host factors including CD81, SR-BI, CLDN1, OCLN, EGFR, EphA2 and potentially NPC1L1 [21,40,50,51]. As this entry route is resistant to the majority of neutralizing antibodies described so far, direct cell–cell transmission probably represents the main process of viral spread [50,51].

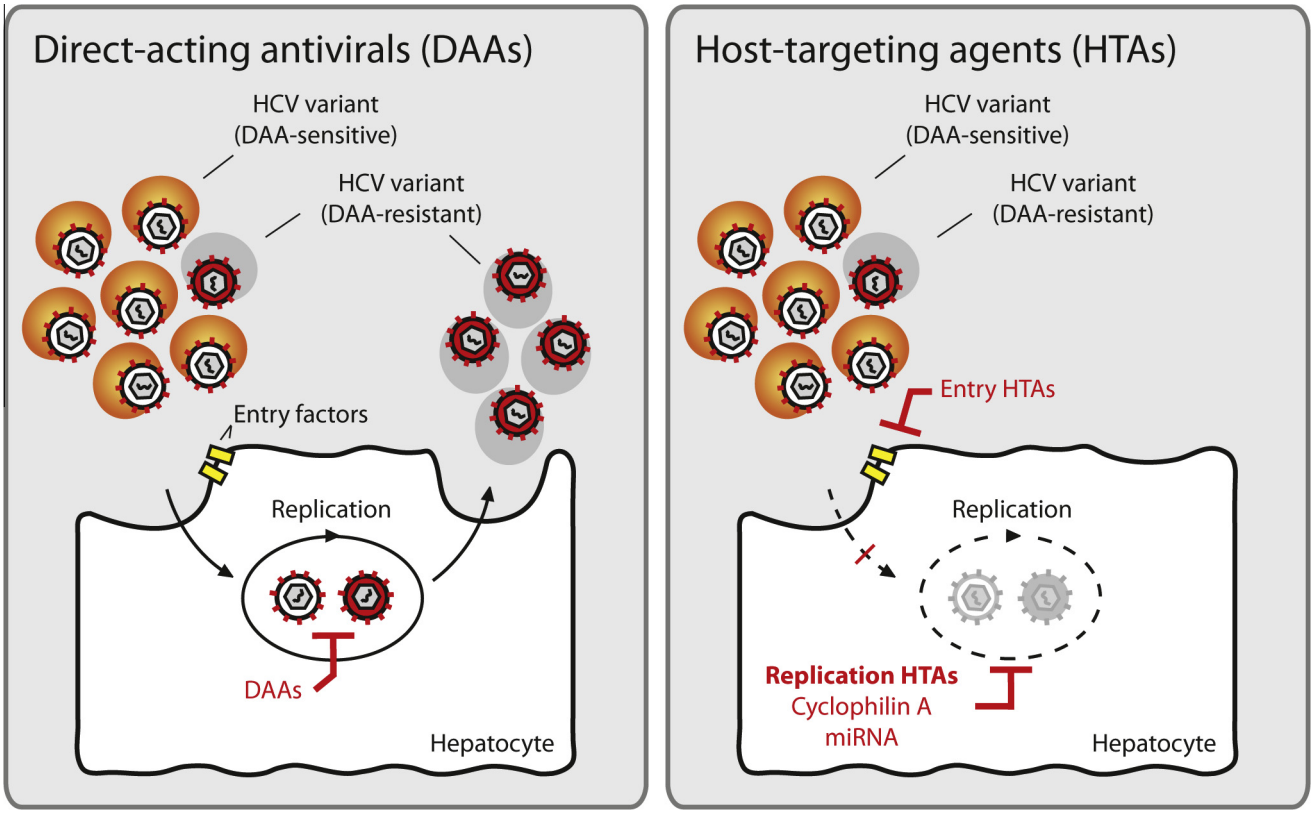

Fig. 3. Host-targeting agents exhibit a high genetic barrier of resistance. HCV lipoviral particles circulate as quasispecies of viral variants that infect and replicate in hepatocytes. The mechanism of viral escape to drug therapy differs between direct-acting antivirals (DAAs) and host targeting agents (HTAs). (Left panel) DAAs efficiently inhibit the replication of DAA-sensitive HCV variants. An HCV variant that is resistant to DAA treatment becomes the predominant HCV variant escaping the antiviral treatment. (Right panel) Targeting host factors required for HCV entry and infection inhibits a broader spectrum of variants and genotypes since the host factor usage is usually highly conserved for all viral variants. As a consequence, the genetic barrier of viral resistance to HTAs can be higher compared to many DAAs.

It is worth noting that there is an overlap of host factors required for cell-free and cell–cell transmission, as most of the host factors involved in cell-free entry have also been described to play a role in cell–cell transmission.

Targeting HCV entry factors may thus allow preventing initiation of HCV infection, such as after LT, and also reduce viral spread and thus maintenance of infection. However, while cell-free HCV entry is strictly dependent on CD81, CD81-independent routes of cell–cell transmission have been described [52,53]. This has to be taken into account for the development of HTA directed against HCV entry factors.

Viral entry has been shown to play an important role in the pathogenesis of HCV infection, especially during HCV reinfection of the graft after LT [14,15]. Viral entry is thus a very promising target for prevention of HCV infection of the liver graft (Fig. 2). Numerous HTAs directed against host entry factors demonstrated a potent antiviral activity *in vitro* (reviewed in [54]). Proof-of-concept studies of HTAs targeting HCV entry have been conducted *in vivo* using the chimeric uPA-SCID mouse model. Antibodies directed against CD81 and SR-BI have both been investigated in prophylactic and post-exposure treatment studies. Administration of 400 μg of either anti-CD81 or anti-SRBI monoclonal antibodies (mAbs) completely protected mice from challenge with HCV [55–57]. Noteworthy, only the administration of anti-SRBI mAb was able to reduce viral dissemination [56,57]. The clinically approved EGFR inhibitor erlotinib, preventing the formation of CLDN1-CD81 complexes, and NPC1L1 inhibitor ezetimibe, that decreases systemic cholesterol in patients, markedly impaired the establishment of HCV infection in the uPA-SCID mouse model [21,40]. Indeed, administration of erlotinib (50 mg/kg/day for 10 days) or ezetimibe (10 mg/kg/day for 2 weeks) prior to viral inoculation significantly delayed the kinetics of HCV infection [21,40]. The clinical potential of kinase inhibitors has been emphasized in a recent case report describing rapid virologic response (RVR) after erlotinib monotherapy (150 mg/day for 12 months) in a HCV-positive HCC patient after LT and viral recurrence due to a discontinued SOC treatment [58]. A clinical trial investigating safety and toxicity of erlotinib in chronically HCV infected patients will soon be conducted to further assess the potential of kinase inhibitors as anti-HCV drugs in combination with DAAs. A phase 1b study assessing the safety of ITX 5061 [26], a small molecule inhibitor targeting the HCV entry factor SR-BI, in HCV-treatment naive patients, is ongoing and an open-label, proof-of-concept phase 1b study assessing the safety and tolerability of ITX 5061 in LT patients has been initiated (Table 1).

Although HCV entry inhibitors are still at a very early step of clinical development, it has been demonstrated that combinations of entry inhibitors with IFN-α, DAAs, or other HTAs *in vitro* result in an enhanced antiviral activity, compared to each compound used in monotherapy, in a synergistic manner [28,29]. This holds promise for entry inhibitors as part of SOC

Table 1. Host-targeting agents against hepatitis C virus infection.

Step	Target	Compound with *in vivo* proof of concept or in clinical development	Stage of development	Reference
Entry	CD81	Anti-CD81 mAbs	Mouse model	[55]
	SR-BI	Anti-SR-BI mAbs	Mouse model	[56, 57]
		ITX 5061	Phase 1b	ClinicalTrials.gov identifier: NCT01165359
	EGFR	Erlotinib	Mouse model	[21]
	NPC1L1	Ezetimibe	Mouse model	[40]
Replication	miR122	Miravirsen/SPC3649	Phase 2a	[23]
	HMGCoA reductase	Statins	Phase 2	[75, 76]
	Cyclophilin A	SCY-635	Phase 1	[87]
		Alisporivir/Debio 025	Phase 3	[91]
Assembly	Glucosidase	MX-3253	Phase 2	[108]

as well as future IFN-sparing regimen(s) for the treatment of HCV infection.

Key Points 1

- With more than 130 million chronically infected individuals, HCV infection is a major cause of chronic liver disease and HCC worldwide
- HCV-induced liver cirrhosis and HCC are major indications for liver transplantation (LT)
- In contrast to hepatitis B virus (HBV), strategies for immunoprevention of HCV reinfection of the graft are absent
- The high variability of HCV represents a challenge for preventive and therapeutic antiviral strategies
- DAAs increase the response to IFN-based antiviral therapy against HCV genotype 1 but also lead to selection of drug-resistant HCV variants
- Given their important side effects and drug-drug interactions, DAAs against HCV are currently not approved for patients undergoing LT, HCV/HIV co-infected patients or pediatric patients
- First generation DAAs are not efficient against all HCV genotypes
- Although early clinical trials have demonstrated impressive outcomes for combinations of DAAs in IFN-free regimens for treatment naïve patients, there will be a need for novel antivirals addressing resistance, treatment of patients with co-morbidity, co-medication or immunosuppression and patients undergoing LT

HCV replication inhibitors

Following HCV entry, the HCV RNA genome is released into the cytosol. Initiation of HCV translation occurs through binding of the 40S ribosomal subunit to the HCV IRES and this association can be enhanced by miR-122, a liver-specific microRNA (miRNA) [59,60]. miR-122 is also an important host factor for HCV replication [24] and miR-122 sequestration using 122–2′OMe oligomers or miR-122 antisense locked nucleic acid SPC3649 reduces HCV replication in a genotype-independent manner *in vitro* [24,25]. Interestingly, weekly intravenous administration of miR-122 antisense locked nucleic acid miravirsen/SPC3649 (5 mg/kg) for 12 weeks to chronically genotype 1 infected chimpanzees led to sustained suppression of HCV viremia, with no evidence of viral resistance [61]. Given the physiological role of miR-122 in cholesterol metabolism, miravirsen/SPC3649 led to markedly lowered serum cholesterol in animals but no important adverse effects were observed [61–63]. Recently, the safety, tolerability and efficacy of miravirsen/SPC3649 have been assessed in a phase 2a study (Table 1). Miravirsen/SPC3649 given as a four-week monotherapy (3, 5 and 7 mg/kg) to treatment-naïve genotype 1 patients was well tolerated and provided robust, dose-dependent antiviral activity that was maintained for more than four weeks after the end of therapy [23]. Four out of nine patients treated at the highest dose with miravirsen/SPC3649 (7 mg/kg) became HCV RNA undetectable during the study. Although markedly decreased pretreatment miR-122 levels had been reported in livers of chronic HCV infected patients who did not achieve virological response during IFN therapy [64], data from this first clinical trial indicate that targeting miR-122 *in vivo* offers a high barrier to viral resistance and the potential for combination in a future IFN-free regimen [23]. Most recently, an allosteric self-cleavable ribozyme capable of releasing antisense sequence to miR-122 only in the presence of HCV NS5B was developed in order to minimize potential side effects related to targeting physiological miR-122 functions [65]. The safety and efficacy of this strategy will next have to be assessed *in vivo*.

HCV RNA replication depends on viral protein association with altered intracellular membranes, probably derived from the endoplasmic reticulum (ER), in a so called membranous web (reviewed in [66]). The HCV replication complex, i.e., viral RNA and viral proteins associated to altered host cell membranes, is dependent on the host cell lipid metabolism. Indeed, this complex requires elements of cholesterol and fatty acid synthesis and

geranylgeranylation of host proteins, as *in vitro* HCV replication can be disrupted by treatment with inhibitors of 3-hydroxy-3-methyglutaryl CoA (HMGCoA) reductase – such as the statin lovastatin, L-659,699 or ZA – or with an inhibitor of protein geranylgeranyl transferase I [67,68]. This is in line with data indicating that HCV replication during acute infection of chimpanzees is associated with the modulation of several genes involved in lipid metabolism [69]. Noteworthy, not all HMGCoA reductase inhibitors also inhibit HCV replication as the statin pravastatin exhibits no anti-HCV activity while fluvastatin has the strongest antiviral effect [70]. While initial clinical studies indicated that statin monotherapy did either not significantly modulate HCV RNA levels or only modestly reduced HCV RNA in chronic HCV patients [71–73], statins may represent interesting adjuvants to SOC. Indeed, fluvastatin (20 mg/day) increased the response to PegIFN-α/RBV, especially in aged women who respond poorly to SOC [74]. Moreover, in two recent large retrospective analyses, statin use was associated with an improved sustained virological response (SVR) in patients receiving combination antiviral therapy [75,76]. However, the addition of fluvastatin (80 mg/day) to PegIFN-α/RBV did not significantly increase SVR rates in HIV/HCV genotype 1 co-infected patients (also receiving highly active antiretroviral (HAART) therapy with a complete suppression of HIV replication) although it did significantly improve the RVR [77]. Taken together, these clinical trials indicate that, with the exception of HIV/HCV co-infected patients, statins may increase the efficacy of SOC in chronic HCV infected patients. Interestingly, most recently small molecule inhibitors of SKI-1/S1P, a lipogenic pathway regulator upstream of HMGCoA reductase, have been described [78]. The most potent inhibitor, PF-429242, inhibited HCVcc replication more efficiently than statins and, in contrast to statins, also reduced infectious particle production [78]. SKI-1/S1P inhibitors may thus also be considered for development of novel antivirals.

Cyclophilins are also important host factors for HCV replication and CypA has been demonstrated to interact with HCV NS5A [79,80]. Cyclophilins had been identified as host targets for antiviral therapy more than 20 years ago as cyclosporine, a widely used immunosuppressive drug, was demonstrated to inhibit non-A non-B hepatitis virus [81]. More recently, cyclosporine analogs lacking immunosuppressive activity and displaying higher *in vitro* antiviral activity, e.g., alisporivir/Debio 025, NIM811 and SCY-635, have been developed [82–84]. These compounds disrupted CypA-NS5A interaction [85,86]. Moreover, SCY-635, currently in phase 1 clinical study, enhanced secretion of type I and type III IFNs in replicon cells and increased the expression of IFN response genes [87]. These data suggest that in addition to inhibiting viral replication, CypA inhibitors may restore the host innate immune responses to HCV inhibitors and thereby enhance their antiviral activity [87]. Interestingly, alisporivir/Debio 025 has also proven anti-HIV activity *in vitro* as this molecule inhibits CypA-HIV capsid protein binding [88,89]. CypA inhibitors may thus have an additional benefit in HIV/HCV co-infected patients. In a phase 1 study, 14-day oral alipsorivir/Debio 025 (1200 mg twice daily) treatment significantly reduced HCV RNA serum levels in HIV/HCV co-infected patients independently of the HCV genotype (1, 3 and 4) [90]. However, a potent synergy between alisporivir/Debio 025 (200, 600 and 1200 mg twice a day for one week and then once daily) and PegIFN-α was also observed in a subsequent phase 2 study demonstrating that addition of alisporivir/Debio 025 increased RVR [91]. Further phase 2 trials also demonstrated improved efficacy and good tolerance adding alisporivir/Debio 025 to PegIFN-α/RBV without selection of resistant variants (reviewed in [92]). This CypA inhibitor is thus characterized by a high barrier to resistance and is the first HTA that reached phase 3 studies (Table 1). Given three cases of acute pancreatitis, the FDA recently put a clinical hold on this trial before proceeding to the next steps. The fact that the combination of alisporivir/Debio 025 with DAAs resulted in additive antiviral activity in short-term *in vitro* antiviral assays [93] holds promise for HTAs as part of future IFN-sparing regimen(s) for the treatment of HCV infection.

HCV assembly/release inhibitors

Following HCV replication, new infectious virions are assembled in the vicinity of lipid droplets and ER [94–97]. The HCV particle is composed of an encapsidated RNA genome that is surrounded by an envelope composed of the envelope glycoproteins E1 and E2 [98,99]. E1 and E2 associate as a non-covalent heterodimer and are essential for viral infectivity as they mediate interactions with different host cell factors during viral binding and entry. E1 and E2 are heavily N-glycosylated, contain ER retention signals and are processed within the ER by glucosidases I and II to ensure proper folding and assembly [98]. HCV assembly has been suggested to parallel VLDL assembly [100–102]. Microsomal triglyceride transfer protein (MTP), the rate limiting enzyme of VLDL assembly [103], probably also contributes to HCV particle assembly [101].

Targeting host glucosidases thus represents a promising strategy to interfere with viral infectivity (Table 1). MX-3253/celgosivir (reviewed in [104]), an alpha-glucosidase I inhibitor, induces misfolding of HCV envelope glycoproteins and leads to reduced viral infectivity *in vitro* [105,106]. MX-3253/celgosivir demonstrated modest antiviral efficacy in a phase 2a monotherapy study (200 and 400 mg/day for 12 weeks) in treatment-naive and IFN-intolerant genotype 1 HCV patients [107]. While MX-3253/celgosivir (400 mg/day for 12 weeks) demonstrated clinical benefit in combination with PegIFN-α/RBV in chronic HCV genotype 1 infected patients [108], the further development of MX-3253/celgosivir for HCV infection has subsequently been halted.

Compounds inhibiting VLDL assembly, such as MTP inhibitors, also reduce HCV release from infected cells [100–102]. MTP inhibitors have been developed for treatment of dyslipidemia and currently several MTP inhibitors are in clinical trials for the treatment of hypercholesterolemia or hyperlipidemia (reviewed in [109]). However, whether MTP inhibitors display an antiviral effect against HCV infection *in vivo* remains to be determined. Moreover, recent screens revealed that several approved drugs display antiviral activity against HCV by targeting HCV assembly and/or release: these studies identified two anti-cancer drugs, pterostilbene (a methylated form of resveratrol) and torimefene (a derivative of tamoxifene) [110] as well as quinidine, a class I antiarrhythmic agent [111] as potential antivirals against HCV. Taken together, these data indicate the further potential of clini-

cal development of HCV assembly inhibitors for the treatment of chronic hepatitis C.

Clinical perspectives of HTAs interfering with the HCV life cycle

To date, the main issue of anti-HCV SOC is to avoid viral resistance and severe side effects. Generally speaking, the use of DAAs against different potential highly variable viruses, such as HCV, HIV or influenza virus, is associated with the development of resistance, while HTAs, acting on cellular targets that are less prone to mutations, may impose a higher genetic barrier for resistance (Fig. 3) [112,113]. On the other hand, the principle theoretical drawback of using HTAs is their potential greater cellular toxicity. Nevertheless, it has to be pointed out that the development of several DAAs targeting HCV, such as BILN 2061, had to be stopped due to severe side effects [114]. Moreover, the majority of current drugs widely used for cardiovascular, neurological or endocrine diseases as well as cancer, target host proteins [115–117]. Thus, side effects have to be carefully evaluated for novel antiviral strategies against hepatitis C irrespective of the drug target.

While DAAs allow increasing the virological response of HCV genotype 1 infected patients, a large fraction of chronic HCV patients, especially HIV/HCV co-infected patients and patients undergoing LT, will not be eligible for DAAs given the important drug–drug interactions with anti-retroviral therapy and immunosuppressive agents. Noteworthy, synergy between IFN-α, DAAs and HTAs allowing to decrease the concentrations of the individual compounds [28,29] holds promise for a variety of possibilities of future combination therapy treatments of hepatitis C infection that may be adapted to the individual patient. Furthermore, given (i) the importance of host entry factors for HCV reinfection of the graft during LT [15], (ii) the broad antiviral activity of entry inhibitors against viral escape variants selected during LT [14,20,21], and (iii) the synergy between entry inhibitors and neutralizing anti-HCV envelope antibodies [27], entry inhibitors also represent a promising strategy to prevent viral reinfection of the liver graft (Fig. 2).

tremendous progress has been made in the characterization of the HCV life cycle

Key Points 2

- The HCV life cycle offers several well characterized host targets for antiviral therapy
- Due to low genetic variability of host factors, HTAs may impose a higher genetic barrier to resistance than DAAs
- Most HTAs have a pan-genotypic antiviral activity
- Given their complementary mechanism of action, HTAs may inhibit viral infection in a synergistic manner in combination with IFN-α and/or DAAs
- As for DAAs, host-related adverse effects need to be carefully addressed
- Pan-genotypic antivirals alisporivir/Debio 025, a specific HTA targeting cyclophilin A, and miravirsen/SPC3649, a miR-122 antisense locked nucleic acid, have completed proof-of-concept in humans
- Many other HTAs targeting the HCV life cycle are at different stages of development
- Synergy between IFN-α, DAAs and HTAs holds promise for a variety of possible combination therapies for prevention and treatment of hepatitis C
- HTAs offer the perspective to improve antiviral treatment by decreasing resistance, shortening of treatment duration and ameliorating adverse effects
- Given the importance of host entry factors for HCV reinfection of the graft during LT, entry inhibitors represent a promising strategy to prevent viral reinfection of the liver graft

Conclusions and perspectives

The goal of current anti-HCV SOC is sustained viral eradication. However, due to the high variability of HCV, viral resistance and subsequent treatment failure remain major challenges. Moreover, therapeutic strategies for a large fraction of patients, especially HIV/HCV co-infected patients, patients with immunosuppression and co-morbidity and patients undergoing LT remain limited [7,118]. Although early clinical trials have demonstrated impressive outcomes for combinations of DAAs in IFN-free regimens for treatment naïve patients [11] there will be a need for antivirals addressing resistance, treatment of patients with co-morbidity, co-medication or immunosuppression and patients undergoing LT [10].

Alternative or complementary approaches to current anti-HCV therapies are to boost the host's innate immunity or interfere with host factors required for pathogenesis. HTAs act on cellular targets and thus may impose a higher genetic barrier for resistance than DAAs. Moreover, HTAs are usually characterized by a pan-genotypic antiviral activity. In the past years, and several host targets for specific antiviral therapy have been uncovered. Alisporivir/Debio 025 and miravirsen/SPC3649, two HTAs inhibiting HCV replication, recently completed proof-of-concept in humans [23,92]. Many other HTAs targeting the HCV life cycle are at different stages of preclinical and clinical development suggesting that the therapeutic arsenal against chronic HCV infection may widen within the next years. Furthermore, recent studies underscored the importance of host factors during HCV liver graft infection and highlighted the potential of HCV entry inhibitors for prevention of graft infection during LT [15,20,21,57,119].

The recent preclinical and clinical development of HTAs for HCV as well as novel HTA-based strategies for other pathogens including other viruses and bacteria [120] highlights the promise of this approach to address unmet medical needs in the prevention and treatment of virus-induced liver disease.

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Conflict of interest

The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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