Chapter

Host-Targeting Antivirals for Treatment of Hepatitis C

Bouchra Kitab, Michinori Kohara and Kyoko Tsukiyama-Kohara

Abstract

Treatment of chronic hepatitis C virus (HCV) infection has been revolutionized during last years with the development of highly potent direct-acting antivirals (DAAs) specifically targeting HCV proteins. DAAs are the current standard of care for patients with chronic hepatitis C, leading to high cure rates. However, some hurdles exist including the high cost of these therapies restricting access to patients, their inability to protect against the risk of developing hepatocellular carcinoma in patients with advanced fibrosis, and emergence of resistant variants resulting in treatment failure. New therapeutic options should be essential to overcome DAAs limitations and improve survival. By targeting host-cell factors involved in HCV life cycle, host-targeting antivirals (HTAs) offer opportunity for promising anti-HCV therapy with low mutational rate and may act in a synergistic manner with DAAs to prevent viral resistance and reduce viral replication. Moreover, HTAs could be effective in difficult-to-cure patients by acting through complementary mechanisms. In this chapter, we will focus on the latest and most relevant studies regarding the host-cell factors required in HCV infection and explored as targets of antiviral therapy, we will also discuss the HTAs evaluated in preclinical and clinical development and their potential role as alternative or complementary therapeutic strategies.

Keywords: chronic hepatitis C, direct-acting antivirals, cell factors, host-targeting antivirals, antiviral therapy

1. Introduction

Hepatitis C virus (HCV) is a major causative agent of chronic liver diseases. Globally, it is estimated that 71 million people have chronic HCV infection, defined as the persistence of HCV genome in the blood for at least six months after the onset of acute infection [1, 2]. Patients with chronic HCV infection are at high risk of developing liver cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC), which are the most common indications for liver transplantation [3]. Until 2011, the standard-of-care therapy for HCV infection consisted on pegylated-interferon alpha in combination with the nucleotide analogue ribavirin (peg-IFNα/RBV) leading to sustained virologic response (SVR) in 54–63% of patients with substantial side effects [4, 5]. The great advances in HCV research allowed the development of direct-acting antivirals (DAAs) which have dramatically improved the standard-of-care for HCV-infected patients [6, 7]. As their name suggests, DAAs are class of antivirals that directly target viral proteins required in HCV replication.

The first-generation DAAs used in combination with peg-IFNα/RBV improve SVR rates by approximately 70% [8, 9]. Subsequently, IFN-free DAAs regimens, based on the use of highly potent and well-tolerated DAAs combinations were introduced and currently used for treatment, providing SVR in more than 95% of patients, with minimal side effects [10, 11].

Although DAAs offer the chance of viral cure for most of HCV patients, there are some limitations that restrain their full potential, including their high cost limiting access to treatment, the high mutation rate of HCV which may lead to the selection of DAA-resistant HCV variants resulting in treatment failure, and the low SVR rate in difficult-to-treat patients such as those with advanced liver cirrhosis [12–14]. Recent studies reported the inability of DAAs to protect against the risk of HCV re-infection of liver graft in transplanted patients, or the risk of developing HCC in patients with advanced liver fibrosis [15, 16]. Consequently, there is a need for other therapeutic options with better affordability, high rate of viral cure, and fewer cases of viral resistance. HCV requires host-cell factors to establish productive infection and propagation, thus development of host-targeting antivirals (HTAs) that interfere with these factors provides promising antiviral candidates, which may help to improve the current landscape of hepatitis C therapy [14, 17]. Several HTAs have been evaluated for preclinical and clinical development with some of them showing promising results. In this chapter, we provide an overview on recent advances in antiviral therapies against HCV and highlight the most important host factors explored as therapeutic targets. We also discuss the different HTAs evaluated in preclinical and clinical development and their potential impact as alternative or complementary therapeutic options to cure HCV infection and associated liver diseases.

2. Molecular virology of HCV

HCV is an enveloped, positive-sense single-stranded RNA virus, classified in the *hepacivirus* genus of *Flaviviridae* family [18]. HCV genomic RNA (~9.6 kb in length) contains highly structured 5′- and 3′-untranslated regions (UTRs) flanking a single open reading frame [19, 20]. The 5'-UTR is highly conserved and contains an internal ribosome entry site (IRES) essential to initiate viral RNA translation [21]. The high error prone of HCV NS5B RNA-dependent RNA polymerase leads to frequent mutations across the viral genome, resulting in high intra-patient variability (1–5%) represented in the form of quasispecies, and high inter-patient variability manifested by the existence of 7 genotypes, and 67 confirmed subtypes [22, 23]. HCV genotypes differ from each other by 31–33% at nucleotide level, compared with 15–25% between subtypes within a given genotype [24]. A global survey showed that HCV genotypes 1 and 3 are the most prevalent worldwide accounting for 46% and 30% of global HCV cases, respectively. Genotypes 2, 4, 5, and 6 are responsible for the majority of remaining HCV cases: 9%, 8%, <1%, and 5.4%, respectively [25]. Genotype 7 has been identified in Canada in few patients originating from Central Africa [26]. HCV genotypes have distinct geographic distributions throughout the world, which reflect differences in mode of transmission and ethnic variability. While genotype 1a is predominant in USA, genotype 1b dominated in Europe and Japan, genotype 2 dominated in West Africa and parts of South America, genotype 3 in south Asia, genotype 4 in middle East and Central/North Africa, genotype 5 in South Africa, and genotype 6 in Southeast Asia [25].

HCV replication cycle initiates through viral attachment and entry into the hepatocyte by clathrin-mediated endocytosis [27, 28]. The acidic pH of the early endosomes is essential to trigger fusion leading to nucleocapsid uncoating and release of the viral RNA genome in the cytosol [29]. At the rough endoplasmic reticulum (ER), HCV genomic RNA is translated via an HCV-IRES mediated

mechanism to produce a single polyprotein of ~3010 amino acids [30]. This polyprotein is cleaved by cellular and viral proteases into three structural proteins that build up the HCV particle (Core and envelope glycoproteins E1 and E2) and seven nonstructural (NS) proteins permitting viral RNA replication, and viral particle assembly (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [31]. The viroporin p7 and the cysteine protease NS2 are involved in viral particle assembly. NS3, NS4A, NS4B, NS5A, NS5B and HCV genomic RNA template form the viral replicase complex for HCV RNA replication [31, 32]. As all positive-strand RNA viruses, HCV induces massive rearrangements of cytoplasmic membranes in the host cell to generate a replication-favorable compartment called "the membranous web" in the case of HCV [33]. The membranous web is mainly composed of double membrane vesicles (DMVs) derived from the ER and may serve to increase the local concentration of viral proteins and relevant host cell factors required for efficient viral RNA replication [33, 34]. Within the replicase complex, the plus-strand RNA genome is replicated into a minus-strand RNA intermediate, which then gives rise to multiple plus-stranded HCV RNA copies [35]. The importance of specific lipids in HCV RNA replication has been highlighted. Indeed, HCV infection induces synthesis of specific sphingolipids that enhance NS5B-mediated RNA replication [36].

The newly progeny plus-strand RNAs can either be used for translation, therefore production of new viral proteins, or synthesis of new minus-strand RNAs, or packaged into viral particles. It has been shown that HCV assembly initiates at the ER membrane in close proximity to lipid droplets where the viral RNA is packaged into capsids [37]. HCV proteins NS5A and core have been reported as key players in the translocation of viral structures from the replication complex to lipid droplets [38]. The nascent nucleocapsids bud into the ER thereby acquiring a ER-derived lipid bilayer envelope in which the viral glycoproteins E1 and E2 are anchored as heterodimers [39]. Interestingly, a peculiar feature of HCV is its association with host lipoproteins and apolipoproteins such as ApoE, ApoB, and ApoA1, leading to the formation of lipo-viroparticles (LVPs) [40, 41]. Incorporation of host lipoproteins into HCV virions plays an essential role in virus infectivity and immune escape [40]. Next, LVPs traffic through the Golgi secretory pathway for final egress [42]. Several key components of the endosomal transport system are necessary for the egress of HCV LVPs, including the endosomal-sorting complex required for transport (ESCRT) pathway and Rab proteins [43, 44]. Estimations showed that approximatively 1.3×10^{12} HCV virions are produced per day in each infected patient [45].

3. Impact of current antiviral therapies in the clinical outcome of hepatitis C

There are three critical points in the natural history of HCV infection including development of chronic hepatitis C, development of liver cirrhosis, and development of cirrhosis-related complications including portal hypertension and hepatocellular carcinoma (HCC) [46]. Chronic hepatitis C is a slowly progressive disease. It is estimated that 20–30% of chronic HCV patients develop liver cirrhosis over a 20 years period [46]. A deep inter-individual variability exists in the progression of hepatitis C and response to antiviral treatment, mainly related to viral factors such as HCV genotype, viral load, or coinfection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), and host factors such as patient's genetic background including interleukin-28B (IL-28B) polymorphism, age, gender, and obesity [47, 48].

The ultimate aim of antiviral treatment is to cure chronic HCV infection, in order to prevent the progression to liver cirrhosis and severe hepatic events (decompensation and HCC), and thereby improve patient survival and prevent

HCV transmission. Viral cure, known as sustained virological response (SVR) is defined as undetectable HCV RNA in blood 12–24 weeks after completing antiviral treatment [49, 50]. Current anti-HCV treatment consists on all-oral, IFN-free regimens combining highly potent, and well tolerated DAAs achieving SVR rates over 95% [11, 50]. DAAs specifically target HCV nonstructural proteins resulting in disruption of HCV replication (**Figure 1**). According to the therapeutic target and mechanism of action, DAAs are divided into four categories: NS3/4A protease inhibitors (e.g. simeprevir, paritaprevir, glecaprevir), NS5A protein inhibitors (e.g. velpatasvir, daclatasvir, pibrentasvir), NS5B nucleoside polymerase inhibitors and NS5B non-nucleoside polymerase inhibitors (e.g. sofosbuvir and dasabuvir, respectively) [11, 49, 50] (**Figure 1**). Combination of DAAs targeting different viral proteins regularly each of them with high potency and high genetic barrier, allows a high success of treatment regimens. Also, combination regimens comprising two drugs are preferred to triple combination regimens, to minimize the risk of side-effects and drug–drug interactions [11, 49, 50].

The introduction of DAAs has many positive impacts, through decreasing the incidence of severe hepatic complications and extra-hepatic diseases and reducing hepatitis C-related mortality [51, 52]. However, the high cost of DAAs still a barrier to access to therapy [12, 53]. According to recent estimations, the overall access to DAA is less than 10% of the HCV-infected patients on a global level [54]. Moreover, the potency of DAAs can be impaired by the emergence of specific amino acid substitutions designated resistance-associated substitutions (RASs). As an RNA virus, HCV easily develops a resistance to antiviral treatments due to its error-prone replication property and drug pressure. Risk of treatment failure is low in patients receiving 2 different categories of highly potent DAAs [13]. NS3/4A protease inhibitors are generally unaffected earlier by RASs, but many NS5A inhibitors continue to have overlapping resistance profiles. Furthermore, large studies have shown that a higher proportion of patients failed by an NS5A inhibitor-based regimen developed RASs than patients failed by NS3/4A protease inhibitor-based regimens [14, 55, 56]. The prevalence of RASs varied among HCV genotypes. HCV genotype 3 exhibits the

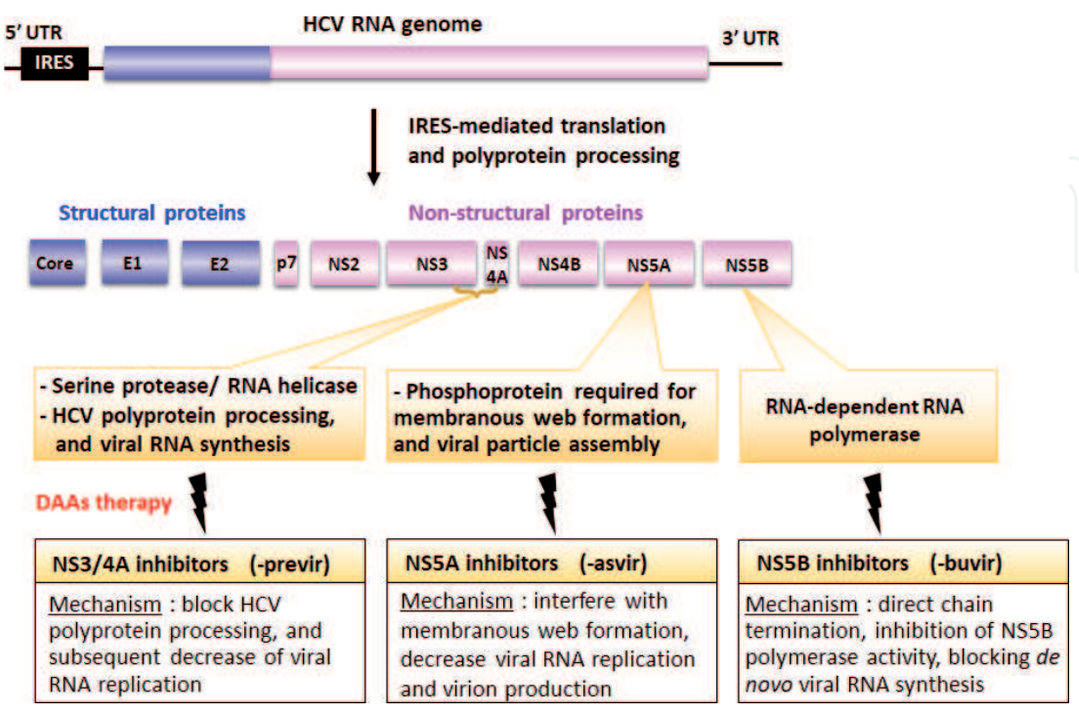

Figure 1.
HCV genomic RNA and encoded viral proteins; virological functions of targeted non-structural proteins for direct-acting antivirals (DAAs) therapy. UTR, untranslated region; IRES, internal ribosome entry site.

highest resistance to DAAs therapy, with lower SVR rates compared to other genotypes [57]. Moreover, the debate continues about DAAs treatment and development of HCC. Some studies have shown that DAAs treatment was not associated with an increase in the development of HCC [58, 59]. Other studies have shown conflicting results, indicating that DAAs therapy is associated with an increase in the recurrence of HCC in patients previously cured by liver transplantation [60]. Collectively, these findings indicate that surveillance for HCC should be continued especially for patients with advanced fibrosis and cirrhosis. Interestingly, the persisting risk for HCC development following SVR in patients treated with DAAs raises questions about the mechanisms that maintain HCC risk in these patients after viral cure.

4. Role of host-targeting antivirals in therapy of HCV infection

HCV exploits the host cell extensively to complete replication cycle and establish persistent infection. The unveiling of HCV-host cell interactions at both structural and functional levels has been investigated intensely, in relation with great progress in HCV cell culture systems and experimental animal models, and also advances in functional genomics screening, including genome-wide small interfering RNA (siRNA) screens and genome-scale CRISPR–Cas screens [61–63]. These tools paved the way for the identification of host-encoded factors involved in each step of HCV life cycle [63, 64]. Characterization of these factors, also known as host dependencies factors, provides not only critical insights into mechanisms of HCV pathogenesis, but also novel candidates for antiviral therapy.

To cure HCV infection, a therapeutic drug should combine a potent antiviral activity and a high genetic barrier to viral resistance. Unlike DAAs, which target viral proteins of high variability, most of host-targeting antivirals (HTAs) are expected to have a high genetic barrier to viral resistance since host factors are less prone to mutations [65]. In addition, HTAs are usually genotype-independent and thus exhibit a pan-genotypic antiviral activity. Nonetheless, a major concern in the usage of HTAs is their interference with physiological functions of targeted host factors, which may induce cellular toxicity and side effects mutations [65, 66]. In the following sections, we discuss recent advances in HTAs against HCV that have potential as new therapeutic options and are in preclinical/clinical development. We also discuss their potential to overcome the current challenges of anti-HCV treatment. **Table 1** summarizes the different HTAs evaluated against HCV with their targeted host-cell factors and current development phase.

4.1 HTAs involved in HCV entry

HCV entry is the first step of virus–host cell interactions required for spread and maintenance of infection. HCV enters the hepatocyte through a highly orchestrated process involving HCV envelope glycoproteins E1 and E2 and four main host-cell receptors: the scavenger receptor class B type I (SR-BI), the human cluster of differentiation 81 (CD81) and the tight-junction proteins Claudin-1 (CLDN-1) and Occludin (OCLN) [67–69]. A genome-scale CRISPR/Cas9 knockout screening in human cells demonstrated that cellular receptors CD81, CLDN1, and OCLN are particularly critical for HCV infection *in vitro*, and thus determine the tropism of HCV for human cells [63]. HTAs targeting HCV entry offer the advantage of blocking HCV life cycle before the beginning of viral genome translation and replication, which might block cell–cell transmission, virus spread, and thus persistent infection [70]. Interestingly, because viral entry plays an important role during HCV re-infection of the graft in end-stage patients undergoing liver transplantation,

Host factor	Host-targeting antiviral	HCV life cycle step	Phase of development	References
Scavenger receptor BI (SR-BI)	ITX-5061	Entry	Phase 2	[73, 74]
Human cluster of differentiation 81 (CD81)	Anti-CD81 mAbs	Entry	Preclinical	[75]
Claudin-1 (CLDN1)	Anti-CLDN1 mAbs (OM-7D3-B3, H3L3)	Entry	Preclinical	[76–79]
Occludin (OCLN)	Anti-OCLN mAbs (Xi 1-3, Xi 37-5)	Entry	Preclinical	[80]
Epidermal growth factor receptor (EGFR)	Erlotinib	Entry	Preclinical	[82]
Nieman-Pick C1-Like 1 (NPC1L1)	Ezetimibe	Entry	Phase 1	[81, 83]
Cyclophilin A	- Alisporivir	RNA replication	Phase 3 (halted)	[87, 90–93]
	- NIM811	RNA replication	Phase 2	[88]
	- SCY-635	RNA replication	Phase 2a	[89, 94]
micoRNA-122	-Miravirsen/ SPC3649	RNA replication	Phase 2a	[102–105]
	-RG-101	RNA replication	Phase 1b	[106–108]
Diglyceride acyltransferase I (DGAT-I)	-siRNA	Assembly	Preclinical	[109]
	-Quercetin	Assembly	Phase 1	[110, 111]
	-LCQ908/Pradigastat	Assembly	Phase 2	[112]
Acyl coenzyme A:cholesterol acyltransferase (ACAT)	Avasimibe	Assembly/Egress	Preclinical	[114, 115]
Adaptor-associated kinase 1 (AAK1)	Sunitinib	Assembly/Egress	Preclinical	[116]
Cyclin-associated kinase (GAK)	-Erlotinib	Assembly/Egress	Preclinical	[117]
	-Isothiazolo [5,4-b] pyridine	Assembly/Egress	Preclinical	[118]

Table 1.
Host-targeting antivirals against HCV with their targeted host-cell factors and current phase of clinical development.

entry inhibitors represent an interesting strategy to prevent graft reinfection [71]. Numerous compounds have been evaluated, the most advanced was ITX-5061, an antagonist of SR-BI that reduces SR-BI-mediated HDL lipid transfer [72]. SR-BI binds HDL and delivers lipids into the cell membrane. The lipid transfer activities of SR-BI play an important role in HCV entry, thus reducing cholesterol transfer into the cell membrane may be one possible mechanism by which ITX-5061 reduces HCV entry [72]. An *in vitro* study indicated that ITX-5061 functions synergistically with the protease inhibitor telaprevir, and no cross-resistance is expected between ITX-5061 and HCV polymerase or protease inhibitors [73]. ITX-5061 completed a clinical

phase 1b study. Oral ITX-5061 was safe and well tolerated over 28 days of dosing in noncirrhotic adults with chronic HCV infection [74]. This compound is undergoing phase II clinical trials in HCV-positive patients and appears to be a promising option for treatment [74]. Antibodies targeting CD81 have also been investigated and demonstrated potent antiviral effects in preclinical mouse studies [75]. In the case of CLDN1-targeting inhibitors, a rodent anti-CLDN1 mAb (OM-7D3-B3) demonstrated antiviral potential against HCV infection in primary human hepatocytes (PHHs) and human liver-chimeric mice [76, 77]. Towards a clinical development, Colpitts and colleagues [78] successfully humanized anti-CLDN1 mAb (OM-7D3-B3) into human IgG4 isotype, designed as H3L3. This antibody exhibits pan-genotypic activity against HCV entry without viral escape both *in vitro* and in mouse model [78]. Furthermore, H3L3 demonstrated a synergy with DAAs sofosbuvir and daclatasvir. Such synergy could allow shortening of treatment duration, thus reducing costs and side effects [79]. OCLN may also be considered as a potential target. To date, two successful human-rat chimeric mAbs have been developed against OCLN, and completely inhibit HCV infection *in vitro* and in human liver-chimeric mice without side effects [80]. Other inhibitors of kinases and host-cell pathways involved in HCV entry have been evaluated *in vitro* and significant results have been obtained in mouse models including Nieman-Pick C1-Like 1 (NPC1L1) and epidermal growth factor receptor (EGFR) [81, 82]. The clinically approved EGFR inhibitor Erlotinib, that prevents the formation of CLDN1-CD81 complex, and NPC1L1 inhibitor Ezetimibe, that decreases systemic cholesterol in patients, markedly impaired the establishment of HCV infection in the uPA-SCID mouse model [81, 82]. However, in a phase I clinical trial that enrolled, Ezetimibe elicits only minor effects on HCV viral loads in patients undergoing liver transplantation [83].

4.2 HTAs involved in HCV RNA replication

Targeting HCV RNA replication is a promising approach to eradicate HCV from infected liver cells in patients. The most advanced HTAs against HCV RNA replication are the inhibitors of Cyclophilin A (CypA) and antagomirs of microRNA-122 (miR-122). Cyclophilin A (CypA) is an essential proviral factor for HCV replication, and interacts with HCV NS5A protein to initiate the formation of the replicase complex, and thereby promote viral RNA replication [84, 85]. Specific inhibition of CypA by cyclosporine A destroys the CypA/NS5A complex and suppresses HCV RNA replication *in vitro* [86]. Because cyclosporine A exhibits both antiviral and immunosuppressive activities, non-immunosuppressive antiviral derivatives of cyclosporine A were developed, including Alisporivir/Debio-025, N-methyl-4-isoleucine-cyclosporin (NIM811), and SCY-635 [87–89]. The comparison of CsA-resistant mutants for resistance to Alisporivir and NIM811 demonstrated that Alisporivir has the highest resistant activity against the adaptive mutations [90]. The antiviral effect of Alisporivir on HCV genotypes 1a or 1b has been confirmed in chimeric mice with human hepatocytes [87]. Alisporivir is the most advanced in clinical development. In a phase II clinical trial, administration of Alisporivir in combination with pegIFN-α/RBV to treatment-naïve HCV genotype 1 patients, resulted in SVR rates of 69–76% compared to 55% in the group receiving only pegIFN-α/RBV [91]. Recently, Alisporivir was explored as an interferon-free combination regimen with DAAs in HCV genotype 2 and 3 infected patients, resulting into SVR rates of 80–85% [92]. However, the development of Debio-025 was halted following the report of seven cases of acute pancreatitis [93]. For CypA inhibitor SCY-635, a clinical phase 2a study demonstrated that SCY-635 reduces HCV viral load and increases plasma levels of type I and III IFNs and IFN-stimulated genes, thereby contributes to the activation of innate antiviral immunity [94].

Another important host factor is the liver-specific microRNA-122 (miR-122). microRNAs are small (~22 nucleotides) endogenous noncoding RNAs, which bind to the 3′-untranslated region of the messenger RNAs (mRNAs), resulting in gene silencing through mRNA degradation or translational repression [95]. The replication of HCV in hepatocytes has been shown to be critically dependent on miR-122, as the sequestration of miR-122 in liver cells results in marked loss of replicating HCV RNAs [96]. Several mechanisms by which miR-122 promotes HCV replication have been reported. miR-122 promotes HCV genome replication by direct binding with two adjacent sites in the 5'-UTR of the HCV RNA [97]. miR-122 protects HCV RNA genome from degradation by host 5′-3′ exonucleases Xrn1 and Xrn2, and phosphatases DOM3Z and DUSP11 [98–101]. Therapeutic approaches based on inhibition of miR-122 using modified anti-sense oligonucleotides have been generated. Miravirsen/SPC3649 is a locked nucleic acid-modified DNA antisense oligonucleotide that sequesters mature miR-122 in a highly stable heteroduplex, thereby inhibiting its function. Miravirsen demonstrated antiviral activity against all HCV genotypes *in vitro* [102]. In a phase 2a study, the safety and efficacy of miravirsen were evaluated in 36 patients with chronic HCV genotype 1 infection. The results showed a prolonged and dose-dependent decrease in HCV RNA levels without evidence of viral resistance and serious adverse effects [103]. Miravirsen treatment results in a prolonged reduction in cholesterol levels in line with the effects of miR-122 on cholesterol metabolism [103, 104]. In addition, Miravirsen demonstrated a potent antiviral activity when tested against DAA-resistant HCV variants [105]. Another antimir-122 molecule is RG-101, a hepatocyte targeted N-acetylgalactosamine conjugated oligonucleotide that antagonizes miR-122 [106]. Van der Ree and colleagues [106] performed a phase 1B study that assessed the safety, tolerability and antiviral effect of RG-101 in 32 patients chronically infected with HCV genotypes 1, 3, or 4. The results showed that RG-101 was well tolerated and resulted in substantial decrease in viral load in all treated patients within 4 weeks, and sustained virological response in 3 patients at week 76 of follow-up. However, viral rebound between weeks 5 and weeks 12 was observed in six patients with HCV genotype 1 [106]. Another phase 1B study assessed the effects of dosing RG-101 on antiviral immunity in chronic HCV patients and showed that a single dose of RG-101 led to a decrease in HCV RNA levels in all patients and SVR >76 weeks in 3 patients [107]. The combination of a highly potent DAAs with miravirsen or RG-101 could potentially shorten HCV treatment duration. Recently, two clinical studies were performed to evaluate the potential of combining a NS5B inhibitor (GSK2878175) and RG-101 as a single- visit curative regimen for chronic hepatitis C [108]. GSK2878175 molecule demonstrated acceptable safety, tolerability and pan-genotypic antiviral activity, especially for HCV genotype 3 that is considered difficult to treat. The results showed that daily oral administration of GSK2878175 with a single dose of RG- 101 results in high cure rates if the treatment duration is >9 weeks in noncirrhotic, treatment-naïve patients with HCV genotype 1 and 3 infections [108]. Altogether, these findings highlight the clinical potential of miR-122 inhibitors as complementary therapeutic strategy that especially may be valuable for difficult-to-cure patients with current DAAs.

4.3 HTAs involved in HCV assembly and egress

There is a close relationship between HCV particle biogenesis and host-cell lipid metabolism. HCV circulates as lipo-viralparticles (LVPs) in the blood of infected patients, thus targeting the host-cell factors involved in the lipid metabolism may provide potential therapeutic options. An essential cellular enzyme involved in this process is the diglyceride acyltransferase I (DGAT-I) which directly interacts with the HCV core protein, and is required for the trafficking of core to lipid droplets [109]. Inhibition of DGAT1 activity or RNAi-mediated knockdown of DGAT1

severely impairs infectious viral particles production *in vitro*, implicating DGAT-1 as a new target for antiviral therapy [109]. Interestingly, quercetin, a natural flavonoid that inhibits DGAT-I, was reported to have anti-HCV properties [110]. In a phase I study, quercetin exhibited high safety and potent antiviral activity in patients with chronic HCV infection [111]. Moreover, the antiviral efficacy of the DGAT-I inhibitor LCQ908/pradigastat was assessed in phase II clinical trials in patients with HCV infection, but no significant decrease in HCV viral load was observed in treated patients [112]. Further studies are needed to determine whether the DGAT-I inhibitor could be used in combination with DAAs.

Apolipoproteins (e.g. ApoE, ApoB, and ApoA1) are essential to the formation of infectious HCV particles during viral assembly, and highly infectious HCV particles are usually associated with more lipoproteins [40, 113]. Mechanistic studies demonstrated that Avasimibe, an inhibitor of acyl coenzyme A:cholesterol acyltransferase (ACAT), induced downregulation of microsomal triglyceride transfer protein expression, resulting in reduced ApoE and ApoB secretion [114]. Avasimibe significantly impairs the assembly of infectious HCV virions and exhibits significant pan-genotypic antiviral activity and great potential for combination therapy with DAAs [115]. Furthermore, the adaptor-associated kinase 1 (AAK1) and the cyclin-associated kinase (GAK) are known to regulate core-AP2M1 interaction [116]. Accordingly, Neveu and colleagues showed that AAK1 and GAK inhibitors, including the approved anti-cancer drugs sunitinib and erlotinib, can block HCV assembly [116, 117]. However, these compounds could induce adverse effects due to their lack of specificity. To overcome this limitation, a specific GAK inhibitor, isothiazolo [5,4-b]pyridine was developed [118]. This drug efficiently impairs HCV entry and assembly *in vitro* with limited off-target effects [118].

5. Conclusion and prospects

The great advances in hepatitis C treatment through the development of highly potent DAAs define the intense efforts towards a global eradication of HCV infection. However, most infected people live in low resource countries, which may limit access to treatment and restrain the impact of DAAs on the global burden of HCV infection and associated diseases. Another principal challenge is viral resistance, subsequent treatment failure and emergence of DAA-resistant variants. HTAs against host-cell factors required for HCV pathogenesis are promising candidates for development as alternative or complementary therapeutic options. Intense research on HTAs is needed to develop highly effective drugs with the least side effects. Several HTAs are at different stages of preclinical and clinical development, which promise for enlarged therapeutic arsenal against chronic HCV infection in the future.

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Conflict of interest

The authors declare no conflict of interest.

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