



Current therapeutics against HCV

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Abstract Hepatitis C is a positive stranded enveloped RNA virus belonging to the *Flaviviridae* family. HCV infection leads to severe liver diseases, cirrhosis and hepatocellular carcinoma worldwide. Although treatments have been available for a while, due to its complexity and genetic diversity, only few are reported to be effective against all HCV genotypes. Here, we review the HCV life cycle and its immunogenic potential and various mechanisms via which the virus interferes in the signalling process. A comprehensive overview of current anti-HCV therapeutics, such as, Direct Acting Antiviral (DAA) as well as Host Targeting Agents (HTA), along with their scope, known mechanism of action and limitations are presented.

Keywords HCV · Therapeutics · DAA · HTAs

Introduction

Hepatitis C is a hepatotropic, enveloped, single stranded, positive sense RNA virus belonging to *Flaviviridae* family under the species Hepacivirus. More than 71 million people are infected worldwide and nearly 400,000 people die every year from this viral infection, mostly due to HCV related cirrhosis and hepatocellular carcinoma (HCC). In

India, prevalence of hepatic disease by this virus is 1–1.9% [48]. Viral infection induced liver associated disease symptoms include cirrhosis, steatosis, chronic hepatitis, and hepatocellular carcinoma. Approximately 70% infected patients ultimately develop severe infections and end-stage patients require liver transplantation.

There are eight identified genotypes of HCV [13] with more than 50 subtypes and millions of quasispecies. Globally HCV-1 is the most prevalent genotype (49.1%) followed by HCV-3 (17.9%), HCV-4 (16.8%) and HCV-2 (11%). HCV-5 and HCV-6 together comprise less than 5% of the total infected individuals [42]. HCV genotypes 1, 2 and 3 have wide global distribution but the others such as 4,5,6 and 7 vary from one geographical area to another [42].

As the viral RNA dependent RNA polymerase (RdRp, the polymerase responsible for viral genome synthesis) does not have proof-reading activity, millions of viral quasi species have evolved [56]. This high level of plasticity is due to mutations that continuously allow the virus to adapt to variable environmental conditions. This also leads to escape of the host's immune responses resulting in the viral persistence [56]. Such diversity and complexity of the virus has been the major challenge in therapeutic and vaccine development.

Therapeutics are usually designed to develop more specific compounds which either target the virus or key host cell factors involved in viral pathogenesis. Hence antivirals can be classified into two broad classes: (1) direct acting antivirals (DAA) designed to prevent viral replication by targeting key viral proteins involved in viral life cycle; and (2) host-targeting agents (HTAs) that inhibit cellular factors and receptors involved in viral infection [61]. Unlike anti-HCV DAAs, which inhibit viral proteins that rapidly mutate, the HTAs due to their conserved

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targets are important for avoiding viral escape. However they are associated with high risk of cellular toxicity as they are directed towards a cellular pathway/ protein [16]. In this review, we aim to summarize the current knowledge on viral life cycle and analyse all the predominant HCV therapeutics that target different steps of viral biology.

HCV life cycle

A discussion on the therapeutics is incomplete without an understanding of the life cycle of HCV, a multistep process which includes attachment, entry, endocytosis, uncoating of genetic material and replication, all targets of antiviral therapy. At the initial stage of infection, upon entering the blood circulation, HCV interacts with several lipid molecules, such as, LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein) to become LVPs (lipoviral particles) (Supplementary Fig. 1). Within the host, liver cells are the main target of HCV as it expresses all the entry receptors for HCV infection. The entry steps are all detailed in Fig. 1. Viral and host membrane fusion leads to the viral genome release into the cytosol which results in the initiation of viral replication and translation. The viral RNA produces a single polyprotein of 10 different gene products. Starting from N' terminal end, the proteins are—Core-EI-E2-p7- NS2-NS3-NS4A-NS5A-NS5B-C terminal end. The capsid producing core and the glycoproteins, E1 and E2 are the only structural components, rest being non-structural. Like any other glycoproteins, post-translational modifications (such as N-glycosylation) of E1 and E2, E1E2 heterodimer formation take place in the ER membrane itself. NS2 is a cysteine auto-protease and possibly cleaves and releases itself [14]. NS3 mediates cleavage of NS4A and associates with NS4A to form a NS3/4A serine protease complex. NS3/4A protease complex cleaves NS4B/5A and NS5A/5B junctions to yield the mature proteins [14]. NS4B helps in the production of ER membranous webs, made up of several lipid molecules, essential for the replication process of the virus. NS5A is involved in replication, assembly and release, NS5B is the RdRp which replicates the viral genome [14]. HCV replication is also dependent on microRNA122 (miR-122) [26], a liver-specific micro-RNA. miR-122 molecules form an oligomeric complex at the 5' proximal end of the HCV RNA genome, thereby enhancing viral RNA abundance by stabilizing the viral genome [26]. The miR-122 is believed to protect the HCV RNA genome from degradation by the 5'-3' exo-ribonuclease Xrn2 [26].

Details of the assembly and egress steps of HCV are depicted in Fig. 1. Nucleocapsid of the HCV particle is formed by the core protein which after cleavage and release by the signal peptidase interacts with the genome and the

cytosolic lipid droplets (LDs) [7]. The interaction of core protein and LDs is important for the recruitment of other viral components in virion assembly [7]. Core has two domains: a D1 domain which interacts with the viral RNA during nucleocapsid formation and a D2 domain which binds endoplasmic reticulum (ER) outer leaflet [7]. E1 and E2 and other non-structural proteins also co-localize at close proximity of LDs and core complex. Viral RNA is also found at the proximity of LDs. During virus production core protein resident on lipid droplets recruits viral proteins and RNA, which is an essential prerequisite for virus production.

HCV virion biogenesis is closely related to very low density lipoprotein (VLDL) assembly pathway. In addition, host factors involved in the biosynthesis and secretion of human lipoproteins have emerged to be essential cofactors for virus production. Specifically, apolipoprotein B (ApoB), apolipoprotein E (ApoE) and microsomal triglyceride transfer protein (MTP) were shown to contribute to virus production. Inhibitors of the microsomal triglyceride transfer protein (MTP), a protein involved in VLDL biogenesis, block the production of viral particles. Long chain acyl-CoA synthetase 3 (ACSL3) [58], an enzyme involved in VLDL assembly and hepatocyte nuclear factor 4a (HNF4a) [11], a transcription factor that regulates the VLDL secretory pathway are known to regulate the production of infectious HCV particles [58]. Nascent viral particles enter the ER lumen and bind with pre-VLDLs. The virion then combines with the luminal lipid droplets and Apo E to produce LVPs. Infectious HCV are hence a “lipo-viro particle” rich in cholesteryl esters comprising of ApoB and ApoE (Fig. 1). Consequently apolipoproteins such as apoE, apoB, apoA1, apoC1, apoC2, and apoC3 are part of the circulating HCV particles [47] and the lipid composition of viral particles resembles that of VLDL and LDL with cholesteryl esters accounting for almost half of the total HCV lipids.

HCV particles are released from cells by transit via the secretory pathway [17] Fig. 1. During this process, HCV virions acquire their characteristic low buoyant density [17]. Furthermore, glycans associated with the viral envelope glycoproteins are modified. Finally, during egress, HCV particles presumably depend on p7 to neutralize acidic compartments within the secretory pathway.

Pathophysiology of HCV infection

In response to the presence of HCV, both the innate and adaptive immune responses are activated. Details of the innate signalling are depicted in Fig. 2 [21]. In response to activation by NFκB and JNK pathway, interferons are secreted which aim to create an antiviral state. Type I

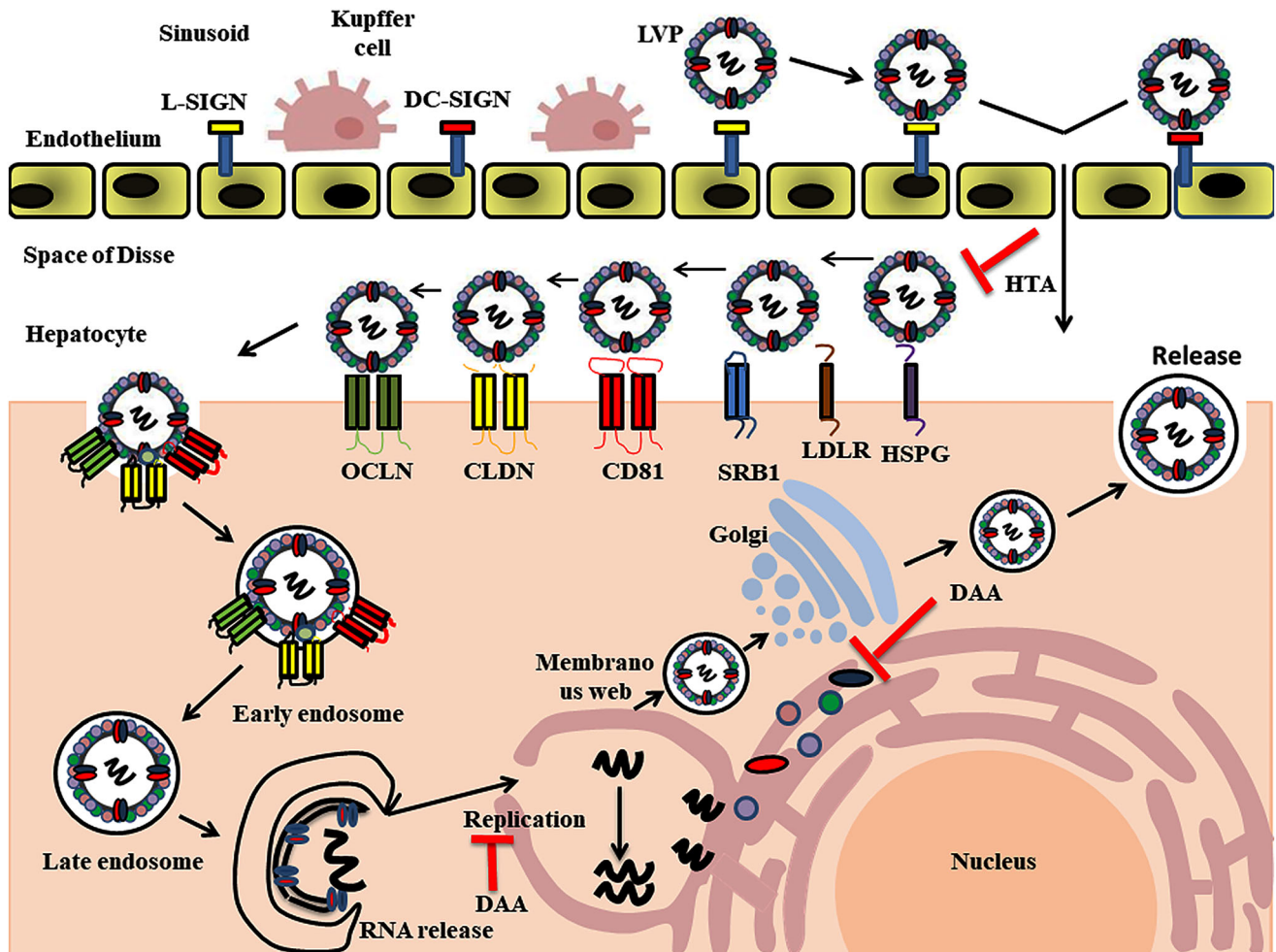


Fig. 1 HCV entry and life cycle. Circulating lipoviroparticles enter hepatocytes in a multi-step process involving multiple interactions as depicted. DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) and L-SIGN (liver/ lymph node-specific intercellular adhesion molecule-3-grabbing integrin) have been shown to bind with HCV envelope glycoprotein E2 and deliver the virus to the liver. Circulating LVPs enter the liver via sinusoidal blood pass through the space of Disse, and interact with the receptors on the hepatocyte surface. LVPs near to the surface of hepatocytes are initially captured by HSPG which enables its interaction with LDLR. Other entry receptors include SRB1 (scavenger receptor class B type 1), CD81, the TJ (tight junction) proteins such as occludin (OCLN) and claudin-1 (CLDN1), EGFR (epidermal growth factor receptor), iron receptor protein TfR1 (transferrin receptor 1), RTKs (receptor tyrosine kinases), EphA2 (ephrin receptor A2) and NPC1L1 (Niemann-Pick C1-like 1 cholesterol uptake receptor). The initial attachment of LVPs is with SRB1 which rearranges lipoprotein on HCV particles and exposes the hidden E1E2 epitopes, which enable E1E2 binding to other receptors. Following attachment with CD81, CLDN1 and OCLN join to form a complex and the whole is internalized via clathrin and dynamin mediated endocytosis. Viral and host membrane fusion leads to the viral genome release into the cytosol which results in the initiation of viral replication and

translation. Viral envelope proteins E1 and E2 play the key role in the membrane fusion where E1 acts as a chaperone. A fusion pore is formed due to the conformational changes in the glycoproteins and the viral genome is released in the cytoplasm. The acidic pH along with the optimum temperature within the endosomal compartment triggers this process of penetration of host cell membrane and uncoating. Host membrane protein NPC1L1 modulates and rearranges the lipid composition in the membrane which leads to membrane fusion. After entry, the virus undergoes replication within a membranous web adjacent to the ER. Viral replication machinery consists of NS3/4A, NS4B, NS5A, and NS5B. A negative strand intermediate is synthesized by NS5B using the RNA genome as template. A microenvironment is created in the cytoplasm by NS4B which involves massive rearrangements of intracellular membranes to form a 'membranous web', where viral replication takes place. Upon translation, the HCV proteins are associated with membranes derived from the endoplasmic reticulum (ER). Nascent RNA genomes are translated to produce new viral proteins and also serve as new/additional RNA templates for further RNA replication which are progressively assembled to form infectious virions. Thereafter the virus assembles at the ER surface and egresses via the secretory pathway. Red stop dash arrows indicate points of intervention by DAAs

interferons (IFN- α , IFN- β and IFN- ω), secreted by a wide variety of immune cells, bind to their specific receptors and activate a subset of genes broadly called interferon-

sensitive genes (ISGs). ISG may include PKR which induces shutdown of mRNA translation and thereby may induce apoptosis. Another ISG includes OAS (2', 5'-

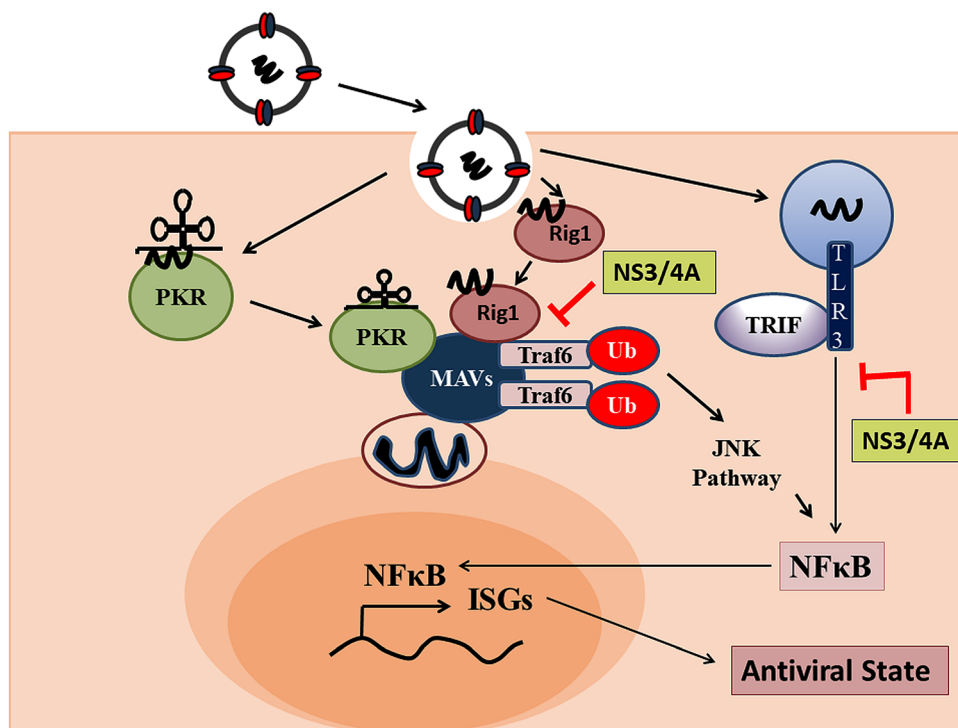


Fig. 2 Innate immune response to HCV infection and sites of intervention. Innate immune response to trigger interferon signalling upon HCV infection is depicted. Presence of viral RNA activates PRR (pattern recognition receptors) on the cell surface, cytoplasm, and endosomes and help to initiate an immunogenic response via PAMP (pathogen-associated molecular pattern). IRES (internal ribosome entry site) of HCV is recognized by RNA-dependent PKR (protein kinase R) whereas RIG-I (retinoic acid inducible gene I), another PRR recognizes unique features of sequences present in 3' and 5' region of HCV RNA. After viral recognition by RIG-I and PKR, longer HCV dsRNA intermediates activate endosomal TLR 3 (toll-like-receptor 3). Upon activation, PKR and RIG-I bind to MAVS (Mitochondrial antiviral signalling protein, also called VISA, IPS-1 and CARDIF) which via ubiquitinylation of Traf6 ultimately results in upregulation of interferon synthesis via the JNK pathway and activation of NFκB. Simultaneously, TLR3 signals are transmitted via the adapter molecule IFN-β/TRIF (Toll/IL-1 receptor domain-containing adapter inducing interferon-β). ds RNA activated protein kinase R (PKR)

plays a major role in host antiviral defence, by phosphorylating and thereby inhibiting eIF2α (eukaryotic elongation initiation factor 2α subunit). Inhibition of eIF2α decreases cellular mRNA translation which may ultimately culminate in induction of apoptosis. HCV can however escape via induced phosphorylation of protein kinase R thereby inactivating it and is one of the probable reason for HCV persistence. Either way, viral protein synthesis is not affected by PKR induced stall in mRNA translation due to an IRES (internal ribosome entry site) dependent manner. HCV can also escape immune response by NS3-4A serine protease complex mediated cleavage of MAVS and TRIF. This blocks the production of IFN-β in infected liver cells. Circulating dendritic cells and macrophages are also activated to produce an interferon response upon activation by PAMPs in the hepatocytes. Ultimately in response to activation by NFκB and JNK pathway, interferons are secreted which aim to create an antiviral state. Red stop dash arrows indicate points of intervention by viral proteins to stop the onset of immune response

oligoadenylate synthase) which help to cleave viral and host ssRNA. Type II interferons (IFN-γ) secreted by T-lymphocytes, macrophages and dendritic cells helps to clear acute and chronic infection by HCV. Type III interferons (IFN-λ) bind to receptors on hepatocytes and activate many ISG similar to type I interferons. In liver biopsy of HCV-infected patients IFN-β is observed to be expressed in liver macrophages and Kupffer cells [28]. IFN-λ has also been detected at high concentration in HCV infected chimpanzees and patients with chronic HCV infection. Like other interferons, IFN-λ can be produced by non-parenchymal cells and which leads to the escape from HCV induced attenuation of type I IFN production in HCV infected patients. However, HCV can persist in presence of

the immune response by inhibiting interferon stimulated genes (ISG) (Fig. 2).

Therapeutics against HCV

Since 1990 use of interferon α (INF-α) monotherapy has been administered against HCV [32]. As mentioned previously, IFNs are cellular immune proteins that are secreted in response to pathogens and are the most significant antiviral response in our body. Administration of recombinant interferons help to exacerbate their effects which although classified as creation of antiviral state, specifically remains uncharacterised. The antiviral state however includes

inhibition of cell growth and induction of apoptosis of the infected cell and sometimes also the adjacent uninfected cells. In addition, IFN- α also interacts with both the adaptive and innate immune response of the host, promotes T-helper cell differentiation leading to increased production of interleukin (IL)-2 and IFN- γ . The response to IFN treatment is determined by measuring sustained viral response (SVR), where SVR is defined as no trace of virus after 24 weeks of completion of any antiviral therapy [41].

The effectiveness of IFN is significantly enhanced by the addition of RBV which results in higher sustained virological response (SVR) rate. Ribavirin, a guanosine analogue has been a potent antiviral agent since the late 1980s (Table 1). Once absorbed, RBV undergoes phosphorylation to form Ribavirin monophosphate (RMP) and further into Ribavirin triphosphate (RTP) [36]. Broadly, there are four mechanisms by which ribavirin acts as an antiviral agent. First, RTP is incorporated into the replicating genome which when acts as template to help incorporation of either a CTP or a UTP with equal efficiency and thus induces mutations that can extend towards catastrophe for the virus. Secondly, RTP can also bind to the nucleotide binding region of the RdRp thus preventing incorporation of the correct nucleotide. Thirdly, RMP acts an inhibitor for the enzyme inosine monophosphate dehydrogenase (IMPDH) which is a rate limiting enzyme for guanosine production. Finally, although incorporation of RTP occurs at a lower rate compared to the natural nucleotide, reduction of GTP pool causes an increased probability of RTP incorporation, thus enhancing the mutagenic effect of RBV. In essence ribavirin is responsible for defective viral particle production. Furthermore, RBV has also been shown to exhibit immunomodulatory effects where it enhances Th1 (T helper) phenotype from Th2 [46]. Most of the actions of Ribavirin has been studied in cell culture, in vitro and animal models but in humans, monotherapy isn't significantly effective against HCV although many physiological parameters of the liver do improve. However simultaneous administration of RBV with interferon vastly exacerbates the effects of each other.

Pegylated IFN (PEG-IFN- α) used in place of IFN- α along with RBV increases the rate of SVR up to about 40–50% in case of genotype-1 and approximately 80% in genotype-2, 3. For patients, infected with genotypes 4, 5 and 6, SVR is not well documented due to lack of sufficient clinical trials. A combined application of interferon, with PEG, increases its half-life and decreases the rate of drug clearance while maintaining cellular activity [44].

As interferon targets the HCV virus by activating the immune system, which has a broad spectrum of activities, IFN treatment comes with many side effects which lead to poor patient compliance to antiviral therapy. Hence recent

advances in therapeutics are aimed towards INF free, shorter duration regimen with minimum side effects.

Direct acting antivirals (DAAs)

Direct Acting Antivirals are the class of drugs that have been designed against an essential pathway/ process that is unique to the viral life cycle. Since Ribavirin, advancement of research has brought many other drugs into the market. Broadly, there are those that act as NS3/4 protease inhibitors, those that act as the protease NS5a inhibitors and those that act as the NS5B polymerase inhibitors. To inhibit HCV RdRp, two approaches have been pursued for antiviral therapy. A) Non-nucleoside inhibitor (NNI) [10] and B) Nucleos(t)ide inhibitors (NI) [4]. Non-nucleoside inhibitors (NNI) binds with RdRp allosterically, away from the active site and affects enzyme–substrate binding [10]. Unlike NNIs, Nucleotide Inhibitors (NIs) compete with the incoming nucleotide triphosphate for binding and incorporation to the growing polypeptide chain.

IFN dependant therapeutics

According to consensus guidelines from CASL (“Canadian Association for the Study of the Liver, 2015”) and AASLD (“American Association for the Study of Liver Diseases, 2017”), RBV is recommended for all HCV genotypes. As Ribavirin monotherapy does not give an efficient SVR, it is always used to treat HCV infections as a part of combination therapy. In various first and second-line combination therapies, ribavirin is typically used as an adjunct therapy. First line therapy refers to the initial or primary treatment for a particular disease whereas second-line treatment is prescribed for whom the first one does not work adequately.

Since the time of Ribavirin and PEG-IFN, many DAAs and HTAs have been developed. Details of current drugs that have been approved by FDA along with the reported side effects are listed in Table 1. Many drugs that are not approved as monotherapy by FDA but are often administered as combination with other drugs are also enlisted in Table 1.

As it is evident from the Table 1 that all the drugs need to be administered either as a combination with each other or with ribavirin. All of these drugs except TECHNIVIE and DAKLINZA are effective against Genotype 1, the best studied and understood genotype. Genotype 4 follows genotype 1 in the number of therapeutics available. Options include drugs against the NS3/4A protease, NS5A protease as well as NS5B polymerase. Of these drugs, SOF, VEL and hence their combination as well the combination of Glecapravir and Pibrentasvir (MAVYRET) are reported to

Table 1 Common Therapeutics against HCV-DAA's

Type of therapeutic (DAA)	Brand name	Company	Composition	Active against genotype	Function	Adverse effect	IFN dependency (IFN dependent or not)	FDA approval status
<i>FDA approved DAAs</i>								
Nucleoside Analog Antiviral ³	RIBAVIRIN	Schering-Plough ¹	C ₈ H ₁₂ N ₄ O ₅ ²	Genotype 1, 2, 3, 4, 5, 6 ³	Metabolised into nucleoside analogs that blocks viral RNA synthesis and viral mRNA capping ³	Anaemia, Fatigue, nausea, rash, itching ¹	Both ¹	Approved July 2001 ¹
NS3/4A inhibitors:	VICTRELIS	Merk ⁴	Boceprevir (or) BOC ⁴	Genotype 1 ⁴	acts by binding to the active site of NS3 serine proteases ⁴	Fatigue, anaemia, nausea, headache, dysgeusia ⁴	IFN Dependent ⁴	Approved May 2011 ⁴
	INCIVEK	Vertex Pharmaceuticals ⁵	Telaprevir (or) TVR ⁵	Genotype 1 ⁵	Inhibiting protease NS3/4A ⁵	Pruritus, anaemia, nausea, hemorrhoids, diarrhea, anorectal discomfort, dysgeusia, fatigue, vomiting, anal pruritus ⁵	IFN Dependent ⁵	Approved May 2011 ⁵
	OLYSIO	Janssen Therapeutics ⁶	Simeprevir	Genotype 1	Inhibiting protease NS3/4A	Rash, nausea, muscle pain and increased bilirubin ⁶	IFN Independent	Approved November of 2013 ⁶
NS5A inhibitor	DAKLINZA	Bristol-Myers Squibb ⁷	Daclatasvir	Genotype1 and 3 ⁷	Inhibiting protease NS5A	Fatigue, headache, nausea and diarrhea ⁷	IFN Independent	Approved July 2015 ⁷
NS5B inhibitor:	SOVALDI	Gilead ⁸	Sofosbuvir (or) SOF ⁶	Genotypes 1-4 ⁸	Uridine nucleotide analog replication inhibitor ⁸	Fatigue, headache, nausea, insomnia, anemia ⁸	Both ⁸	Approved December of 2013 ⁸
Combination drugs:	OLYSIO (with or without ribavirin)	Janssen Therapeutics ⁶	Simeprevir + Sofosbuvir ⁶	Genotype 1 ⁶	NS3/4A protease inhibitor ⁶	Rash, nausea, muscle pain and increased bilirubin ⁶	Both ⁶	Approved November of 2013 ⁶
	OLYSIO + RIBAVIRIN	Janssen Therapeutics ⁶	Simeprevir	Genotype 1/4	NS3/4A protease inhibitor	Rash, nausea, muscle pain and increased bilirubin ⁶	INF dependent	Approved November of 2013 ⁶
	HARYONI	Gilead ⁹	Combination of ledipasvir and sofosbuvir. (ledipasvir requires low gastric pH for absorption, it should be administered very carefully with acid suppression therapies) ⁸	Genotype 1, 3, 4, 5 and 6 ⁹	ledipasvir-NS5A inhibitor sofosbuvir-nucleotide analogue HCV polymerase inhibitor ⁹	Fatigue, headache ⁹	Both ⁹	Approved October 2014 ⁹
	VIKERA PAK	Abbvie ¹⁰	Ritonavir-boosted Paritaprevir, Ombitasvir, and Dasabuvir (PTOD) ¹⁰	Genotype 1 ¹⁰	Ombitasvir-NS5A inhibitor, Paritaprevir-inhibits NS3/4A serine protease and Ritonavir-increases the bioavailability of Paritaprevir Dasabuvir: non-nucleoside NS5B palm polymerase inhibitor ¹⁰	Fatigue, nausea, pruritus, other skin reactions, insomnia, asthenia ¹⁰	IFN Independent ¹⁰	Approved December 2014 ¹⁰

Table 1 continued

Type of therapeutic (DAA)	Brand name	Company	Composition	Active against genotype	Function	Adverse effect	IFN dependency (IFN dependent or not)	FDA approval status
	TECHNIVIE	Abbvie ¹²	Ombitasvir, Paritaprevir and Ritonavir ¹¹	Genotype 4 ¹¹	Ombitasvir: NS5A inhibitor, Paritaprevir: NS3/4A protease inhibitor and Ritonavir: CYP3A inhibitor ¹¹	Asthenia, fatigue, nausea, insomnia ¹¹	IFN Independent ¹¹	Approved July 2015 ¹¹
	DAKLINZA	Bristol-Myers Squibb ⁷	Daclatasvir and combination of Sofosbuvir ⁷	Genotype 3 ¹³	NS5A replication complex inhibitor ⁷	Fatigue, headache, nausea and diarrhea ⁷	IFN Independent ⁷	Approved July 2015 ⁷
	ZEPATIER	Merck ¹²	Combination of Elbasvir (MK-8742), Elbasvir with ribavirin and Grazoprevir (MK-5172) ¹²	Genotypes 1 or 4 ¹²	Elbasvir-NS5A inhibitor ⁷ prevents transcription of HCV RNA and virion assembly	Feeling tired, nausea, and headache low red blood counts (anemia), fatigue, shortness of breath, and rash or itching	IFN Independent ¹²	Approved January 2016 ¹²
	EPCLUSA	Gilead Sciences ¹³	Contains sofosbuvir ¹³ and Velpatasvir (VEL) ¹³	All Genotypes 1–6 ¹⁵	Grazoprevir- NS3/4A protease inhibitor ¹² SOF is a NS5B nucleotide polymerase inhibitor ¹³ VEL is a NS5A inhibitor ¹³	Fatigue, anaemia, nausea, headache, insomnia, diarrhoea	IFN Independent ¹³	Approved June 2016 ¹³
	MAVYRET	AbbVie ¹⁴	combination of Glecaprevir and Pibrentasvir ¹⁴	Genotype 1–6 ¹⁴	Glecaprevir is HCV NS3/4A protease inhibitor, and Pibrentasvir is HCV NS5A inhibitor ¹⁴	8 weeks headache and fatigue with most serious reasons reported for HBV reactivation in HCV/HBV co-infected patients who were undergoing or had completed treatment with HCV-DAA, and who were not receiving HBV antiviral therapy, resulting hepatic failure and death ¹⁵	IFN Independent ¹⁴	Approved August 2017
	VOSEVI	Gilead Sciences, Inc. ¹⁶	Sofosbuvir, Velpatasvir, and Voxilaprevir ¹⁶	All genotype ¹⁷	Voxilaprevir is a NS3/4A inhibitor Velpatasvir is a NS5B inhibitor Sofosbuvir is a NS5B inhibitor	Headache, fatigue, diarrhoea, and nausea ¹⁸	IFN Independent ¹⁸	Approved July, 2017 ¹⁶
<i>FDA not approved DAAs</i>								
NS3/4A inhibitors:	PARITAPREVIR	Enanta Pharmaceuticals ¹⁹	Veruprevir ²⁰	Genotype 1 ²¹	Inhibiting protease NS3/4A ritonavir-increases the bioavailability of paritaprevir		IFN Independent	Not approved as monotherapy

Table 1 continued

Type of therapeutic (DAA)	Brand name	Company	Composition	Active against genotype	Function	Adverse effect	IFN dependency (IFN dependent or not)	FDA approval status	
NS5A inhibitor	GRAZOPREVIR		(MK-5172) ²⁰	Genotypes 1 or 4 ¹⁹	NS3/4A protease inhibitor ¹ prevents cleavage of the necessary polyproteins for viral replication ²⁰		IFN Independent	Not approved as monotherapy	
	GLECAPREVIR				NS3/4A protease inhibitor ²²		IFN Independent ²³	Not approved as monotherapy	
	VOXLAPREVIR				NS3/4A protease inhibitor ²⁴		IFN Independent ²⁴	Not approved as monotherapy	
	LEDIPASVIR		Ledipasvir	Genotype 1, 3, 4, 5 and 6 ⁹	Inhibiting protease NSSA	fatigue, headache ²⁵	IFN independent	Not approved as monotherapy	
	OMBITASVIR		Ombitasvir	Genotype 1 ²⁶	Inhibiting protease NSSA		IFN Independent	Not approved as monotherapy	
	PIBRENTASVIR		Pibrentasvir		Inhibiting protease NSSA		IFN Independent	Not approved as monotherapy	
	VELPATASVIR		(VEL) ²⁷	All Genotypes 1–6 ²⁷	Inhibiting protease NSSA		IFN Independent	Not approved as monotherapy	
	ELBASVIR		(MK-8742)	Genotypes 1 or 4	NS5A inhibitor ²⁸ prevents transcription of HCV RNA and virion assembly		IFN Independent ¹⁹	Not approved as monotherapy	
	NS5B inhibitor:	DASABUVIR		Dasabuvir	Genotype 1 ²⁶	non-nucleoside NS5B palm polymerase inhibitor ²⁶		IFN Independent	Not approved as monotherapy

Table 1 continued

Type of therapeutic (DAA)	Brand name	Company	Composition	Active against genotype	Function	Adverse effect	IFN dependency (IFN dependent or not)	FDA approval status
CYP3A inhibitor	RITONAVIR						IFN Independent ²⁹	Not approved as monotherapy
			¹ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/708/rebetol-ribavirin					
			² https://pubchem.ncbi.nlm.nih.gov/compound/ribavirin					
			³ https://www.drugbank.ca/drugs/DB00811					
			⁴ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/1149/victrelis-boceprevir					
			⁵ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/1150/ncivek-telaprevir					
			⁶ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/1296/olyzio-simeprevir					
			⁷ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100086/daklinza-daclatasvir					
			⁸ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/1298/sovaldi-sofosbuvir					
			⁹ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100039/harvoni-ledipasvir-and-sofosbuvir					
			¹⁰ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100055/viekira-pak-ombitasvir-paritaprevir-ritonavir-and-dasabuvir-tablets					
			¹¹ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100087/technivie-ombitasvir-paritaprevir-and-ritonavir					
			¹² https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100130/zepatier-elbasvir-and-grazoprevir					
			¹³ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100157/epclusa-sofosbuvir-and-velpatasvir					
			¹⁴ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100217/mavyret-glecaprevir-and-pibrentasvir					
			¹⁵ https://www.rxlist.com/mavyret-drug.htm#description					
			¹⁶ https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209195_toc.cfm					
			¹⁷ Link et al.[30]					
			¹⁸ Bourliere et al.[8]					
			¹⁹ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100130/zepatier-elbasvir-and-grazoprevir					
			²⁰ https://adinsight.springer.com/drugs/800029722					
			²¹ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100055/viekira-pak-ombitasvir-paritaprevir-ritonavir-and-dasabuvir-tablets					
			²² https://pubchem.ncbi.nlm.nih.gov/compound/Glecaprevir					
			²³ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100217/mavyret-glecaprevir-and-pibrentasvir					
			²⁴ Bourliere et al.[8]					
			²⁵ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100039/harvoni-ledipasvir-and-sofosbuvir					
			²⁶ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100055/viekira-pak-ombitasvir-paritaprevir-ritonavir-and-dasabuvir-tablets					
			²⁷ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100157/epclusa-sofosbuvir-and-velpatasvir					
			²⁸ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100086/daklinza-daclatasvir					
			²⁹ https://www.centerwatch.com/drug-information/fda-approved-drugs/drug/100087/technivie-ombitasvir-paritaprevir-and-ritonavir					

Table 2 Common Therapeutics against HCV (HTA)

Type of therapeutic (HTA)	Brand name	Company	Composition	Active against genotype	Function	Adverse effect	IFN dependency (IFN dependent or not)	FDA approval status
MIRAVIRSEN SPC3649	SantarisPharma ¹		Locked nucleic acid (LNA) ribonucleotide interspaced throughout a DNA ^{2,3,4}	All Genotypes ^{2,3,4}	Inhibits viral RNA by blocking mir-122	No evidence of side effects ^{2,3,4}	IFN Independent ^{2,3,4}	Not approved
ITX5061					Inhibits HCV entry by increasing high-density lipoproteins (HDLs) in both humans and mice by blocking the interaction of virus with SRBI ⁵		IFN Independent ⁵	Not approved
EZETIMIBE	OHM LABS INC				Inhibits cholesterol absorption receptor NPC1L1, reduces the absorption of cholesterol from the intestine		IFN Independent	Not approved
ERLOTINIB	OSI/Genentech		Quinazoline derivatives		EGFR inhibitor, prevents the formation of CLDN1-CD81 complexes and thereby endocytosis	Overdose causes rash, diarrhoea, anorexia, fatigue	IFN Independent	Not approved
CEGALOSVIR MX3253			Derivative of castanospermine ^{6,7,8}	Genotype 1 ^{6,7,8}	Alpha-glycosidase I inhibitor ^{6,7,8} leads to reduced viral infectivity in vitro ^{6,7,8}		Both ^{6,7,8}	Not approved
PTEROSTILBENE			stilbenoid, chemically related to resveratrol ⁸		Serves as defensive phyto-alexin role ⁸			Not approved
TORIMEFENE			A first generation nonsteroidal selective estrogen receptor modulator ⁹		An estrogen agonist ⁹			Not approved
QUINIDINE			optical isomer of quinine, extracted from the bark of the Cinchona tree and similar plant species ¹⁰		This alkaloid dampens the excitability of cardiac and skeletal muscles by blocking sodium and potassium currents across cellular membranes. It prolongs cellular action potential, and decreases automaticity. Quinidine also blocks muscarinic and alpha-adrenergic neurotransmission ¹⁰			Not approved

Table 2 continued

Type of therapeutic (HTA)	Brand name	Company	Composition	Active against genotype	Function	Adverse effect	IFN dependency (IFN dependent or not)	FDA approval status
Combination drugs:	PTEROSTILBEN, TORIMEFENE and QUINIDINE		Pterostilbene-resveratrol Torimefene-Quinidine ⁹		Class I antiarrhythmic agent ²⁶ act as a potential antiviral agent for HCV ⁹		IFN Independent ⁹	Not approved

¹<https://www.nejm.org/doi/full/10.1056/NEJMoa1209026>

²Lacek K, Vercauteren K, Grzyb K, Nadddeo M, Verhoye L, Slowikowski MP, et al. Novel human SR-BI antibodies prevent infection and dissemination of HCV in vitro and in humanized mice. *J Hepatol.* 2012;57(1):17–23

³Scheel TKH, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. *Nat Med.* 2013;19(7):837–49

⁴Gastaminza P, Whitten-Bauer C, Chisari FV. Unbiased probing of the entire hepatitis C virus life cycle identifies clinical compounds that target multiple aspects of the infection. *Proc Natl Acad Sci USA.* 2010;107(1):291–96

⁵Koseki M, Ishibashi M, Larson CJ, Miller SG, King BD, Tall AR. Increased HDL cholesterol and apoA-I in humans and mice treated with a novel SR-BI inhibitor. *Masson D. Arterioscler Thromb Vasc Biol.* 2009;29(12):2054–60

⁶<https://adisinsight.springer.com/drugs/800029722>

⁷<https://pubchem.ncbi.nlm.nih.gov/compound/Gilecaprevir>

⁸Langcake P, Pryce RJ. A new class of phytoalexins from grapevines. *Experientia.* 1977;33(2):151–2

⁹<https://www.drugbank.ca/drugs/DB00539>

¹⁰<https://www.drugbank.ca/drugs/DB00908>

be effective against all genotypes. These are the only drugs that are reported to be effective against genotypes 2, 5 and 6. Patients infected with genotype 3 however can be treated with Ledipasvir and Daclatasvir and combination containing these in addition to those available for genotypes 2, 5 and 6.

In many of the therapies, along with Ribavirin, interferon is administered as well, often approved as triple combination therapy [1]. In naive genotype 1 patients, the rate of SVR can be improved by more than 70% by using of a protease inhibitor along with the standard IFN/RBV treatment [1]. Two of the first generation protease inhibitor DAAs, VICTRELIS (boceprevir or -BOC-) [24] and INCIVEK (telaprevir or TVR) [23], are used in combination with Peg-IFN and ribavirin therapy. Use of these protease inhibitors is however currently not recommended by clinicians due to several side effects.

IFN independent therapeutics

The most broad spectrum and successful drug is SOVALDI (sofosbuvir or SOF) [18] which has shown higher efficacy in combination with other DAA along with or without PEG-IFN. It has shown high therapeutic potency via oral administration with high barrier to resistance. SOF inhibits HCV replication by mimicking a nucleotide and blocks the viral NS5B [18]. In clinical trials, Sofosbuvir has exhibited good results [18]. Consequently all combination drugs that include Sofosbuvir such as HARVONI, OLYSIO, EPCLUSA show good profile against HCV infection [12] and are effective against many genotypes and can also be administered without accompanying interferon. In patients infected with genotype 1, a combined injection of the second-generation protease inhibitor, OLYSIO (simeprevir) with peg-IFN, ribavirin and simeprevir is no longer recommended, because of toxicity and adverse effects. However the combination of simeprevir and sofosbuvir, with or without ribavirin in patients with genotype 1 has been very well tolerated and has provided an excellent alternative to the older first-generation protease inhibitors [6]. However, those with genotype 1a- associated Q80K polymorphisms [49], where alteration of NS3/4A protease has occurred, a substantially reduced efficacy of simeprevir has been reported.

Interferon- free, three-drug (3D) regimen is 'VIKERA PAK' [43] and 'TECHNIVIE' [53], which comprises of ritonavir-boosted paritaprevir, ombitasvir, and dasabuvir (ProD). This is introduced with or without RBV. The adverse side effects of these drugs have still not been clearly evaluated in large populations.

DAKLINZA ("a combination of sofosbuvir and Daclatasvir") along with or without ribavirin is effective in patients infected with genotypes 1–4. In patients with liver

transplantation, Daclatasvir has given efficient result when combined with sofosbuvir. It can also be safely used in people with renal insufficiency. In Japan combination of daclatasvir and asunaprevir, has also been approved against genotype 1b infected patients [38]. In studies conducted thus far, it has been generally well tolerated [38]. It is not however known whether daclatasvir passes into breast milk or if it could cause harm to a nursing baby.

Recently, ZEPATIER has been approved to treat chronic HCV infection in both treatment-native and treatment-experienced patients. Initially in patients with HIV co-infection and patients undergoing dialysis, this combination showed efficacy. However from the combination of grazoprevir and elbasvir some populations may not benefit due to the presence of baseline NS5A resistance thus exhibiting decreased SVR [5].

A combination medicine, EPCLUSA comprises of SOF and velpatasvir. Across genotypes 1–6, the pangenotypic activity of EPCLUSA is effective because it targets a highly conserved catalytic site of the NS5B [27]. VEL is another novel second-generation NS5A inhibitor. A new pan-genotypic, fixed dose combination medicine is MAVYRET (a combination of glecaprevir and pibrentasvir). It is used to treat adult patients with chronic HCV infection without or with mild liver cirrhosis, including patients with moderate to severe kidney disease and those who are on dialysis [31]. Another combination drug is VOSEVI which comprises of Sofosbuvir, Velpatasvir and Voxilaprevir [8].

Host targeting antivirals (HTAs)

HTAs target different host proteins which play important role in viral life cycles. It also regulates the host immune system and other host cellular processes. Every step of viral life cycle, starting from viral entry to HCV progeny release, are dependent on several host cell factors. Till date two main concepts for HTAs have been discussed in detail, (1) interfering with host factors that are indispensable for the viral life cycle inside the host cell; (2) boosting host's innate immunity by administering IFN- λ or TLR (Toll like receptor) antagonist [20]. In this context miRNA-122, CypA (cyclophilin A) and FASN (fatty acid synthase), key cellular factors required for HCV lifecycle are also alternate treatment options of HCV infection [37].

HTAs against HCV entry

A number of compounds have been developed to block SR-B1, one of the major receptors for viral entry. It is the main HDL receptor present in the hepatocytes which probably modulates viral attachment to the hepatocytes [35]. In humanized mouse model, monoclonal antibody based SR-

B1 targeted compounds have been tested and were found to reduce both HCV infection and dissemination [35]. Recently, there is an alternative use of an anti SR-B1 small molecule, ITX5061.

Mature HDL delivers cholesterol to the peripheral tissues where other than steroidogenic organs, none are able to catabolise cholesterol. Hence, they are removed and transported to the hepatocytes by SRBI (reverse cholesterol transport; RCT). Thus, SRBI inhibiting compounds can alter cholesterol transport mechanism from peripheral tissues and increase cholesterol levels in sera which might be lead to cardiovascular diseases, especially atherosclerosis. Moreover, several reports indicate that in the absence of serum lipoproteins, HCVcc (cell culture adaptable HCV) can efficiently infect Huh7.5 cells and deficiency of SRBI cannot entirely block HCV entry. Recent studies suggest that administration of 400 µg monoclonal antibodies (mAbs) of either anti-CD81 [33] or anti-SRBI protect mice completely from HCV infection [34]. Ezetimibe is a potent inhibitor of cholesterol uptake [2] which is also a possible candidate. Ikeda et al. have reported in their 2006 study that statins, commonly used as cholesterol-lowering medication can inhibit *in vitro* HCV replication. Using OR6 cells infected with HCV, the authors evaluated the antiviral activity of five statins: atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin. All statins, except pravastatin, were tested as monotherapy and inhibited viral replication. Fluvastatin exhibited the strongest antiviral effect, whereas atorvastatin and simvastatin showed moderate inhibitory effects, and lovastatin exhibited a weak inhibitory effect on HCV replication. All of these statins exert a cholesterol-lowering effect by inhibiting the activity of 3-hydroxy3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol synthesis. Pravastatin, unlike the other tested statins, has no antiviral activity; hence, it inhibits HCV replication not by a direct action on HMG-CoA reductase, but by a specific antiviral mechanism [22]. It is evident from the above reports that HTAs although found to be effective in animal models however can only be marketed after extensive and through clinical trials as most of these drugs are expected to manifest widespread side effects. This is because HTAs target key host molecules in HCV life cycle which may be have vital physiological roles. Hence it is expected that HTAs will have stronger side effects compared to DAAs.

HTAs to boost host's innate immunity

In the last 25 years, IFN α is used for the treatment of HCV patients to activate the immune response which is among all one of the best studied and tested HTAs. However, the administration of IFN α alone has not given good results in cure of genotype 1 patients, resulting in the production of

viral escape mutants, and several side effects. Toll-like-receptor (TLR) agonist shows enhancement of the antiviral response and HCV elimination. TLR 7/ TLR 9 agonists both demonstrated activation of the immune response [40]. However, IMO-2125 is the TLR9 agonist which lowers the viral loads in the patient population.

HTAs against HCV replication

Mir-122 is liver specific micro RNA that plays a key role in cholesterol and fatty acid metabolism in the liver. It also plays a crucial role in liver development and differentiation [29]. Several reports indicate that Mir-122 has a positive effect on different stages of HCV life cycle. Unlike other miRNAs, Mir-122 enhances viral replication by directly binding with two conserved adjacent sites present in 5' UTR of HCV RNA. Targeting molecules for Mir-122 has been shown to be a new era in the field of HCV therapeutic research. Miravirsin, a mir-122 antisense blocker, is the latest HTA from Santaris Pharma. In chimpanzee a weekly intravenous administration of 5 mg/kg has lowered serum cholesterol without adverse effects [29].

Cyclosporine A (CsA) is a cyclophilin inhibitor (CyoI) with reported anti-HCV activity *in vitro* [55]. Several studies in hepatoma cell lines indicate that this compound prevents HCV replication and protein synthesis. In combination with NS5A and other protease inhibitors, EDP-546 (Cyclophilin inhibitor) is a potential drug because of its high barrier to HCV resistance [39].

HTAs against HCV assembly

Targeting host glucosidases has been indicated as potential and effective strategy to interfere with HCV assembly. MX-3253/ celgosvir is a potential α -glucosidase I inhibitor which prevents hepatitis C virus infection [15]. VLDL assembly inhibitory substances, such as MTP (microsomal triglyceride transfer protein) inhibits HCV release from infected cells. Torimefene (a derivative of tamoxifene), Pterostilbene (a methylated form of resveratrol) and quinidine [25] also act as the potent inhibitors of HCV.

A number of these drugs which could be possible HTAs have been listed in Table 2 although none of them have been approved by FDA.

Consideration of gender in HCV therapy

According to some clinical evidences, gender has also been shown to play a major role in HCV infection and treatment. Males are more prone to hepatic fibrosis and have been shown to develop hepatocellular carcinoma [60] whereas, females have been observed to clear HCV spontaneously

and exhibit slower progression to cirrhosis. It is possible that this viral clearance among women is associated with genetic [50] and hormonal factors, especially the protective role of estrogen. In support of this hypothesis, it was seen that there is a greater chance of progression to hepatic cirrhosis among asymptomatic women older than 50 years of age [54].

Similarly, the influence of gender has been investigated in treatment as well. One report suggests that treatment for HCV genotype 1 patients' response to combined therapy with Peg-IFN and RBV was not satisfactory among females compared to males aged 50 years or older [51]. However, the gender influence on HCV treatment is not well-known for all genotypes.

Other considerations for therapy

With newer antiviral agents becoming available for clinical use, data regarding resistance profile of these new generation drugs are also emerging, therefore prior to treatment viral genome sequencing may be necessary to detect such resistance associated variants (RAV), especially for certain agents that may help in choice and duration of antivirals [45].

Natural therapeutics agents against HCV

Natural or ethnobotanical medicine against many diseases, can be easily available and often used as first line treatment especially in tropical countries. The broad spectrum activity at multiple targets with minor side-effects and cost effectiveness may be the reason why most low economy countries rely upon these traditional remedies for primary healthcare. For example, in Burkina Faso, West Africa more than 90% of people prefer to use ethnobotanical medicines to treat many diseases [19]. Ayurveda in India and the Traditional Chinese Medicine (TCM) in China, have both been used to treat infectious diseases for many decades [9]. Several bioactive compounds that have been found to be present in different parts of plants which acts against several viral infection including dengue virus (DENV), coronavirus (CoV) and hepatitis C virus (HCV) [59]. Some examples of such compounds in use include Silymarin, a flavonoid compound present in *Silybum marianum* (L.) plant has been tested against HCV and is found to be effective in inhibiting HCV core protein expression in genotype 3. [52]. Green tea derived polyphenol compound (–)-epigallocatechin-3-gallate (EGCG) has been found to inhibit HCV replication in Huh7 cell line [57]. Another plant derived flavonoid quercetin found in *Syzygium cumini* acts as a protease inhibitor (NS3) of HCV and inhibit viral

replication [3]. There are many reports of anti HCV properties in many different plants reported from a number of areas of this world. In many cases however although anti-HCV effect has been detected, the exact mechanism of action has not been worked out. However, the discussion on plant extracts in the treatment of HCV is vast and can be dealt with in a separate review altogether.

Concluding remarks

Novel and very efficient therapies are available since 2014 and according to the WHO's declaration, HCV eradication will be possible by the year 2030. Although there are many drugs available in the market, these treatments are still very expensive in low income countries and none are free from side effects. In addition, DAAs does not protect people from reinfection and unknowingly they can transmit the virus, as initial HCV infection is asymptomatic. Data on severe drug–drug interactions with other treatments are also scanty. The appearance of resistant strains due to the use of these antiviral drugs are also expected, thereby increasing the number of HCV infected patients in the future. Furthermore more studies on genotype specific differences and similarities are needed to further understand and develop pangenotypic therapeutics.

Author's contribution CB: conceptualization, data curation, formal analysis. MS: data curation, formal analysis, writing-review and editing. DD: data curation, writing original draft the initial stages, imaging. SC: writing-review and editing. AM: funding acquisition, project administration, supervision, visualization.

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Declarations

Conflict of interest The author(s) declare that there are no conflicts of interest.

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