

Review

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# Molecular targeting of antiviral drugs used against hepatitis C virus infection

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## Abstract

Present study reports an update on the molecular interaction of antiviral drugs with viral and host cell components during hepatitis C virus (HCV) infection. In addition to the traditional therapeutic drug regimen, termed as standard of care, some recent drugs have been added in the existing regimen used for HCV infection. These drugs were categorized as direct-acting antivirals (DAAs) agents and "other agents", with their efficacious impact in the control of HCV infection. They target both viral proteases and host cell receptor proteins/enzymes involved in HCV entry into the cell, replication, and assembly to check their propagation both *in situ* as well as in cell to cell transmission. Recent studies have reported a significant rise in sustained virological response after the use of these drugs both alone and in combination with pegylated interferon- $\alpha$  (PegIFN- $\alpha$ ) plus ribavirin. Recently, DAAs have been reported to be highly effective in eradication of HCV infection, especially liver cirrhosis, reducing but not avoiding the occurrence of liver cancer. Some studies have demonstrated that the presence of resistant HCV variants, arising during viral replication, may be controlled by the new drug regimen. It is important to note here that all these drugs are influenced by viral as well as host factors including basic viral load, HCV genotypes, IFN action, interleukin 28B polymorphism and some liver and metabolic diseases, *etc.* This is an area with on-going investigations to explore more antiviral agents that may address new challenges in HCV therapy.

**Keywords:** Hepatitis C virus, interferon, pegylated interferon, direct-acting antivirals, sustained virological response, drug-resistance

## INTRODUCTION

Hepatitis C virus (HCV) infection is a known cause of serious liver diseases recorded worldwide. Majority of infections are asymptomatic and in about 80% of cases, the virus persists without the patient's



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awareness. HCV infection causes both acute as well as chronic liver diseases including cirrhosis of liver and hepatocellular carcinoma. Globally, HCV infection affects nearly 180 million people<sup>[1]</sup> which account for 3% population of the world. Approximately 3 million new cases are added to this population every year<sup>[2,3]</sup>. A high proportion of HCV infected patients develop chronic liver diseases and nearly 20% of them progress to cirrhosis and about 10% to liver cancer<sup>[4,5]</sup> in later stage. The presence of HCV infection, though varies from region to region, has been noted throughout the world. Hepatitis B virus (HBV)-based prevention and control measures for viral hepatitis have achieved remarkable results, and hepatitis C has relatively little awareness. Efforts have been made to develop effective prophylactic and therapeutic measures for treatment of chronic HCV infection. There is a common belief now that HCV infection needs more attention even than human immunodeficiency virus (HIV) infection in terms of its early detection and timely remedies since both of them do not have any vaccine for prevention. Moreover, the disease burden caused by HCV is also more serious even than HIV. A high genomic variability in HCV has led to development of at least seven genotypes and many isotypes.

HCV is an RNA virus with about 9.6 kb genome. This is a single stranded, enveloped virus with positive polarity and has been categorized under flaviviridae family. Its genome has a single ORF encoding for polypeptides of 3011 amino acids. The 5'UTR region has an internal ribosomal entry site (IRES) which is involved in HCV replication. Using host and viral proteases, HCV polyprotein is cleaved into three structural proteins (Core, E1 and E2) and seven non-structural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B)<sup>[6]</sup>. HCV-core forms viral nucleotide that has significant role in viral pathogenesis<sup>[7]</sup> and E1 and E2 proteins are involved in viral entry into the cell<sup>[8]</sup>. The P7, a 63-amino acid protein, helps in translocation of NS2 into endoplasmic reticulum and also in viral assembly and release of HCV virions<sup>[9,10]</sup>. The NS2 peptide is a transmembrane protein which plays role in viral replication. The NS3, on the contrary, is a protease and acts as ATPase/helicase<sup>[11,12]</sup>. Usually, HCV protease disrupts interferon (IFN) and toll-like receptor-3 (TLR-3) signaling pathways. The NS4A acts as a cofactor for NS3 protease, the NS4B is needed to recruit other viral proteins<sup>[13,14]</sup> and NS5A, a phosphoprotein, plays role in viral replication<sup>[15,16]</sup>. The last non-structural protein i.e. NS5B is an HCV RNA dependent RNA polymerase (RdRp) which also participates in RNA replication<sup>[17]</sup>.

The studies available in last few decades have elucidated the virus specific events in infected cells. In order to use these events as targets for chemotherapy, some antiviral agents were developed and used to treat HCV infection on a line similar to the one used for other viral infections. This targeting is aimed to suppress virus reproduction without an adverse effect on the host-cell. There are a number of virus specific processes within virus replicative cycle in an infected cell that may be targeted for chemotherapeutic intervention. The major target steps include virus entry into the cell, reverse transcription, viral DNA/RNA polymerization and the reactions involved in viral DNA/RNA synthesis *etc.* At present, a variety of agents including nucleosides and non-nucleosides entities have been developed which interact with virus targets and inhibit virus replication. In case of treating HCV infection, today a variety of agents are available for use. In addition to the virus-specific events, there are several host enzymes and processes that are closely associated with viral DNA, RNA or protein synthesis. These processes may also be the targets for antiviral agents.

The recommended treatment for HCV infection includes a combination therapy with PegIFN and ribavirin<sup>[18]</sup>. However, recently several new regimens have been evolved for treatment of HCV infection. The drugs including direct-acting antiviral agents (DAAs) like boceprevir or telaprevir as protease inhibitors have provided a new promise to aim the HCV treatment. This therapy improves sustained virological response (SVR) in patients infected with HCV genotype-1 by more than 70%. Moreover, it has an additional significance of little chances of development of drugs resistant variants<sup>[19-21]</sup>. Several other DAAs are in clinical trials today and have been evaluated for combination therapy<sup>[22]</sup>. The emerging new antivirals need a new trial for serious liver diseases, particularly, in those cases with poor response to current regimens<sup>[23,24]</sup>.

Present study gives an update on the availability and action of therapeutic agents targeting various steps of HCV viral life cycle and infected host cell processes that may be disrupted to check viral reproduction and underlying pathological reaction cascade. It also describes the comparative efficacies of different agents and the future of HCV- treatment under the use of these agents.

## TYPES OF DRUGS

In a common practice, the combination of pegylated interferon- $\alpha$  (PegIFN- $\alpha$ ) and ribavirin, is used for the treatment of HCV infection<sup>[18]</sup>. The addition of DAAs, like boceprevir, ortelaprevir, in the drug regimen, has brought a new change in the status of HCV treatment. This regimen improves the SVR to a significant level even in genotype-1 infected patients<sup>[19]</sup>. IFN and ribavirin can cause patients with flu-like symptoms, cognitive dysfunction, thyroid dysfunction and other adverse reactions, leading to premature termination of treatment in some patients. However DAAs were found to develop drug resistant variants<sup>[20,21]</sup>. Subsequent studies introduced the next generation DAAs like simeprevir and sofosbuvir, that were approved by FDA for treatment of HCV infection<sup>[21,25]</sup>. An interferon free drug regimen comprising ombitasvir, paritaprevir, ritonavir and dasabuvir has been approved for HCV genotype-1 infected patients. Now it is believed that the new drug combination may consist of interferon free regimen with high viral killing efficiency, short therapy time and less adverse effect. The development of drugs and their different combinations for an effective therapy against HCV is under investigation for last several years. Some new drugs developed and used in recent past are described in [Table 1](#). These drugs are used both alone as well as in combination to other drugs. Based on their nature, action and host response, these drugs have been classified under different categories:

### Interferon

PegIFN- $\alpha$ , a commonly used drug increases the SVR rate by causing a delay in renal clearance. Human albumin-IFN- $\alpha$  (Albinterferon) is a fusion protein. This protein is used for the treatment of HCV infection. Different reports have shown that the SVR rate arising from the use of Albinterferon and Ribavirin was nearly the same as noted with use of the SOC treatments<sup>[26,27]</sup>. Similarly, IFN- $\lambda$  which is a class-III interferon, is also used for the treatment of HCV infection. The receptors of IFN- $\lambda$  are mainly present in the liver and therefore very minimal extrahepatic adverse effects were recorded with the use of IFN- $\lambda$  in comparison to IFN- $\alpha$ <sup>[28]</sup>.

### Direct acting antiviral agents

This is the class of drugs acting against viral and host proteins involved in HCV life cycle. The major inhibitors of NS3 viral protein are telaprevir and boceprevir. Telaprevir was approved and recommended for use with PegIFN- $\alpha$  and ribavirin in genotype-1 patients. This was classified as triple therapy. Since telaprevir treatment is reported to be effective against the resistant mutants in the short term duration, it was decided to use it for long-term and subsequently approved for the treatment<sup>[29]</sup>. It is important to note here that the long term use of these drugs often leads to drug resistance including T54A/S, R155K/T, V36A/M, V55A, and A156/S/T/V, *etc.* Simeprevir is another NS3 protease inhibitor classified as second generation drug. This drug is a reversible inhibitor of NS3/4A protease<sup>[30]</sup>. Danoprevir and faldaprevir are also second-generation HCV NS3/4A protease inhibitors and used in patients infected HCV genotype-1. In addition to these drugs, there are various other NS3 protease inhibitors like Vaniprevir (MK-7009), Narlaprevir (SCH 900518), Asunaprevir (BMS 650032), VX 985, and MK-5172 which are used for treatment of HCV infection. There is every possibility that these drugs may be approved for therapeutic use against HCV infection<sup>[29]</sup>.

Daclatasvir (BMS) 790052 was found to inhibit NS5A, a protein involved in HCV replication and therefore used as a drug for control of HCV infection. This particular drug has a broad genotype antiviral activity. In addition, other NS5A inhibitors include Ledipasvir (GS-5885), ABT 267, IDX791, and ACH-2928 *etc.* NS5B is a RNA-dependent RNA polymerase (RdRp) involved in HCV replication. This NS5B enzyme activity is inhibited by two categories of inhibitors that are nucleoside/nucleotide derivative inhibitors (NIs) and non-

**Table 1. Mechanism of drug action to control HCV infection**

Site of action (target)	Drugs	Mechanism of action
Viral entry		
Attachment	Lectin cyanovirin-N, BA-LNC, Ficolin, Heparin and heparin-derived compounds, Heparanase, EGCG and its derivatives, Lactoferrin, A p7 ion channel-derived peptide H2-3	Inhibits attachment factors reducing concentration of virions on cell surface
Post-binding interactions with entry factors		
CD81	Imidazole-based compounds, Anti-CD81 mAbs, Soluble CD81 LEL	Inhibits viral binding with entry factors
SRB1	Serum amyloid A, Anti-SRB1 pAb and mAb, ITX5061	
CLDN1	Anti-CLDN1 peptides, Anti-CLDN1 pAb and mAb	
EGFR	Erlotinib	
EphA2	Dasatinib	
TfR1	Anti-TfR1 mAbs, Ferristatin	
NPC1L1	Anti-NPC1L1 mAbs, Ezetimibe	
Clathrin-mediated endocytosis	Chlorpromazine, Arbidol	Restrict endocytosis of virions
Fusion and uncoating		
Endosome acidification	Concanamycin A, Bafilomycin A Chloroquine, Ammonium chloride	Reduces acidification of endosome required for membrane fusion between virus and host cell
Lipid composition of virus or host cell	Arbidol, Phenothiazines, RAFIs (aUY11), LJ001, Silymarin	Reduced fusion efficiency of HCV particles
Unclear mechanism	Ferroquine, PS-ONs	
Natural compounds and small molecules	Flavonoids, Terpenoids, Tannic acid, Gallic acid, PF-429242	Exact mechanism not elucidated
Viral replication		
	Interferon	
	PegIFN- $\alpha$ , Human serum albumin IFN- $\alpha$ , PegIFN- $\lambda$ -1a	IFN-alpha declines HCV RNA level
	Ribavirin (Nucleoside analogue)	Mechanism unclear
	DAA's	
Viral protein		
NS3/4A	Telaprevir, Boceprevir, Faldaprevir, Simeprevir, Asunaprevir, Paritaprevir, Danoprevir, Grazoprevir, Vaniprevir, TMC435	Inhibits NS3/4A proteases involved in viral replication
NS5A	Daclatasvir, Ledipasvir, Ombitasvir, Elbasvir, Velpatasvir	Inhibits binding of NS5A to viral RNA required for RNA replication and viral assembly of HCV
NS5B	Sofosbuvir, Dasabuvir, Mericitabine B1207127, Lomibuvir/VX-222, Setrobuvir	Inhibits NS5B, RNA-dependent RNA polymerase inhibitor
NS3	3-bromo-4-hydroxyl derivative 4,5,6,7 - tetrobromo benzotriazole (TBBT), 30-methylpiperidine-10-YI QU663	NS3 helicase inhibitor Protein kinase-2 inhibitor Helicase inhibits NS3 helicase inhibitor
NS4B	Clemizole	Inhibits HCV RNA replication by blocking binding of viral RNA to NS4B
Host factors		
Cyclophilins	Cyclosporin A	Inhibit HCV replication
miRNA	Miravirsin	Reduces HCV replication
Viral assembly		
Alpha-glucosidase	UT-231B (Immino sugar) and Celgosivir (MX-3253-a castano- spermine prodrug)	Inhibits alpha glucosidase involved in HCV assembly
DGAT-1 (Cellular factor) (Diacylglycerol O- acyltransferase-1)	DGAT-1 inhibitor	Inhibits DGAT-1 needed for core protein localization around LDs
DGAT-2 (Cellular factor) (Diacylglycerol O- acyltransferase-2)	DGAT-2 inhibitor	DGAT-2 involved in LD biogenesis
VLDL biogenesis	Grapefruit flavonoid naringenin	Inhibitor of VLDL secretion disturbing viral assembly

nucleotide inhibitors (NNIs). It has been found that NIs have a similar effect for different HCV genotypes and also show low incidence of resistant genes. Sofosbuvir, a NIs, has been used in cases of HCV infection caused by non-genotype-1 HCV<sup>[31,32]</sup>. However, DAAs are well tolerated and adverse reactions are significantly lower

than IFN, but there are still a few cases of adverse reactions and reactivation of HBV during DAAs anti-HCV treatment<sup>[31]</sup>.

### Cyclosporine and miravirsen

Cyclophilins including cyclophilins A, B, and C are involved in HCV replication. An immunosuppressive compound cyclosporine A is involved in the inhibition of HCV RNA replication by interfering with cyclophilins A functions. Alisporivir (Debio-025) which is a derivative of cyclosporine A acts as antiviral agent against many HCV genotypes. The antiviral effect of cyclophilin inhibitors is increased when used in combination with PegIFN- $\alpha$ . Thus, in addition to many other benefits, these agents may be used as effective antiviral agents<sup>[33,34]</sup>. Miravirsen is another drug that targets miRNA-122. It inhibits several HCV genotypes *in vitro*. Its effect lasts long simultaneous with non-appearance of resistant mutations.

### Other antiviral agents

In addition to antiviral agents described above, vitamin B12 was also reported to act as an inhibitor of HCV replication. The use of vitamin B12 with SOC drugs raised the SVR rate to the level higher than the rate noted in patients treated with SOC alone<sup>[35]</sup>. Recently, it has been observed that vitamin D also acts against HCV *in vitro*. The SVR rate of patients infected with HCV genotype-1 or 2/3 is improved once vitamin D is added to PegIFN- $\alpha$  and ribavirin therapy<sup>[36,37]</sup>. A comparison of study using PegIFN- $\alpha$  and RBV with supplement of L-carnitine group vs. the PegIFN- $\alpha$  plus RBV group has shown an increase in SVR rate<sup>[38]</sup>. This substantiates that L-carnitine may be useful for the treatment of HCV infection.

## MECHANISM OF DRUG-ACTION

### Targets of drugs

The basic aim of designing the drugs against HCV infection is to develop agents that can check the entry of virus into cells, blocks its replication and disrupts the viral assembly inside the cell. As such, drugs do not kill the virus or its components but prevent their formation and reproduction. In case of HCV infection, attempts were made to develop drugs that can check viral entry and replication process. Since the discovery of HCV, a number of experimental studies were conducted which reported detailed analysis of HCV life cycle and its interaction with human host. These studies revealed several targets for therapeutic intervention in HCV infection. Recent improvements in the SOC therapy have raised the hope that HCV infection can be managed with adequate medical intervention. However, the current treatment is not effective for all seven genotypes. The basic aim for HCV therapy is to achieve high SVR using traditional drugs in combination with direct acting antivirals (DAAs), without any chance of escape mutations.

### *HCV entry as target*

The drugs inhibiting HCV entry into cells target receptors and enzymes helping in viral entry process. These entry inhibitors have prophylactic properties and show synergistic effect when combined with other agents<sup>[39]</sup>. Circulating virions bind with glycosaminoglycans (GAGs) and LDLA<sup>[40]</sup>. The lectin cyanovirin-N (CV-N) impairs viral binding by its interaction with E1/E2 HCV proteins to check entry<sup>[41]</sup>. Similarly, L-ficolin proteins can neutralize HCV particles through their binding to E1/E2 proteins<sup>[42]</sup>. Epigallocatechins gallate (EGCG), a natural polyphenol compound and abundant in green tea extract regulate lipid metabolism impairs HCV binding to host cell by interfering with HCV E1/E2 function and also block cell-to-cell transmission *in vitro*<sup>[43-45]</sup>. This is the reason that green tea is considered as an effector against HCV infection. Lactoferin, present in milk, also blocks HCV attachment<sup>[46]</sup>. Like E1/E2, the P7 protein also inhibits HCV entry by directly effecting virus binding to cell surface and interfering with host-virus interaction<sup>[47]</sup>.

After attachment of virus with cell surface, its entry requires different host factors like CD81, SRB1, CLDN1 and OCCDN1, jfRI, EGFR, EphA2 and NPC1-L1, *etc*. CD81 interacts with HCV E2 helping HCV infection. Specific NTCD81 monoclonal antibodies like JS-81 or KO4 counteract HCV E2-CD81 interactions and

interfere with HCV entry during post binding process<sup>[48-53]</sup>. SRB1 proteins, related to lipid metabolism, also affect HCV entry to host cells<sup>[54]</sup>. Serum amyloid A, an acute phase protein and produced by liver, inhibits HCV entry<sup>[55-57]</sup>. Similarly, ITX5061, a small molecule, also blocks uptake of HCV and functions synergistically with DAAs, thus giving a promise for future use. CLDNs and OCLNs form complex with CD81 and contribute to efficient HCV internalizations. Since CLDN1 is highly expressed in hepatocytes, it may be a potential target for antiviral agents. Antibodies vs. CLDN1 show inhibitory effect on HCV infection<sup>[58-60]</sup>. OCLN is also a main entry factor for HCV. Recently, it has been found that mi R-122 can decrease HCV entry by inhibiting OCLN. The EGFR and EphA2, the receptor tyrosine kinases (RTKs), act as cofactors for HCV entry<sup>[61]</sup>. These are expressed in liver and inhibited by anticancer drugs like Erlotinib and Desatinib. These drugs impair HCV cell-entry. RTKs interfere with CD81-CLDN1 complex association and block cell to cell transmission of HCV<sup>[61]</sup>. However, their efficiency needs further authentication. After interaction with various receptors, HCV particles are internalized through clatherin-mediated endocytosis<sup>[62]</sup>. CD81-CLDN1 complex facilitates virus entry and fusion simultaneously<sup>[58]</sup>. The compound chlorpromazine interferes with clatherin, thus impairing HCV endocytosis<sup>[63]</sup>. Arbidol, used as an anti-influenza drug, impairs clatherin mediated endocytosis of HCV<sup>[64]</sup>. The fusion of virus membrane to host cell is followed by viral replication inside the cell. The indole derivative arbidol also inhibits HCV membrane fusion<sup>[65]</sup>. Silymarin is a mixture of several flavonolignans and flavonoid taxifolines and inhibits fusion as done by arbidol<sup>[66]</sup>. Other fusion inhibitors include feroquine and aclorocquin, *etc.*

#### *HCV replication as target*

The HCV replication cycle presents another important target for antiviral therapy. The successful use of protease inhibitors for the treatment of HIV infections prompted researchers to focus on the HCV associated enzymes including NS3-4A protease and NS5B polymerase, *etc.*<sup>[67,68]</sup>. The HCV RdRp also became an attractive drug target. Finally, inhibitors targeting NS5A have also been developed. Simultaneous with viral proteins, several host cellular components were also used as targets while developing drugs against them.

NS3 is a component of HCV encoded polyprotein which together with NS4A, constitutes the protease NS3-4A. Its carboxy-terminal region shows RNA helicase and NTPase activity<sup>[69]</sup>. Both these proteases are essential for HCV replication and have been pursued as drug targets. Since NS3-4A binds with its substrate by weak interactions, this restricts the development of drugs targeting NS3-4A. However, later studies could be successful in developing certain DAAs targeting NS3-4A<sup>[70]</sup>. These drugs were put under three different categories on the ground of their properties and action<sup>[71]</sup>. The DAAs in category I include linear peptidomimetics that bind proteases enzymes through covalent bonds. For example, telaprevir and boceprevir, the drugs of class I bind to the active-site Ser (Serine) forming a covalent enzyme - inhibitor adduct. This not only shows antiviral activity but also uses strong forces to bind the target site. DAAs under category II and III are NS3-4A specific drugs. These are linear peptidomimetics or macrocyclic inhibitors and do not bind with their target by covalent bonds. It has been reported that these drugs do not target all HCV genotypes. These NS3-4A inhibitors are two macrocycles MK-5172<sup>[72]</sup> and ACH-2684<sup>[73]</sup>.

The NS5A replicase is the most enigmatic HCV protein. On the basis of molecular masses, their predominant forms are p56 and p58, respectively<sup>[74]</sup>. The phosphorylation in NS5A replicase is reported to be mediated by different kinases<sup>[75,76]</sup>. It has several sites identified as targets in the central and c-terminal part of NS5A and LCS1 region. The RdRp-NS5B is another enzyme regulating viral RNA synthesis. Several studies have demonstrated the candidate NS5B inhibitors which are nucleoside and nucleotide inhibitors (NIs) in nature and bind at active site of the enzyme. The non-nucleoside inhibitors (NNIs) bind at allosteric sites to bring conformational changes and inhibit polymerase activity<sup>[67,68,71]</sup>. These NIs have been reported to be effective against several HCV genotypes.

HCV replication is a complex process involving many other viral proteins simultaneous with NS3-4A, NS5A and NS5B. These proteins have been pursued as drug targets. Moreover, there are some non-enzymatic

proteins which also make a suitable intervention point. Although the exact function of NS4B is not very clear, it has been found as a good drug target<sup>[77]</sup>. NS4B also plays an important role in HCV RNA replication by forming membranous replication complexes. It has been observed that the C-terminal portion of NS4B is needed for functional HCV replication complexes<sup>[78]</sup>. Clemizole has been found as a potent inhibitor of HCV RNA replication. This agent blocks the binding of viral RNA to NS4B<sup>[79]</sup>.

Apart from viral proteins, some host cell factors also emerged as promising targets for antiviral therapy. Among host factors contributing to the viral replication cycle, we describe here two main factors that have been studied in detail, which are cyclophilins and miR-122. Cyclophilins A (CYPA) is the primary host factor and targeted by immunosuppressive drug cyclosporin A (CsA)<sup>[80,81]</sup> which inhibits HCV replication in cell culture<sup>[82]</sup>. The CYPA-CsA complex also inhibits calcineurin, involved in activation of T cells. Some CYPA antagonists have been developed. These compounds are Alisporivir, NIM811 and SCY635. miRNA-122 is another important host factor that was targeted for the treatment of chronic HCV infection. miRNA-122 stimulates HCV replication by stabilizing HCV RNA<sup>[83,84]</sup>, translates of the viral genome<sup>[85]</sup> and enhances RNA replication<sup>[83]</sup>. Naturally, targeting miRNA-122 by antagonist disrupts HCV replication *in vitro* and *in vivo*<sup>[86,87]</sup> and therefore becomes an effective target of therapy. miRNA-122 also shows the important role in hepatocyte lipid homeostasis and it may be taken into account when considering the therapeutic use of miRNA-122 antagonists.

#### *HCV assembly as target*

The experimental studies indicated that antiviral molecules act at different steps of HCV lifecycle. Also many cellular factors act as candidate targets. The inhibition of  $\alpha$ -glucosidases disrupts HCV assembly<sup>[88,89]</sup>. The  $\alpha$ -glucosidase inhibitors including UT-231B and Celgosivir (MX-3253-a castano-spermine prodrug), were used as assembly antagonists<sup>[90,91]</sup>. Identification of diacylglycerol O-acyltransferase-1 (DGAT1), the factor needed for core protein localization around LDs, indicates that DGAT1 may be a target for therapeutic intervention<sup>[92]</sup>. Although diacylglycerol O-acyltransferase-2 (DGAT2) is also involved in LD biogenesis<sup>[93]</sup>, HCV targets only DGAT1. Furthermore, DGAT2-generated LDs form normally in DGAT1 inhibitor treated cells. This shows a limited effect of DGAT1 inhibitors on the cellular functions<sup>[92]</sup>.

## **EFFECT OF VIRAL AND HOST COMPONENTS ON DRUG ACTION**

### **Baseline viral load**

When baseline viral load is less than 400,000-800,000 IU/mL, the course of treatment may be reduced to 24 weeks in genotype-1/4 patients and to 12-16 weeks in genotype-2/3 patients. Many studies have shown that low viral load (HCV-RNA, 600,000-800,000 IU/mL is a good predictor of SVR<sup>[94-96]</sup>. An increase in viral load decreases SVR rate.

### **Viral genotypes**

HCV has a total of seven genotypes with more than 50 subtypes and several quasispecies. Genotypes play very important roles in deciding the host response to anti-viral treatment. Patients infected with genotype-1, -4, -5, -6 respond worse than those with genotype-2/3 infection. Although, it is not fully established, it is believed that DAAs have better effect on non-responder genotypes like genotype-1. Using sofosbuvir drug it has been altered that when it is combined with the SOC regimen, there is a good impact on SVR, both in genotype-1 and genotype-2/3 patients<sup>[97,98]</sup>.

### **Interferon action**

Interferons are involved in host natural immune response against various pathogens including HCV<sup>[99]</sup>. Interferon binds with receptors on the target cells and activates signaling pathways like JAK-STAT pathway. This upregulates IFN-stimulated genes (ISGs) with expression of several types of antiviral effector protein<sup>[100-102]</sup>. This has been a basis of using IFN- $\alpha$  as an antiviral agent in chronic HCV infection<sup>[103]</sup>. However,

some studies have demonstrated that IFN- $\alpha$  based treatment of HCV infection is influenced by several factors including viral as well as host factors. Viral load and HCV genotypes were found to be important factors influencing IFN-therapy. HCV genotype-1 responded poorly to IFN therapy achieving SVR to near about 50% in comparison to HCV genotype-2 and -3 where SVR reached up to 85%<sup>[104]</sup>. It has been found that many HCV proteins interfere in the antiviral action of IFN- $\alpha$ <sup>[105]</sup>. Subsequently, it was noted that various HCV proteins including Core, E2, NS3/4A, NS5A/5B, antagonize antiviral effect of IFN- $\alpha$ . It may be illustrated more specifically in reference to individual HCV viral proteins. For example, HCV core induces expression of Suppressor of cytokine signaling-3 and -1 (SOCS-3 and SOCS-1), which antagonize IFN- $\alpha$  action by blocking JAK/STAT-pathway and ISGs expression<sup>[106,107]</sup>. HCV core also inhibits IFN induced phosphorylation and nuclear translocation of STAT-1. Binding of HCV core to STAT-1 decreases its phosphorylation and ISGs transcription<sup>[108,109]</sup>. Another important structural protein HCV E2 was also found inactivating IFN- $\alpha$  through inhibition of PKR<sup>[110]</sup>. This effect of E2 was detected prominently in patients infected with HCV-1 isolate. HCV genotype-2 and -3 could not show the same effect<sup>[110]</sup>. Of the nonstructural proteins, HCV NS3/4A was found to disrupt the IFN induction pathway. HCV NS3/4A protease cleaves various proteins including antiviral signaling proteins (MAVs)<sup>[111,112]</sup>, TIR domain containing adaptor inducing IFN- $\alpha$  (TRIF)<sup>[113]</sup> and adapter protein of RIG-1 TLR-3 signaling pathways *etc.* This cleavage disrupts not only innate immune response but also IFN-induction pathway, ultimately resulting in down regulation of the transcription of IFN-alpha inducible genes<sup>[114,115]</sup>. In addition, HCV NS4B and NS5A were also found to inhibit protective action of IFN- $\alpha$ . NS4B reduces IFN- $\alpha$  induced phosphorylation of STAT-1 and expression of IFN receptors. On the other hand NS5A binds and inactivates PKR<sup>[116-118]</sup>. Several studies have shown inhibitory effect of NS5A on IFN induced JAK-STAT signaling pathway<sup>[119-121]</sup>. NS5A usually blocks IFN-1 induced STAT-1 phosphorylation and its nuclear translocation resulting in downregulation of ISGs induced expression.

### **IL28B polymorphism**

Single nucleotide polymorphism (SNP) in IL28B gene present on chromosome 9 has an impact on HCV treatment response. The SVR rate of SOC in HCV patients carrying CC genotypes was 2-3 times higher as compared to the one with its clearance. There is high frequency of CC genotypes<sup>[122]</sup> in comparison to European and African. IL28B polymorphism is the best predictor of treatment response, better even than viral load, liver fibrosis, glucose level *etc.* EASL guidelines showed that IL28B polymorphism can be used to give a predictive value. Thus IL28B gene has a better predictive value in comparison to SOC and DAAs.

### **Hepatic steatosis**

Patients with hepatic steatosis usually do not respond well to HCV infection treatment. The presence of steatosis does not allow the EVR or SVR to attain in genotype-1 infected patients when treated with SOC. Similarly, steatosis affects negatively in patients infected with other genotypes. It causes relapse after discontinuation of treatment in patients with genotype-3. This all indicates that pathogenesis of steatosis differs in different genotypes and influences the treatment. In addition to all above factors influencing the treatment response, other conditions like age, insulin resistance, and metabolic syndrome *etc.* also have negative impacts on treatment.

### **Virological response to therapy**

The therapy of HCV infection is basically aimed to eradicate the virus and prevent the ensuing disease complications. The success of therapy is monitored by SVR rate which is defined as the absence of the HCV RNA in serum post 24 weeks of stoppage of treatment<sup>[123]</sup>. The value of SVR indicated not only eradication of virion from circulation but also correlates with symptoms<sup>[124-127]</sup>. The combination of PegIFN and ribavirin has been the SOC for all patients infected with HCV irrespective of viral genotypes<sup>[123]</sup>. This regimen produces SVR to 70%-80% in patients with HCV genotype-2 or -3 infection. However, SVR reached only 45%-70% in patients infected with other genotypes<sup>[123]</sup>. In recent trials of boceprevir and telaprevir in patients with cirrhosis it was noted that SVR was low in comparison to that in non-cirrhotic patients.

## Drug resistance

HCV is a highly variable virus with a large viral population and numerous quasispecies turnover in an infected individual. Its life cycle remains confined to the cytoplasm in cell with little possibility of its genome integration with host genome. Treatment of chronic HCV infection is based on the combination of PegIFN- $\alpha$  and ribavirin. The use of DAAs against HCV demonstrates that these agents may give rise to drug resistant viral species. These viral variants have different amino acid composition on target sites and so, are less susceptible to drug action<sup>[128]</sup>. In fact, the variants preexist before treatment, possibly arising from error prone activities of HCV-RNA dependent RNA polymerase (RdRp)<sup>[129]</sup> and rarely detected by current techniques. Drug exposure inhibits replication of the dominant drug-sensitive viral population to the level of appearance of resistant variants. *In vivo*, viral resistance is influenced by three major factors including the genetic barrier to resistance, *in vivo* fitness of the viral variant population and drug exposure. Different studies have indicated that the variants show resistance to NS3/4A protease inhibitors, nucleoside/nucleotide analogues, non-nucleoside RNA-dependent RNA polymerase inhibitors, NS5A as well as cyclophilin inhibitors<sup>[130]</sup>. In view of these alterations, the drug resistant variants may cause a serious challenge to infection and therefore, this problem needs a solution by more extensive investigations.

## CONCLUSION

This study concludes that the use of PegIFN- $\alpha$  and ribavirin is still a major part of standard of care (SOC) and the control of HCV infection. The addition of new drugs including DAAs, cyclophilins and miravirsin, *etc.* has made a significant improvement in SVR even in those patients where HCV genotypes remain resistant to PegIFN- $\alpha$  plus ribavirin drug regimen. These drugs target and inhibit viral proteases and cell receptor proteins as well as enzymes facilitating viral entry into the cell and viral replication and assembly inside the cell. A check on viral entry as well as their cell to cell transmission or further replication by the use of these drugs achieves the aim of treatment. In spite of an increase in SVR, the effect of DAAs is altered by the viral and cellular factors. Basic viral load and viral genotypes were found to show a significant effect on therapeutic outcome. Similarly, some disease conditions or cellular genomic polymorphism like IL28B polymorphism also have an impact on drug therapy. The development of drug resistant HCV variants during viral propagation still remains a serious challenge and needs to be resolved by different combination or development of new drugs. Studies are in progress looking towards new aspects of drug therapy against HCV infection.

## DECLARATIONS

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### Authors' contributions

All authors have made equal contributions in the performance of study, compilation of data and preparation of the manuscript.

### Availability of data and materials

Not applicable.

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None.

### Conflicts of interest

The authors declare that there are no conflicts of interest related to this study.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

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