

The Molecular Architecture of HCV: A review

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Abstract

Hepatitis C is an intricate liver disease. Its causative agent, hepatitis C virus (HCV) has been studied intensively for many years to spot out new therapeutic approaches. Even after more than 20 years since its discovery, HCV poses uninterrupted public health problem around the globe. HCV infection is present in approximately in 200 million people in world which includes Pakistan also. The treatment therapy of interferon alpha (IFN- α) plus ribavirin has failed to eliminate the virus completely from the body of patients. So no ideal treatment for HCV available that is equally effective against all type of HCV genotypes. The improved therapy is peginterferon and ribavirin (PR). Recently with combined therapy additional direct acting antiviral agents has been approved by FDA, which is recommended for genotype 1a are telaprevir and boceprevir. The molecular mechanism by which it is show resistance is still subject of investigation. HCV genotype 1a shows greater hindrance to treatment compare to other genotypes. This treatment regimen is not an ideal treatment for HCV, because the success rate is only 40 to 50 percent in case of infection of genotype 1 or 4, develops a SVR. The main key problem in the management of HCV is high mutation rates which produces diverse population of mutants called quasispecies. Variation in the quasi-species population confers remarkable potential on HCV to adjust according to the changing host situation and helps in evading the host immune system and in differential sensitivity to IFN treatment. NS5A protein is associated with interferon resistance. We have travelled through the era of Interferon (1991), ribavirin (1998) pegylated interferon plus ribavirin (2002) to triple therapy pegylated interferon + ribavirin + boceprevir and telaprevir (2011), quadruple therapy (2013) and now moving ahead to all oral quad therapy.

Keywords: Hepatitis C, oral quad therapy, HCV infection

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INTRODUCTION

HCV is a foremost public health worry affecting around 200 million persons around the world and over 4 million in the US, being a common blood-borne infection (Alter 2007). In 60-85% cases HCV extend to cirrhosis and hepatocellular carcinoma (HCC) (Shepard et al. 2005). In Pakistan almost 17 million persons are having it and 8-10% individuals are HCV carriers. The virus is mainly transmitted during exposure to, injection drug use, contaminated blood, blood transfusion and

insecure medical practices. Some other percutaneous exposures like ear piercing, skin scarification and tattooing are also linked with its transmission. (Alter et al. 1999). If HCV is not cleared during its acute phase, it progresses to chronic infection and causes rigorous liver injuries that may ultimately cause liver cirrhosis and hepatocellular carcinoma (HCC) (Hoofnagle 2002; Zoulim et al. 2003). To date, we lack a therapeutic or prophylactic vaccine available against this virus. The standard treatment for the chronically

infected HCV patients is polyethylene glycol-conjugated interferon (PEG-IFN- α) and ribavirin. However, treatment outcome of this therapy varies from person to person and also depends on the HCV genotype and Host IL28B genotype. Apart from this, the treatment also has severe side effects (McHutchison et al. 1998). HCV belongs to the Flaviviridae family and genus hepacivirus and was originally reported in 1989 (Choo et al. 1989). Its genome is positive sense single stranded RNA of almost 9.6 kb and holds a single open reading frame (ORF) that codes for the poly-precursor protein of almost 3000 amino acids. The ORF is flanked by un-translated regions that are 5'-3'. These un-translated regions are reported to be involved in virus replication and translation. The precursor poly protein produced is afterward sliced by viral and cellular peptidases into 10 different proteins. Out of these ten proteins, three are structural proteins and seven are non-structural proteins. The structural proteins are Core, Envelope1 and Envelope 2 and non-structural are P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. The NS5B gene codes for viral RNA-dependent RNA polymerase (RdRp). The RdRp does not have the capacity of proof reading during the process replication of virus and mutations are introduced across all the HCV genome. This accumulation of mutation is the basis for HCV genetic diversity. It is estimated that 10¹² virions are produced in chronically infected patients with HCV per day (Bartenschlager and Lohmann 2000).

The HCV viral RNA sequence variation provides the means for HCV classification. It is classified into genetically separate groups known as genotypes. They are more classified into subtypes, isolates and quasispecies (Martro et al. 2008). Currently HCV is classified into six main HCV genotypes approved i.e. Genotypes 1, 2, 3, 4, 5 and 6. The genetic variation among these genotypes at nucleotide and amino acid level is 30% (Martell et al. 1992). The further sub classification into subtype has nucleotide variation of 20 to 25 %. These genotypes vary in geographical division, transmission route

and treatment response. Genotypes 3a, 1a and 1b appear to have worldwide distribution due to their transmission through use of injectable drugs, blood transfusion and use of improperly sterilized surgical and medical equipments (Simmonds 2004).

Genotype 3a is common genotype in South Asia and Pakistan 1b and 1a in the Japan, US A and Europe, genotype 4 in Middle East, North and Central Africa, genotypes 5 in South Africa and genotypes 6 in Hong Kong (Simmonds et al. 1993). The prevalence of genotype 1a is at increase without any increase in genotype 3a. The Balochistan province has the highest percentage of 1a (4.03%). A recent study has reported from Lahore city, (23.6%) prevalence of genotype 1a. The best method of classification and genetic characterization is by entire genome sequence for subtype. The HCV Genotype is important because it determines the dose and duration of the current antiviral therapy against HCV. During replication, mutations arise randomly across the HCV genome due to lack of proof reading ability of RNA polymerase (Bukh et al. 1995). The quasispecies nature is associated with chronic infection caused by HCV and has significant inference for viral resistance to antiviral therapy, persistence, and pathogenicity (Farci and Purcell 2000). Variation in the quasi-species population confers remarkable potential on HCV to become accustomed with changing host immune system and in degree of difference sensitivity to IFN treatment. Sequence difference is not equally circulated alongside the entire HCV genome. Core and 5'UTR are greatly conserved, NS2, 3, 5b and 3'UTR are comparatively variable, while E1, E2, NS4 and NS5A genes show the maximum sequence diversity.

Hepatitis

Hepatitis is inflammation of liver and may be due to a group of viruses known as hepatitis viruses or because of toxins (notably alcohol), injury, drugs, genetic disorder, autoimmune process or autonomic condition and non-characteristic immune factors attack the

body's own liver cells. The common cause of which is hepatotropic viruses, Hepatitis A, B, C, D & E. These viruses basically infect the hepatocytes and starts replication in the liver cells, viral hepatitis is almost as primitive as known human history. Viruses that infect primarily hepatocytes (liver cells) and begin replication are in most cases hepatitis viruses where viral hepatitis is almost as old as known human history. Viral hepatitis is serious public health issue in most the parts of the world. It has high impact of morbidity and mortality in human being. Hepatitis may acute, chronic (persistent or chronic active). Some time the acute hepatitis develops into chronic states.

The treatment of hepatitis varies from specific medication to liver transplantation.

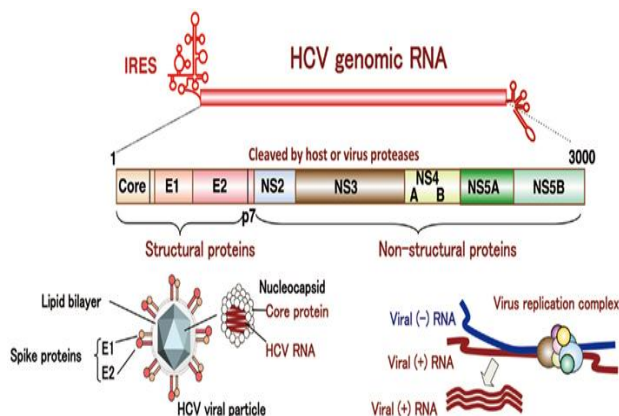


Figure 1- Structure of HCV (Moriishi and Matsuura 2012).

HCV is the key agent of acute and chronic hepatitis around the World. According to the WHO figures approximately that 200 million people, three percent of population is infected with HCV. Ten percent of Pakistani residents are infected with hepatitis. Mostly 75% of individuals having the HCV virus develops chronic infection at some stage, which later on converts into complex liver disease such as cirrhosis and fibrosis, hepatocellular carcinoma (Bostan and Mahmood 2010). The virus indirectly damages the liver, by interplay role between the virus and immune system, as a result of that the normal healthy liver tissue is damaged.

The earlier period of HCV

In middle of 1970s term of non-non-B hepatitis was introduced. Which illustrated inflammation of liver, but was not associated with hepatitis A virus or hepatitis B Virus. In 1975 reported by serological analysis 90%

cases at that time were neither HAV nor HBV and its transmission was linked with chimpanzee. After the development of animal model, it was known as tubule-forming agent, may be toga like or flavi like virus. The virus was characterized as an agent which less than 80 nm, having enveloped and sensitive to chloroform. The identification of HCV was made by direct molecular cloning from its genome. It was a team effort of seven years to report a positive stranded RNA virus (Shimizu et al. 1979).

Distribution and Mode of transmission

Around 200 million across the world are infected with HCV, including almost 10 millions of Pakistani. The most prevalent genotype is 3a and second to that is 1a. Prevalence depends on number of factor in various areas and different groups within a population.. Almost 49.3–64.0 million adults in Australia, Egypt and Asia are anti- HCV positive. China (13 million), Pakistan (10 million) and Egypt (6.5 million) number of HCV infected persons. Whereas the prevalence in Taiwan (4.4%), Pakistan (4.7%) and Egypt (15%), represented by figure 1 (Sievert et al. 2011). Some factor among them is poverty, sexual behavior, low rate of literacy, life style, use of drug but some of its association still needs explanation. The number of HCV infected cases are increases nearly 3% every year, whereas the cirrhosis infected patients are 20% and approximately 2-5% people dies of it.

Hepatitis C is transmitted by exposure of contaminated blood. Common use of injective therapies and illicit drug use has increased the rate of HCV infection in 20th Century. Besides that, dental exposure, occupational exposure to blood, sexual exposure, body piercings and tattoos from mom to offspring and common use of personal care products and blood transfusions (Idrees et al. 2008).

The genetic architecture of HCV

The HCV genome has a similarity with F Flaviviridae family. It has little sequence homology with flaviviruses and pestivirus, hence classified into genus hepacivirus, which different from flaviviruses and pestivirus of the Flaviviridae family .The size of HCV is 55-65nm, composed of core shell that has RNA as genetic material. The core

protein on its surface contains lipid envelope of cellular origin as presented in. Its basic six genotypes are further classified into 100 phylogenetically-distinct subtypes. The genome of HCV is of 9.6 Kb, linear, single strand RNA (ssRNA) and uncapped. The open reading frame is about 9,024 base pair translated into a polyprotein of 3010 amino acids. Processing of this polyprotein requires a combination of host cellular machinery and viral machinery resultant of structural and nonstructural protein. The nonstructural proteins are, NS2, NS3, NS4A, NS4B, NS5A, NS5B (Smith and Simmonds 1999). HCV is a member of genus Hepacivirus and is grouped in family Flaviviridae. In this family ssRNA is harbored in at nucleocapsid. The 9.6kb positive stranded RNA is translated into 3010 amino acids. The 5' and 3'UTRs play a vital role in signal transduction for RNA translation and replication. At the level of endoplasmic reticulum (ER) member pre polyprotein undergo co-translationally and post translationally changes to produce multiple structural and nonstructural proteins. The translation is needs internal ribosome entry spot present at 341-nucleotide of 5'UTR. The 5'UTR of ~341bp nucleotides are highly conserved. 5'UTR holds IRES that kick off translation of polyprotein in a cap- independent style. IRES combine separately to the 40S ribosomal subunit and instruct ribosome to initiate codon of the HCV mRNA in order to make easy translation in a cap-independent mode. The key facilitation of HCV RNA translation is made by structural domain I-IV. Whereas domain I and II has role in the HCV several replication, so 125 nucleotides of the HCV 5' N TR has vital role in RNA replication. The binary complex of small 40S ribosomal subunit is the initial step of translation start, through cross linking of ribosomal proteins. Recent studies of mouse hepatitis virus (MHV) has show that 5' 140 nucleotides contain three loops, stem- loop 1 (S L1), S L2, and SL4. S L1 and SL2 are necessary for subgenomic RN A synthesis. (Choo et al. 1991)

The 3'UTR of ~200-235bp is separated into three motifs/domains: a short variable region at the last part of the O RF (40 nucleotide), a poly- U/UC tract of heterogeneous length and a highly conserved 98 nucleotides X-tail

region. The 3'UTR has a function in replication. The polypyrimidine tract-binding protein (PTB) connects with 3'-end 98 nucleotides of HCV RNA tail region. The X region is highly conserved in all genotypes. Any deletions or a substitution in 3' UTR has impact on RNA stability, translation and replication. The stem- loop help in the identification of 3' end of the viral RNA by the viral RNA replicase (Friebe and Bartenschlager 2002).

Structural Protein

The cellular peptidases present inside the lumen of the endoplasmic reticulum (ER) slice the polyprotein precursor to set free three structural proteins: two envelope glycoproteins (E1 and E2) and the core protein. The first quarter of N-terminal of the polyprotein produces structural proteins respectively, Core (C) with basic nature basic, non-glycosylated nucleocapsid protein (191 amino acids- 21kDa) and E1 and E2 transmembrane glycoproteins (190, 370 amino acids, 33, 72kDa respectively). The ion channel protein p7 is of 63 amino acid s polypeptide monomer of two transmembrane alpha-helices, TM1 and TM2 in between the junction of structural and nonstructural gene. It oligomerizes, functions as ion channel and role in assembly and release of HCV Core protein has multi- functional viral protein, which interacts with number of viral and cellular protein . The processing of HCV core gene switched on by host signal peptidase, which converts it to functional protein. It also promotes the movement of core protein from ER membrane to the exterior lipid droplets, where the HCV particle is given finishing touch to produce virions (Li et al. 2012). Number of studies suggests that cellular lipid droplets are crucial for HCV. Core protein and lipid droplets are very important for morphogenesis of HCV infection . Functional core protein 1-169 (C(HCV)169) is dimeric alpha- helical protein, which can be divided into N-terminal domain (D1) with hydrophilic nature 1-117 (C(HCV)117) and the other C-terminal hydrophobic domain (D2), respectively two third and one third ratio. The D1 is of highly of basic charachteristic, homo-oligomerizatic and RNA binding protein and forms nucleocapsid. The D2 domain is compulsory for the folding of D1

and strength after the maturation at the site of endoplasmic reticulum membrane. The physical linkage of core protein with envelope glycoproteins E1 and E2 takes place at ER membrane showed by immunofluorescence study in transfected cells (Boulant et al. 2005; Boulant et al. 2006). In case of genotype 3 the core protein expression accumulation of fat is three times higher than genotype 1. The growth of hepatocytes is regulated by cellular proto-oncogenes and tumor suppressor genes. The N-terminal of core protein is potent inducer of apoptosis and necrosis compared to carboxyl terminal. Whereas the central region has less impact compared to both ends. So the different domains of core have separate function. The activation and deactivation of anti- or pro-apoptotic effect has also been reported through binding of HCV core protein to p53 (Kao et al. 2004).

E1-E2 are indispensable machinery of the virion and obligatory designed for viral entry. E1 and E2 are type-I transmembrane proteins with N-terminal ectodomain and C-terminal hydrophobic anchor that bring together as noncovalent heterodimers yielding the envelope glycoproteins of HCV. The glycosylated protein is product of amino acid (192-383, E1) and (384-746-E2) of predecessor polyprotein, respectively molecular weights (33-35kDa & 70-72kDa). The physical heterodimers of E1-E2 is the building blocks of viral envelope. Ectodomain of E1-E2 translocate to the ER lumen by transmembrane domains (TMDs), which undergo extensive modification by extensive N-linked glycosylation up to 6 and 11 potential glycosylation sites (Deleersnyder et al. 1997). TMD contains a signal sequence in C-terminal with a function of localizing of E1-E2 to the ER and assembling of the envelope proteins. Some of Glycan helps in the HCV glycoprotein folding during the virus entry. Residues 384-661 of E2 protein is a receptor binding domain; to extracellular loop of CD81 a tetraspanin located on hepatocytes and epithelial cells. The amino terminal of E2 is highly heterogeneous fragment recognized as the hypervariable region-1 (HVR1). The HVR1 domain which is 27 amino acids in length has an immunological importance of having epitopes for B and T Cells. So it

provides a possible target for antiviral molecules that may block its entry (Goffard and Dubuisson 2003; Dubuisson and Rice 1996).

The domain composed of amino acids 659-670 known as PePHD (PKR-eIF2 α phosphorylation homology domain) is responsible for inhibiting PKR indirectly cellular interferon. The envelope protein E2 has the capacity to induce apoptosis as of Flaviviruses, which induces apoptosis in cultured mammalian cells for persistent infection or by a mitochondrial damage-mediated caspase pathway. Lee and his colleague has also reported that E2 has the capability to lessen apoptosis by inhibiting TRAIL-induced cytochrome c discharge from the mitochondria, which may afterward increases persistent HCV infection (Lee et al. 2005).

The virus attaches with cellular membrane by E1-E2 glycoproteins by the configuration of non-covalent heterodimer or large disulphide linkages. The heterodimer glycoprotein complex has a function in the entry of host cells. These proteins are targeted to the endoplasmic reticulum (ER) by signal sequence in the preceding polypeptide and cotranslationally alienated from one and other by host signal peptidase cleavage (Forns et al. 2000). They stay behind fastened to endoplasmic reticulum membrane by a hydrophobic sequence present at their COOH-terminus (Carrere-Kremer et al. 2002; Steinmann et al. 2007). The p7, polypeptide composed of 63 amino acids is present between the structural and nonstructural protein. It links both structural and nonstructural region. It oligomerizes to give rise a heptameric cation channel in vitro. It consists of two trans-membrane domains at both its N- and C-terminals oriented towards the lumen of ER having a role in virus assembly or release in vivo. These two TMDs are kept apart from each other by a conserved loop important for ion channel function. The p7 has shown the ability to cross link as hexamers in HepG2 Cells]. The recombinant HCV p7 can also give rise to ion channels in artificial lipid bilayer membrane with a role in the ion transport between ER to the cytoplasm of HCV infected cells. The capacity of transportation has been blocked with a number of

iminosugar derivatives linked to long chains of alkyl groups, amantadine, viroporin inhibitors. It reflects a possible target for antiviral compounds. The nucleotide variability is present in p7 sequence with respect to genotype. For the reason amantadine was ineffective to check the HCV assembly in cultured cells compare to iminosugar (Griffin et al. 2003; Steinmann et al. 2007).

Non structural proteins

The HCV p7 and NS2 has a role in the assembly and discharge of nascent virions. NS2 has role in virus morphogenesis and infectivity. NS2 has a role in protease activity, which slices the NS2/NS3 at junction. The crystal structure of NS2(Pro), forms a homodimer with a role in dimerization. The role of NS2 is unidentified; however, before cleavage NS2 takes part in a protease activity accountable for the breakdown of NS2/NS3 junction. The dimeric cysteine proteinase (NS2-3) has two active sites and every active site has catalytic histidine (His952) and glutamate residues by a monomer and the nucleophilic cysteine (Cys993) of other monomer. The cellular chaperone HSP90 is important for the activity of NS2-3 proteinase (Lindenbach 2011; McPhee et al. 2012; Gallo et al. 2010). NS2 is composed of various stretches of hydrophobic amino acids having polytopic membrane protein by nature. The topology of NS2 is still unclear, only four transmembrane segments having documented. The processing of proteinase occurs in cytosolic region. It is short life protein and degradation takes place in phosphorylation pattern, by protein kinase-CK2. It also interacts by pro-apoptotic protein CIDE-B and blocks the CIDE-D directed apoptosis (Kim et al. 1996; Gouttenoire et al. 2006; Yao et al. 1999).

Non protease is a multi- functional protein with, C-terminal RNA helicase/NTPase domain and N-terminal serine protease domain. The amino terminal domain is chymotrypsin- like fold consisting of two beta barrel domains and a catalytic triad of His-57, Asp-81, Ser-139 (Li et al. 2005). For an increased activity it requires NS4A cofactor. The cofactor contributes one beta strand located at N-terminal protease domain, with tetrahedrally coordinated metal ion at active

site. The cofactor intercalates within the beta sheet of enzyme core. The NS4A make stable the protease from proteolytic degradation. The protease has shallow substrate binding sites and requires more surfaces for interaction with the substrate (Bartenschlager et al. 2004). Hence a good tool for inhibitors designs. Further this protease also participate in blocking the host cell capacity to initiate the innate antiviral activity (De Francesco and Migliaccio 2005) and interfere with dsRNA signaling pathway mean of proteolysis through interferon regulatory factor-III (IRF-III) activation, as result it is detached from the mitochondrial membrane and stoppage of signals regarding the anti- viral immune response (Li et al. 2005). In addition to NS3-4A also breaks the TRIF protein by cutting the signals of IRF-III activation (Tai et al. 1996).

RNA helicase

The carboxy-terminus of NS3 encodes RNA helicase, able of unwinding RNA-RNA duplexes in the presence of ATP. The enzyme is of Y-shape composed of three subdomains. With single domain it can bind with RNA and rest of the two domains are required for unwinding of RNA molecule (Yao et al. 1997). Kinetics studies have shown that the process of dsRNA unwinding takes place through highly coordinated cycles. The activity of helicase is less in isolated domains where as high in full length NS3 protein. Serine protease can modulate the helicase activity by interacting with its domains. This activity is enhanced in the full length NS3-4A complex. Helicase contribute in removing stable RNA secondary structure or displacing the attached protein which may interfere with RNA synthesis. It is possible aim for the design of anti-HCV molecules like manoalide, Asunaprevir and ACH-1625 (linear peptidomimetic inhibitor) (Serebrov and Pyle 2004; Pang et al. 2002). T324 amino acid linking domain 1 and 2 of the helicase is portion of flexible hinge for opening of ATP attachment. Number of assay is used for screening of anti-helicase molecules, e.g., fluorescence polarization, homogeneous time- resolved fluorescence assays and FP-based assay. NS3 protein cooperates with number of cellular proteins and is involved in carcinogenesis (Mukherjee

et al. 2012; Hanson et al. 2012; Tellinghuisen and Rice 2002).

It is NS4B transmembrane 27-kDa protein with hydrophobic in nature. It has a key role in viral replication. It consists of four domains. The N and C terminal are present in cytoplasm, whereas a portion of N-terminus is present in the ER lumen. NS4B protein can activate the transcription factor (ATF6) and inositol-requiring enzyme 1 (IRE1), to support the HCV viral replication. Further it induces intracellular membrane changes supporting the RNA replication (Welker et al. 2010; Hugle et al. 2001).

It contains nucleotide binding motif which binds and hydrolyzes GTP. Mutations in the motif alter the replication. Domains at both sides of ER membrane helps in cross linking between the ER lumen and the cytosol. The c-terminal part of the protein is very important for the replication and so a probable site for HCV replication inhibitors. NS4B restrains RIG-I- mediated IFN-beta creating signaling by protein interface with S TING]. This non structural protein may be another aim for Direct-acting Antiviral Agents (DAAs) (Einav et al. 2008; Einav et al. 2004). It is a NS5A phosphoprotein composed of 447 amino acid, (56-58 kd) with an amphipathic, alpha helix, with three domains, that is membrane binding domain, zinc binding domain and protein interacting domain. This protein exists as multiple phospho iso-forms present at cytoplasmic or peri- nuclear partitions of the cell, such as ER and Golgi bodies function such as viral replication, interferon response, apoptosis, pathogenesis and cell signaling are associated with this protein. The non structural 5b protein is allied through membrane by C-terminal trans- membrane domain (Bressanelli et al. 1999), vital for RNA replication. It is RNA dependent RNA polymerase associated with ER, key player for HCV RNA replication machinery. RNA template is used for the synthesis of RNA, initiated in de novo manner (Lesburg et al. 1999). The crystal structure shows a structural fold relevant to other polymerase with finger, palm and thumb sub-domains (Ago et al. 1999). The active site is present at palm domain, whereas modulation is made through finger and thumb subdomains. The enzyme is fully surrounded active site,

because of manifold connections between the finger and thumb sub-domains making a tunnel in single stranded RNA molecule is directly guided toward the active site. Another positively charged tunnel makes easy the entry of NTPs to the active site. Binding to the RNA template to start of RNA synthesis are synchronized by stretchy beta hairpin loop present at thumb domain, directed towards the active site. Rd- RNA polymerase activity is transformed by interaction of other viral protein, like NS3 and NS4. RNA binding activity is catalyzed at C-terminal by cyclophilin B, a peptidyl-prolyl- cis- trans- isomerase. Whereas cyclosporin A, inhibited it replication in cell culture. The cyclophilin B activity is dependent of genotype. N umber of various nucleotide, Nucleoside, and non nucleoside inhibitors are under investigation at various stages to inhibit HCV-NS5B-RNA-dependent RNA-polymerase (Sofia et al. 2012), such as Filibuvir (PF-00868554) (Troke et al. 2012), indole-based C-3 (Watashi et al. 2005).

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