Hepatitis C virus: Molecular biology & current therapeutic options

Suresh D. Sharma

Department of Biochemistry & Molecular Biology, Pennsylvania State University, Pennsylvania, USA

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Hepatitis C virus (HCV) is a small (~55 to 65 nm), spherical, enveloped, hepatotropic RNA virus that causes acute and chronic hepatitis in humans. Persistent virus infection with HCV often leads to cirrhosis and hepatocellular carcinoma (HCC). At present there is neither a selective antiviral therapy nor a preventive vaccine. The only available treatment option is a long-acting pegylated-interferon-alpha, given in combination with nucleoside analog ribavirin, which is not very effective. Molecular studies of HCV began with the successful cloning of its genome in 1989. For many years, research to develop therapeutics was stalled by the inability to grow virus in tissue culture. A major milestone was achieved with the recent development of a robust cell culture system for HCV propagation. HCV proteins assemble and form replication complexes on modified host membranes, called as membranous webs. Even though HCV is detected and targeted by host immune mechanisms, it establishes and maintains a life-long persistent infection. HCV has evolved multiple strategies to survive and persist in hostile cellular environments; and the viral population is known to rapidly change during the course of a natural infection thereby escaping immune surveillance. Rapid mutations also help virus to survive by selecting for the variants which are resistant to antiviral drugs. Although precise mechanisms regulating HCV entry into hepatic cells via receptors remain unknown, HCV also has the capability of direct cell-to-cell transmission. The extremely complex and incompletely understood nature of the HCV lifecycle has complicated the discovery of new therapies. A complete understanding of the functional roles played by the HCV proteins during HCV lifecycle is vital for developing a successful cure. This review deals with current status of efforts in addressing these daunting tasks and challenges in developing therapeutics against chronic and rapidly changing hepatitis C virus.

**Key words** HCV - hepatocellular carcinoma (HCC) - novel HCV therapeutics - pegylated-Interferon - ribavirin - structural and nonstructural proteins

**Introduction**

Hepatitis C is a complex liver disease. Its medical importance and the need to rapidly identify new therapeutic approaches has resulted in intensive study of its causative agent, hepatitis C virus (HCV). Humans are the only known natural hosts of HCV. Even after two decades since its discovery, HCV continues to be a major cause of concern and a huge burden on public health systems worldwide. The WHO estimates that a minimum of 3 per cent of the world’s population is chronically infected with HCV\(^1\,^2\).

HCV is a prototype member of the Hepacivirus genus (from the Greek hepar, hepatos, liver) and is further classified into at least seven major genotypes.
that differ by about 30 per cent in their nucleotide sequence. These genotypes (1, 2, 3, 4, 5, 6 & 7) show differences with regard to their worldwide distribution, transmission and disease progression3, 4. These genotypes have been further classified into sub-types (a, b, c, d, etc). In fact, HCV circulates in infected individuals as a population of diverse but closely related variants referred to as “quasispecies”.

HCV is most commonly spread by direct contact with infected blood and blood products. Availability of injectable therapies and drugs has had a remarkable influence on HCV epidemiology. The incubation period of HCV, though ranging up to several months, averages 6-8 wk. HCV infection is often asymptomatic, making it a very difficult to detect it at an early stage. This is the major reason why early treatment is difficult. Therefore, hepatitis C is often referred to as a “silent disease”. In a majority of infected people, virus infection does not resolve naturally. Neutralizing antibodies appear to be produced during the course of a natural infection, yet the virus mutates to escape surveillance5. When liver fails to clear the virus, the individuals become chronic carriers. However, within this chronically infected population the disease outcomes vary, it can be mild (minimal inflammation of the liver) or severe and can lead to scar tissue formation (Fig. 1). Chronic infection eventually causes cirrhosis leading to hepatocellular carcinoma (HCC) and ultimately death. Currently, there is no vaccine to prevent hepatitis C.

Liver steatosis occurs in more than 50 per cent of the patients with chronic hepatitis C (CHC) infection. Some individuals with CHC may report non specific symptoms such as fatigue, muscle aches, nausea and pain in the right upper quadrant. Antibodies directed against several HCV proteins can be detected in chronic patients. A variety of autoimmune and immune complex-mediated diseases have also been associated with chronic HCV infection, autoimmune thyroiditis being one of them6.

**Molecular Biology of HCV**

For many years the failure to replicate and produce authentic infectious hepatitis C virus in cell culture remained a major obstacle for innovative and cost-effective therapy. A breakthrough in the field came with the development of a complete in vitro cell culture system for HCV (JFH1) in 20057,8. JFH1 viral genome (genotype 2a), cloned from a Japanese patient with fulminant hepatitis could not only replicate efficiently in cell culture but could also generate viral particles. HCVcc (for HCV grown in cell culture) has allowed researchers for the first time to study the complete life cycle of HCV. Yet, owing to the limited host range of HCV, the development of a small animal model (to study viral replication and pathogenesis) is still a big challenge in the field.

HCV virions exhibit a wide range of densities, although the most infectious fraction has a density of 1.15-1.17 g/ml8,9. Present inside the outer envelope, there is a (30-35 nm) inner core which encapsulates the single-strand viral RNA (positive-sense), which is approximately 9.6 kb (Fig. 2). The HCV genome does not enter the cell nucleus. HCV- RNA replication occurs in the cytoplasm of hepatocytes. The genomic organization of HCV is shown schematically in Fig. 3. The viral-RNA genome harbours a single ORF (open reading frame) which is flanked by 5’ and 3’ non translated RNA segments (NTRs). The cis-acting replication elements or CREs are located in both the 5’ and 3’ NTRs and in the NS5B coding sequence10. RNA
The 5′- and the 3′-NTRs of the genome are highly conserved and contain control elements for translation of the viral polyprotein and replication. The 5′ UTR (+) is ~341 nucleotides in length and contains an internal ribosomal entry site (IRES). The HCV IRES is folded into four stem-loop motifs which are called as I, II, III and IV. The IRES is required for cap-independent translation of viral RNA, which is carried out by host cell ribosome. The domain IIIId of the IRES constitutes the key anchoring site for the 40S subunit11. The IRES domains III-IV have also been shown to be an activator of protein kinase R (PKR)12. However, this activation does not interfere with cap-independent translation of HCV viral proteins. HCV core protein was reported to interact with the 5′-NTR of plus-strand RNA13. However, recent work with JHF1 viral RNA suggested that its 5′-NTR (+) does not contain RNA packaging signals14 and authors further speculate that it may reside in the RNA region encoding the replicase.

The 3′-UTR (+) is around ~200nt and is involved in RNA replication. Three different domains can be recognized in this UTR: (i) a poly (U/UC) tract with an average length of 80 nucleotides (nt), (ii) a variable region, and (iii) a virtually invariant 98-nt X-tail region made up of 3 stem-loops (3′SL1, 3′SLII and 3′SLIII). The 3′-UTR can robustly stimulate IRES-dependent translation in human hepatoma cell lines15. Recent studies have recognized that various stem-loop structures exist in the negative strand 3′-NTR. This region is recognized by the viral polymerase as the initiation site for plus-strand synthesis of the HCV genome16. A recent study identified a cellular factor called Far-upstream element (FUSE) binding protein (FBP) which binds to 3′NTR by interacting with the poly(U) tract17. The importance of long-range RNA-RNA interactions in the modulation of HCV lifecycle has been well documented. Within the 3′-end of the non-structural protein 5B (NS5B) coding sequence, a cis-acting replication element (CRE) was discovered18. This CRE is called as SL9266 (or 5BSL3.2) and its disruption blocks RNA replication19. Mutual long-range binding with both 5′ and 3′ sequences is suggested to stabilize the CRE at the core of a complex pseudoknot20.

Non-coding RNA molecules or microRNAs (miR) are important in the control of gene expression and regulation. MicroRNA, miR-122 is specifically expressed and is found to be abundant in the human liver20. A recent discovery showed binding of a miRNA (miR-122) to the 5′-UTR of HCV. Sequestration of miR-122 in liver cell lines strongly reduced HCV translation, whereas its addition stimulated translation via direct interaction of miR-122 with two sites in the 5′-UTR21. These studies have generated a lot of interest in the role of miR-122 in HCV multiplication and its potential as a therapeutic target. A role for proteasome alpha-subunit PSMA7 in regulating HCV IRES-mediated translation has also been demonstrated22. These host factors require further scrutiny to be considered as candidates for drug targets.

HCV Structural Proteins

HCV encodes a single polyprotein (NH2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH) which is approximately 3010 amino acids (Fig. 3). The structural proteins (core, E1 and E2) and the p7 protein are released from the polyprotein after cleavage by host endoplasmic reticulum (ER) signal peptidase(s). The non structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are cleaved by viral proteases NS2-3 and NS3-4A. This proteolytic processing of the polyprotein during and after translation by host and viral proteases yield at least 10 mature viral proteins.

Core: HCV core is a multifunctional protein which is highly basic in nature23. It forms the structural component of the virus particle. Core has been implicated in the development of hepatocellular
steatosis and oncogenesis. The core protein is generated from a polyprotein encoded by the viral genome and is processed by cellular proteases in the endoplasmic reticulum (ER)\textsuperscript{24}. A recent study showed that core protein can self-assemble in HCV-like particles (HCV-LPs) in ER-membranes\textsuperscript{25}. A region of core protein (spanning amino acids 112 to 152) is essential for association, not only with the ER but also with the outer mitochondrial membrane\textsuperscript{26}. The HCV core protein is known to pass from the ER into mitochondria and is involved with Ca\textsuperscript{2+} regulation and apoptotic signals\textsuperscript{27}. The HCV core protein was shown to affect the steady state levels of a subset of mitochondrial proteins, including prohibitin (functions as a chaperon of mitochondrial proteins)\textsuperscript{28}. This interaction of core with the chaperon protein resulted in an increased oxidative stress due to perturbation of normal interactions between cytochrome c oxidase and prohibitin.

Core has many intriguing regulatory functions with one of the most important being recruitment of non structural proteins to the lipid droplet-associated membranes\textsuperscript{29}. Lipid droplets (LDs) are intracellular organelles involved in lipid storage and also take part in intracellular vesicular trafficking\textsuperscript{30}. HCV makes use of lipid droplets for replication. In infected Huh-7 cells, the core protein is associated with the surface of lipid droplets\textsuperscript{31}. Besides its structural and regulatory function, core plays an imperative role in the pathogeneses of liver steatosis. Upregulation of de novo fatty acid biosynthesis by HCV core protein in Huh7 cells has been reported. Core protein also interacts with apolipoprotein AI, a component of lipid droplets. HCV core protein is targeted to lipid droplets by its domain 2 (D2) and this association with lipid droplets is required for virus production. Disrupting the association of core protein with lipid droplets is deleterious for HCVcc production and this interaction is thought to contribute to steatosis, via deposition of triglycerides in the liver\textsuperscript{30}. These data are further supported by studies showing that the expression of core protein can lead to the development of steatosis in transgenic mice\textsuperscript{28}.

Clinical studies have reported that virus-induced steatosis is very severe with HCV genotype 3 than with other genotypes\textsuperscript{32}. Interestingly, core protein derived from genotype 3a induced higher fatty acid synthase activity than core protein derived from genotype 1b. However, no genetic or functional differences were observed between genotype 3a core proteins from patients with and without HCV-induced steatosis, thus, suggesting a possible role of other viral proteins in the development of hepatocellular steatosis\textsuperscript{33}. HCV-subgenomic replicon systems (which allow HCV-RNA to replicate autonomously), have shown subcellular localization of core to be both cytoplasmic and nuclear\textsuperscript{26,34}. A study with chronically HCV-infected liver also revealed a similar distribution, with core localized to both cytoplasm and the nucleus\textsuperscript{35}. In the cytoplasm the core protein is mostly localized to the endoplasmic reticulum (ER). HCV core protein, circulating ‘free’ in non enveloped state has also been detected in HCV-infected patients\textsuperscript{36}.

Transgenic mice expressing the core protein develop HCC, indicating a direct part played by the core protein in this process\textsuperscript{37}. A study found two mutations in the core gene (36G/C and 209A), which were linked with increased HCC risk\textsuperscript{38}. The core gene sequence data may provide useful information about HCC risk and more studies should be performed to develop it further. However, it is important to consider the type of genotype involved, as it is clear that gene expression profile in hepatocytes is dependent on the HCV-core-genotype sequence\textsuperscript{39}. Tumour suppressor protein promyelocytic leukaemia (PML) is known to be involved in antiviral response. Interestingly, a recent study suggests a potential mechanism for the development of liver cancer via the HCV-core mediated inactivation of the PML tumour suppressor pathway\textsuperscript{40}.

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**Fig. 3.** A schematic representation of HCV genome, structural and nonstructural proteins: The viral RNA consists of a 5' untranslated region (UTR) containing the internal ribosome entry site (IRES), followed by the genomic region for structural and nonstructural genes and the 3' UTR. HCV is translated as a polyprotein that is proteolytically processed by host and viral proteases\textsuperscript{20}. The structural proteins (C, core; E1, envelope protein 1; E2, envelope protein 2) are located at the amino-terminal end while the nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are located at the carboxyl-terminal end\textsuperscript{20}. The p7 protein (ion channel or viroporin) is located at the junction of structural and nonstructural proteins\textsuperscript{30}.
**ARFPs:** Alternate reading frame proteins or ARFPs are encoded by the +1 reading frame of the viral genome which overlaps with the core protein coding sequence. These additional proteins of unknown function are also called as core+1 or F1 and were recently identified. Since then multiple mechanisms have been proposed for the expression of ARFPs, which include (i) frame shifting (ii) form transcriptional slippage, or (iii) from internal initiation in the +1 open reading frame (ORF) of the core protein coding sequence. ARFPs have been shown to be associated with the ER and mitochondria. Interestingly, these proteins are labile and very short lived. Bases on the ability of ARFPs to bind to the proteasome subunit alpha3, it was suggested that it may regulate protein degradation in cells. However, the functional role of ARFPs in the HCV lifecycle is not clear yet.

**Envelope glycoproteins:** The HCV envelope glycoproteins, E1 and E2 are structural components of the virion. They constitute the outer coat of fine spike-like projections of the HCV particle (Fig. 2). They undergo post-translational modifications (N-linked addition of carbohydrate chains) while being translated in the endoplasmic reticulum (ER). Both, E1 and E2 envelope glycoproteins are required for host-cell entry via receptor binding. Insight into the mechanisms by which HCV gains entry into host cells is vital to understand primary HCV infection and re-infection post-transplantation.

The E2 protein has the binding sites for human CD81, a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes. The binding of E2 was further mapped to the major extracellular loop of CD81. CD81 along with human scavenger receptor SR-BI, and tight junction molecules claudin-1 (CLDN) and occludin (OCLN) are the most important receptors that mediate HCV entry. In addition, it is thought that HCV may utilize glycosaminoglycans and low density receptors on hepatocytes as initial attachment factors. Both CD81 and SR-BI were identified as candidate HCV receptors based on their physical interaction with a soluble version of E2. The HCVpp system was subsequently used to prove that they are required for viral entry. HCVpp system (also called as HCV pseudoparticle) generates virus particles, which display E1-E2 glycoproteins of HCV on their surface. Cell entry of HCVpp is HCV glycoprotein mediated. HCVpp are generated by co-transfection of 3 plasmids into 293T cells: (i) gag-pol genes of HIV or MLV, (ii) HCVgp, and (iii) a retrovirus genome with LTRs, packaging signals and a reporter gene. HCVpp system does not require productive HCV replication and hence is not restricted to Huh7 or Huh7.5 that support robust replication. HCVpp closely resembles the cell entry properties of genuine HCV virions and was used to identify CLDN and OCLN. The discovery of OCLN provides an vital advance towards efforts to develop small animal models for HCV.

HCV-like particles (HCV-LPs) were isolated from insect cells, infected with recombinant baculovirus expressing HCV structural proteins (E1, E2 and C). Further analysis of HCV-LPs by cryoelectron microscopy (CryoEM) revealed that they are spherical particles with smooth surfaces. They were found to be consistent with the native HCV isoforms isolated from HCV infected patients. HCV-LPs for the first time allowed 3D structural analysis of HCV particles, which further allows studying the complex mechanisms of HCV assembly and maturation.

A hypervariable domain near the amino terminus of E2 is the most variable part of the viral polyprotein and called as HVR-1. It has been shown to be the target for neutralizing antibodies. It is interesting to note that HCV can associate with LDL and VLDL from infected patient serum. Amazingly, HDLs play a very active role in HCV entry. A complex interplay between HDL, SR-BI and HCV envelope glycoproteins leads to enhanced HDL-mediated HCVpp entry in cells. In addition, HDL can inhibit HCV-neutralizing antibodies in serum of acute and chronic HCV-infected patients. E2 has also been shown to bind with CD81 receptors which are expressed on thyroid cell and induce a cascade of signaling pathway leading to IL-8 release. It is thought that E2 protein may induce thyroidal inflammation, thereby triggering thyroiditis by a bystander activation mechanism. Finally, E2 protein has been shown to bind PKR and as a consequence perturb innate immune pathways.

**HCV Non Structural Proteins**

The non structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B are thought to be to be required for replication of the viral genome. The non structural protein, p7, can form ion channels, required for the production of infectious virus particles. It is now recognized that the cross-talk between structural and the non structural proteins of HCV is required for efficient virus particle production.
The p7 ion channel: The HCV p7 is a small 63 amino acid protein, positioned at the junction of the structural and non structural proteins. The p7 protein belongs to a family of viral proteins called as viroporins that form ion channels. It can oligomerize following its inclusion into a lipid membrane creating ion channels. The cleavages mediated by signal peptidases between p7 and NS2 occurs slowly and are partial at the E2/p7 and p7/NS2 sites. This results in the formation of an E2p7NS2 precursor. It is thought that this precursor may have a role in the regulation of HCV lifecycle. Many models have been proposed to explain the potential significance of these precursor forms, the simplest being, the necessity for a timely release of the individual proteins at the appropriate time in the viral lifecycle. However, the precise role and existence of these precursors in a natural HCV infection is not known.

The p7 protein is highly hydrophobic in nature. It is localized in the ER-membranes when encoded by a replication-competent genome. It has two amphipathic, transmembrane regions, TM1 and TM2 (spanning amino acids 19-32 and 36-58) which are embedded in the ER-membrane. The N- and C-termini are exposed to the extracellular environment. The 3-dimensional structure of p7-complex was determined by using single-particle electron microscopy. This hexameric (42 kDa) protein complex was found to depict flower-shaped architecture with protruding petals oriented toward the ER lumen. The p7 ion channel protein serves an essential role in the production of infectious virus particles during HCV lifecycle. It appears to be dispensable for viral RNA replication, as replicons lacking the p7 gene replicate or make viral RNA efficiently.

The ion channel blocker, amantadine, was thought to interfere with the ion channel activity of p7 based on studies with artificial lipid bilayer system. The p7 protein could form amantadine-sensitive ion channels in this artificial system. However, it became clear after clinical trials, that p7 ion channel function is not affected by amantadine. Another study also reported similar findings, where HCV strains were found resistant to the ion channel blocker, amantadine. Nonetheless, dose-dependent reductions of virus titres were achieved with iminosugars. The absolute dependence of HCV on- ion channel function of p7 protein for infectivity makes it an attractive candidate target for antiviral intervention.

HCV NS2: The non structural protein 2 (NS2) is a 23-kDa transmembrane hydrophobic protein. The membrane association of NS2 is p7-independent and occurs co-translationally. NS2 is a membrane-associated cysteine protease, required for HCVcc infectivity. The cleavage between NS2 and NS3 is absolutely required for persistent viral infection in a chimpanzee. NS2 followed by only 2 amino acids of NS3 produces a basal proteolytic activity in vitro. However, the N-terminal 180 aa of NS3 are required besides NS2 for a robust protease activity. Interestingly, all the active site residues (H952, E972 and C993), needed for the catalytic activity of the NS2/3 cysteine protease, are located entirely in NS2. This requirement of NS3 remained intriguing until the recent discovery showing that the zinc binding domain of NS3 could in fact stimulate the protease activity of NS2. The functional sub-domains in NS3 essentially function as its regulatory cofactor again highlighting tight regulation of the proteolytic processing. This process is undoubtedly vital for virus multiplication.

NS2 interacts with itself forming homo-dimers. Moreover NS2 has also been shown to interact with all the other HCV non structural proteins. Until recently the only known function for NS2 was its autocleaving activity at the NS2/3 junction. Expression of NS2 in Huh7 cells resulted in upregulation of transcriptional factor sterol regulatory element-binding protein 1c and fatty acid synthase. These studies implicate a role of NS2 in promoting steatosis. NS2 protein can be phosphorylated by casein Kinase II on Ser residue at position 168. This phosphorylation event appears to regulate the stability of NS2 protein (at least from genotype 1a). NS2 also appears to be involved in a particle assembly step which happens post core, NS5A, and NS3 assembly. The search for molecules or inhibitors targeting NS2, should without a doubt, advance the development of new therapeutics against HCV.

HCV NS3: The non structural protein 3 (NS3) is a member of the superfamily 2 DExH/D-box helicases and its crystal structure has been determined. It is a 67 kDa tri-functional protein with a serine protease, an RNA helicase and NTPase activities. The NS3 enzyme has a chymotrypsin-like serine protease activity. Along with its cofactor NS4A is responsible for cleavage at the NS3/4A, NS4A/4B, NS4B/5A and NS5A/5B junctions. NS3/4A are localized in ER cisternae surrounding mitochondria. However when co-expressed with p53 (tumour suppressor), it is
localized along with p53 in the nucleus. NS3 unwinds both RNA and DNA substrates, although there is no DNA intermediate involved in HCV lifecycle. It couples unwinding of RNA strands (with extensive secondary structures) to ATP hydrolysis. A direct interaction between NS3 and NS5B occurs through the protease domain of NS3. RNA unwinding activity of NS3 helicase is modulated by this interaction with NS5B polymerase. In addition to its role in viral polyprotein processing and HCV multiplication, another important function of NS3 involves antagonizing host innate-immune pathways.

The induction of type I interferon (IFN) genes (Type I IFNs include several IFN-α subtypes and a single IFN-β subtype) is regulated at the step of transcription and is best understood for the IFN-β promoter. Innate immune defense mechanisms activated by alpha/beta IFNs represent an essential first line of protection against viral infections. Retinoic acid inducible gene or RIG-I is a cytoplasmic RNA helicase. It is an essential pathogen recognition receptor (PRR) for HCV. RIG-I upon binding viral RNA undergoes changes in conformation and can then interact with IFN-β promoter stimulator-1 or IPS-1 (IPS-1 is also known VISA, Cardif or MAVS)49. This interaction of RIG-I with IPS-1 can signal downstream activation of IRFs and NFkB to trigger alpha/beta-IFNs.

The localization of MAVS to the mitochondria is critical for its ability to induce IFNs. This function of MAVS is abolished if the mitochondrial-targeting domain of MAVS is deleted55. HCV NS3/4A protease has remarkably evolved to target and cleave ISP-1 (or MAVS at Cys-508), thereby halting alpha/beta interferon expression56. This activity may be necessary but does not appear to be sufficient for long-term viral persistence since there is (cytotoxic) T cell-mediated clearance of NS3/4A-expressing hepatocytes in vivo57. Therefore, other HCV proteins are most likely responsible for interfering with the adaptive immunity.

A recent study utilizing sub-genomic replicon demonstrated that besides methylation (which regulates helicase activity), NS3 undergoes other post-translational modifications, such as phosphorylation and N-terminal acetylation58. The multifunctional roles played by NS3 in the lifecycle of HCV, makes it indispensable for virus multiplication. NS3 therefore represents a very promising target for anti-HCV therapy.

**HCV NS4A:** Non-structural protein 4A (NS4A) is a cofactor for NS3 serine protease activity. The N-terminal portion of 4A is responsible for membrane association of the NS3-4A complex. NS4A also associates with other HCV proteins on the ER-derived membranous webs, where viral replication complex is assembled. Interestingly, NS4A is localized not only on the ER, but also on mitochondria44. Thus, in addition to its essential role in HCV replication it is also involved in viral pathogenesis by affecting cellular functions. Further, a study reported altered intracellular distribution of mitochondria and its damage due to NS4A expression in Huh7 cells55. The role played by this small polypeptide during HCV replication is very significant.

**HCV NS4B:** The HCV non structural protein 4B (NS4B) is a 27-KDa polypeptide. It is a highly hydrophobic integral ER-membrane protein66. It contains four transmembrane domains and is palmitoylated at two C-terminal cysteine residues. Palmitoylation of 4B protein facilitates oligomerization which appears to be essential for HCV replication67,68. It has an amphipatic helix at the N-terminal and a C-terminal domain which are both associated with membranes69. Allelic variation in the NS4B sequence between closely related HCV isolates was found to drastically impact HCV replication in cell culture60. A characteristic feature of Plus-strand RNA viruses is their ability to induce alterations in cellular membranes and then utilize it to replicate their own genomes.

Back in the 1980s, cytoplasmic changes were seen in hepatocytes of infected chimpanzees with the help of electron microscope. Sponge-like inclusion composed of a dense matrix and irregularly arranged membranes were reported71. Recently, Egger et al72 showed that NS4B induced a distinct membrane alteration when expressed in cultured human osteosarcoma (U-2 OS) cells. Thus, a protein which had no known function until then was shown to induce a tight structure, designated membranous web, consisting of vesicles in a membranous matrix. The immunoelectron microscopy of Huh7 cells supporting replication of sub-genomic
replicon revealed a loss of the organization and other morphological alterations of the ER showing convoluted cisternae and paracrystalline structures\textsuperscript{77}.

The replication of HCV is a very intricate process, occurring through protein–RNA and protein–protein interactions. HCV-replication-complex was first identified in Huh7 cells supporting sub-genomic replicon in 2003\textsuperscript{93}. These membranous webs are made up of small vesicles (80-180 nm in diameter) embedded in a membranous matrix and are found closely associated with the rough endoplasmic reticulum. NS4B co-localized within this web together with other structural and NS proteins\textsuperscript{92}. Interestingly, NS4B alone could induce the formation of membranous web. In vitro expression studies of NS4B from all major genotypes have demonstrated the importance of N terminus of NS4B\textsuperscript{84}. Disrupting the amphipathic-helix in the N terminus of NS4B abolishes the ability of NS4B to rearrange membranes. Even before an important role in formation of membranous web like structures was discovered for this interesting protein, a role for NS4B in malignant transformation of NIH3T3 cell in association with the Ha-ras oncogene had been proposed\textsuperscript{95}. It is an important protein for HCV lifecycle.

\textit{HCV NS5A:} The non structural protein 5A (NS5A) has RNA-binding activity\textsuperscript{96}. NS5A protein can be found in basally phosphorylated (56 kDa) and hyper-phosphorylated (58 kDa) forms in cells. P58 form of NS5A is hyperphosphorylated at additional sites that remain unmodified in the p56 form by casein kinase I-alpha\textsuperscript{97, 98}.

The first of its kind, the sub-genomic replicon utilized a HCV genotype-1b clone, called as Con1. This clone was engineered such that HCV-structural genes were replaced by a neomycin resistance gene. Following transfection of \textit{in vitro} transcribed RNA into Huh-7 cells, antibiotic G418-resistant cells could be obtained in which the sub-genomic RNA replicated autonomously. Mutations that enhance the capacity of sub-genomic HCV RNA to replicate in cell culture (Huh-7 cells) were mapped to the NS5A-coding sequence and were called as adaptive mutations. The adaptive mutations alter the phosphorylation state of the NS5A protein. Loss of hyperphosphorylation (or NS5A-p58 form) stimulates RNA replication of the HCV genotype 1b replicon in Huh-7 cells. Human vesicle-associated membrane protein (hVAP) subtype A is known to be a host factor essential for HCV replication by binding to both NS5A and NS5B. Phosphorylation status of NS5A is also known to affect the interaction of NS5A with VAP-A\textsuperscript{99}. NS5A inhibitor (BMS-824) and the NS3 protease inhibitor block hyperphosphorylation of NS5A; however, the mechanism of inhibition remains unknown. Further, the involvement of a protease inhibitor in inhibiting p58 is quite intriguing\textsuperscript{100}.

NS5A has an amphipathic alpha-helix at its amino terminus with which it is anchored to the ER membrane. NS5A is further divided into three-domains which are separated by linker regions. The crystal structure of the conserved domain I (DI) was solved recently and revealed a unique fold. DI has a zinc binding motif and also forms a nucleic acid-binding domain. NS5A specifically binds to the G/U rich sequences in the 3’ ends of HCV genomic RNA\textsuperscript{96}. Domains II (DII) and III (DIII) are more variable among HCV genotypes. Crystal structures for both DII and DIII are not available yet. NMR studies have shown DII of NS5A to be flexible and disordered. NS5A-DII contains the PKR and the HCV-NS5B binding domains. A role for NS5A-DIII in virus assembly was shown via phosphorylation of a serine residue at position 2433 by CKII\textsuperscript{101}. NS5A domain III is not required for RNA replication as sub-genomic replicon lacking DIII, replicate in Huh7 cells. However, a recent study with JFH1 virus showed that DIII of NS5A could influence RNA replication. Genotype 2 HCV isolates have a 19 residue insertion near the C terminus of DIII. Upon its deletion, authors observed a delay in both RNA replication and particle assembly\textsuperscript{102} thereby suggesting a role in viral multiplication.

Full length HCV NS5A has a cytoplasmic localization. Cleavage by caspase 3 and 6 leads to generation of N-terminally truncated NS5A fragments which get localized within the nucleus. The C-terminal domain of NS5A can associate with c-Raf kinase\textsuperscript{103}. This interaction appears to be essential for HCV replication in Huh7 cells, as sequestration of c-Raf by truncated NS5A into the nucleus, negatively impacts HCV replication\textsuperscript{104}. Oxysterol binding protein (OSBP) can interact with the N-terminal region of DI. OSBP is co-localized to the golgi with NS5A and this functional interaction is suggested to play a role in HCV particle release\textsuperscript{105}. A significant association between variations in sequences between 2209-2248 nucleotides of NS5A-gene, and response to interferon treatment has been proposed. This region of NS5A (amino acids 237–276) is called as ISDR or interferon sensitivity determining region. The ISDR was also found to associate with the antiviral molecule, PKR. Patients with a mutant-type
NS5A-ISDR had a higher rate of sustained virologic response (SVR) than those with the wild-type NS5A-ISDR. However, recent studies have questioned the existence of an ISDR in NS5A. Existence of an ISDR is an ongoing debate and controversial. Nevertheless, NS5A can repress the PKR pathway. NS5A also has the ability to inhibit IFN-gamma production. A remarkable study with NS5A transgenic mice suggested that NS5A protein could impair both the innate and adaptive hepatic immune response. Indeed, NS5A has attracted tremendous attention in the HCV field and represents a very promising target for anti-HCV therapy.

**HCV NS5B:** The HCV non structural protein 5B (NS5B) is a RNA dependent RNA polymerase (RDRP) containing the GDD motif in its active site. It belongs to a class of integral membrane proteins termed tail-anchored proteins. NS5B initiates synthesis of complementary negative-strand RNA using the HCV genome (positive polarity) as a template. Subsequently it generates positive-strand RNA from this negative-strand RNA template. The NS5B crystal structure shows the typical fingers, palm and thumb sub-domains. NS5B is anchored to the ER-derived “membranous webs” via its C-terminal 21 amino-acid residues. Studies with sub-genomic replicon revealed that the membrane association is indispensable for viral RNA replication. In vitro NS5B cannot distinguish between natural and synthetic templates. NS5A can directly bind to NS5B and modulate its polymerase (poly A template/poly U primer) activity. NS5A (at sub-stoichiometric levels) stimulates replication by NS5B on templates derived from the 3’ end of the positive strand. NS5A stimulates NS5B during elongation; however the mechanism of action is not clear. It could either be due to the stimulation of its catalytic activity or due to a conformational change induced by NS5A binding. There appears to be a difference in polymerase specific activity in vitro, depending on the type of HCV genotype. The JFH1 NS5B enzyme shows a 10-fold higher specific activity when compared to J6 NS5B. The termination of RNA synthesis in vitro is not understood well.

NS5B lacks a “proofreading” function. Due to a high rate of error-prone replication, complex mutant swarms are generated. However, HCV must also maintain highly conserved genomic segments and a balance between conserved and variable viral elements is above all important to avoid “error catastrophe”. Over the past few years numerous nucleoside and non-nucleoside inhibitors of the polymerase have been discovered and demonstrated clinical efficiency and advanced to clinical trials. NS5B nucleoside inhibitors (NIs) or analogs (NM-107/NM-283, PSI-6130/R7128, IDX184, MK-0608) compete with cellular ribonucleoside triphosphates act as functional chain terminators. Non-nucleoside inhibitors (NNIs) or analogs (ABT-333, GSK625433, VCH-759, PF-868554, GS-9190) act by allosteric mechanism. They bind to allosteric sites on NS5B and thereby inhibit RNA synthesis.

An important lead was provided by a study which established that NS5B associates with cyclophilin A (CypA) via its enzymatic pocket and exploits the isomerase/chaperone activity of CypA to replicate in cells. NS5B is phosphorylated by the protein kinase C-related kinase 2 (PRK2) in cell culture. It was further discovered that inhibitors of NS5B phosphorylation, HA1077 (fasudil) and Y27632 suppressed the activation of PRK2. The treatment of liver cell lines (stably replicating HCV sub-genomic replicon) with these inhibitors cleared viral RNA suggesting that PRK2 inhibitors act by suppressing HCV replication via inhibition of NS5B activity. However, it would be interesting to see if resistant mutants emerge over a period of time. More critical would be the normal functions affected which are regulated by PRK2.

The continuous generation and selection of resistant variants allows HCV to escape control by these inhibitors/antiviral drugs. A study where patients infected with genotype 1a were treated with ribavirin, led to the emergence of a Phe-to-Tyr (F415Y) mutation in the viral polymerase. There is also evidence for recombination events leading to genetic variation in HCV. Thus, very high rates of genetic variation ensure the survival of hepatitis C viruses under every changing hostile cellular environment.

**Current Therapy**

The very first treatment shown to be effective against HCV involved systematic administration of INF-α. INFs are produced naturally by host’s immune system in response to a viral infection. INF was actually discovered in 1957, during studies with influenza virus. Through the years HCV treatment has improved with the development of modified pegylated-interferon (PEG-INF), which is a stabilized version, has longer biological half-life. A SVR is defined as negative HCV RNA, six months after treatment. INF is known to activate several direct and indirect antiviral
mechanisms (such as viral RNA degradation, halt viral translation). However, the antiviral mechanism of IFN \textit{in vivo} remains unknown.

Ribavirin was first used in treating children with respiratory syncytial virus infection\textsuperscript{122}. It was discovered more than 30 yr ago and is effective against HCV, only when administered along with interferon in combination therapy. Ribavirin (1-beta-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) is a purine-analog with broad-spectrum antiviral activity\textsuperscript{123}. Administration of ribavirin in combination with PEG-INF-alfa-2a (40kDa) to HCV infected patient’s results in an increased SVR rate\textsuperscript{124,125}. The mechanisms or pathways involved, leading to anti-HCV effects are not fully understood yet. Some of the proposed mechanisms include: modulation of interferon-stimulated gene expression, inhibition of host inosine monophosphate dehydrogenase (by ribavirin monophosphate), modulation of the host immune responses, direct inhibition of the viral RNA polymerase (by ribavirin triphosphate) and lethal mutagenesis of HCV-RNA genomes caused via incorporation of ribavirin triphosphate by NS5B\textsuperscript{126,127}. Thus, ribavirin can be a substrate for the HCV RNA polymerase although surprisingly it is not very effective in monotherapy\textsuperscript{128}. Therefore, pegylated interferon (PEG-INF) and ribavirin combination has become the current “gold standard” regimen or treatment of choice for hepatitis C.

Unfortunately, both these compounds are toxic and their administration causes side effects such as headache, fever, severe depression, myalgia, arthralgia and haemolytic anaemia. Moreover, the cost of therapy is very high. Several months of therapy is required for eradication of chronic HCV infection, despite which SVR is often not achieved. In summary, the existing treatment is not very effective and causes significant side effects. Development of better and improved treatment is required urgently and represents a major challenge for virologists.

In addition, responses to treatment vary widely depending on the genotype of HCV. Genotypes 1 and 4 are the most difficult-to-treat. Only 40-50 per cent of the patients achieve SVR with genotype 1 infections. Age is also an important factor as older patients exhibit lower response to therapy than younger patients. Moreover, current available treatment options for non responders are very few. The factors determining the triumph or failure of the antiviral therapy are not well understood. A clear understanding of the function of genes involved in the IFN-α pathway could possibly explain some of these leading questions. This insight and further research exploiting a relationship between therapy response and host genetic polymorphisms in the IFN-α pathway may lead to the development of new therapeutic strategies\textsuperscript{129}.

**Current State of Antivirals**

At present, there is no approved (HCV specific) antiviral therapy. However, search for “specific targeted antiviral therapy for HCV” (STAT-C) has been ongoing for a long time. Advances in the model systems such as: (i) sub-genomic and genomic replicons, (ii) HCVpp, (iii) HCVcc-JHF1, (iv) HCV-LPs to study HCV have further improved our understanding of HCV lifecycle and potential therapeutic targets. The latest development being, the ability to generate HCV particles that are infectious both in cell culture and \textit{in vivo} with the Con1 wild type genome\textsuperscript{34}. We are approaching a very productive as well as challenging phase in the field of HCV virology. Wide varieties of inhibitors or directly acting antiviral agents are at different phases of development and some are currently in clinical trials. These inhibitors target HCV receptors, HCV-RES, NS3/4A, NS5A and NS5B. In addition molecules targeting host factors which play a role in HCV replication, are also being pursued.

HCV enters into hepatocytes by interacting with cell surface receptors. These initial events in virus lifecycle offer an attractive target for antiviral development. The lectin cyanovirin-N (CV-N) was originally discovered against HIV and subsequently also shown to be a potent inhibitor of HCV. It binds to envelope glycoproteins and inhibits the interaction between E2 and CD81\textsuperscript{130}. This suggests that targeting the glycans of viral envelope proteins holds potential in the development of antiviral therapies. Another interesting class of inhibitors such as celgosivir, n-butyl deoxynojirimycin and N-(n-nonyl) deoxynojirimycin target host alpha-glucosidase enzyme and may offer alternative treatment options\textsuperscript{131,132}. Alpha-glucosidase enzyme plays a critical role in HCV maturation by initiating the processing of the N-linked oligosaccharides of viral envelope glycoproteins\textsuperscript{133}. Thus their inhibitors can affect HCV morphogenesis. This was further confirmed by using HCV-LPs, in the presence of alpha-glucosidase inhibitors, viral glycoproteins were misfolded, retained in the ER and also impaired in their interaction with calnexin\textsuperscript{134}. Thus, this novel class of inhibitors can target host-directed glycosylation and may prevent the appearance of drug
resistance when given in combination with the current standard of care\textsuperscript{132}. Celgosivir (rapidly converted to castanospermine) is currently undergoing clinical trials.

A study where phosphatidylinositol 4-kinase type III-alpha (PI4KIII-\(\alpha\)) was knocked down prevented infection by HCVpp or by HCVcc (JFH-1). In addition, the susceptibility to HCVpp infection and replication in cells was dependent on the presence of phosphatidylinositol 4-kinase type III-beta (PI4KIII-\(\beta\))\textsuperscript{135}. Another study showed that PI4K-III-\(\alpha\) is involved in events vital for membrane alterations, which are essential for the formation of HCV replication complexes\textsuperscript{136}. PI4K-III-\(\alpha\) also plays an essential role in membrane alterations leading to the formation of HCV replication complexes. PI4KIII-\(\alpha\) and \(\beta\) are very important host factors for HCV replication. These kinases are potential therapeutic targets.

Other recent studies have demonstrated that iridoids (such as glycones of shanzhiside methyl ester: loganin, verbenalin) and amphipathic DNA polymers (APs) are novel antiviral molecules which inhibit HCV entry. Inhibitors loganin and verbenalin were shown to have anti-HCV entry and anti-infectivity activities\textsuperscript{137}. APs inhibit HCV internalization owing to their amphipathic nature. Thus APs target the cell entry step of HCV. A recent study showed that CD81 has no role in the cell-to-cell spread of HCVcc. In addition, this mode of transmission appears to be shielded from neutralizing antibodies. This study highlights the urgent need to develop strategies aimed at disrupting direct cell-to-cell HCV transmission as targeting cell-free virus alone may not be enough to halt a chronic infection and a dual approach is required\textsuperscript{138}.

The significance of targeting NS3-4A protease is clearly evident from its vital function, required to process viral polyprotein. Targeting NS3-4A has additional advantage, since NS3-4A protease also functions to inhibit host membrane response by cleaving IFN-\(\beta\) promoter stimulator-1 (IPS-1) and TRIF\textsuperscript{139}. The latter activity of NS3 affects signaling of the RIG-1 and Toll-like-receptor-3 pathways, thereby perturbing the interferon response. A specific inhibitor of NS3, telaprevir (VX-950) was put to clinical test recently with some success. The current thought in the field being that a combination therapy along with inhibitors which simultaneously inhibit multiple steps in the virus lifecycle (protease and replicase functions) may yield better SVR. Indeed, recent clinical trials where PEG-IFN and ribavirin were administered along with telaprevir, showed significantly improved SVR rates in patients with genotype 1, which happens to be the most difficult to treat\textsuperscript{140}. However, there were higher rates of discontinuation in the treatment because of the adverse effects such as pruritus, rash, and anaemia.

Another study which involved administration of interferon with telaprevir highlighted the importance of retaining ribavirin during the treatment. As response rates were lowest when ribavirin was not present in the treatment\textsuperscript{141}. There is supporting evidence of a decreased SVR and relapse when ribavirin was prematurely discontinued or ribavirin doses were frequently missed. However, the major drawback of ribavirin is side effects. Further research is required to advance, refine and develop non-toxic versions of these inhibitors. There is some progress in this direction and several ribavirin analogues (such as 4-iodo-1-beta-D-ribofuranosylpyrazole-3-carboxamide, 4-propynyl-1-beta-D-ribofuranosylpyrazole-3-carboxamide, and 4-phenylethynyl-1-beta-D-ribofuranosylpyrazole-3-carboxamide) are being evaluated and could offer an alternative and better therapeutic option\textsuperscript{142}.

NS5A plays a vital role in HCV replication and is emerging as an important target for the therapy of HCV infection. BMS-824 is a small molecule which targets NS5A and inhibits hyperphosphorylation or p58 formation\textsuperscript{100}. Inhibitors that disrupt phosphorylation with a concomitant decrease in virus replication are indeed very attractive and should be developed further. Another molecule, zinc mesoporphyrin (a synthetic heme analogue with a central zinc of the mesoporphyrin macrocycle) causes a dramatic downregulation of the NS5A protein by enhancing its polyubiquitination and proteasome-dependent degradation. Zinc mesoporphyrin holds immense potential as a novel drug to treat HCV infection\textsuperscript{143}.

HCV polymerase is an attractive target owing to its essential role in viral RNA replication. It is beyond any doubt the most validated target for the development of antiviral therapy. Valopicitabine (NM-283) was the first NS5B analogue to demonstrate proof of concept for 5B nucleoside inhibitors in the clinic. Currently only Roche-1728 NI is under active development and clinical trials\textsuperscript{144}. Idenix NI IDX-184 is under phase I trial in patients infected with genotype 1. Another NI from Merck, MK-0608 is in preclinical trials. The NIs can be utilized by the polymerase once converted to the 5'-triphosphate form. NNIs on the other hand
are non-competitive inhibitors which target the viral polymerase, preferentially in the initiation phase. A NNI, PF-00868554 (Pfizer) is currently in phase II clinical trials where it is given along with PEG-INF and ribavirin. GS-9190 (Gilead) is under development and currently a study with VCH-759 (ViroChem Pharma) is ongoing. Several NNIs such as VCH-916 (ViroChem Pharma), GSK-625433 (GlaxoSmithKline) and ABT-333 (Abbott) have reached phase I clinical trials.

A further advance came with the concept of administering inhibitors of both, viral and host proteins. This seems a very intelligent choice for a rapidly mutating virus like HCV and might be vital to success in forthcoming therapies. Remarkable work is being done in this regard with a specific host factor cyclophilin (Cyp), which belongs to a family of cellular peptidyl-prolyl isomerases. It is required for HCV replication. Cyclosporine A inhibits HCV replication by binding to cyclophilin. It has now been established that cyclosporine A and other non-immunosuppressive Cyp inhibitors (such as Debio 025, NIM811, and SCY-635) effectively block HCV replication in vitro. A recent study investigated the effects of combining NIM-811 (host inhibitor) with specific inhibitors of viral protein using replicon system. Interestingly, a combination of specific viral inhibitors (viral protease or polymerase inhibitors) with NIM811 prevented the development of resistance very effectively. Cyclosporine A derivatives such as Debio 025 that lack the immunosuppressive function, are currently in clinical trials.

HCV has developed complex mechanisms to use the variety and intricacy of the host lipidome. A promising candidate for anti-HCV therapy emerged with the recent development of statins, a drug that interferes with lipid metabolism. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Statins have been approved clinically for the treatment of hypercholesterolaemia. These cause a reduction in intracellular mevalonate, LDL and geranylglycerol, thereby inhibiting cholesterol biosynthesis and also causing a decrease in prenylated proteins. This drug has also been used to further enhance the antiviral effects of INF. An interesting study utilizing replicon system showed that statins (mevastatin or simvastatin) in combination with polymerase or protease inhibitors could effectively clear the cultures from HCV replicons. In addition, mevastatin was found to prevent selection of replicons resistant to the non nucleoside inhibitor HCV-796. These cell culture studies suggest that a combination of specific viral inhibitors with statins may delay or prevent the development of resistance to viral inhibitors. However, this remains to be tested in clinical trials.

MicroRNAs (miR) are (~22 nucleotide) naturally occurring noncoding RNAs. These belong to a family of small RNA molecules that perform and regulate critical cellular functions. MicroRNAs serve as gene regulators controlling the cell cycle and tissue differentiation. These are also implicated in apoptosis and oncogenesis. The miRNA (miR-122) is required for HCV replication and development of antiviral therapies targeting specific miRNA has great potential for exploration. However, a better understanding of the role for miR-122 binding to the HCV is required for effective design of antiviral therapies. Attempts are underway utilizing RNAi technology and one such attempt is the designing of TT-033, which contains three separate RNAi molecules to shut down replication of all strains of the hepatitis C virus. This strategy sounds promising and could potentially stop the generation of viral escape mutants.

Another promising area for exploring therapeutic options are targeted at processing bodies (P bodies). These P bodies are important for many viruses in their lifecycles. Recent studies have further highlighted the importance of miRNAs and P bodies in modulating host cell interactions with viruses. An interesting study established the role of miR-29a and P bodies in regulating HIV-1 production and infectivity. A link between HCV and P bodies has also been observed. The RNA helicase DDX3 is a component of P bodies and is required for HCV RNA replication. HCV core protein binds and colocalizes with DDX3 in P bodies. The significance of core-DDX3 helicase interaction in HCV pathogenesis is an active area of research.

There are also reports indicating progress with therapeutic vaccines for HCV. IC41, a synthetic peptide vaccine, containing HCV T-cell epitopes has been recently shown to be safe and is currently in clinical trial. IC41 can induce HCV-specific INF-gamma-secreting CD4+ and CD8+ T cells in healthy individuals. It was shown to induce HCV-specific Th1/Tc1 responses in a subset of difficult to treat HCV non-responder patients. There is also evidence that broadly neutralizing antibodies against antigenic regions of E2 can protect Alb-uPA/SCID mouse (human liver-chimeric), against HCV challenge.
Another interesting candidate for a peptide vaccine is the HCV-specific HLA-A2-restricted NS3 (1073) epitope. As with any RNA virus, rapidly mutating genomes such as HCV pose a huge obstacle to antiviral/vaccine development and represents a major challenge to the field. In addition, HCV acquires a lipid envelope, which originates from host membranes. Thus, the lipid composition of the HCV envelope resembles that of the host cell membrane and this clever mimicry may allow HCV to avoid detection by the immune system.

Conclusions

Recent advances in our understanding of HCV structure, genome and its lifecycle have revealed numerous target sites for potential pharmacological intervention. These should help in further improving HCV treatment. Efforts by investigators across the globe have led to detailed molecular characterization of the virus, but despite these advances in our understanding, many fundamental questions still remain unanswered. For example, we are yet to find the mechanisms by which HCV replicates in human liver? What are the molecular mechanisms by which viral proteins initiate hepatocarcinogenesis? What are the underlying defense mechanisms that play a role in the natural resolution of HCV infection in a very small percentage of people. The membrane-associated RNA replication complex presumably involves various host proteins. However, the precise host components and mechanisms for replication are not entirely understood yet. Current efforts to develop antiviral therapeutics are haunted by the unique nature of the HCV, i.e., the error-prone nature of NSSB, generation of resistance mutants which presents a selective growth advantage. To circumvent this major hurdle in HCV therapy, the current research efforts are aimed at combination therapy targeting multiple proteins both viral and host. Numerous novel HCV specific inhibitors such as nucleoside and non nucleoside polymerase inhibitors, protease inhibitors, as well as non HCV specific compounds with antiviral activity such as nitazoxanide, cyclophilin inhibitors (Debio 025), silibinin are under development in clinical trials. An immunotherapeutic vaccine along with the current therapy may also be beneficial especially in immunocompromised patients. In the meantime, combination therapy utilizing PEG-INF and ribavirin will remain standard of care. A recent estimate projects the current treatment market for HCV to be around US$ 3 billion per year. This market is expected to grow rapidly and reach around US$ 8 billion by 2010.

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