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REVIEW ARTICLE

Pharmaceutical Approaches for Treatment of Hepatitis C virus

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Abstract: Many studies have been performed to develop an antiviral therapy against the hepatitis C virus (HCV) infections. The usual treatment for HCV infection is a combination of PEGylated interferon and ribavirin which offer restricted efficiency and major side effects. Thus, recent development in molecular biology of HCV and its life cycle led to the design of many drugs that target viral proteins and host factors required for viral replication. These drugs were named as direct-acting antivirals (DAAs) that were specifically designed for inhibition of viral life cycle, promising tolerability, short duration of treatment, higher barrier to resistance, and fewer drug interactions. The use of DAAs for the treatment of HCV infection resulted in high virological cure rates in patients. However, the use of combined DAA regimens may present drug interactions especially in patients under treatment for other co-morbidities. On the other hand, drug resistance against virus infection determines the success of long-term therapy. High genetic diversity among HCV virions due to error-prone polymerase activity led to the reduced susceptibility to DAA-therapy. Therefore, preclinical and clinical analysis of HCV resistance to novel drugs is needed. In this review, we describe pharmaceutical approaches for HCV treatment, structural and functional properties of DAAs, the principles of HCV drug-drug interaction, and finally HCV resistance to DAAs.

Keywords: HCV, Direct-acting antivirals, Drug resistance, Drug-drug interaction, Inhibition of viral life cycle, Error-prone polymerase activity.

1. INTRODUCTION

Hepatitis C virus (HCV) is an enveloped virus with a 9.6 kb positive-sense single-strand RNA. This virus was classified in the *Flaviviridae* family and the *Hepacivirus* genus causing both acute and chronic infections [1, 2]. Approximately 15%-45% of patients with acute infection spontaneously clear hepatitis C virus during several months after viral infection. In contrast, about 55%-85% of patients suffer from chronic infection leading to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) which can be asymptomatic for many years [1, 2]. Moreover, HCV infection is involved in various extra-hepatic diseases affecting several organs and systems such as immune system [3, 4]. The rapid replication of virus and the lack of error editing by the viral RNA polymerase are often reasons of mutations in the HCV genome resulting in the generation of six genotypes and more than 50 subtypes [5]. No effective vaccine was found for HCV infection. Thus, the HCV therapy relies on antiviral drugs [1]. Indeed, HCV treatment eradicates the virus for the prevention of both liver and extra-hepatic symptoms. Nowadays, some novel and effective agents are available to treat chronic HCV infection including the direct-acting antivirals (DAAs) that specifically target viral proteins (*i.e.,* NS3, NS5A and NS5B). Although DAAs are proposed as a potent HCV treatment; but about 5-10% of people still fail to clear virus [6-8]. Furthermore, it was observed that some compounds can influence HCV replication in the cells. For instance, supercharged GFP protein (+36 GFP) significantly decreased HCV replication up to 75% in HCV-infected Huh7.5 cells [9]. Other study indicated that the heat shock proteins (Hsps) could suppress virus entry and/ or interact with viral proteins. For example, overexpression of small Hsp20 in HCV-infected Huh7.5 cells reduced HCV replication, but in contrast, overexpression of small Hsp27 did not affect virus replication [10]. These data will help to find some effective natural compounds against HCV infections.

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2. GLOBAL EPIDEMIOLOGY AND PROGNOSIS OF HCV INFECTION

HCV is a blood-borne virus that can be transmitted through sex, intravenous drug use, the transfusion of infected blood, and delivery from an infected mother to baby [11]. The studies showed that the transmission approach, optimal treatment and geographical distribution changes among various genotypes [3]. Epidemiology of HCV infection is different worldwide and the risk of disease is high in certain groups such as people with other infections (*e.g.,* HIV), hemodialysis patients or individuals who inject drugs [12, 13]. The HCV prevalence is mainly high (~3%) in Central Asia, Eastern Europe, Central Europe, North Africa, Central Africa, West Africa, the Middle East and Australasia. In contrast, the prevalence of HCV is less than 1% in other regions of the world such as Western European countries [14, 15]. The Chronic Hepatitis Cohort Study indicated that the risk of mortality and progression to cirrhosis or hepatocellular carcinoma (HCC) were significantly augmented by fibrosis stage in the lack of a potent treatment [3].

3. HCV GENOME ORGANIZATION

HCV genome includes a 9.6 kb positive-strand RNA composed of a 5′-nontranslational region (NTR) containing the internal ribosome entry site (IRES) and a 3′-NTR [16]. The structural proteins (*i.e.,* Core, E1 & E2 envelope glycoproteins) along with p7 and NS2 have an important role in the virus assembly. The nonstructural (NS) proteins make the replication complex. About 10^{12} viral particles (diameter: ~ 50 nm) are produced daily in chronic HCV-infected patients [17-19]. After virus entry through receptor-mediated endocytosis, HCV particles release their RNA genome into the cytoplasm. HCV genome translation generates a single polyprotein precursor (~3000 amino acid) that is further processed by cellular (*i.e.,* signal peptidases) and viral proteases (*i.e.,* NS2, NS3/4A) to produce ten different viral proteins (*i.e.,* core, E1 and E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [18, 20]. Fig.

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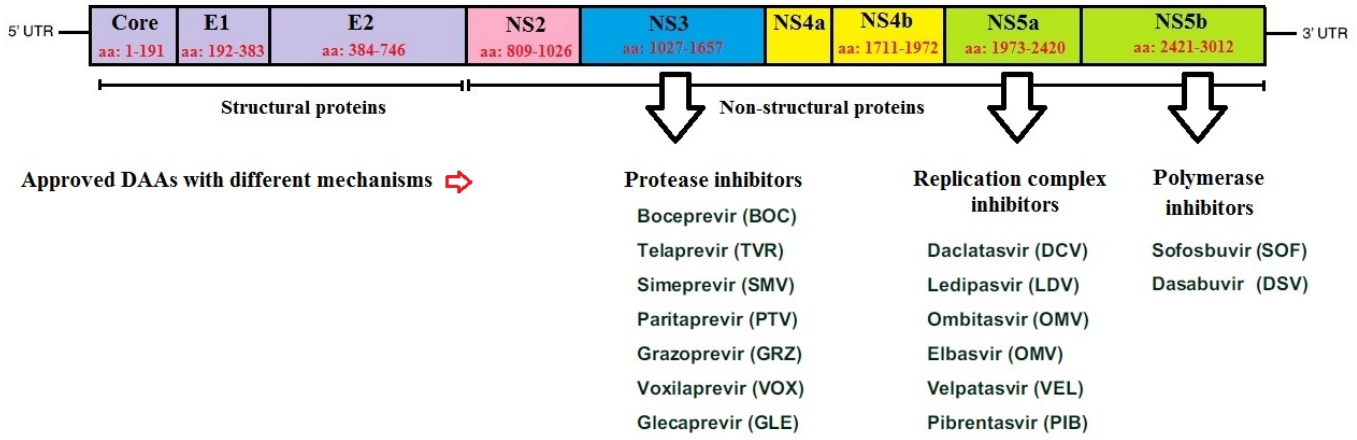


Fig. (1). The main proteins encoded by HCV genome as targets for DAAs: The HCV open reading frame (ORF) encodes at least 11 proteins including 3 structural proteins (Core, E1 and E2), a small protein (p7), 6 nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B), and the so-called "F" protein which results from a frameshift in the core coding region. The 5' untranslated region (UTR) consists of four highly structured domains and the internal ribosome entry site (IRES). The 3'UTR consists of stable stem-loop structures and an internal poly (U)-poly (U/C) tract. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

(1) shows the main proteins encoded by HCV genome as targets for DAAs.

4. HCV-ENCODED PROTEINS

HCV-encoded proteins include structural (S) proteins and nonstructural (NS) proteins as follows.

4.1. Structural Proteins

4.1.1. Core Protein

The core protein is the first structural protein encoded by the HCV open reading frame making the viral nucleocapsid [16]. This protein is generated as a 191 aa precursor of ~ 23 kDa (P23). Although proteins of various sizes (17-23 kDa) were detectable, the 21 kDa core protein (P21) appeared to be the predominant form. The N-terminal domain of the core protein is highly basic, whereas its C-terminal domain is hydrophobic [21]. The core protein is necessary for viral replication and maturation, and pathogenesis. It has various regulatory functions such as cellular and viral gene expression, cell transformation, apoptosis, modulation of signaling pathways, and lipid metabolism [21].

4.1.2. Envelope Proteins

The E1 and E2 glycoproteins form a non-covalent complex. The transmembrane domains located at their C-terminal regions have main roles in heterodimerization and endoplasmic reticulum (ER) retention [16].

4.2. Non-structural Proteins

4.2.1. p7

The p7 protein is a small hydrophobic polypeptide (63 aa) that increases membrane permeability. Moreover, HCV p7 is necessary to generate infection *in vivo* [16, 21].

4.2.2. NS2-3 Protease

This protease system is important for viral replication cycle *in vitro* and *in vivo*. NS2 is linked to intracellular membranes [16]. NS3 is a multifunctional protein with a serine protease located in the N-terminal, and an RNA helicase/NTPase located in the C-terminal of the protein [16]. The NS3 protein possesses antigenic properties and plays an important role in viral clearance [22]. Indeed, NS3 was known as a proper candidate antigen for improvement of HCV therapeutic vaccine [23, 24].

4.2.3. NS4A

NS4A is a small hydrophobic protein (54 aa), which acts as a cofactor for the NS3 serine protease as well as regulates NS5A phosphorylation [25].

4.2.4. NS4B

The NS4B (~27 kDa) protein induces the formation of a specific membrane compartment where viral RNA replication may occur [21].

4.2.5. NS5A

NS5A is a phosphoprotein with different forms including basally phosphorylated (56 kDa) or hyperphosphorylated (58 kDa) types [16]. NS5A can interact with other HCV nonstructural proteins. Furthermore, some cellular proteins interact with NS5A leading to the regulation of virus replication or assembly of the viral replication complex [21].

4.2.6. NS5B

NS5B protein (~ 68 kDa) has the conserved sequence motifs of viral RNA-dependent RNA polymerases (RdRps). Interaction of NS5B with NS3 and NS4A sequentially leads to the formation of complexes with NS4B and NS5A proteins. These interactions are important for the assembly of viral replication complexes [25]. This viral enzyme was determined as a main target for antiviral intervention [16].

5. IMMUNOLOGY OF HCV

Mature dendritic cells (DCs) play a major role in presenting viral antigen, activating T-cells (*i.e.,* release of different cytokines), and inducing anti-viral immunity [26]. Moreover, natural killer (NK) cells are known as the first line of host defense against viruses through stimulation of antiviral immunity in the liver [26]. Generally, cellular immune responses play a major role in the control of HCV infection. The infected hepatocyte cells interact with lymphocytes directly and/or indirectly via the secretion of cytokines and chemokines leading to the death of infected hepatocytes or inhibition of viral replication, and to the death of T lymphocytes or down-regulation of their function [27]. Indeed, activation of lymphocytes in lymphoid organs resulted in the generation of effector T cells (positive loop), while at the same time, these responses were decreased by presentation of antigen on hepatocytes or other specific antigen presenting cells (APCs) (negative loop). Thus, the result of HCV infection is likely determined by the balance between these loops [27]. After HCV infection, the serum ALT levels were in-

creased about 8-14 weeks, when the intrahepatic expression of genes encoding the components of the adaptive immune responses (*e.g.,* MHC class II molecules and chemokines) was up-regulated [28, 29]. Although this phase is clinically asymptomatic for patients, it is interesting that symptomatic patients have a higher chance of recovery than asymptomatic patients [30]. HCV infection in chimpanzees indicated that the induction of IFN-γ by T-cells in the liver was exactly related to a decrease in HCV RNA titers. Direct antiviral effects showed that IFN-γ suppressed the replication of HCV *in vitro* [31]. First screening for HCV antibodies (HCV Ab) was performed by immunoassay. For patients with positive results of HCV Ab, HCV RNA testing was necessary to discriminate those who have spontaneously cleared the infection from those who have become chronically infected and need further medical care [2]. The patients with detectable HCV RNA may need genotyping to determine their type of therapy, and also dose/duration. Furthermore, monitoring of treatment requires additional HCV RNA testing for a sustained virologic response (SVR) [2].

6. HCV TREATMENT

The goal of HCV treatment is eradication of the virus to achieve a sustained virological response (SVR), defined as undetectable HCV RNA in blood, 12 weeks (SVR12) or 24 weeks (SVR24) after the end of therapy [3]. In 1989, IFN-based therapy (sofosbuvir, faldaprevir and simeprevir) was the backbone of HCV therapy (genotype 1) as the standard of care [32-34]. In these tests, only 10-25% of patients achieved a treatment response. In 1990, the combination therapy of IFN-α and ribavirin (~38%) led to a higher percentage of treated patients compared with IFN monotherapy (~13%) [35]. In 2001, the pegylated IFN (PEG-IFN) showed a longer half-life and more favorable pharmacokinetics in combination with ribavirin leading to the improvement in SVR rates (~55%) [36]. Significant advances in understanding of the molecular and structural virology and pathogenesis as well as the ability to produce the recombinant infectious HCV by tissue culture resulted directly in the development of the first direct-acting antivirals (DAAs) [36]. The direct-acting antivirals include: a) NS3/4A protease inhibitors (with -previr suffixes) have a high antiviral potency against genotypes 1 and 4 but not against genotypes 2 or 3. The resistant strains improve quickly and side effects and drug-drug interactions are common [3]; b) NS5B polymerase inhibitors (with -buvir suffixes) contain nucleoside and non-nucleoside analogue inhibitors. Nucleoside analogues have high efficiency, broad genotypic coverage (pangenotypic), low probability of drug resistance, minimal drug-drug interactions but possess several adverse effects. Also, non-nucleoside inhibitors have low to medium efficacy, narrow genotypic coverage, high probability of resistance, side effects dependent on specific drugs, and minor drug-drug interactions [3]; c) NS5A inhibitors (with -asvir suffixes) have high efficiency, medium genotypic coverage, low probability of drug resistance (genotype-dependent), modest side effects and low drug-drug interactions [3]. Some important drugs were discussed as follows.

7. DRUGS

7.1. Type I Interferon

The type-1 IFNs including interferons-α, β, ω and λ play a major role in the innate antiviral immune response [37]. The current commercial forms of IFN-α used for hepatitis C (α 2a, α 2b and consensus IFN) possess similar response rates in treated patients. Interestingly, IFN-λ has anti-HCV activity *in vitro* but without effect on HCV RNA levels in humans [37]. On the other hand, the circulating IFN-α binds to IFN cell-surface-receptor subunits leading to their dimerization and the activation of the receptor-associated Janus-activated kinase 1 (Jak1) and tyrosine kinase 2 (Tyk2) [38, 39]. This activation upregulates IFN-stimulated genes (ISGs) with the expression of several types of antiviral effector protein and stimulates a multidirectional response by increasing the concentrations of 2', 5'-oligoadenylate synthase and β2-microglobulin in serum [38, 39]. Side effects of IFN-α containing flu-like symptoms, fatigue, depression, skin reactions and hematological disorders limit tolerability and treatment in some patients. Moreover, IFN-based HCV treatment was related to high allograft rejection and low rates of viral eradication in kidney transplant recipients [3]. Viral load and HCV genotypes are key factors in an effective IFN-therapy. For example, HCV genotype-1 responded poorly to IFN therapy (SVR: ~50%) in comparison with HCV genotypes 2 and 3 (SVR: ~85%) [39]. Furthermore, some HCV proteins interfere in the antiviral action of IFN-α. The reports indicated that various HCV proteins including core, E2, NS3/4A and NS5A/5B antagonize antiviral effect of IFN-α [39]. For example, HCV core induced the expression of cytokine signaling-3 and -1 (SOCS-3 and SOCS-1) suppressors, which antagonize IFN-α action by blocking JAK/STAT-pathway and ISGs expression. HCV core also inhibited IFN-induced phosphorylation and nuclear translocation of STAT-1 [39]. In addition, HCV E2 was found to inactivate IFN-α through inhibition of protein kinase R (PKR), especially in patients infected with HCV genotype-1. HCV NS3/4A protease was also found to disrupt the IFN induction pathway through cleavage of different proteins (*e.g.,* antiviral signaling proteins (MAVs), the Toll/interleukin-1 receptor (TIR) domain containing adaptor inducing IFN-α (TRIF) and adapter protein of RIG-1 TLR-3 signaling pathways), and finally down-regulation of the transcription of IFN-α-inducible genes [39-42]. In addition, HCV NS4B and NS5A suppressed the protective action of IFN-α. NS4B reduced IFN-α-induced phosphorylation of STAT-1 and expression of IFN receptors. On the other hand, NS5A inactivates PKR and inhibits IFN-induced JAK-STAT signaling pathway leading to down-regulation of interferon-stimulated genes (ISGs)-induced expression [39-42].

7.2. Ribavirin

In 1990, ribavirin (a nucleoside analogue containing a 1, 2, 4-triazol ring) monotherapy was used for the treatment of HCV infection [37]. Ribavirin 5'-triphosphate specifically inhibits viral polymerase activity [43]. Ribavirin has antiviral effects through direct inhibition of HCV replication, competitive inhibition of IMPDH enzyme (participating in the de novo synthesis of guanine nucleotides), enhancement of the frequency of viral mutations due to the mutagenic activity of ribavirin leading to HCV replication errors and subsequently virus death, and immunomodulation by inducing a T-helper 1 (Th1) immune response [43]. It was reported that after oral administration of Ribavirin, its half-life in plasma is 9 h [38, 43]. The adverse effects of ribavirin include hemolytic anemia, CNS disturbances, gastrointestinal/ respiratory tract disturbances, rash, allergy, hyperbilirubinemia, increased levels of iron and reticulocytes in the blood, hyperuricemia as well as its teratogenic and embriotoxic action [38, 43].

7.3. Interferon-α and Ribavirin

Ribavirin monotherapy improved serum aminotransferase levels in at least half of patients, but patients did not clear HCV even with prolonged treatment [37]. In contrast, the addition of ribavirin to IFN-α therapy led to a major improvement in SVR rates. Thus, Ribavirin was approved for use in chronic hepatitis C, but only as a combination therapy with IFN-α [37]. Some studies showed that ribavirin could increase or stabilize the intracellular mediators of IFN activity against HCV, *e.g.,* up-regulation of several ISGs, enhancement of STAT1 binding to DNA, and/ or down-regulation of IL-8 in ribavirin therapy [37, 44, 45]. Further improvement has recently been achieved by the development of pegylated interferon, in which a large molecule of poly(ethylene glycol) (PEG) was covalently attached to recombinant IFN-α, resulting in an active molecule with a longer half-life, and better pharmacokinetic profile and virological response rate [37]. Prior to 2011, treatment was based on the use of a combination of pegylated interferon-α and ribavirin for a period of 24 or 48 weeks, depending on the HCV

genotype [38]. Genotype 1 is more resistant to interferon therapy than other HCV genotypes. The success rates were 70% and 80% for genotypes 2 and 3, respectively, and 45-70% for genotypes 1 and 4 [38]. In general, PEG-IFN with ribavirin can eradicate HCV infection in 40-90% of patients; however, there are some serious hematologic, dermatologic, neurologic, or immunologic side effects [46]. It is interesting that in the all-oral DAA treatments, there is still a major role for ribavirin, especially in the DAAs, with a low barrier to resistance. Ribavirin is relatively well tolerated as part of all-oral DAA regimens and delays (/ prevents) the appearance of resistance, leading to a lower relapse rate and a higher chance of SVR [47]. It was reported that the severity of anaemia was significantly reduced by ribavirin in the absence of interferon. Indeed, the safety profile of ribavirin was improved when co-administered with all-oral DAA combinations in the lack of interferon [47].

7.4. Direct Acting Antivirals (DAAs)

Recently, novel virus-specific direct acting antivirals (DAAs) were developed following the design of an HCV replication system similar to virus replication in cell culture [3]. In 2011, the first generation of DAAs (*i.e.,* boceprevir and telaprevir) was approved for the treatment of chronic HCV genotype 1-infected patients [3]. These two drugs were non-structural protease inhibitors (NS3/4A PIs), which significantly decreased viral replication. Then, they were combined with PEG-IFN-α and RBV to prevent the occurrence of drug resistance. SVR rates were increased to about 75% in some patients with the potential to reduce treatment duration to 24-28 weeks [3]. Recently, three classes of DAAs targeting HCV proteins were considered for their structural and functional properties such as NS3-NS4A protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors [48]. Up to now, five NS3 protease inhibitors (*i.e.,* boceprevir, telaprevir, simeprevir, asunaprevir, paritaprevir), three NS5A inhibitors (*i.e.,* daclatasvir, ledipasvir, ombitasvir), one non-nucleoside (*i.e.,* dasabuvir), and one nucleotide NS5B inhibitor (*i.e.,* sofosbuvir) were approved for the treatment of chronic hepatitis C in the world [48]. Protease inhibitors (PIs) inhibited the processing step, and drugs such as the nucleoside inhibitors (NIs), non-nucleoside inhibitors (NNIs), and the NS5A inhibitors (NS5AIs) targeted HCV RNA replication. Moreover, IFNs (IFN-α, and IFN-λ1) inhibited at least the steps of translation and replication [48]. Table **1** shows important DAAs with their pharmaceutical properties. Mechanistically, DAAs inhibit specific HCV non-structural proteins (NS) that are vital for its replication [49]. Up to now, many promising DAA candidates have been identified; but however, several mutations were associated with viral resistance to DAAs. Thus, combination of drugs with various mechanisms was necessary to prevent the development of drug-resistant HCV and to generate a good sustained virological response [50]. Moreover, a DAA may have differential activity against different HCV genotypes or subtypes; thus, sponsors can target drug development to a specific genotype or to regimens that are optimized for specific subtypes **[**51], **(**Table **2**). Indeed, due to the error prone nature of HCV NS5B polymerase, there is a large diversity of genomic variants of the virus. As known, Genotypes 1 and 3 are the most prevalent HCV genotypes in the world. Genotype 3 is more dangerous than other genotypes because it has a high tendency to cause liver cirrhosis and hepatocellular carcinoma [52]. All new DAAs were primarily developed to eradicate genotype 1 infections and thus possess a limited potency against genotype 3 [53].

7.4.1. NS3/4A Protease Inhibitors

The NS3/4A serine protease is a non-covalent, heterodimer complex formed by the N-terminal serine protease domain of NS3 (catalytic subunit), and the NS4A cofactor (activation subunit). NS3/4A plays a key role in host immune evasion [54]. The NS3/4A protease inhibitors can be divided into two chemical classes: macrocyclic inhibitors, and linear tetrapeptide α-ketoamid derivatives [32]. In 2011, the first generation of NS3/4A protease inhibitors, telaprevir and boceprevir, were approved by the US Food and Drug Administration (FDA) and then by the European Medicines Agency (EMA) for the treatment of patients with chronic genotype 1 HCV infection [55]. Although protease inhibitors were potent against HCV infection but they were restricted by adverse effects and their low barrier to resistance. Moreover, their activity varied among different genotypes. For instance, in a small clinical trial, telaprevir was effective against HCV genotype 2 but not HCV genotype 3 [55]. The next reports showed that with the addition of boceprevir or telaprevir to PEG-IFN/RBV, cure rates for HCV genotype 1 increased to 65%-75% [1]. Subsequently, the second generation of NS3/4A protease inhibitors, simprevir, resulted in similar SVR rates after addition to PEG-IFN/RBV. By 2014, IFN-free regimens had broadly replaced interferon-based therapy [1].

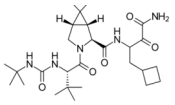
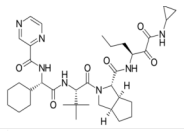
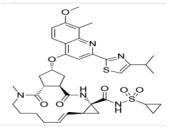
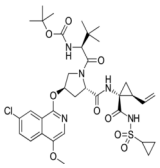
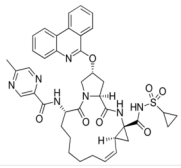
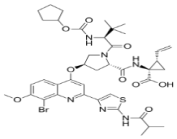
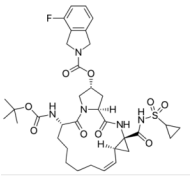
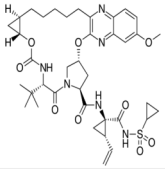
7.4.2. NS5B Polymerase Inhibitors

Unlike the NS3-4A protease, the RNA-dependent RNA polymerase (RdRp) within the NS5B protein is an excellent drug discovery target [56]. For example, sofosbuvir is converted in hepatocytes to an active nucleoside triphosphate form that competes with uridine triphoshate for incorporation into the new positive RNA strand leading to premature chain termination, and interruption of RNA synthesis [56]. Drug binding to HCV NS5B polymerase's active site (This site is highly conserved) demonstrated antiviral activity across all HCV genotypes referred to pan-genotypic DAAs. Some of them also showed a high genetic barrier to the development of drug resistance [32, 38]. By 2013, the second generation of DAA drugs such as sofosbuvir enhanced SVR rates (~90%-100%). In clinical trials of sofosbuvir/ PEG-IFN/RBV, patients with genotype 1 or 4 HCV infection increased SVR rates (~92%), as well [1]. The combination of sofosbuvir and RBV achieved SVR rates of 100% and 91% for genotypes 2 and 3, respectively. Sofosbuvir/ledipasvir and sofosbuvir/simeprevir/RBV resulted in genotype 1 SVR rates of 92%-100% [1]. In 2013, FDA approved sofosbuvir (brand name: Sovaldi) for use in the treatment of chronic HCV genotypes 1, 2, 3 and 4 combined with PEG-IFN and RBV, or with RBV alone (depending on the genotype). Sofosbuvir was also highly effective in HCV patients who are co-infected with HIV [1]. Compared to the nucleoside HCV polymerase inhibitors, there are many non-nucleoside HCV polymerase inhibitors that have progressed into clinical trials [57]. Among them, dasabuvir was the only approved non-nucleoside inhibitor of the NS5B polymerase with a low to medium antiviral activity [58, 59]. However, resistance was more frequent with NNI compared with NI [32].

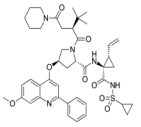
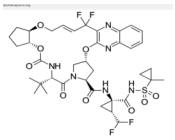
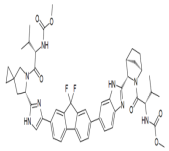
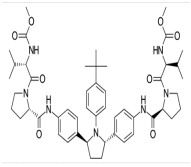
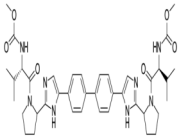
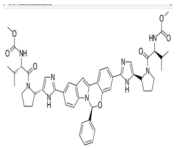
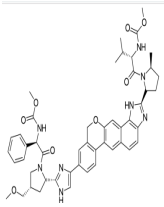
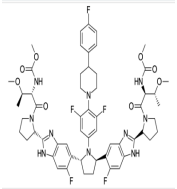
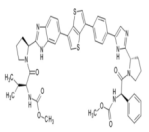
7.4.3. NS5A Inhibitors

A novel class of DAAs was developed to inhibit the viral NS5A including ledipasvir, daclatasvir, ombitasvir, elbasvir and velpatasvir. NS5A is a phosphorylated protein that plays a major role in viral replication, assembly and secretion [60]. HCV-NS5A inhibitors are interesting because of their high efficiency, safety and high barrier to resistance. Some *in vitro* data indicated that their activity changes against different genotypes, but however, two of them showed a pan-genotypic activity. NS5A inhibitors significantly act through two various mechanisms such as suppression of HCV-RNA replication, and inhibition of intracellular virion assembly quickly [60]. The antiviral activity of the first approved NS5A inhibitor "daclatasvir" was extensive with little differences between all HCV genotypes except for HCV genotype 3 [59]. A broad genotypic coverage was also observed for antiviral activity of ledipasvir except for HCV genotypes 2 & 3. Ombitasvir was also characterized by coverage of HCV genotypes 1 to 5 with high antiviral activities except for HCV genotype 6 [59]. Ombitasvir was available only in a fixed dosed combination with the protease inhibitor paritaprevir indicating high antiviral activities in HCV genotypes 1 and 4 [59]. Combinations of ombitasvir/paritaprevir/ritonavir/dasabuvir with or without RBV increased SVR rates (~100%) [1].

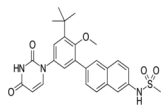
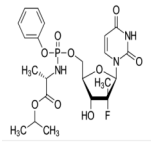
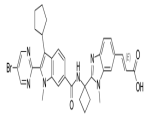
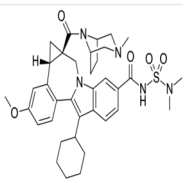
Table 1. Important DAAs with their pharmaceutical properties.

General name	Mechanism	Status	Genetic barriers	Drug-Drug Interactions	Pharmaceutical company	Structure	*In vitro* potency IC_{50}* or EC_{50} (nM)**	Active against HCV Genotype	Reference
Boceprevir	NS3/4A protease inhibitor	Victrelis: Approved in 5/2011, to be discontinued in 12/2015	low	moderate	Merck	Linear 	200-280 nM	1, 2	[1, 32, 75]
Telaprevir	NS3/4A protease inhibitor	Incivek: Approved in 5/2011, discontinued in 10/2014	low	high	Vertex, Janssen	linear 	200-280 nM	1, 2	[1, 32, 75]
Simeprevir	NS3/4A protease inhibitor	Olysio: FDA/EMA approved (October 24, 2013)	moderate	low	Tibotec, Sovriad	Macrocyclic 	1-10 nM	1, 2, 4, 5, 6	[1, 32, 76]
Asunaprevir	NS3/4A protease inhibitor	Dual regimen of asunaprevir and daclatasvir was approved in Japan, 2014	moderate	low	Bristol-Myers Squibb (BMS)	Macrocyclic 	1-10 nM	1, 4	[32, 77]
Paritaprevir	NS3/4A protease inhibitor	Viekira Pak (in combination with ombitasvir, ritonavir and dasabuvir); Technivie/ Viekirax (in combination with ombitasvir and ritonavir): approved in the United States in 2014-2015	moderate	low	Abbvie	Macrocyclic 	1-10 nM	1,4	https://en.wikipedia.org
Faldaprevir	NS3/4A protease inhibitor	Phase III	moderate	low	Boehringer, Ingelheim	Macrocyclic 	1-10 nM	1, 2	[32]
Danoprevir	NS3/4A protease inhibitor	approved in China	moderate	low	Roche	Macrocyclic 	1-10 nM	1, 2, 4	[32]
Grazoprevir	NS3/4A protease inhibitor	Elbasvir/grazoprevir approved by FDA in January 2016	high	low	Merck	Macrocyclic 	1-10 nM	1, 2, 4, , 5, 6	[32]

(Table 1) Contd....

General name	Mechanism	Status	Genetic barriers	Drug-Drug Interactions	Pharmaceutical company	Structure	*In vitro* potency IC_{50}* or EC_{50} (nM)**	Active against HCV Genotype	Reference
Sovaprevir	NS3/4A protease inhibitor	*Sovaprevir* combined with atazanavir and ritonavir in a phase 1 study	moderate	low	Achillion	Macrocyclic 	1-10 nM	1	[32]
Glecaprevir	NS3/4A protease inhibitor	FDA approved (2017)	high	low	Abbvie & Enanta		3.5-11.3 nM	pangenotypic	[78]
Ledipasvir	NS5A protein inhibitor	Approved (February 10, 2014)	low	low	Gilead Sciences		0.11-1.1 nM	1	[79]
Ombitasvir	NS5A protein inhibitor	Technivie (ombitasvir, paritaprevir and ritonavir): Approved July 24, 2015	low	low	AbbVie		For genotypes 1,2,3,4,5: 0.82-19.3 pmol/L; For genotype 6a: 366 pmol/L	pangenotypic	https://www.drugs.com/history/technivie.html; https://en.wikipedia.org/wiki/Ombitasvir
Daclatasvir	NS5A protein inhibitor	Daklinza: Approved (Europ:e in 2014 and the United States and India: in 2015)	moderately high	low	Daklinza (trade name) in BMS		0.14–1.25 nM	pan-genotypic	[80]
Elbasvir	NS5A protein inhibitor	Approved by the FDA in January 2016	high	low	Merck		4-690 pM	1a,1b,2a,3a,4a	https://en.wikipedia.org/wiki/Elbasvir
Velpatasvir	NS5A protein inhibitor	Epclusa, Sofosvel, Velpanat (all in combination with sofosbuvir) approved by US FDA in June 2016	high	low	Gilead		0.36-3.3 µM	pangenotypic	Application for inclusion of sofosbuvir/velpatasvir (Epclusa®) tablets on the WHO Model List of Essential Medicines; 2016
Pibrentasvir	NS5A protein inhibitor	MAVIRET (glecaprevir/pibrentasvir) approved by FDA in December 2016	high	low	abbvie		4.3 pM	pangenotypic	[81]
Samatasvir	NS5A protein inhibitor	Phase II	high	low	Merck		2-24 pM	1,2,3,4	[82]

(Table 1) Contd....

General name	Mechanism	Status	Genetic barriers	Drug-Drug Interactions	Pharmaceutical company	Structure	*In vitro* potency IC_{50}* or EC_{50} (nM)**	Active against HCV Genotype	Reference
Dasabuvir	Nonnucleoside HCV polymerase inhibitor	approved for medical use in 2014	low	low	Exviera (trade name) in BMS		1a: 2.2 nM; 1b: 7.7 nM	1	[66]
Sofobuvir	NS5B polymerase inhibitor	Sovaldi: Approved in 12/2013	high	low	Gilead Science		0.92 nM	1,2,3,4	[1]
Deleobuvir	RNA polymerase inhibitor	Phase III	high	low	Boehringer Ingelheim		50 nM	1	http://www.probechem.com/products_Deleobuvir.aspx; https://en.wikipedia.org/wiki/Deleobuvir
Beclabuvir	Nonnucleoside HCV polymerase inhibitor	Phase II	high	low	BMS		50 nM	1	https://en.wikipedia.org/wiki/Beclabuvir
Sofobuvir (GS-7977)(400 mg) + Ledipasvir (GS-5855)(90 mg)	NS5B polymerase inhibitor + NS5A protein inhibitor	Harvoni: Approved in 10/2014	high	low	Gilead Sciences	Mixture	For 1a: 0.018 nM For 1b: 0.006 nM	1	[1]
Ombitasvir (ABT-267) + Paritaprevir (ABT-450) + Ritonavir + Dasabuvir (BT-333)	NS5A protein inhibitor + NS3/4A protease inhibitor + a cytochrome P450 3A4 inhibitor + NS5B polymerase inhibitor	Viekira Pak: Approved in 12/2014	high	low	AbbVie	Mixture	0.18-0.43 nM	1	[1]
Asunaprevir (BMS-650032) + Daclatasvir (BMS790052) + Beclabuvir (BMS791325)	NS3/4A protease inhibitor + NS5A protein inhibitor + NS5B polymerase inhibitor	Phase III	high	low	Bristol-Myers Squibb	Mixture	0.9 nM	1	[1]
Grazoprevir (MK-5172) + Elbasvir (MK-8742)	NS3/4A protease inhibitor + NS5A protein inhibitor	Phase III	high	high	Merck	mixture	25 μM	1	[1]
Ombitasvir (ABT-267) + Paritaprevir (ABT-450) + Ritonavir	NS5A protein inhibitor + NS3/4A protease inhibitor + a cytochrome P450 3A4 inhibitor	Viekirax: Phase III	low	low	AbbVie	mixture	2.4-4.2 nM	1,4	[1]

*IC50 is the half maximal inhibitory concentration of a drug

** EC50 is the concentration of a drug that gives half-maximal response

Table 2. The commonly DAA combinations in experimental phase [49].

DAA combination	Genotype
Sofosbuvir/ Daclatasvir +/- Ribavirin	1, 2, 3, 4
Sofosbuvir / Ledipasvir +/- Ribavirin	1, 4, 5, 6
Sofosbuvir/ Velpatasvir	Pan-genotypic
Sofosbuvir/ Velpatasvir/ Voxilaprevir	Pan-genotypic
Grazoprevir/ Elbasvir	1, 4
Glecaprevir/ Pibrentasvir	Pan-genotypic
Paritaprevir/ Ritonavir/ Ombitasvir/ Dasabuvir +/- Ribavirin	1
Sofosbuvir/ Simeprevir +/- Ribavirin	1, 4
Paritaprevir/ Ritonavir/ Ombitasvir +/- Ribavirin	4
Sofosbuvir/ Ribavirin	2, 3

8. DRUG-DRUG INTERACTIONS

The risk of morbidity and mortality related to HCV infection was significantly reduced in patients who achieve a sustained virologic response (SVR) with antiviral therapy [61]. At present, improvement of direct-acting antiviral agents targeting different steps in the HCV life cycle has led to further efficiency and reduction in toxicity compared to previous interferon-based therapies [61]. However, the use of these DAAs required special attention to drug-drug interactions because all HCV combination regimens interact with drug metabolizing enzymes, drug transporters, or both of them [61]. For every drug, there is a range of doses that balances its efficiency with toxicity. Many factors can influence drug doses including organ dysfunction, body weight, diet, host genetics, and drug interactions [62]. Drugs with a broad therapeutic range have a high tolerance to drug-drug interactions. Drug-drug interactions possess important clinical implications for drugs with a narrow therapeutic range [62]. Drug interactions increased concentration-dependent toxicities due to the use of higher doses. On the other hand, sub-therapeutic doses led to the development of drug resistance, which can prevent the success of current and future treatments [62]. A large number of membrane transporters were identified, but only few of them were used in clinically relevant drug-drug interactions such as P-glycoprotein (P-gp), organic anion transporting polypeptide 1B1 (OATP1B1), and breast cancer resistance protein (BCRP) [62, 63]. Drug interactions with transporters have the potential to influence drug bioavailability by either increasing elimination or decreasing cellular absorption. The studies showed that the NS3 protease inhibitors, boceprevir and telaprevir, were inhibitors of the transporter P-glycoprotein and the cytochrome P450 enzyme 3A4, and thus were prone to clinically relevant drug interactions [49, 62]. Most of DAA agents are mainly metabolized by CYP450 enzyme [49]. It was reported that first generation of protease inhibitors (DAA agents) was metabolized through the CYP3A system. They showed different drug interactions. The newer DAA agents such as sofosbuvir and ledipasvir which were only slightly affected by CYP450 enzymes were relatively less susceptible to major hepatic pharmacokinetic interactions [49]. Drug interactions should be carefully evaluated in patients with HIV/HCV co-infection. All HCV DAA regimens have the potential for drug interactions with some HIV antiretroviral (ARV) combinations [63]. In general, progress in DAA-based regimens has led to pan-genotypic combinations with high efficacy and minimal adverse effects. However, it is important to prevent side effects of drug-drug interactions. For example, the nucleotide polymerase inhibitor sofosbuvir was the backbone of treatment for many DAA regimens. Sofosbuvir is a p-glycoprotein substrate, and thus strong p-glycoprotein inducers should not be co-administered with sofosbuvir-based therapy [64].

9. HCV RESISTANCE TO DAAS

The high rate of HCV replication and low fidelity of the HCV polymerase (*i.e.,* the lack of proofreading and post-replication repair) led to heterogeneous virus populations [17, 65]. The genetic barrier to resistance was defined as the number of amino acid substitutions required to confer full resistance to a drug. Usually, DAA with a low genetic barrier to resistance needed only one or two amino acid substitutions for high resistance [32]. DAA with a high barrier to resistance usually required three or more amino acid substitutions in the same region to cause loss of activity [32]. The term "resistance-associated variant" or RAV that was used to characterize substitutions reduced susceptibility to a drug or drug class. The substitutions (*i.e.,* amino acid changes) that confer resistance were called "resistance-associated substitutions" (RAS), whereas the viral variants that carry these RASs were named "resistant variants" [66]. In general, HCV resistance to DAAs was affected by three main parameters: a) the genetic barrier to resistance: The genetic barrier to resistance changed with the drug class and the HCV genotype/subtype. It determines the probability of resistant viruses generated during replication [66]; b) the ability of resistant virus populations which is independent of the level of resistance conferred by the RASs. It determines the probability of resistant viruses generated in minor or major populations [66]; c) drug exposure as compared to the 50% and 90% inhibitory concentrations of drug *in vitro*. It determines the ability of the drug to suppress replication of resistant variants [66]. The genetic barrier to protease inhibitors was usually low, and the resistance changed broadly between HCV genotypes [67]. Viral resistance to telaprevir occurred significantly in genotype 1a compared to genotype 1b due to nucleotide differences at position 155 in HCV subtype 1a (AGA, encodes R) versus 1b (CGA, also encodes R). Indeed, the major limitation of the first-generation PIs was their low genetic barrier to resistance. Second generation of PIs had a higher barrier to resistance, better activity against several genotypes, more suitable dosing programs, and improved safety and tolerance. The use of RAS testing depends on patients and DAA regimen [67-70].

The role of the PEG-IFN/ribavirin backbone in the DAAs and host-targeting agents (HTAs) was to effectively suppress RAVs that emerged during therapy [71]. Protease inhibitors (PI)-triple therapy containing the PEG-IFN/RBV backbone showed high potency to

inhibit HCV replication and consequently overcome resistance to DAAs. The PROVE 1/2 [69, 70] and SPRINT-2 [71] studies reported no significant effect of baseline RAVs on SVR in patients that responded to PEG-IFN/RBV. Interferon exerted its antiviral effect through several interferon-stimulated genes (ISGs) with the best host immune response in non-cirrhotic patients that are IL28 CC-genotype [72]. In contrast, patients with inherent unresponsiveness to interferon were at high-risk for treatment failure in triple regimens containing PEG-IFN/RBV plus only one DAA. Thus, prior PEG-IFN/RBV null responders were considered for 'quad regimens' containing two DAAs plus PEG-IFN/RBV backbone or interferon-free regimens with multiple DAAs with or without RBV [72]. The anti-HCV mechanisms of RBV remained unclear likely due to lethal mutagenesis and/or immunomodulatory functions, but its simultaneous administration was clearly shown to reduce viral breakthrough in PI-containing triple versus PEG-IFN/PI dual therapy (2% versus 26%) [72]. Generally, HCV DAAs have difference in the resistance barrier, and DAA treatment is clearly genotype dependent. HCV resistant variants were observed in most patients who did not achieve SVR. These resistance-associated mutations were dependent on the class of direct-acting antiviral drugs used, and also varied between HCV genotypes and subtypes. It was shown that NS5A, NS3/4A inhibitors and non-nucleoside inhibitors had relatively low barriers to resistance [73]. Thus, the understanding of resistance-associated mutations showed a clear clinical implication in terms of choice and combination of drugs used [74].

CONCLUSION

Among the non-structural proteins, NS3/4A, NS5A and NS5B proteins were known as HCV drug targets because they initiate the replication process inside the host cells. Recently, DAAs with or without pegylated IFN and RBV treatment has been recommended to the patients based on the genotypes. The combination therapies with DAAs were found to be effective for chronic hepatitis. However, HCV resistance was a common phenomenon related to DAA failure. Natural resistance and clinical conditions (*e.g.,* cirrhosis or high viral load) may contribute to virological failure in some genotypes (*e.g.,* genotype 1a and 3). Thus, finding the novel DAAs is useful and can be accelerated with the use of chemogenomics approaches. On the other hand, drug-drug interaction is the modification of the action of one drug by another and may be pharmacodynamic or pharmacokinetic in nature. Thus, the use of the DAAs needs special attention to drug-drug interactions because all HCV combination regimens interact with drug metabolizing enzymes, drug transporters, or both of them.

LIST OF ABBREVIATIONS

DAAs	=	Direct-acting antivirals
HCV	=	Hepatitis C virus
HCC	=	Hepatocellular carcinoma
NTR	=	Nontranslational region
IRES	=	Internal ribosome entry site
NS	=	Nonstructural
ER	=	Endoplasmic reticulum
DC	=	Dendritic cell
NK	=	Natural killer
APC	=	Antigen presenting cell
SVR	=	Sustained virological response
	=	IFN-stimulated gene
TRIF	=	Toll/interleukin-1 receptor domain containing adaptor inducing IFN-α
Th1	=	T-helper 1
FDA	=	Food and Drug Administration
RdRp	=	RNA-dependent RNA polymerase

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Zhang X. Direct anti-HCV agents. Acta Pharm Sin B 2016; 6(1): 26-31. http://dx.doi.org/10.1016/j.apsb.2015.09.008 PMID: 26904396

[2] Tolan NV, Horowitz GL, Graham CS, Hillyard D, Osburn W, Ray S. New therapies for treating hepatitis C virus: Impact on laboratory testing recommendations and clinical management. Clin Chem 2017; 63(12): 1799-805. http://dx.doi.org/10.1373/clinchem.2016.266569 PMID: 29018029

[3] Puchades Renau L, Berenguer M. Introduction to hepatitis C virus infection: Overview and history of hepatitis C virus therapies. Hemodial Int 2018; 22(Suppl. 1): S8-S21. http://dx.doi.org/10.1111/hdi.12647 PMID: 29694724

[4] Bassendine MF, Nielsen SU, Bridge SH, *et al.* Hepatitis C virus and atherosclerosis: A legacy after virologic cure? Clin Res Hepatol Gastroenterol 2017; 41(1): 25-30. http://dx.doi.org/10.1016/j.clinre.2016.09.008 PMID: 27840032

[5] Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci 2006; 3(2): 47-52. http://dx.doi.org/10.7150/ijms.3.47 PMID: 16614742

[6] Alazard-Dany N, Denolly S, Boson B, Cosset FL. Overview of HCV life cycle with a special focus on current and possible future antiviral targets. Viruses 2019; 11(1): 30. http://dx.doi.org/10.3390/v11010030 PMID: 30621318

[7] Kim S, Han KH, Ahn SH. Hepatitis C virus and antiviral drug resistance. Gut Liver 2016; 10(6): 890-5. http://dx.doi.org/10.5009/gnl15573 PMID: 27784846

[8] Bartenschlager R, Lohmann V, Penin F. The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection. Nat Rev Microbiol 2013; 11(7): 482-96. http://dx.doi.org/10.1038/nrmicro3046 PMID: 23748342

[9] Vahabpour R, Basimi P, Roohvand F, *et al.* Anti-viral effects of superpositively charged mutant of green fluorescent protein. Protein Pept Lett 2019; 26(12): 930-9. http://dx.doi.org/10.2174/0929866526666190823145916 PMID: 31441722

[10] Vahabpour R, Soleymani S, Roohvand F, Zabihollahi R, Bolhassani A. In vitro anti-viral effects of small heat shock proteins 20 and 27: A novel therapeutic approach. Curr Pharm Biotechnol 2019; 20(12): 1011-7. http://dx.doi.org/10.2174/1389201020666190729104648 PMID: 31362669

[11] Roberts EA, Yeung L. Maternal-infant transmission of hepatitis C virus infection. Hepatology 2002; 36(5)(Suppl. 1): S106-13. PMID: 12407583

[12] Ozaras R, Leblebicioglu H. Global epidemiology of chronic hepatitis C virus infection.Viral Hepatitis: Chronic Hepatitis C. Springer 2019; pp. 1-24. http://dx.doi.org/10.1007/978-3-030-03757-4_1

[13] Organization WH. Global hepatitis report 2017. World Health Organization 2017.

[14] Pradat P, Virlogeux V, Trépo E. Epidemiology and elimination of HCV-related liver disease. Viruses 2018; 10(10): 545. http://dx.doi.org/10.3390/v10100545 PMID: 30301201

[15] Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An

up-date of the distribution and circulation of hepatitis C virus genotypes. World J Gastroenterol 2016; 22(34): 7824-40. http://dx.doi.org/10.3748/wjg.v22.i34.7824 PMID: 27678366

[16] Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. Nat Rev Microbiol 2007; 5(6): 453-63. http://dx.doi.org/10.1038/nrmicro1645 PMID: 17487147

[17] Lynch SM, Wu GY. The molecular basis of anti-HCV drug resistance. Update on Hepatitis 2017; C: 217. http://dx.doi.org/10.5772/intechopen.70775

[18] Kim CW, Chang KM. Hepatitis C virus: virology and life cycle. Clin Mol Hepatol 2013; 19(1): 17-25. http://dx.doi.org/10.3350/cmh.2013.19.1.17 PMID: 23593605

[19] Dubuisson J, Helle F, Cocquerel L. Early steps of the hepatitis C virus life cycle. Cell Microbiol 2008; 10(4): 821-7. http://dx.doi.org/10.1111/j.1462-5822.2007.01107.x PMID: 18081727

[20] Penin F, Dubuisson J, Rey FA, Moradpour D, Pawlotsky JM. Structural biology of hepatitis C virus. Hepatology 2004b; 39(1): 5-19. http://dx.doi.org/10.1002/hep.20032 PMID: 14752815

[21] Suzuki T, Ishii K, Aizaki H, Wakita T. Hepatitis C viral life cycle. Adv Drug Deliv Rev 2007; 59(12): 1200-12. http://dx.doi.org/10.1016/j.addr.2007.04.014 PMID: 17825945

[22] Alizadeh S, Irani S, Bolhassani A, Sadat SM. Simultaneous use of natural adjuvants and cell penetrating peptides improves HCV NS3 antigen-specific immune responses. Immunol Lett 2019; 212: 70-80. http://dx.doi.org/10.1016/j.imlet.2019.06.011 PMID: 31254535

[23] Basirnejad M, Bolhassani A. Development of HCV therapeutic vaccines using Hp91 peptide and small heat shock protein 20 as an adjuvant. Protein Pept Lett 2018; 25(10): 924-32. http://dx.doi.org/10.2174/0929866525666180925145304 PMID: 30255740

[24] Basirnejad M, Bolhassani A, Sadat SM. The distinct role of small heat shock protein 20 on HCV NS3 expression in HEK-293T cell line. Avicenna J Med Biotechnol 2018; 10(3): 152-7. PMID: 30090208

[25] Reed K, Rice C. Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties.The Hepatitis C Viruses. Springer 2000; pp. 55-84. http://dx.doi.org/10.1007/978-3-642-59605-6_4

[26] Irshad M, Khushboo I, Singh S, Singh S. Hepatitis C virus (HCV): a review of immunological aspects. Int Rev Immunol 2008; 27(6): 497-517. http://dx.doi.org/10.1080/08830180802432178 PMID: 19065353

[27] Willberg C, Barnes E, Klenerman P. HCV immunology--death and the maiden T cell. Cell Death Differ 2003; 10(S1)(Suppl. 1): S39-47. http://dx.doi.org/10.1038/sj.cdd.4401122 PMID: 12655345

[28] Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. J Virol 2001; 75(15): 7059-66. http://dx.doi.org/10.1128/JVI.75.15.7059-7066.2001 PMID: 11435586

[29] Su AI, Pezacki JP, Wodicka L, *et al.* Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci USA 2002; 99(24): 15669-74. http://dx.doi.org/10.1073/pnas.202608199 PMID: 12441396

[30] Gerlach JT, Diepolder HM, Zachoval R, *et al.* Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 2003; 125(1): 80-8. http://dx.doi.org/10.1016/S0016-5085(03)00668-1 PMID: 12851873

[31] Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005; 5(3): 215-29. http://dx.doi.org/10.1038/nri1573 PMID: 15738952

[32] Schinazi R, Halfon P, Marcellin P, Asselah T. HCV direct-acting antiviral agents: the best interferon-free combinations. Liver Int 2014; 34(Suppl. 1): 69-78. http://dx.doi.org/10.1111/liv.12423 PMID: 24373081

[33] Davis GL, Balart LA, Schiff ER, *et al.* Hepatitis Interventional Therapy Group. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. N Engl J Med 1989; 321(22): 1501-6. http://dx.doi.org/10.1056/NEJM198911303212203 PMID: 2509916

[34] Di Bisceglie AM, Martin P, Kassianides C, *et al.* Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989; 321(22): 1506-10. http://dx.doi.org/10.1056/NEJM198911303212204 PMID: 2509917

[35] McHutchison JG, Gordon SC, Schiff ER, *et al.* Hepatitis Interventional Therapy Group. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med 1998; 339(21): 1485-92. http://dx.doi.org/10.1056/NEJM199811193392101 PMID: 9819446

[36] Li DK, Chung RT. Overview of direct-acting antiviral drugs and drug resistance of hepatitis C virus.Hepatitis C Virus Protocols. Springer 2019; pp. 3-32. http://dx.doi.org/10.1007/978-1-4939-8976-8_1

[37] Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature 2005; 436(7053): 967-72. http://dx.doi.org/10.1038/nature04082 PMID: 16107837

[38] Zając M, Muszalska I, Sobczak A, Dadej A, Tomczak S, Jelińska A. Hepatitis C - New drugs and treatment prospects. Eur J Med Chem 2019; 165: 225-49. http://dx.doi.org/10.1016/j.ejmech.2019.01.025 PMID: 30685524

[39] Irshad M, Gupta P, Irshad K. Molecular targeting of antiviral drugs used against hepatitis C virus infection. Hepatoma Res 2018; 4: 23. http://dx.doi.org/10.20517/2394-5079.2018.25

[40] Gong GZ, Cao J, Jiang YF, Zhou Y, Liu B. Hepatitis C virus nonstructural 5A abrogates signal transducer and activator of transcription-1 nuclear translocation induced by IFN-α through dephosphorylation. World J Gastroenterol 2007; 13(30): 4080-4. http://dx.doi.org/10.3748/wjg.v13.i30.4080 PMID: 17696225

[41] Lan KH, Lan KL, Lee WP, *et al.* HCV NS5A inhibits interferon-α signaling through suppression of STAT1 phosphorylation in hepatocyte-derived cell lines. J Hepatol 2007; 46(5): 759-67. http://dx.doi.org/10.1016/j.jhep.2006.11.013 PMID: 17275127

[42] Kumthip K, Maneekarn N. The role of HCV proteins on treatment outcomes. Virol J 2015; 12(1): 217. http://dx.doi.org/10.1186/s12985-015-0450-x PMID: 26666318

[43] Paul D, Madan V, Bartenschlager R. Hepatitis C virus RNA replication and assembly: living on the fat of the land. Cell Host Microbe 2014; 16(5): 569-79. http://dx.doi.org/10.1016/j.chom.2014.10.008 PMID: 25525790

[44] Zhang Y, Jamaluddin M, Wang S, *et al.* Ribavirin treatment upregulates antiviral gene expression via the interferon-stimulated response element in respiratory syncytial virus-infected epithelial cells. J Virol 2003; 77(10): 5933-47. http://dx.doi.org/10.1128/JVI.77.10.5933-5947.2003 PMID: 12719586

[45] Fiedler MA, Wernke-Dollries K, Stark JM. Inhibition of viral replication reverses respiratory syncytial virus-induced NF-kappaB activation and interleukin-8 gene expression in A549 cells. J Virol 1996; 70(12): 9079-82. http://dx.doi.org/10.1128/JVI.70.12.9079-9082.1996 PMID: 8971048

[46] Sulkowski MS, Cooper C, Hunyady B, *et al.* Management of adverse effects of Peg-IFN and ribavirin therapy for hepatitis C. Nat Rev Gastroenterol Hepatol 2011; 8(4): 212-23. http://dx.doi.org/10.1038/nrgastro.2011.21 PMID: 21386812

[47] Feld JJ, Jacobson IM, Sulkowski MS, Poordad F, Tatsch F, Pawlotsky JM. Ribavirin revisited in the era of direct-acting antiviral therapy for hepatitis C virus infection. Liver Int 2017; 37(1): 5-18. http://dx.doi.org/10.1111/liv.13212 PMID: 27473533

[48] Koizumi Y, Ohashi H, Nakajima S, *et al.* Quantifying antiviral activity optimizes drug combinations against hepatitis C virus infection. Proc Natl Acad Sci USA 2017; 114(8): 1922-7. http://dx.doi.org/10.1073/pnas.1610197114 PMID: 28174263

[49] Geddawy A, Ibrahim YF, Elbahie NM, Ibrahim MA. Direct acting anti-hepatitis C virus drugs: Clinical pharmacology and future direction. J Transl Int Med 2017; 5(1): 8-17. http://dx.doi.org/10.1515/jtim-2017-0007 PMID: 28680834

[50] Chae HB, Park SM, Youn SJ. Direct-acting antivirals for the treatment of chronic hepatitis C: Open issues and future perspectives.The Scientific World Journal. 2013; 2013: pp. 1-9.

[51] U.S. Department of Health and Human Services; Food and Drug Administration. Center for Drug Evaluation and Research (CDER) Chronic hepatitis C virus infection: Developing direct-acting antivi-

ral drugs for treatment guidance for industry. Clinical/Antimicrobial 2017; pp. 1-40.

[52] Kanwal F, Kramer JR, Ilyas J, Duan Z, El-Serag HB. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. Hepatology 2014; 60(1): 98-105. http://dx.doi.org/10.1002/hep.27095 PMID: 24615981

[53] Abdurakhmanov E. Discovery and evaluation of direct acting antivirals against hepatitis C virus. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 2015; pp. 1-49.

[54] Vermehren J, Sarrazin C. The role of resistance in HCV treatment. Best Pract Res Clin Gastroenterol 2012; 26(4): 487-503. http://dx.doi.org/10.1016/j.bpg.2012.09.011 PMID: 23199507

[55] Schaefer EA, Chung RT. Anti-hepatitis C virus drugs in development. Gastroenterology 2012; 142(6): 1340-1350.e1. http://dx.doi.org/10.1053/j.gastro.2012.02.015 PMID: 22537441

[56] De Francesco R, Migliaccio G. Challenges and successes in developing new therapies for hepatitis C. Nature 2005; 436(7053): 953-60. http://dx.doi.org/10.1038/nature04080 PMID: 16107835

[57] Li H, Shi ST. Non-nucleoside inhibitors of hepatitis C virus polymerase: current progress and future challenges. Future Med Chem 2010; 2(1): 121-41. http://dx.doi.org/10.4155/fmc.09.148 PMID: 21426049

[58] Gaultier I. 8-week efficacy and safety of ABT-333 or ABT-972 with standard-of-care, following 3-day monotherapy in genotype 1 HCV-infected treatment-naive subjects. Hepatol Int 2011; 5.

[59] Sarrazin C. The importance of resistance to direct antiviral drugs in HCV infection in clinical practice. J Hepatol 2016; 64(2): 486-504. http://dx.doi.org/10.1016/j.jhep.2015.09.011 PMID: 26409317

[60] Gitto S, Gamal N, Andreone P. NS5A inhibitors for the treatment of hepatitis C infection. J Viral Hepat 2017; 24(3): 180-6. http://dx.doi.org/10.1111/jvh.12657 PMID: 27925362

[61] Menon RM, Badri PS, Wang T, *et al.* Drug-drug interaction profile of the all-oral anti-hepatitis C virus regimen of paritaprevir/ritonavir, ombitasvir, and dasabuvir. J Hepatol 2015; 63(1): 20-9. http://dx.doi.org/10.1016/j.jhep.2015.01.026 PMID: 25646891

[62] Kiser JJ, Burton JR Jr, Everson GT. Drug-drug interactions during antiviral therapy for chronic hepatitis C. Nat Rev Gastroenterol Hepatol 2013; 10(10): 596-606. http://dx.doi.org/10.1038/nrgastro.2013.106 PMID: 23817323

[63] El-Sherif O, Khoo S, Solas C. Key drug-drug interactions with direct-acting antiviral in HIV-HCV coinfection. Curr Opin HIV AIDS 2015; 10(5): 348-54. http://dx.doi.org/10.1097/COH.0000000000000185 PMID: 26248122

[64] Ahmed A, Lutchman GA, Kwo PY. Drug-drug interactions in hepatitis C virus treatment: Do they really matter? Clin Liver Dis (Hoboken) 2017; 10(5): 111-5. http://dx.doi.org/10.1002/cld.668 PMID: 30992768

[65] Romano KP, Ali A, Aydin C, *et al.* The molecular basis of drug resistance against hepatitis C virus NS3/4A protease inhibitors. PLoS Pathog 2012; 8(7): e1002832. http://dx.doi.org/10.1371/journal.ppat.1002832 PMID: 22910833

[66] Pawlotsky JM. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology 2016; 151(1): 70-86. http://dx.doi.org/10.1053/j.gastro.2016.04.003 PMID: 27080301

[67] McCown MF, Rajyaguru S, Kular S, Cammack N, Nájera I. GT-1a or GT-1b subtype-specific resistance profiles for hepatitis C virus inhibitors telaprevir and HCV-796. Antimicrob Agents Chemother 2009; 53(5): 2129-32. http://dx.doi.org/10.1128/AAC.01598-08 PMID: 19273674

[68] Sorbo MC, Cento V, Di Maio VC, *et al.* Hepatitis C virus drug resistance associated substitutions and their clinical relevance: Update 2018. Drug Resist Updat 2018; 37: 17-39. http://dx.doi.org/10.1016/j.drup.2018.01.004 PMID: 29525636

[69] Hézode C, Forestier N, Dusheiko G, *et al.* PROVE2 Study Team. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009; 360(18): 1839-50. http://dx.doi.org/10.1056/NEJMoa0807650 PMID: 19403903

[70] McHutchison JG, Everson GT, Gordon SC, *et al.* PROVE1 Study Team. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009; 360(18): 1827-38. http://dx.doi.org/10.1056/NEJMoa0806104 PMID: 19403902

[71] Poordad F, McCone J Jr, Bacon BR, *et al.* SPRINT-2 Investigators. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011; 364(13): 1195-206. http://dx.doi.org/10.1056/NEJMoa1010494 PMID: 21449783

[72] Welsch C, Zeuzem S. Clinical relevance of HCV antiviral drug resistance. Curr Opin Virol 2012; 2(5): 651-5. http://dx.doi.org/10.1016/j.coviro.2012.08.008 PMID: 23006585

[73] Paolucci S, Premoli M, Novati S, *et al.* Baseline and breakthrough resistance mutations in HCV patients failing DAAs. Sci Rep 2017; 7(1): 16017. http://dx.doi.org/10.1038/s41598-017-15987-1 PMID: 29167469

[74] Ahmed A, Felmlee DJ. Mechanisms of hepatitis C viral resistance to direct acting antivirals. Viruses 2015; 7(12): 6716-29. http://dx.doi.org/10.3390/v7122968 PMID: 26694454

[75] González-Colominas E, Broquetas T, Retamero A, *et al.* Drug-drug interactions of telaprevir and boceprevir in HCV-monoinfected and HIV/HCV-coinfected patients can modify the adherence. Liver Int 2015; 35(5): 1557-65. http://dx.doi.org/10.1111/liv.12729 PMID: 25385188

[76] Izquierdo L, Helle F, François C, Castelain S, Duverlie G, Brochot E. Simeprevir for the treatment of hepatitis C virus infection. Pharm Genomics Pers Med 2014; 7: 241-9. PMID: 25206310

[77] Eley T, Garimella T, Li W, Bertz RJ. Asunaprevir: a review of preclinical and clinical pharmacokinetics and drug–drug interactions. Clin Pharmacokinet 2015; 54(12): 1205-22. http://dx.doi.org/10.1007/s40262-015-0299-6 PMID: 26177803

[78] Ng TI, Tripathi R, Reisch T, *et al.* In vitro antiviral activity and resistance profile of the next-generation hepatitis C virus NS3/4A protease inhibitor glecaprevir. Antimicrob Agents Chemother 2017; 62(1): e01620-17. http://dx.doi.org/10.1128/AAC.01620-17 PMID: 29084747

[79] Cheng G, Tian Y, Doehle B, *et al.* In vitro antiviral activity and resistance profile characterization of the hepatitis C virus NS5A inhibitor ledipasvir. Antimicrob Agents Chemother 2016; 60(3): 1847-53. http://dx.doi.org/10.1128/AAC.02524-15 PMID: 26824950

[80] McCormack PL. Daclatasvir: a review of its use in adult patients with chronic hepatitis C virus infection. Drugs 2015; 75(5): 515-24. http://dx.doi.org/10.1007/s40265-015-0362-5 PMID: 25721433

[81] Real LM, Macías J, Pérez AB, *et al.* Baseline resistance-guided therapy does not enhance the response to interferon-free treatment of HCV infection in real life. Sci Rep 2018; 8(1): 14905. http://dx.doi.org/10.1038/s41598-018-33367-1 PMID: 30297726

[82] Bilello JP, Lallos LB, McCarville JF, *et al.* In vitro activity and resistance profile of samatasvir, a novel NS5A replication inhibitor of hepatitis C virus. Antimicrob Agents Chemother 2014; 58(8): 4431-42. http://dx.doi.org/10.1128/AAC.02777-13 PMID: 24867983