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Current therapy for chronic hepatitis C: The role of direct-acting antivirals



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ABSTRACT

One of the most exciting developments in antiviral research has been the discovery of the direct-acting antivirals (DAAs) that effectively cure chronic hepatitis C virus (HCV) infections. Based on more than 100 clinical trials and real-world studies, we provide a comprehensive overview of FDA-approved therapies and newly discovered anti-HCV agents with a special focus on drug efficacy, mechanisms of action, and safety. We show that HCV drug development has advanced in multiple aspects: (i) interferon-based regimens were replaced by interferon-free regimens; (ii) genotype-specific drugs evolved to drugs for all HCV genotypes; (iii) therapies based upon multiple pills per day were simplified to a single pill per day; (iv) drug potency increased from moderate (~60%) to high (>90%) levels of sustained virologic responses; (v) treatment durations were shortened from 48 to 12 or 8 weeks; and (vi) therapies could be administered orally regardless of prior treatment history and cirrhotic status. However, despite these remarkable achievements made in HCV drug discovery, challenges remain in the management of difficult-to-treat patients.

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Contents

1. Introduction	84
2. Literature selection	84
3. Overview of hepatitis C virus	84
3.1. HCV origin, epidemiology, and diversity	84
3.2. HCV clinical syndrome and pathogenesis	85
3.3. HCV proteins and drug targets	86
4. Approved antiviral therapies	88
4.1. Boceprevir (Victrelis [®]) + PegIFN α + RBV	90
4.2. Telaprevir (Incivek [®]) + PegIFN α + RBV	92
4.3. Sofosbuvir (Sovaldi [®]) + PegIFN α + RBV	94
4.4. Simeprevir (Olysio [®]) + sofosbuvir or PegIFN α /RBV	96
4.5. Ledipasvir + sofosbuvir (Harvoni [®])	97
4.6. Ombitasvir + paritaprevir + ritonavir + dasabuvir (Viekira Pak [™])	100
4.7. Ombitasvir + paritaprevir + ritonavir (Technivie [™])	101
4.8. Daclatasvir (Daklinza [™]) + sofosbuvir (Sovaldi [®])	101

Abbreviations: BID, twice a day; CDC, Centers for Disease Control and Prevention; DAA, direct-acting antiviral; EC₅₀, half maximal effective concentration; FDA, U S Food and Drug Administration; GT, genotype; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IC₅₀, half maximal inhibitory concentration; PegIFN α , pegylated interferon alfa; QD, once a day; RBV, ribavirin; SVR12, sustained virologic response after the treatment for 12 weeks; SVR24, sustained virologic response after the treatment for 24 weeks; TID, three times a day; WHO, World Health Organization.

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4.9.	Elbasvir + grazoprevir (Zepatier™)	104
4.10.	Sofosbuvir + velpatasvir (Epclusa®)	105
5.	New agents under development	106
5.1.	NS3/4A inhibitors	106
5.2.	NS4B inhibitors	109
5.3.	NS5A inhibitors	110
5.4.	NS5B inhibitors	111
5.5.	E1/E2 and p7 inhibitors	112
5.6.	Immuno-stimulators and cellular protein inhibitors	112
6.	Conclusions and future perspectives	113
	Acknowledgements	114
	References	114

1. Introduction

Over the past two decades, tremendous attempts have been made to discover antiviral drugs that effectively treat hepatitis C virus (HCV) infection (De Clercq, 2015; De Clercq, 2012; De Clercq, 2014; Welzel et al., 2014; De Clercq and Li, 2016). The first generation of FDA-approved HCV drugs includes: interferon alfacon-1 (approval year: 1997, discontinued in 2013 due to severe adverse events), ribavirin (1998), pegylated interferon alfa-2b (2001), and pegylated interferon alfa-2a (2002). These drugs had low cure rates and may cause severe adverse events (Manns et al., 2006); yet they have been the only standard-of-care treatments over a decade and are still popular in several countries. Subsequently, direct-acting antivirals (DAAs) represented a breakthrough in *in vitro* experiments and in clinical trials. In May 2011, telaprevir and boceprevir became the first FDA-approved drugs in the DAA class. The advent of DAAs marks a new era of anti-HCV drugs that directly target HCV proteins, offering promising cure rates and minimum adverse events. Similar to cocktail therapies against human immunodeficiency virus, combination therapies that target different stages of the HCV life cycle have been conceived to avoid cross-resistance. Importantly, their cure rates could attain more than 90% in clinical trials and real-world cohorts. Forthcoming therapies are endowed with higher cure rates, shorter treatment duration, lesser side effects, while being based upon all-oral regimens.

This review aims to summarize the latest trend of HCV drug development, focusing on FDA-approved therapies and newly discovered agents against HCV genotype 1 to 6 infections. We first provide a general overview of HCV infections. Subsequently, we summarize the approved HCV drugs and highlight their efficacy in clinical trials. Recent development of new agents is described. Challenges in HCV drug development are discussed at the end. To endorse this review, movies and teaching slides that highlight HCV drug actions are available from our online platform (<http://www.virusface.com>).

2. Literature selection

Here, our procedure for literature selection is described. We searched literature in PubMed using the keywords of HCV drugs within the publication period from 2013/01/01 to 2016/10/01. To search the most recent publications that are unavailable in PubMed, similar queries were also performed by visiting the websites of eleven journals (NEJM, JAMA, Lancet, Lancet infectious diseases, Gut, Clinical Infectious Diseases, Gastroenterology, Hepatology, Journal of hepatology, The American Journal of Gastroenterology, Annals of Internal Medicine). Only clinical trials in the phase 2, 3 and 4 stages were considered, while case reports were excluded from our literature review because of their small samples

and rare subjects. We also extracted information about clinical trials from [ClinicalTrials.gov](http://www.clinicaltrials.gov) (<http://www.clinicaltrials.gov/>) or the European Clinical Trials Database (EudraCT, <https://eudract.ema.europa.eu/index.html>). Clinical studies in the drug labeling of the FDA (<https://www.accessdata.fda.gov>) were also included in our literature search. Three exclusion criteria were undertaken. First, data from the meta-analysis or reports that summarized clinical trials in other publications were not considered so as to avoid duplicates. Second, publications (e.g. HCV-TARGET (Terrault et al., 2016), real-world study (Backus et al., 2016)) that reported the coinfections with HCV and other infectious diseases (e.g. HIV, HBV) were not collected because coinfections were beyond the focus of our review. Third, in order to highlight the SVR rates of antiviral drugs in each HCV genotype, only publications that clearly addressed SVR rates of HCV therapies in individual genotypes were summarized. Moreover, we communicated with every corresponding author if SVR rates in certain patient groups were not addressed in a publication. By doing so, we managed to obtain SVR data from most publications except for a phase 2 study (Osinusi et al., 2013), the ANRS C023 CUPILT study (Coilly et al., 2016), the A1444040 study (Sulkowski et al., 2014a), the C-EDGE Head-2-Head study (Sperl et al., 2016), and the C-EDGE treatment-experienced study (Kwo et al., 2017).

3. Overview of hepatitis C virus

3.1. HCV origin, epidemiology, and diversity

Hepatitis C virus is a *hepacivirus* in the *Flaviviridae* family, and it was first discovered from the serum of a person with non-A, non-B hepatitis in 1989 (Choo et al., 1989; Kuo et al., 1989). Now, HCV can be found in worldwide populations (Shepard et al., 2005), while the immediate sources of HCV associated with its pandemic spread have been traced to the circulation in Central and West sub-Saharan Africa and South and Southeast Asia over hundreds of years (Simmonds, 2013). Although its origin remains unclear, HCV might have originated from zoonotic sources such as non-human primates (e.g. monkeys, apes) and mammals (e.g. dogs, horses) (Simmonds, 2013). As of today, HCV has been recognized as one of the most lethal infectious diseases next to measles, influenza, respiratory syncytial virus, rotavirus, hepatitis B, and human immunodeficiency virus (Mortality, 2016; Disease et al., 2016). According to the WHO global health survey, 130 to 150 million people are currently living with HCV, causing approximately 700,000 deaths every year. Importantly, more than 95% of HCV-infected patients are unaware of their status in worldwide populations (Cox, 2015).

A single HCV particle is approximately 68 nm (range: 45–86 nm) in diameter (Catanese et al., 2013), and it contains a linear positive-sense single-stranded RNA genome encoding 10

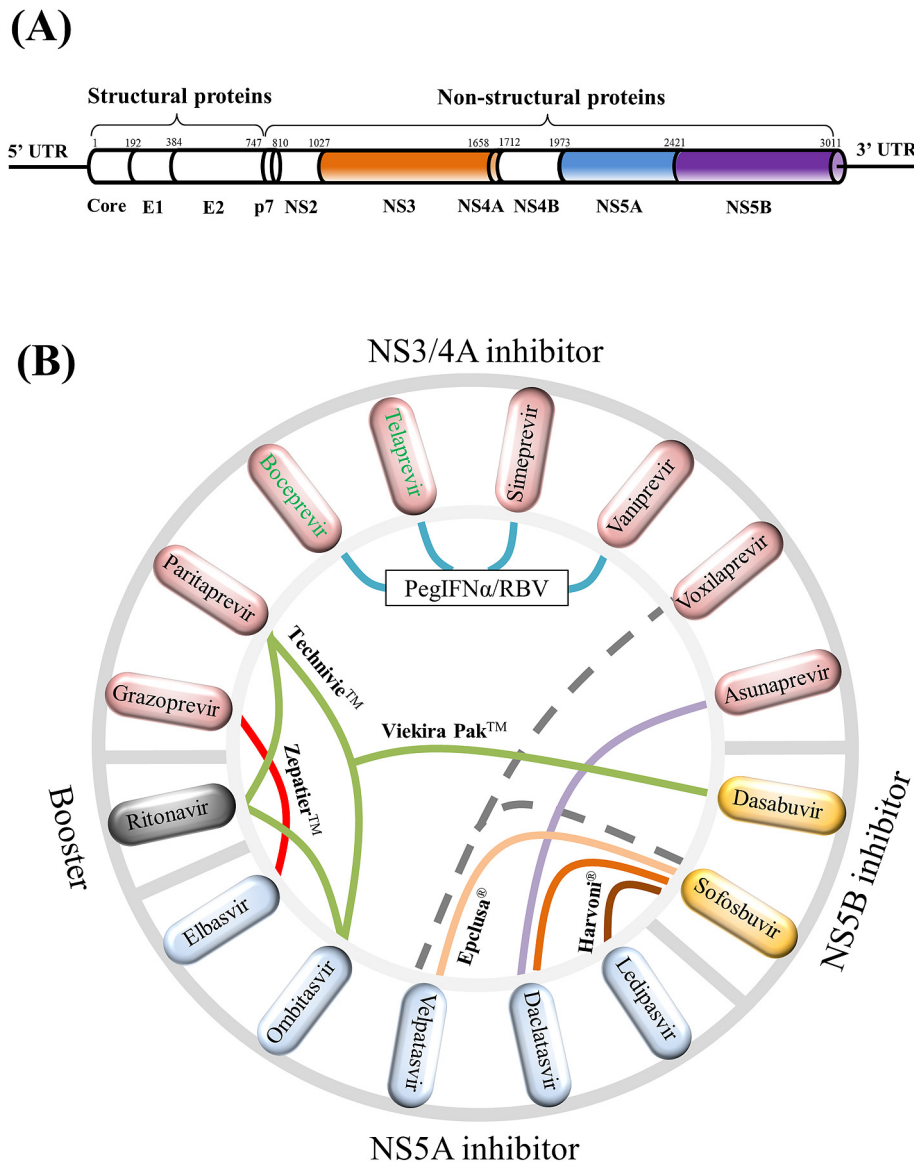


Fig. 1. HCV genome structure and schematic view of HCV combination drugs. (A) HCV genome structure. In the length of approximately 3011 amino acids, the HCV genome codes for three structural proteins (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) whose amino acid positions are mapped accordingly. The 5' untranslated region (5' UTR) and the 3' untranslated region (3' UTR) are also indicated. Approved antiviral agents directly target to NS3/4A, NS5A, and NS5B for effective inhibition of HCV replications. (B) A total of 15 NS3/4A, NS5A, NS5B compounds plus ritonavir are displayed in the circle. Colored links in the center visualize 13 drug combinations: (i) boceprevir (Victrelis®) + PegIFN α /RBV, (ii) telaprevir (Incivek®) + PegIFN α /RBV, (iii) sofosbuvir (Sovaldi®) + PegIFN α /RBV, (iv) simeprevir (Olysio®) + PegIFN α /RBV, (v) ledipasvir + sofosbuvir (Harvoni®), (vi) ombitasvir + paritaprevir + ritonavir + dasabuvir (Viekira Pak™), (vii) ombitasvir + paritaprevir + ritonavir (Technivie™), (viii) daclatasvir (Daklinza™) + sofosbuvir (Sovaldi®), (ix) elbasvir + grazoprevir (Zepatier™), (x) sofosbuvir + velpatasvir (Epclusa®), (xi) vaniprevir (Vanihep®) + PegIFN α /RBV, (xii) asunaprevir (Sunvepra®) + daclatasvir (Daklinza®), (xiii) voxilaprevir + velpatasvir + sofosbuvir. Notably, (i) to (x) were approved by the FDA and could be used with or without ribavirin; (xi) and (xii) were approved in Japan; and (xiii) is currently under assessment by the FDA. Two discontinued drugs boceprevir and telaprevir are indicated by green texts. This figure shows that HCV combination drugs are composed of anti-HCV inhibitors from different drug classes.

viral proteins (Moradpour and Penin, 2013) (Fig. 1A). Due to the sequence variation of different HCV strains, 7 genotypes (genotypes 1 to 7) have been recognized and their global prevalence is unequally distributed. Among 7 HCV genotypes, genotype 1 (46.2%) and 3 (30.1%) dominate the global infections; genotypes 2, 4, and 6 circulate in approximately 22.8% of HCV infections; genotype 5 accounts for the remaining less than 1% (Messina et al., 2015); genotype 7 has been identified so far in very few patients originating from Central Africa (Murphy et al., 2015). It is worth mentioning that the nucleotide diversity of HCV genomes is approximately 32.4% between HCV genotypes, and 14.6% within HCV genotypes (Cuypers et al., 2015). An average of 1.3×10^{12} virions are produced per day in each HCV-infected patient (Neumann

et al., 1998). Moreover, HCV evolves so fast that the HCV mutation rate is approximately 1.92×10^{-3} nucleotide substitutions per genomic site per year (Ogata et al., 1991).

3.2. HCV clinical syndrome and pathogenesis

As a blood-borne virus, HCV can be transmitted by blood transfusions, needle sharing, sexual contacts, or maternal transmissions. Although HCV can circulate in many human organs, it infects predominantly hepatocytes in the liver and evades the host innate and adaptive immune system (Rehermann, 2009). With an incubation period of 2–12 weeks, HCV infection begins with an acute phase that usually goes undiagnosed, during which

Table 1
FDA-approved HCV therapies with their recommended use.

Drug name	FDA-approved HCV therapy	Genotype	Date approved
Victrelis [®]	PegIFN α /RBV for 4w + 200 mg boceprevir TID plus PegIFN α /RBV for 44 weeks.	GT-1	May 2011
Incivek [®]	1125 mg telaprevir BID plus PegIFN α /RBV for 12w + PegIFN α /RBV for 12w or 36w.	GT-1	May 2011
Sovaldi [®]	400 mg sofosbuvir QD + ribavirin for 12w or 24w; or 400 mg sofosbuvir QD + PegIFN α /RBV for 12w.	GT-1, GT-2, GT-3, GT-4	Dec. 2013
Olysio [®]	150 mg simeprevir + 400 mg sofosbuvir QD for 12w or 24w; 150 mg simeprevir QD for 12w + PegIFN α /RBV for 24w or 48w.	GT-1, GT-4	Nov. 2013
Harvoni [®]	A fixed-dose combination tablet of 90 mg ledipasvir + 400 mg sofosbuvir, QD with or without ribavirin for 12 or 24 weeks.	GT-1, GT-4, GT-5, GT-6	Oct. 2014
Viekira Pak [™]	Two fixed-dose combination tablets of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir QD plus 250 mg dasabuvir BID with or without ribavirin for 12 or 24 weeks.	GT-1	Dec. 2014
Technivie [™]	Two fixed-dose combination tablets of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir with or without ribavirin, QD for 12 weeks.	GT-4	July 2015
Daklinza [™] + Sovaldi [®]	60 mg daclatasvir QD + 400 mg sofosbuvir QD with or without ribavirin for 12 weeks.	GT-1, GT-3	July 2015
Zepatier [™]	A fixed-dose combination tablet of 100 mg grazoprevir QD + 50 mg elbasvir QD for 12 weeks, or with ribavirin for 12 or 16 weeks.	GT-1, GT-4	Jan. 2016
Eplclusa [®]	A fixed-dose combination tablet of 400 mg sofosbuvir + 100 mg velpatasvir with or without ribavirin, QD for 12 weeks.	GT-1 to GT-6	June 2016

Victrelis[®] and Incivek[®] were discontinued. Except for Technivie[™], all the HCV therapies above could be applied to patients with cirrhosis. Sunvepra and Vanihep approved in Japan are not listed in Table 1.

symptomatic infections (10%–15%) and asymptomatic infections (85%–90%) are observed (Maheshwari et al., 2008). Approximately, 25%–52% of symptomatic infections and 10%–15% of asymptomatic infections undertake spontaneous viral clearance, indicating that HCV is cleared from HCV-infected patients by specific immune responses (Maheshwari et al., 2008). If not cleared, acute HCV evolves into chronic HCV. Without proper treatment, patients with chronic HCV are threatened by serious complications such as cirrhosis, liver cancer, and liver failure (Thrift et al., 2017). Of note, liver damage is the consequence of long-lasting inflammation when host immune responses are activated to fight HCV infections (Antonelli et al., 2008).

According to Centers for Disease Control and Prevention (CDC), HCV-related cirrhosis is often observed in 5%–20% of patients with chronic infections over a period of 20–30 years. Advanced cirrhosis eventually causes end-stage liver disease and liver failure with debilitating symptoms such as hepatic encephalopathy, portal hypertension, hepatorenal syndrome, and hepatocellular carcinoma (Ge and Runyon, 2016). As the most common type of liver cancer, hepatocellular carcinoma can be found in 1%–3% of HCV-infected patients over three decades (El-Serag, 2012). Nevertheless, successful anti-HCV treatments can reduce the overall mortality and the risk of death among HCV patients with compensated cirrhosis (Nahon et al., 2016).

3.3. HCV proteins and drug targets

HCV genome encodes 3 structural proteins (core, E1, E2) and 7 nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) (Fig. 1A). Similar to genome-wide protein interactions in human immunodeficiency virus (Li and De Clercq, 2016), HCV proteins cooperatively play multiple functions that take part in different stages of the viral life cycle (Dubuisson and Cosset, 2014; Bartenschlager et al., 2013). Major functions of HCV proteins are briefly described herein. (i) The core protein is the key element to form viral nucleocapsid that encapsulates HCV RNA genome inside viral particles. (ii) Envelope glycoproteins E1 and E2 constitute a non-covalent complex that takes part in receptor binding and fusion processes during the viral entry. (iii) The non-structural protein p7 in the form of hexamers has a cation

channel activity and plays multiple roles in viral particle maturation and release (Scull et al., 2015). (iv) The NS2-NS3 auto-protease cleaves the link between NS2 and NS3 in the same polyprotein during the HCV polyprotein processing. (v) NS3 protease with its cofactor NS4A in a structural complex cleaves four links (NS3-NS4A, NS4A-NS4B, NS4B-NS5A, NS5A-NS5B) in the polyprotein to release NS3, NS4A, NS4B, NS5A and NS5B for their maturation. (vi) NS4B induces the formation of the membranous web which serves as the principle site of viral replication and assembly (Egger et al., 2002; Gosert et al., 2003). (vii) The NS5A phosphoprotein is a replicase factor that interacts with many other viral and host proteins to regulate HCV replication (Bartenschlager et al., 2013). (viii) The NS5B polymerase is the key enzyme that synthesizes viral RNA to produce new viral particles. Overall, ten HCV proteins play important roles in the viral life cycle, making them potential drug targets to inhibit HCV replication and infection. More details about HCV life cycle can be found in other reviews (Bartenschlager et al., 2013; Moradpour et al., 2007; Scheel and Rice, 2013; Lindenbach and Rice, 2005; Bartenschlager et al., 2011).

Discovery of the multifunctional aspects of HCV proteins has shed light on the novel mechanisms of anti-HCV drugs. Known drug targets include one non-structural protein (NS5A) and two HCV enzymes (NS3/4A, NS5B) (De Clercq, 2014). Three drug targets therefore offer ideal drug binding pockets for the design of HCV combination therapies, leading to the tremendous success of pan-genotypic therapies (e.g. Eplclusa[®]) with more than 90% of sustained virologic response. Approved DAA combination therapies are commonly composed of two or three compounds that inhibit either NS3/4A, NS5A, or NS5B with different mechanism of drug action (Fig. 1). In comparison to individual compounds, combination therapies offer much higher efficacy to inhibit HCV infections.

Four classes of drug actions have been discovered. First, NS3/4A inhibitors (e.g. asunaprevir, boceprevir, telaprevir, paritaprevir, grazoprevir, simeprevir, vaniprevir, voxilaprevir) can inhibit the enzymatic activity of NS3/4A. Second, NS5A inhibitors (e.g. daclatasvir, ledipasvir, ombitasvir, elbasvir, velpatasvir) might inhibit replication complex formation at the endoplasmic reticulum and sequester NS5A in lipid droplets to inhibit virus formation and

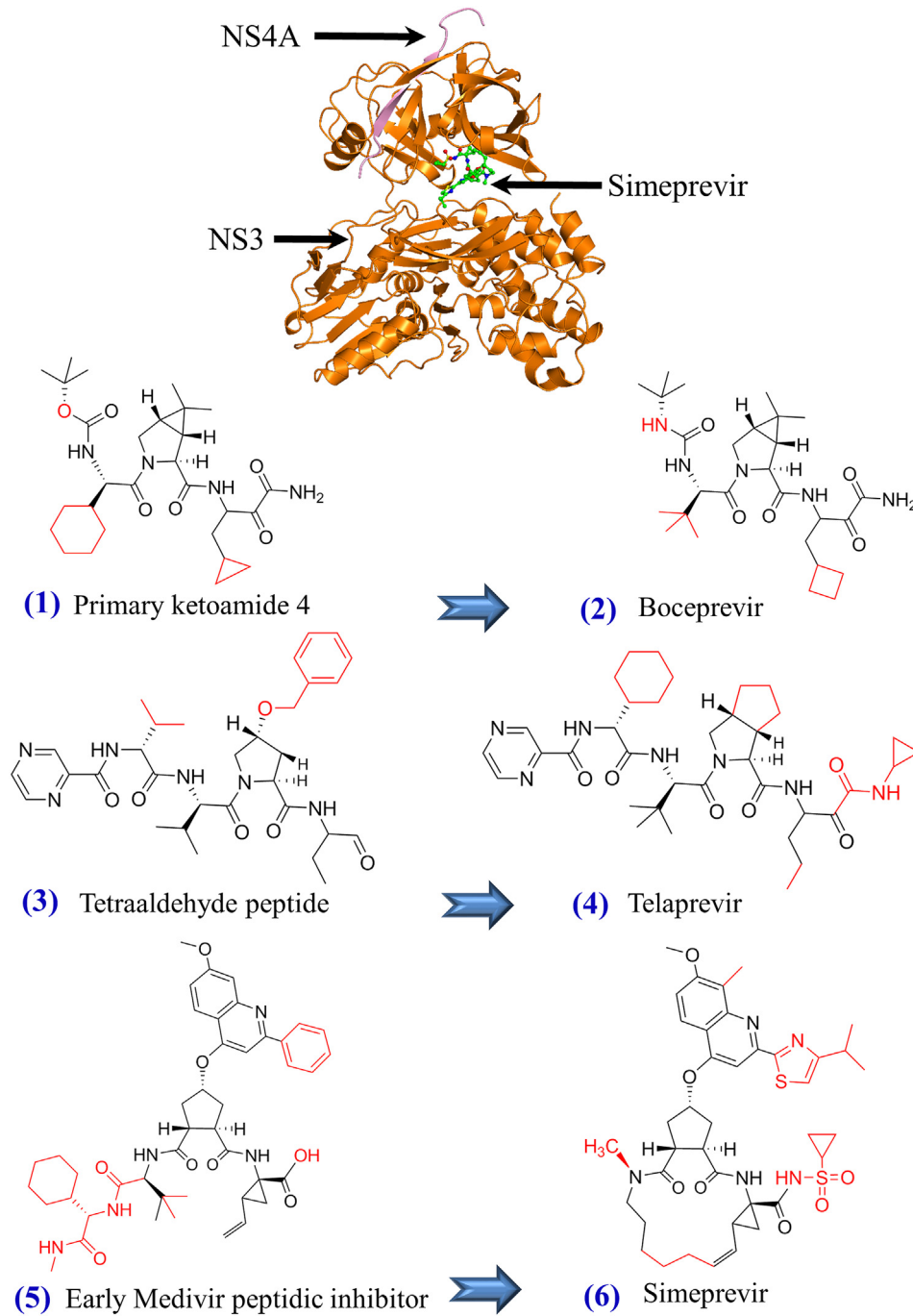


Fig. 2. Tertiary structure of HCV NS3/4A protease and structural formulas of approved or experimental NS3/4A inhibitors from (1) to (18). The tertiary structure of NS3/4A protease in complex with simeprevir (PDB codes: 3KEE and 4B76) is shown on top. HCV NS3 and NS4A proteins are displayed in orange and pink, respectively. Beneath the NS3/4A protein, chemical structures of approved and experimental NS3/4A inhibitors (Table 20) are demonstrated. Blue arrows indicate the optimization from initial compounds to FDA-approved compounds such as boceprevir (Njoroge et al., 2008), telaprevir (Kwong et al., 2011), simeprevir (Rosenquist et al., 2014), and grazoprevir (Harper et al., 2012; Liverton et al., 2008). Red structures indicate the differences between initial compounds and FDA-approved compounds.

release (Nettles et al., 2014). Without affecting the stability and dimerization of NS5A, NS5A inhibitors not only block HCV RNA synthesis at the stage of membranous web biogenesis (Berger et al., 2014), but also impair viral assembly by inhibiting the delivery of HCV genomes to assembly sites (Boson et al., 2017). In spite of their high potency, NS5A inhibitors slowly inhibit HCV RNA synthesis in comparison to HCV protease or polymerase inhibitors (McGovern et al., 2014). It is also worth mentioning that the cooperation interaction between pairs of NS5A inhibitors may enhance drug resistance barrier and restore antiviral activity against NS5A

resistance variants (Sun et al., 2015). Third, nucleotide inhibitors (e.g. sofosbuvir triphosphate) bind to the catalytic site of NS5B and compete with incoming nucleoside triphosphates to interrupt the RNA synthesis. Fourth, non-nucleotide inhibitors (e.g. dasabuvir) bind to the allosteric binding pockets outside the NS5B catalytic site based on the non-competitive mechanisms of action that inhibit the RNA synthesis (Gotte and Feld, 2016). In addition to these known drug binding sites, alternative drug targets in other viral and host proteins have been explored by experimental drugs. The following sections provide more details about the approved and

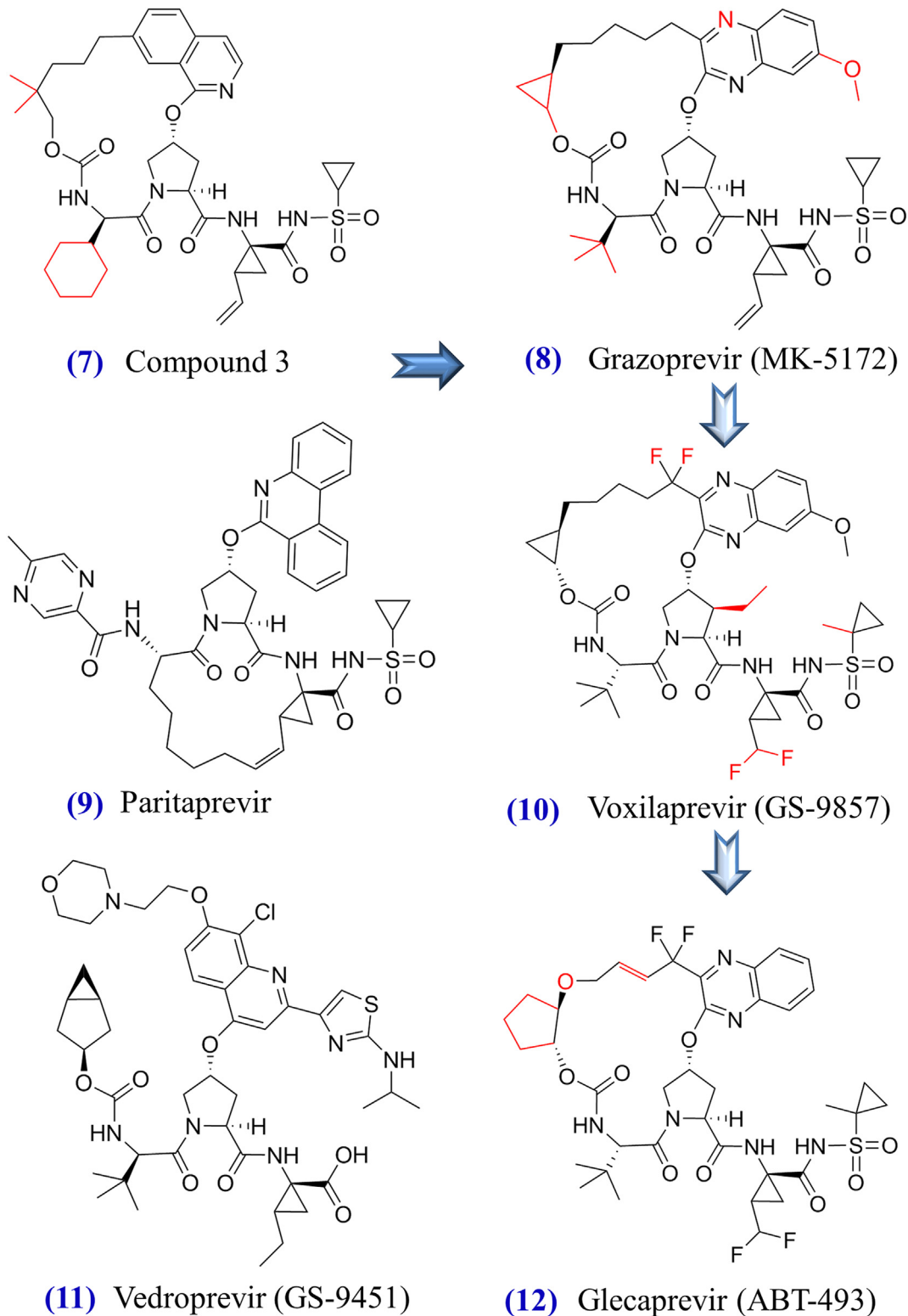


Fig. 2. (continued).

experimental drugs.

4. Approved antiviral therapies

Interferon alfacon-1 (Infergen[®]) was the first FDA-approved drug against HCV infections. Later, ribavirin (Copegus[®], Rebetol[®],

Virazole[®]), pegylated interferon alfa 2b (PegIntron[®], Intron[®]-A), and pegylated interferon alfa 2a (Pegasys[®], Roferon[®]-A) joined to combat HCV infections. Before 2011, interferon and ribavirin were the standard of care therapies, whereas they offered rather low clinical effectiveness and caused serious side effects (see reviews elsewhere (Selzner et al., 2011; Aghemo et al., 2010; Webster et al.,

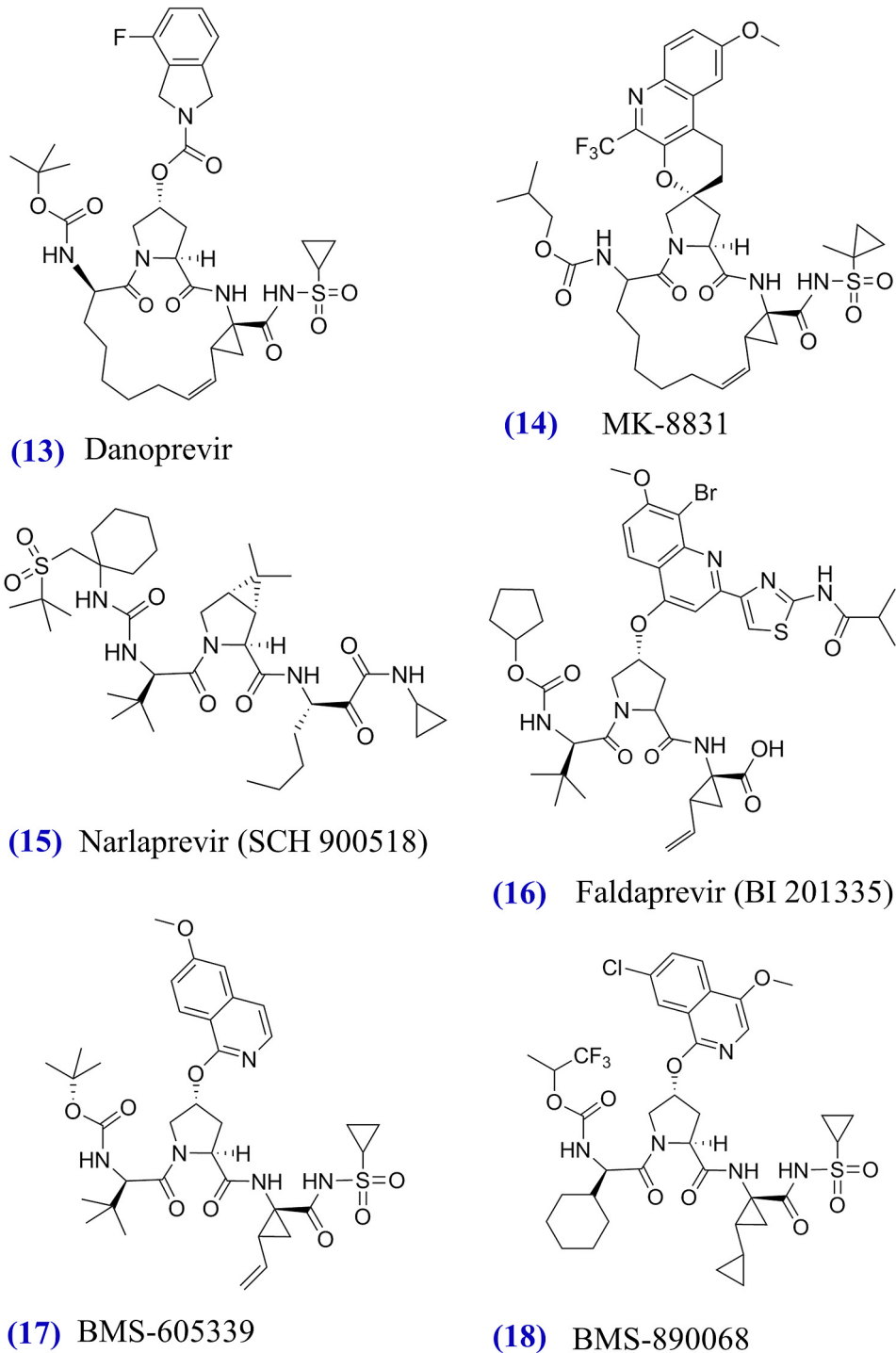


Fig. 2. (continued).

2009)). The advent of DAAs opened an era to potentially cure HCV in most patients (De Clercq, 2015; De Clercq, 2012; De Clercq, 2014). Between January 2011 and November 2016, there were ten therapies approved by the FDA (Table 1), as well as two approved drugs (asunaprevir + daclatasvir, vaniprevir + ribavirin + PegIFN α) in Japan. Due to severe side effects and commercial reasons, telaprevir and boceprevir have been discontinued.

It is worth mentioning that treatment success of HCV therapies is commonly evaluated by the sustained virologic response (SVR) at the post-treatment for 8 weeks (SVR8), 12weeks (SVR12) or 24

weeks (SVR24). For instance, SVR rates may vary profoundly when HCV therapies are used against different genotypes, patients with or without compensated cirrhosis, and patients experienced with or without previous treatments. Resistance aspects of HCV DAAs also play a role in the treatment success (Cuyper et al., 2016; Sarrazin, 2016). Therefore, American and European guidelines for Hepatitis C treatments have been proposed for different scenarios (AASLD/IDSA HCV Guidance Panel, 2015; European Association for Study of Liver, 2015). In this section, we aimed at summarizing the effectiveness of FDA-approved therapies against HCV mono-

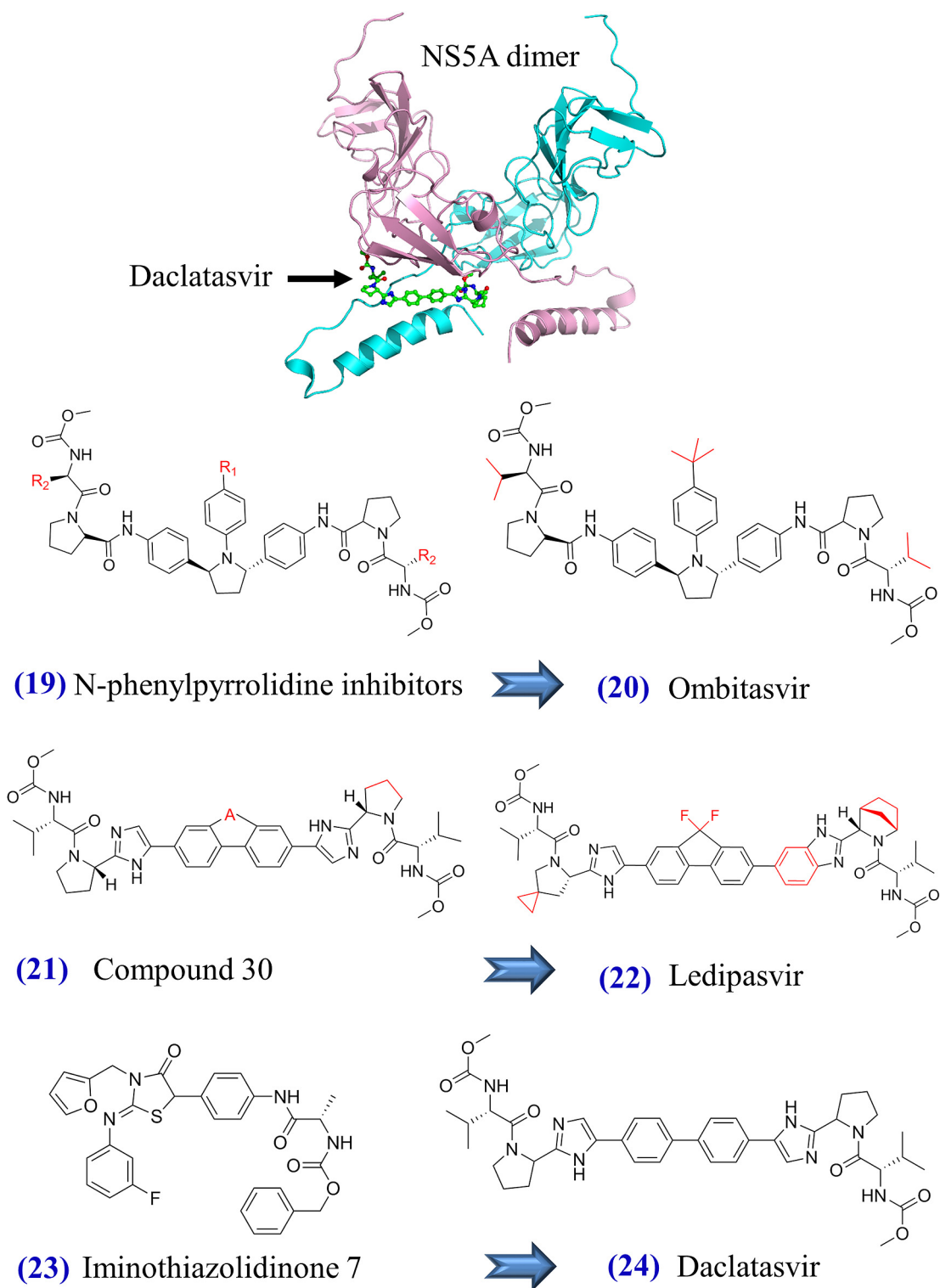


Fig. 3. Tertiary structure of HCV NS5A and structural formulas of approved or experimental NS5A inhibitors from (19) to (36). Two units of an NS5A dimer are colored by pink and cyan, respectively. NS5A inhibitor daclatasvir is also illustrated. Blue arrows indicate the optimization from initial compounds to FDA-approved compounds such as ombitasvir (DeGoey et al., 2014), ledipasvir (Link et al., 2014), daclatasvir (Belema and Meanwell, 2014), and elbasvir (Coburn et al., 2013). Other NS5A inhibitors are summarized in Table 20.

infections in clinical trials or real-world studies. Chemical formulas of the approved NS3/4A inhibitors, NS5A inhibitors, NS5B nucleoside inhibitors, and NS5B non-nucleoside inhibitors are highlighted in Figs. 2–5, respectively.

4.1. Boceprevir (Victrelis®) + PegIFN α + RBV

Boceprevir (formerly SCH503034) in combination with PegIFN α /RBV was the first FDA-approved therapy against HCV genotype 1

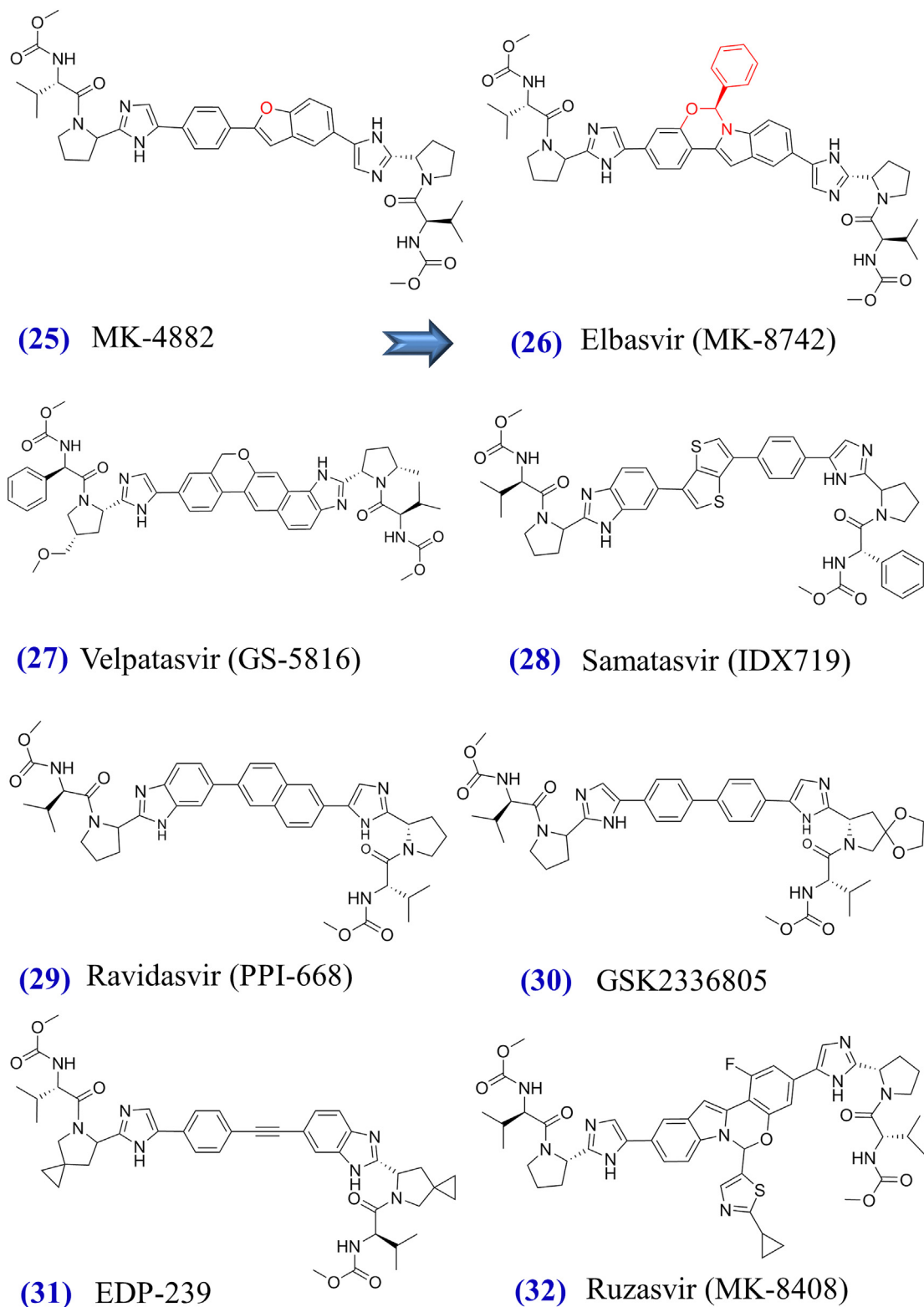
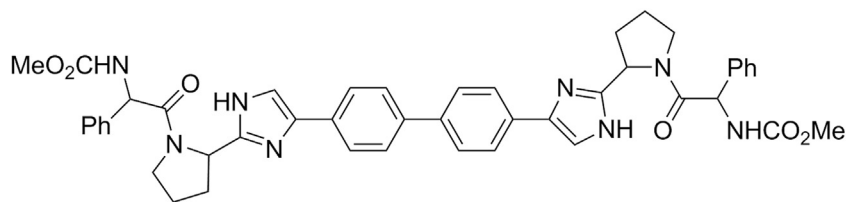


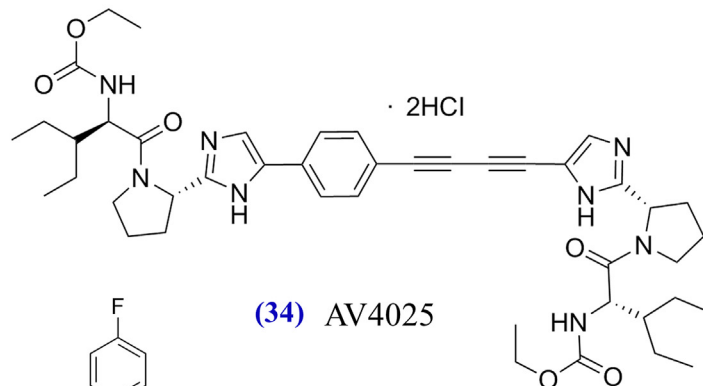
Fig. 3. (continued).

infections (Table 1). After the failure of screening efforts over four million compounds, boceprevir was derived from an α -ketoamide (Fig. 2) using structure-based drug design approaches (Njoroge et al., 2008). The advancement of structure-based drug design was promoted by the discovery of NS3 protease crystal structures revealed in 1996 (Kim et al., 1996; Love et al., 1996). As a potent NS3

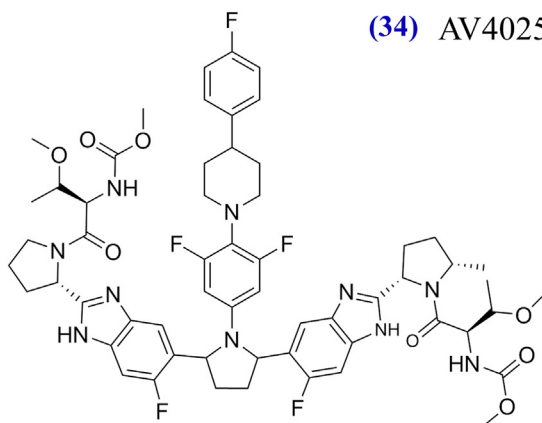
protease inhibitor, boceprevir blocks the serine hydroxyl of HCV NS3/4A protease (Njoroge et al., 2008). According to in vitro experiments, the EC_{50} values of boceprevir were 196 ± 56 nM against GT-1a, 251 ± 71 nM against GT-1b, 283 ± 36 nM against GT-2a, 315 ± 30 nM against GT-2b, and 159 ± 5 nM against GT-3a (Silva et al., 2013). In May 2011, the FDA approved the clinical use of



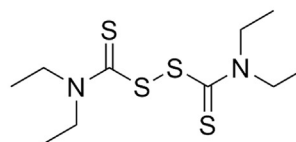
(33) Biarylimidazole chemotype 50b



(34) AV4025



(35) Pibrentasvir (ABT-530)



(36) Disulfiram

Fig. 3. (continued).

boceprevir plus PegIFN α /RBV in the treatment of HCV genotype 1 infection (Table 1).

The efficacy of the approved boceprevir-based therapy (PegIFN α /RBV for 4 weeks followed by 200 mg boceprevir TID plus PegIFN α /RBV for 44 weeks) was demonstrated in phase 2 and 3 clinical trials. Here, we summarize SVR24 rates of this therapy against genotype 1 infections in regard to cirrhosis and prior treatment history (Table 4). For treatment-naïve patients without cirrhosis, SVR24 rates reached 59.2% (87/147) in the HCV-TARGET study (Sterling et al., 2015), 64% (387/604) in a phase 3 study (Poordad et al., 2013), 76.3% (74/97) in the SPRINT-1 study (Kwo et al., 2010), and 67.4% (223/331) in the SPRINT-2 study (Poordad et al., 2011). For treatment-experienced patients without cirrhosis, SVR24 rates were 41.7% (68/163) in the HCV-TARGET study (Sterling et al., 2015), 64.1% (86/134) in a phase 3 study (Flamm et al., 2013), 66.4% (17/22) in the RESPOND-2 study (Bacon et al., 2011), and 64.2% (104/162) in the PROVIDE study (Vierling et al., 2014). For treatment-naïve patients with cirrhosis, SVR24 rates were approximately 32%–55% in phase 2 or 3 studies (Sterling et al., 2015; Poordad et al., 2011, 2013; Kwo et al., 2010). As for treatment-experienced patients with cirrhosis, SVR24 rates were between 31.1% and 77.3% in clinical trials (Table 3). Although

boceprevir plus PegIFN α /RBV was the first FDA-approved therapy, its efficacy was superseded by new DAAs, and its discontinuation was announced by Merck in 2015.

4.2. Telaprevir (Incivek[®]) + PegIFN α + RBV

In May 2011, the FDA approved the clinical use of 1125 mg telaprevir (Incivek[®]) twice daily plus PegIFN α /RBV for 12 weeks, followed by PegIFN α /RBV for 12w or 36w. Telaprevir (formerly VX-950) in combination with PegIFN α /RBV was the second FDA-approved therapy against HCV genotype 1 infections (Table 1). Illustrated in Fig. 2, telaprevir was discovered from a viral NS5A/5B substrate (sequence: EDVVCCMSY) of the HCV protease using structure-based drug design approaches (Kwong et al., 2011). The principle was based on the fact that HCV protease could be inhibited by its own cleavage products (Kwong et al., 2011), a mechanism similar to protease inhibitors explored for HIV (Li and De Clercq, 2016). Telaprevir is a reversible noncovalent NS3 inhibitor with favorable pharmacokinetic profiles in HCV cell cultures and animal models. Moreover, the EC₅₀ values of telaprevir reached 252 ± 54 nM to 953 ± 103 nM in HCV GT1, GT2, and GT3 replicon assays (Silva et al., 2013).

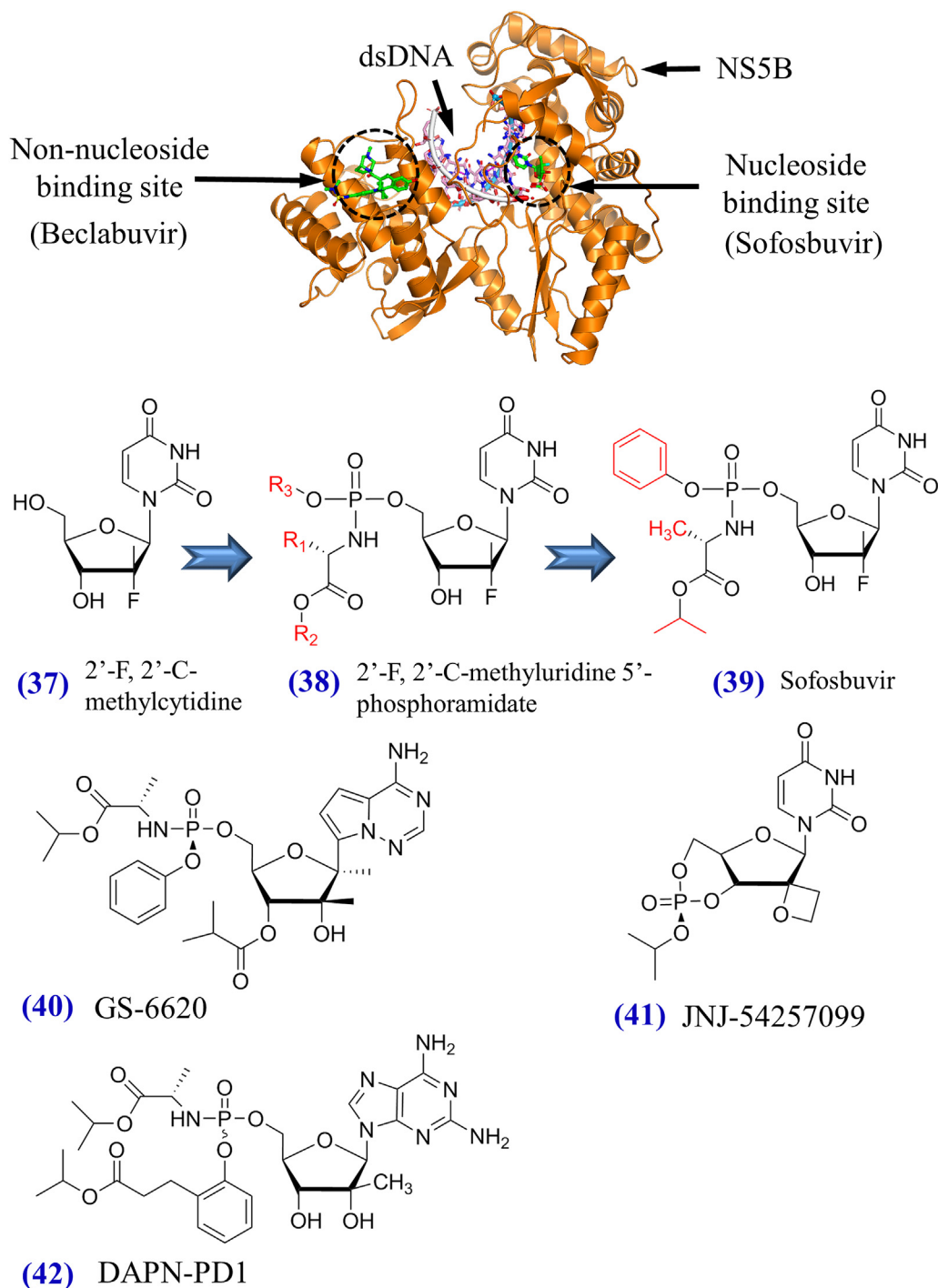


Fig. 4. Tertiary structure of HCV NS5B and structural formulas of approved or experimental nucleoside inhibitors from (37) to (42). NS5B structure in complex with beclabuvir and sofosbuvir diphosphate (PDB codes: 4NLD and 4WTG) is visualized on top. The discovery of sofosbuvir undertakes the optimization path from 2'-F, 2'-C-methylcytidine to 2'-F, 2'-C-methyluridine 5'-phosphoramidate (Sofia et al., 2010). Structural formulas of GS-6620, JNJ-54257099, and DAPN-PD1 are also demonstrated.

Effectiveness of telaprevir plus PegIFN α /RBV against HCV GT1 was demonstrated in phase 2 and 3 clinical trials (Table 4). SVR12 rates of this therapy were between 62.2% and 80% in treatment-naïve patients without cirrhosis (Table 4). In the group of treatment-experienced patients without cirrhosis, SVR12 rates fell approximately between 50% and 75%. For the treatment-naïve patients with cirrhosis, SVR24 rates were 43.5% and 53.7% in the HCV-TARGET (Sterling et al., 2015) and OPTIMIZE (Buti et al., 2014) studies, respectively. For the treatment-experienced patients with

cirrhosis, SVR12 rates were 38.6% and 52.4% in the ATTAIN (Reddy et al., 2015a) and ANRS CO20 (Hezode et al., 2014) studies, respectively.

Although telaprevir showed a favorable efficacy in clinical trials, the telaprevir-based therapy may cause fatal and non-fatal serious skin reactions such as Stevens-Johnson syndrome (SJS), drug reaction with eosinophilia and systemic symptoms (DRESS), and toxic epidermal necrolysis (TEN). Due to the advent of next-generation DAAs, Vertex Pharmaceuticals announced the discontinuation of

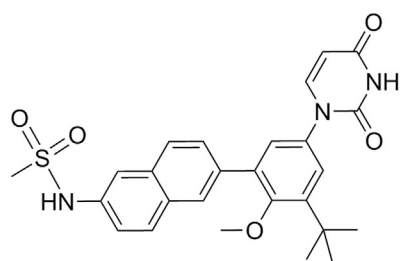
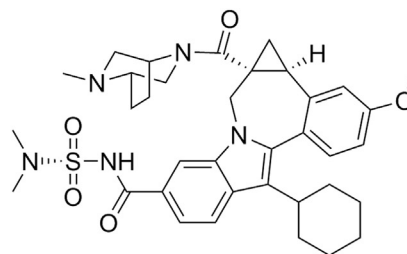
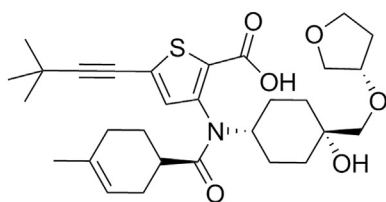
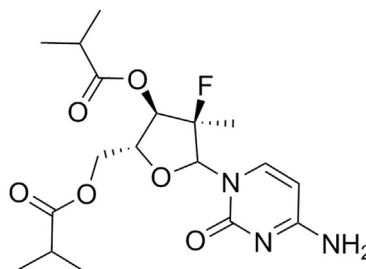
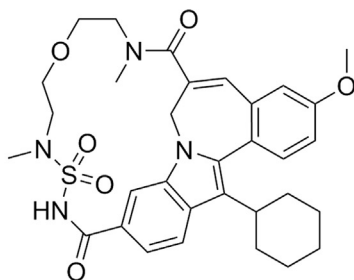
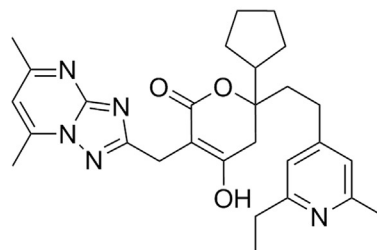
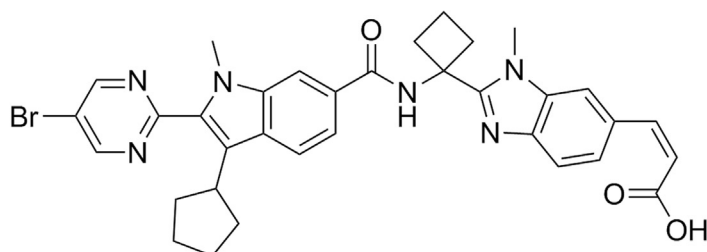
**(43)** Dasabuvir**(44)** Beclabuvir (BMS-791325)**(45)** GS-9669**(46)** Mericitabine (RG7128)**(47)** TMC647055**(48)** Filibuvir (PF-00868554)**(49)** Deleobuvir (BI 207127)

Fig. 5. Structural formulas of NS5B non-nucleoside inhibitors from (43) to (54). FDA-approved dasabuvir and experimental compounds target the non-nucleoside binding site in NS5B (see Fig. 4).

telaprevir in 2014.

4.3. Sofosbuvir (Sovaldi[®]) + PegIFN α + RBV

In December 2013, the FDA approved the clinical use of Sovaldi[®] against HCV genotype 1 or 4 infections: (i) 400 mg sofosbuvir QD plus PegIFN α /RBV for 12 weeks against genotype 1 or 4; (ii) 400 mg sofosbuvir QD plus ribavirin for 12 weeks against genotype 2; (iii)

400 mg sofosbuvir QD plus ribavirin for 24w against genotype 4. Sofosbuvir (formerly GS-7977 or PSI-7977), discovered at Pharmasset in 2007 and acquired by Gilead Sciences in 2011, is the first FDA-approved nucleotide analogue that inhibits HCV NS5B (Lawitz et al., 2015a). As a selective inhibitor of HCV NS5B polymerase (Fig. 4), β -D-2'-deoxy-2'- α -fluoro-2'- β -C-methyluridine nucleosides were selected and the optimization of their phosphoramidate prodrugs led to the discovery of sofosbuvir (Sofia et al., 2010)

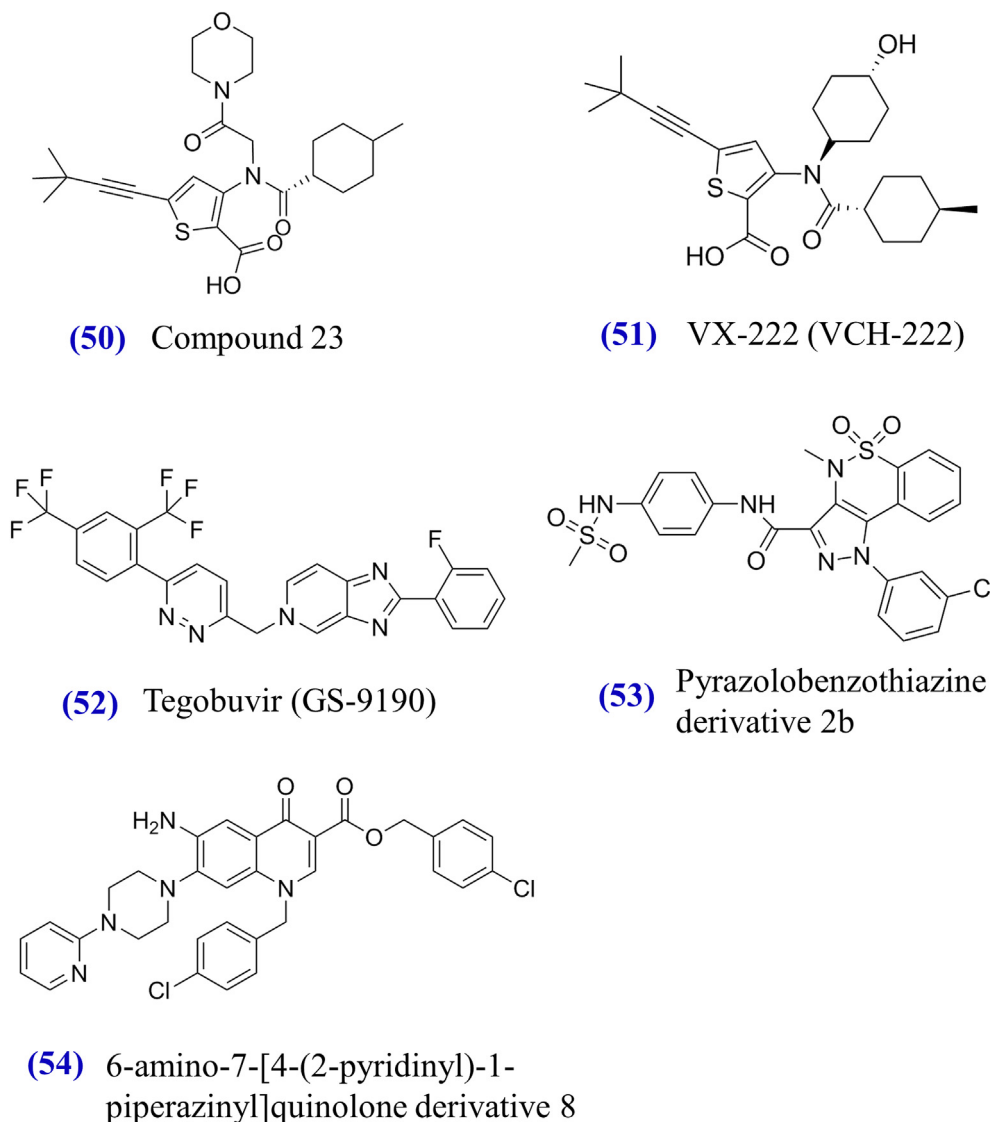


Fig. 5. (continued).

(Fig. 4). Moreover, sofosbuvir demonstrated potent pan-genotypic activity, and its EC_{50} values were between 16 ± 3.4 nM and 48 ± 13 nM in HCV GT1, GT2, and GT3 replicons (Lam et al., 2012). The favorable antiviral activity of sofosbuvir was also found in primary hepatocytes from rat, dog, and monkey (Sofia et al., 2010). Importantly, Sovaldi[®] is the first FDA-approved HCV drug orally administered once-daily, leading to better adherence, excellent tolerability, and safety.

The efficacy of sofosbuvir plus PegIFN α /RBV was demonstrated in a number of phase 2 and 3 clinical trials (Tables 5–7). The efficacy of 400 mg sofosbuvir QD plus PegIFN α /RBV over a period of 12 weeks was demonstrated in clinical studies such as BOSON (Foster et al., 2015a), HCV-TARGET (Feld et al., 2016a), Electron (Gane et al., 2013a), ATOMIC (Kowdley et al., 2013), NEUTRINO (Lawitz et al., 2013a), KULDS (Ogawa et al., 2016), and LONESTAR-2 (Lawitz et al., 2015b). When we merged treatment outcome data to estimate drug efficacy regardless of the distinct nature between different clinical trials, the SVR12 rates of this therapy were approximately 87.6% (659/752) against GT1, 95.6% (482/504) against GT2, 91.3% (220/241) against GT3, 92.3% (36/39) against GT4, 100% (1/1) against GT5, and 100% (11/11) against GT6 (Table 5).

In particular, the use of above therapy against genotype 1 or 4 was approved by the FDA. Table 6 illustrates the efficacy of 400 mg sofosbuvir QD plus ribavirin at 12 weeks in clinical studies such as POSITRON (Jacobson et al., 2013), FUSION (Jacobson et al., 2013), Electron (Gane et al., 2013a), FISSION (Lawitz et al., 2013a), and HCV-TARGET (Feld et al., 2016a; Welzel et al., 2016a). The SVR12 rates were approximately 62.8% (22/35) against GT1, 90.8% (750/826) against GT2, 55% (297/540) against GT3, and 73.4% (61/83) against GT4. Table 7 illustrates the efficacy of 400 mg sofosbuvir QD plus ribavirin at 24 weeks in clinical studies such as ASTRAL-2 (Foster et al., 2015b), ASTRAL-3 (Foster et al., 2015b), VALENCE (Zeuzem et al., 2014a), and BOSON (Foster et al., 2015a). SVR12 rates were approximately 58.7% (37/63) against GT1, 100% (17/17) against GT2, 82.4% (610/740) against GT3, and 90.1% (73/81) against GT4. Moreover, recurrent HCV infections after liver transplantation in patients, including those with fibrosing cholestatic hepatitis and decompensated cirrhosis, might be treated by sofosbuvir plus ribavirin, achieving modest efficacy (SVR12: 59%) (Forns et al., 2015a). The most common side effects of sofosbuvir plus PegIFN α /RBV were fatigue, headache, nausea, insomnia and anemia.

Table 2
Antiviral activity of FDA-approved compounds in HCV cell culture.

Protein	Compound	In vitro activity (EC ₅₀ , nM)								Ref.
		GT-1a	GT-1b	GT-2a	GT-2b	GT-3a	GT-4a	GT-5a	GT-6a	
NS3/4A	Boceprevir (SCH503034)	196 ± 56	251 ± 71	283 ± 36	315 ± 30	159 ± 5	–	–	–	Silva et al., 2013
	Telaprevir (VX-950)	395 ± 16	285 ± 60	252 ± 54	402 ± 52	953 ± 103	–	–	–	Silva et al., 2013
	Paritaprevir (ABT-450)	1.0 ± 0.33	0.21 ± 0.07	5.3 ± 1.2	–	19 ± 5.2	0.09 ± 0.03	–	0.69 ± 0.09	Pilot-Matias et al., 2015
	Grazoprevir (MK-5172)	0.4 ± 0.2	0.5 ± 0.3	2.3 ± 1.2	3.7 ± 1.1	2.1 ± 1	0.3 ± 0.2	6.6 ± 0.6	0.9 ± 0.1	Lahser et al., 2016
	Simeprevir (TMC435)	28.4 (19–39.7)	8.1 (4.5–11.9)	–	–	–	–	–	–	Lin et al., 2009
NS5A	Daclatasvir (BMS-790052)	0.05 ± 0.013	0.009 ± 0.004	0.071 ± 0.017	–	0.146 ± 0.034	0.012 ± 0.004	0.033 ± 0.01	–	Gao et al., 2010
	Ledipasvir (GS-5885)	0.031	0.004	21	16	168	0.39	0.15	1.1	Cheng et al., 2016
	Ombitasvir (ABT-267)	0.0141 ± 0.0068	0.005 ± 0.0019	0.0124 ± 0.0027	0.0043 ± 0.0012	0.0193 ± 0.0058	0.00171 ± 0.00088	0.0043 ± 0.0009	0.415 ± 0.097	DeGoey et al., 2014
	Elbasvir (MK-8742)	0.004 ± 0.002	0.003 ± 0.001	0.003 ± 0.001	3.4 ± 2.6	0.14 ± 0.09	0.0003 ± 0.0001	0.001 ± 0.001	0.009 ± 0.006	Lahser et al., 2016
	Velpatasvir (GS-5816)	0.013	0.015	0.009	0.01	0.013	0.009	0.059	0.007	Cheng et al., 2013
NS5B	Sofosbuvir (GS-7977)	44 ± 4.7	48 ± 13	37 ± 3.6	20 ± 4.4	16 ± 3.4	–	–	–	Lam et al., 2012
	Dasabuvir (ABT-333)	7.7 ± 3.8	1.8 ± 0.98	–	–	–	–	–	–	Kati et al., 2015

--: Unavailable data. Information of HCV strains and experimental settings is available in the literature.

4.4. Simeprevir (Olysio[®]) + sofosbuvir or PegIFN α /RBV

In November 2013, the FDA approved the use of (i) 150 mg simeprevir QD plus PegIFN α /RBV against genotype 1 or 4 infections; (ii) 150 mg simeprevir plus 400 mg sofosbuvir QD against genotype 1 infections (Table 1). Simeprevir (formerly TMC435) is a potent inhibitor targeting HCV NS3/4A (Rosenquist et al., 2014) (Fig. 2). It is worth mentioning that the NS3/4A inhibitor simeprevir in the combination with the NS5B inhibitor sofosbuvir is considered to efficiently inhibit different stages of HCV life cycle – a principle in line with the standard cocktail treatment for HIV. Simeprevir was discovered during the extensive exploration of novel P2 cyclopentane macrocyclic inhibitors using HCV NS3 protease assays, the cellular replicon system, and structure-based

design (Rosenquist et al., 2014). Moreover, EC₅₀ values of simeprevir were 28.4 nM and 8.1 nM in Huh7-derived replicon cells with genotype 1a (strain: H77) or genotype 1b (con1b), respectively (Lin et al., 2009). Indeed, simeprevir showed excellent in vitro and in vivo biological, antiviral and safety pharmacology profiles, which paved its way to clinical trials.

Here, we summarize the efficacy of simeprevir plus sofosbuvir or PegIFN α /RBV in phase 2 and 3 clinical studies such as COSMOS (Lawitz et al., 2014a), OPTIMIST-1 (Kwo et al., 2016), OPTIMIST-2 (Lawitz et al., 2016a), TARGET (El-Khayat et al., 2016; Sulkowski et al., 2016), and ANRS CO22 HEPATHER(112). We attempted to estimate drug efficacy by merging all clinical data regardless of the distinct nature between different clinical trials. (i) For the treatment of 150 mg simeprevir plus 400 mg sofosbuvir QD at 12w, its

Table 3
Efficacy of PegIFN α /RBV for 4 weeks + 200 mg boceprevir TID plus PegIFN α /RBV for 44 weeks.

Cirrhosis	Prior treatment ^a	SVR24 rate in GT-1	Study and trial phase	Ref.
No cirrhosis	Naïve	76.3% (74/97)	SPRINT-1, phase 2	Kwo et al., 2010
	Naïve	67.4% (223/331)	SPRINT-2, phase 2	Poordad et al., 2011
	Experienced	66.4% (85/128)	RESPOND-2, phase 3	Bacon et al., 2011
	Naïve	59.2% (87/147)	HCV-TARGET, phase 3	Sterling et al., 2015
	Experienced	41.7% (68/163)		
	Null response	40.8% (20/49)	PROVIDE, phase 3	Vierling et al., 2014
	Partial response	67% (57/85)		
	Relapse	96.4% (27/28)		
	Naïve	64.1% (387/604)	Protocol 6086, phase 3	Poordad et al., 2013
	Non-response	47.2% (17/36)	P05685AM2, phase 3	Flamm et al., 2013
	Relapse	70.4% (69/98)		
Cirrhosis	Naïve	41.7% (10/24)	SPRINT-2, phase 2	Poordad et al., 2011
	Naïve	50% (3/6)	SPRINT-1, phase 2	Kwo et al., 2010
	Naïve	55% (33/60)	Protocol 6086, phase 3	Poordad et al., 2013
	Naïve	32% (16/50)	HCV-TARGET, phase 3	Sterling et al., 2015
	Experienced	31.1% (28/90)		
	Relapse	53.9% (55/102) ^b	ANRS CO20, phase 3	Hezode et al., 2014
	Partial response	38.3% (36/94) ^b		
	Null response	0% (0/10) ^b		
	Experienced	77.3% (17/22)	RESPOND-2, phase 3	Bacon et al., 2011

^a Naïve: patients were treatment-naïve prior to therapies in clinical trials. Experienced: patients experienced other HCV therapies prior to therapies in clinical trials.

^b SVR12 rates were evaluated in the study.

Table 4Efficacy of 1125 mg telaprevir BID plus PegIFN α /RBV for 12w + PegIFN α /RBV for 12w or 36w.

Cirrhosis	Prior treatment	SVR12 rate in GT-1	Study and trial phase	Ref.
No cirrhosis	Naïve	1a: 82.3% (28/34), 1b: 78% (32/41)	MALACHITE-I, phase 3	Dore et al., 2016a
	Null response	56.5% (13/23)	MALACHITE-II, phase 3	
	Partial response	75% (9/12)		
	Relapse	75% (9/12)		
	Naïve	50% (95/190)	ATTAIN, phase 3	Reddy et al., 2015a
	Partial response	72.3% (86/119)		
	Naïve	62.2% (69/111)	Phase 3	Kumada et al., 2016
	Naïve	77.78% (245/315)	OPTIMIZE, phase 3	
	Naïve	61.8% (272/440) ^a	HCV-TARGET, phase 3	Sterling et al., 2015
	Experienced	59.3% (300/506) ^a		
Cirrhosis	Naïve	53.7% (29/54)	OPTIMIZE, phase 3	Buti et al., 2014
	Naïve	43.5% (103/237) ^a	HCV-TARGET, phase 3	
	Experienced	48.4% (194/401) ^a		Sterling et al., 2015
	Null response	31.3% (15/48)	ATTAIN, phase 3	
	Partial response	51.9% (14/27)		Reddy et al., 2015a
	Relapse	74.2% (92/124)	ANRS CO20, phase 3	
	Partial response	40% (54/135)		Hezode et al., 2014
	Null response	19.4% (6/31)		

1a: HCV genotype 1a, 1b: HCV genotype 1b.

^a SVR24 rates were evaluated in the clinical study.**Table 5**Efficacy of 400 mg sofosbuvir QD + PegIFN α /RBV for 12w.

Cirrhosis	Prior treatment	SVR12 rate						Study and trial phase	Ref.
		GT-1	GT-2	GT-3	GT-4	GT-5	GT-6		
No cirrhosis	Experienced		100% (9/9)	83.3% (10/12)				LONESTAR-2, phase 2	Lawitz et al., 2015b
	Naïve		100% (4/4)	100% (7/7)				Electron, phase 2a	
	Naïve	91.5% (43/47)	93.3% (14/15)	90% (9/10)				Phase 2a	Lawitz et al., 2013b
	Experienced	78.8% (63/80)						Phase 2	
	Naïve	87% (275/316)			81.8% (9/11)		100% (5/5)	ATOMIC, phase 2	Kowdley et al., 2013
	Naïve	91.3% (219/240)			100% (27/27)	100% (1/1)	100% (5/5)	NEUTRINO, phase 3	
	Naïve			95.8% (68/71)				BOSON, phase 3	Foster et al., 2015a
	Experienced			94.2% (49/52)					
	Naïve	100% (16/16)			?			C-EDGE Head-2-Head,	Sperl et al., 2016
	Experienced	100% (1/1)			?			phase 3	
	Naïve			100% (4/4)				HCV-TARGET, phase 4	Feld et al., 2016a
	Experienced			75% (3/4)					
	Naïve		98.1% (252/257)					KULDS, real-world study	Ogawa et al., 2016
	Experienced		95.9% (95/99)						
Cirrhosis	Experienced		92.9% (13/14)	83.3% (10/12)				LONESTAR-2, phase 2	Lawitz et al., 2015b
	Naïve			91.3% (21/23)				BOSON, phase 3	
	Experienced		93.8% (15/16)	85.7% (30/35)					Foster et al., 2015a
	Naïve	80.8% (42/52)			0% (0/1)		100% (1/1)	NEUTRINO, phase 3	
	Naïve			66.7% (2/3)				HCV-TARGET, phase 4	Feld et al., 2016a
	Experienced			87.5% (7/8)					
	Naïve		95.6% (43/45)					KULDS, real-world study	Ogawa et al., 2016
	Experienced		82.2% (37/45)						

?: Question marks indicate that the data was unavailable in literature.

SVR12 reached 93.8% (407/434) in GT1-infected patients without cirrhosis, 97.9% (476/486) in GT4-infected patients without cirrhosis, 81.7% (371/454) in GT1-infected patients with cirrhosis, 84.8% (84/99) in GT4-infected patients with cirrhosis (Table 8). (ii) Regarding the treatment of 150 mg simeprevir plus 400 mg sofosbuvir QD for 24w, its SVR12 reached 96.9% (31/32) in GT1-infected patients without cirrhosis, and 100% (10/10) in GT1-infected patients with cirrhosis (Table 8). (iii) Regarding the treatment of 150 mg simeprevir QD plus PegIFN α /RBV for 12w + PegIFN α /RBV for 12w, its SVR12 reached 76.2% (1087/1426) in GT1-infected patients without cirrhosis, 50.2% (88/175) in GT1-infected patients with cirrhosis (Table 9). (iv) Regarding the treatment of 150 mg simeprevir QD plus PegIFN α /RBV for 12w + PegIFN α /RBV for 36w, its SVR12 reached 83.2% (252/303) in GT1-infected patients without cirrhosis, 65.9% (29/44) in GT1-infected patients with cirrhosis (Table 10). In the RESTORE study,

patients received simeprevir plus PegIFN α /RBV for 12w followed by PegIFN α /RBV for 12w or 36w (Moreno et al., 2015). SVR12 rates were 66.6% (72/108) in GT4-infected patients without cirrhosis, and 40.8% (20/49) in GT4-infected patients with cirrhosis. Furthermore, 12w of simeprevir plus PegIFN α /RBV followed by additional 36w of PegIFN α /RBV was approved for patients co-infected with HIV and HCV infections. In clinical studies, the most common side effects of simeprevir plus sofosbuvir were fatigue, headache and nausea, while simeprevir plus sofosbuvir may have caused common adverse events such as rash, pruritus, nausea, and photosensitivity reactions.

4.5. Ledipasvir + sofosbuvir (Harvoni[®])

In October 2014, the FDA approved the fixed-dose combination tablets of 90 mg ledipasvir + 400 mg sofosbuvir (Harvoni[®]) with or

Table 6
Efficacy of 400 mg sofosbuvir QD + ribavirin for 12 weeks.

Cirrhosis	Prior treatment	SVR12 rate				Study and trial phase	Ref.
		GT-1	GT-2	GT-3	GT-4		
No cirrhosis	Naïve	84% (21/25)	100% (4/4)	100% (6/6)		Electron, phase 2a	Gane et al., 2013a
	Null response	10% (1/10)					
	Naïve				90.9% (10/11)	Phase 2	Ruane et al., 2015
	Experienced				61.5% (8/13)		
	Experienced		92.4% (85/92)	67.9% (57/84)		POSITRON, phase 3	Jacobson et al., 2013
	Experienced		96.2% (25/26)	36.8% (14/38)		FUSION, phase 3	
	Naïve		98.3% (58/59)	61.4% (89/145)		FISSION, phase 3	Lawitz et al., 2013a
	Naïve		91.9% (159/173)	75% (42/56)		HCV-TARGET, phase 4	Feld et al., 2016a; Welzel et al., 2016a
	Experienced		87.5% (42/48)	84.6% (22/26)			
	Naïve		97.6% (80/82)			Phase 3	Omata et al., 2014
	Experienced		96.3% (52/54)				
	Naïve		96.7% (29/30)	0% (0/2)		VALENCE, phase 3	Zeuzem et al., 2014a
	Experienced		93.8% (30/32)	42.9% (3/7)			
	Naïve				86.4% (19/22)	Egyptian study	Doss et al., 2015
Experienced				72.7% (16/22)			
Naïve or experienced			86.4% (19/22)		Real-world study	Maasoumy et al., 2016	
Cirrhosis	Naïve				33.3% (1/3)	Phase 2	Ruane et al., 2015
	Experienced				50% (2/4)		
	Naïve		100% (2/2)			VALENCE, phase 3	Zeuzem et al., 2014a
	Experienced		77.8% (7/9)	0% (0/2)			
	Naïve		76.6% (36/47)			VALOR, phase 4	Ho et al., 2017
	Experienced		84.2% (16/19)				
	Experienced		94.1% (16/17)	21.4% (3/14)		POSITRON, phase 3	Jacobson et al., 2013
	Experienced		60% (6/10)	19.2% (5/26)		FUSION, phase 3	
	Naïve		71.9% (23/32)	55.3% (21/38)		HCV-TARGET, phase 4	Feld et al., 2016a; Welzel et al., 2016a
	Experienced		86.7% (26/30)	37.9% (22/58)			
	Naïve		90.9% (10/11)	34.2% (13/38)		FISSION, phase 3	Lawitz et al., 2013a
	Naïve		100% (8/8)			Phase 3	Omata et al., 2014
	Experienced		88.9% (8/9)				
	Naïve				66.7% (2/3)	Egyptian study	Doss et al., 2015
	Experienced				60% (3/5)		
	Naïve or experienced		90% (9/10)			Real-world study	Maasoumy et al., 2016

Table 7
Efficacy of 400 mg sofosbuvir QD + ribavirin for 24 weeks.

Cirrhosis	Prior treatment	SVR12 rate				Study and trial phase	Ref.
		GT-1	GT-2	GT-3	GT-4		
No cirrhosis	Experienced	1a: 80% (8/10), 1b: 50% (3/6)		100% (5/5)	0% (0/1)	Phase 2	Charlton et al., 2015a
	Naïve				100% (11/11)	Phase 2	Ruane et al., 2015
	Experienced				81.8% (9/11)		
	Naïve	?				SPARE, phase 2	Osinusi et al., 2013
	Naïve			90.4% (141/156)		ASTRAL-2, ASTRAL-3, phase 3	Foster et al., 2015b
	Experienced			70.9% (22/31)			
	Naïve			94.6% (87/92)		VALENCE, phase 3	Zeuzem et al., 2014a
	Experienced			86.7% (85/98)			
	Naïve			90.3% (65/72)		BOSON, phase 3	Foster et al., 2015a
	Experienced			81.5% (44/54)			
	Naïve				90.5% (19/21)	Real-world study	Doss et al., 2015
	Experienced				95.2% (20/21)		
Naïve or experienced		75% (3/4)		76.9% (10/13)	Real-world study	Maasoumy et al., 2016	
Cirrhosis	Experienced	1a: 66.7% (8/12), 1b: 50% (2/4)		100% (1/1)		Phase 2	Charlton et al., 2015a
	Naïve				100% (3/3)	Phase 2	Ruane et al., 2015
	Experienced				100% (4/4)		
	Naïve	?				SPARE, phase 2	Osinusi et al., 2013
	Naïve			73.3% (33/45)		ASTRAL-2, ASTRAL-3, phase 3	Foster et al., 2015b
	Experienced			57.9% (22/38)			
	Naïve			92.3% (12/13)		VALENCE, phase 3	Zeuzem et al., 2014a
	Experienced			61.7% (29/47)			
	Naïve			81.8% (18/22)		BOSON, phase 3	Foster et al., 2015a
	Experienced		100% (17/17)	76.5% (26/34)			
	Naïve				100% (3/3)	Egyptian study	Doss et al., 2015
	Experienced				66.7% (4/6)		
Naïve or experienced		48.1% (13/27)		52.6% (10/19)	Real-world study	Maasoumy et al., 2016	

?: Question marks indicate that the data was unavailable in literature.

Table 8

Efficacy of 150 mg simeprevir plus 400 mg sofosbuvir QD for 12w.

Cirrhosis	Prior treatment	SVR12 rate		Study and trial phase	Ref.
		GT-1	GT-4		
150 mg simeprevir plus 400 mg sofosbuvir QD for 12w					
No cirrhosis	Naïve	100% (4/4)		COSMOS, phase 3	(Lawitz et al., 2014a)
	Null response	94.1% (16/17)		OPTIMIST-1, phase 3	(Kwo et al., 2016)
	Naïve	97.4% (112/115)			
	Experienced	95% (38/40)			
	Naïve		98.3% (285/290)	HCV-TARGET, phase 3	(El-Khayat et al., 2016)
	Experienced		97.4% (191/196)		
	Naïve	100% (11/11)		GALAXY, phase 2	(O'Leary et al., 2017)
	Naïve	1a:88.4% (61/69), 1b:97.4% (37/38)		HCV-TARGET, phase 3	(Sulkowski et al., 2016)
Cirrhosis	Experienced	1a:89.5% (77/86), 1b:94.4% (51/54)			
	Naïve	66.6% (2/3)		COSMOS, phase 3	(Lawitz et al., 2014a)
	Null response	100% (4/4)		OPTIMIST-2, phase 3	(Lawitz et al., 2016a)
	Naïve	88% (44/50)			
	Experienced	79.2% (42/53)			
	Naïve		80.8% (42/52)	HCV-TARGET, phase 3	(El-Khayat et al., 2016)
	Experienced		88.9% (40/45)		
	Naïve	1a:83.9% (73/87), 1b:91.7% (33/36)		HCV-TARGET, phase 3	(Sulkowski et al., 2016)
150 mg simeprevir plus 400 mg sofosbuvir QD for 24w	Experienced	1a:75.2% (97/129), 1b:82.1% (64/78)			
	Experienced	1a:81.8% (9/11), 1b:100% (3/3)	100% (2/2)	ANRS CO22 HEPATHER	(Hezode et al., 2016)
	Naïve or experienced	80.8% (97/120)		Real-world study	(Shiffman et al., 2015)
	Naïve	100% (2/2)			
No cirrhosis	Naïve	100% (2/2)		COSMOS, phase 3	(Lawitz et al., 2014a)
	Null response	100% (18/19)		GALAXY, phase 2	(O'Leary et al., 2017)
	Naïve	100% (11/11)			
Cirrhosis	Naïve	100% (6/6)		COSMOS, phase 3	(Lawitz et al., 2014a)
	Null Response	100% (4/4)			

without ribavirin for the treatment of HCV genotype 1, 4, 5 or 6 infections. Ledipasvir is a potent NS5A inhibitor (Fig. 3), while the nucleotide analogue sofosbuvir targets NS5B to inhibit its activity (Fig. 4). Ledipasvir (formerly GS-5885) was discovered through a series of modifications in an unsymmetric benzimidazole-difluorofluorene-imidazole core and distal [2.2.1] azabicyclic ring system (Link et al., 2014). In HCV replicon assays, the EC₅₀ values of ledipasvir were 0.031 nM against GT1a, 0.004 nM against GT1b, 21 nM against GT2a, 16 nM against GT2b, 168 nM against GT3a, 0.39 nM against GT4a, 0.15 nM against GT5a, and 1.1 nM against GT6a (Cheng et al., 2016). Pharmacokinetic studies in animal models (e.g. rats, dogs, monkeys) further supported the potent antiviral activity of ledipasvir (Link et al., 2014).

The efficacy of ledipasvir plus sofosbuvir with or without

ribavirin was demonstrated in many clinical studies, including LONESTAR (Lawitz et al., 2014b), ION-1 (Afdhal et al., 2014a), ION-2 (Afdhal et al., 2014b), ION-3 (Kowdley et al., 2014a), SOLAR-1 (Charlton et al., 2015b), SOLAR-2 (Manns et al., 2016), NIAID SYNERGY (Kohli et al., 2015a), TRIO (Younossi et al., 2016), GS-US-337-0113 (Mizokami et al., 2015), and SIRIUS (Bourliere et al., 2015) (see summary in Tables 11 and 12). For instance, the SOLAR-1 and SOLAR-2 studies indicated that the ledipasvir + sofosbuvir + ribavirin therapy for 12 weeks led to high rates of SVR12 (>85%) even in patients with decompensated cirrhotic before and after liver transplantation (Charlton et al., 2015b; Manns et al., 2016). Here, we attempted to estimate drug efficacy by merging all clinical data regardless of the distinct nature between different clinical trials. (i) Regarding the treatment of

Table 9Efficacy of 150 mg simeprevir QD plus PegIFN α /RBV for 12w + PegIFN α /RBV for 12w.

Cirrhosis	Prior treatment	SVR12 rate in GT-1	Study and trial phase	Ref.
No cirrhosis	Naïve	80.5% (62/77)	PILLAR, phase 2b	Fried et al., 2013
	Naïve	88.6% (109/123)	CONCERTO-1, phase 3	Hayashi et al., 2014
	Non-response	52.8% (28/53)	CONCERTO-2, phase 3	Izumi et al., 2014
	Relapse	95.9% (47/49)	CONCERTO-3, phase 3	
	Naïve	91.7% (22/24)	CONCERTO-4, phase 3	Kumada et al., 2015a
	Non-response	38.5% (10/26)		
	Relapse	100% (29/29)		
	Relapse	78.2% (230/294)	PROMISE, phase 3	Forns et al., 2014
	Naïve	82.1% (188/229)	QUEST-1, phase 3	Jacobson et al., 2014
	Naïve	81.8% (189/231)	QUEST-2, phase 3	Manns et al., 2014
	Null response	50.3% (87/173)	ATTAIN, phase 3	Reddy et al., 2015a
	Partial response	72.9% (86/118)		
	Cirrhosis	Relapse	74.4% (29/39)	PROMISE, phase 3
Null response		24.6% (15/61)	ATTAIN, phase 3	Reddy et al., 2015a
Partial response		55.6% (15/27)		
Naïve		58.1% (18/31)	QUEST-1, phase 3	Jacobson et al., 2014
Naïve		64.7% (11/17)	QUEST-2, phase 3	Manns et al., 2014

Table 10
Efficacy of 150 mg simeprevir QD plus PegIFN α /RBV for 12w + PegIFN α /RBV for 36w.

Cirrhosis	Prior treatment	SVR12 rate		Study and trial phase	Ref.
		GT-1	GT-4		
No cirrhosis	Relapser	87.3% (55/63)		ASPIRE, phase 2b	Zeuzem et al., 2014b
	Partial responder	74.1% (43/58)			
	Null responder	59.5% (22/37)			
	Naïve		84.4% (27/32)	RESTORE ^a , phase 3	Moreno et al., 2015
	Experienced		59.2% (45/76)		
	Naïve	91% (132/145)			
Cirrhosis	Relapser	73.3% (11/15)		ASPIRE, phase 2b	Zeuzem et al., 2014b
	Partial responder	81.8% (9/11)			
	Null responder	30.8% (4/13)			
	Naïve		66.7% (2/3)	RESTORE ^a , phase 3	Moreno et al., 2015
	Experienced		39.1% (18/46)		
	Naïve	100% (5/5)			
			TIGER, phase 3	Wei et al., 2016	

?: Question marks indicate that the data was unavailable in literature.

^a Patients in the RESTORE study received simeprevir plus PegIFN α /RBV for 12w followed by PegIFN α /RBV for either 12w or 36w (Moreno et al., 2015).

400 mg sofosbuvir + 90 mg ledipasvir QD for 12 weeks, the SVR12 rates were 96.1% (3674/3824) in GT1-infected patients without cirrhosis, and 92.2% (867/940) in GT1-infected patients with cirrhosis, 91.6% (44/48) in GT4-infected patients without cirrhosis, 96.8% (31/32) in GT5-infected patients without cirrhosis, and 95.6% (22/23) in GT6-infected patients without cirrhosis (Table 11). (ii) Regarding the treatment of 90 mg ledipasvir + 400 mg sofosbuvir QD for 24 weeks, its SVR12 reached 99.3% (266/268) in GT1-infected patients without cirrhosis, and 96.9% (127/131) in GT1-infected patients with cirrhosis. (iii) Regarding the therapy of 90 mg ledipasvir + 400 mg sofosbuvir QD with ribavirin for 12 weeks, SVR12 rates were 98.9% (467/472) in GT1-infected patients without cirrhosis, and 93.3% (393/421) in GT1-infected patients with cirrhosis (Table 12). Moreover, the drug resistant mutations at baseline exerted minimal effects on patient responses to the therapy of ledipasvir plus sofosbuvir (Sarrazin et al., 2016). The most common adverse events with the treatment of Harvoni[®] were headache, fatigue and asthenia.

Adding ribavirin to ledipasvir plus sofosbuvir might provide additional efficacy in certain patient populations, but its added value may not compensate for the increased rates of treatment-associated adverse events, for instance, in black patients (Wilder et al., 2016). Ledipasvir plus sofosbuvir for 12w was safe and effective against GT-1 infections in treatment-naïve patients with compensated cirrhosis, but its use for treatment-experienced patients with compensated cirrhosis required the extending treatment duration to 24 weeks (Reddy et al., 2015b).

4.6. Ombitasvir + paritaprevir + ritonavir + dasabuvir (Viekira Pak[™])

In December 2014, the FDA approved the fixed-dose combination tablets of paritaprevir + ombitasvir + ritonavir copackaged with dasabuvir (Viekira Pak[™]). Regarding the mechanisms of drug action, paritaprevir (formerly ABT-450), ombitasvir (ABT-267), and dasabuvir (ABT-333) are antiviral inhibitors against HCV NS3/4A, NS5A, and NS5B proteins, respectively. In addition to its known function as a booster to HIV protease inhibitors (De Clercq and Li, 2016), ritonavir in Viekira Pak[™] effectively inhibits human gene CYP3A encoding important enzymes in the human body that oxidize small foreign organic molecules for drug metabolism. The inhibition of CYP3A-mediated metabolism of paritaprevir thus increases the plasma concentration of paritaprevir. In replicon cell lines, paritaprevir demonstrates potent activities against different genotypes, and the EC₅₀ values of paritaprevir were estimated to be

1.0 ± 0.33 nM against GT1a, 0.21 ± 0.07 nM against GT1b, 5.3 ± 1.2 nM against GT2a, 19 ± 5.2 nM against GT3a, 0.09 ± 0.03 nM against GT4a, and 0.69 ± 0.09 nM against GT6a (Pilot-Matias et al., 2015). Based on cell-culture experiments, it has been shown that EC₅₀ values of ombitasvir were approximately 14.1 ± 6.8 pM against GT1a, 5.0 ± 1 pM against GT1b, 12.4 ± 2.7 pM against GT2a, 4.3 ± 1.2 pM against GT2b, 19.3 ± 5.8 pM against GT3a, 1.71 ± 0.88 pM against GT4a, 4.3 ± 0.9 pM against GT5a, and 415 ± 97 pM against GT6a (DeGoey et al., 2014). Regarding the discovery of ombitasvir, it was optimized from a symmetric series of N-phenylpyrrolidine-based inhibitors with chiral pyrrolidine-based cores (DeGoey et al., 2014) (Fig. 3). As for dasabuvir (Fig. 5), it is a nonnucleoside inhibitor that targets the allosteric site within the NS5B palm region (Liu et al., 2012). The EC₅₀ values of dasabuvir were 7.7 nM against GT1a and 1.8 nM against GT1b in replicon cell lines (Kati et al., 2015).

As the first co-formulated triple-DAA therapy, Viekira Pak[™] offers significant improvement against genotype 1. Here, we summarize the efficacy of Viekira Pak[™] in phase 2 and 3 clinical studies, including PEARL-II (Andreone et al., 2014), PEARL-III (Ferenci et al., 2014), PEARL-IV (Ferenci et al., 2014), SAPPHIRE-I (Feld et al., 2014), RUBY-I (Pockros et al., 2016), AVIATOR (Kowdley et al., 2014b), MALACHITE-I (Dore et al., 2016a), MALACHITE-II (Dore et al., 2016a), TURQUOISE-II (Poordad et al., 2014), TURQUOISE-III (Feld et al., 2016b), and SAPPHIRE-II (Zeuzem et al., 2014c). (i) Table 13 highlights the efficacy of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir once-daily fixed-dose two pills plus 250 mg dasabuvir BID for 12w. We attempted to estimate drug efficacy by merging all clinical data regardless of the distinct nature between different clinical trials. The SVR12 rates of this regimen reached 96.9% (1065/1099) in GT1-infected patients without cirrhosis, and 100% (84/84) in GT1-infected patients with cirrhosis in GT1-infected patients with cirrhosis. (ii) Table 14 shows the efficacy of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir once-daily fixed-dose two pills plus 250 mg dasabuvir BID plus ribavirin for 12w. Based on clinical studies, the SVR12 rates of this therapy reached 96.4% (2518/2612) in GT1-infected patients without cirrhosis, and 95.7% (630/658) in GT1-infected patients with cirrhosis. (iii) Table 15 illustrates the efficacy of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir fixed-dose two pills QD plus 250 mg dasabuvir BID plus ribavirin for 24w. In the TURQUOISE-II study, SVR12 rates reached 95.9% (165/172) in GT1-infected patients with cirrhosis (Poordad et al., 2014). Overall, the SVR12 rates of Viekira Pak[™] achieved above 90% in the treatment of GT1-infected patients with or without cirrhosis. The most common

Table 11
Efficacy of 90 mg ledipasvir + 400 mg sofosbuvir, QD for 12 weeks.

Cirrhosis	Prior treatment	SVR12 rate					Study and trial phase	Ref.
		GT-1	GT-3	GT-4	GT-5	GT-6		
No cirrhosis	Naïve	94.7% (18/19)					LONESTAR, phase 2	Lawitz et al., 2014b
	Experienced	100% (8/8)						
	Naïve	1a:100% (11/11), 1b:100% (9/9)					Phase 2a	Kohli et al., 2015a
	Naïve or experienced			92.9% (13/14)			NIAID, phase 2a	Kohli et al., 2015b
	Naïve or experienced		76.2% (16/21)			95.7% (22/23)	ELECTRON-2, phase 2	Gane et al., 2015
	Experienced	100% (7/7)					SPARE, phase 2a	Osinusi et al., 2014
	Experienced	91.2% (31/34)					NIAID synergy, phase 2a	Wilson et al., 2016
	Naïve			95.2% (20/21)			Phase 2	Abergel et al., 2016a
	Experienced			84.6% (11/13)				
	Naïve				94.4% (17/18)		Phase 2	(Abergel et al., 2016b)
	Experienced				100% (14/14)			
	Naïve	100% (70/70)					GS-US-337-0113, phase 3	Mizokami et al., 2015
	Experienced	100% (60/60)						
	Naïve	100% (179/179)					ION-1, phase 3	Afdhal et al., 2014a
	Experienced	96.2% (50/52)					ION-2, phase 3	Afdhal et al., 2014b
	Experienced	94.3% (33/35)						
	Naïve	1a: 94.8% (163/172), 1b: 97.7% (43/44)					ION-3, phase 3	Kowdley et al., 2014a
Naïve	1a: 94.6% (406/429), 1b: 98.3% (172/175)					TRIO, real-world study	Younossi et al., 2016	
Naïve	1a:95.7% (1352/1413), 1b:95.2% (415/436)					Real-world study	Ioannou et al., 2016	
Experienced	1a:96.7% (472/488), 1b:95.6% (175/183)							
Cirrhosis	Experienced	90.9% (10/11)					LONESTAR, phase 2	Lawitz et al., 2014b
	Naïve			100% (1/1)			Phase 2	Abergel et al., 2016a
	Experienced			100% (9/9)				
	Naïve				100% (3/3)		Phase 2	Abergel et al., 2016b
	Experienced				83.3% (5/6)			
	Null response	70% (7/10)					Phase 2	Gane et al., 2014a
	Naïve or experienced			100% (7/7)			NIAID, phase 2a	Kohli et al., 2015b
	Experienced	70% (7/10)					ELECTRON-1, phase 2	Gane et al., 2013b
	Naïve or experienced		25% (1/4)			100% (2/2)	ELECTRON-2, phase 2	Gane et al., 2015
	Naïve	96.9% (32/33)					ION-1, phase 3	Afdhal et al., 2014a
	PI + PegIFN α +RBV	85.7% (12/14)					ION-2, phase 3	Afdhal et al., 2014b
	PegIFN α +RBV	87.5% (7/8)						
	Naïve	100% (13/13)					GS-US-337-0113, phase 3	Mizokami et al., 2015
	Experienced	100% (28/28)						
	Naïve	1a:91.7% (483/527), 1b:93.6% (162/173)					Real-world study	Ioannou et al., 2016
	Experienced	1a:90.7% (68/75), 1b:100% (38/38)						
	Experienced	76.9% (10/13) ^a	40% (2/5) ^a				Real-world study	Cheung et al., 2016

^a SVR24 rates were evaluated in the clinical study.

side effects of Viekira Pak™ in these clinical studies were fatigue, nausea, skin rash, pruritus, insomnia, and asthenia.

4.7. Ombitasvir + paritaprevir + ritonavir (Technivie™)

In July 2015, the FDA approved the fixed-dose combination of ombitasvir plus paritaprevir plus ritonavir with or without ribavirin for GT4-infected patients without cirrhosis. Technivie™ is the first FDA-approved therapy solely against the GT4 infections. Basically, Technivie™ lacks dasabuvir in comparison to Viekira Pak™, while both products are manufactured by Abbvie.

Several clinical studies such as the PEARL-I study (Lawitz et al., 2015c; Hezode et al., 2015) and the GIFT-I study (Kumada et al., 2015b) were designed to illustrate the efficacy of Technivie™ (Table 16). As for the fixed-dose combination of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir taken two tablets once daily for 12 weeks, the SVR12 rates were 95% (400/421)

in GT1-infected patients without cirrhosis, 95.7% (135/141) in GT1-infected patients with cirrhosis, and 90.9% (40/44) in GT4-infected patients without cirrhosis. In the PEARL-I study (Hezode et al., 2015), the SVR12 rate of Technivie™ plus ribavirin achieved 100% in 91 GT4-infected patients without cirrhosis. The most common adverse events were asthenia, nausea, fatigue, and insomnia.

4.8. Daclatasvir (Daklinza™) + sofosbuvir (Sovaldi®)

In July 2015, the FDA approved the clinical use of 60 mg daclatasvir QD plus 400 mg sofosbuvir QD with or without ribavirin for 12 weeks against HCV genotype 1 or 3 infections. Noteworthy, daclatasvir and sofosbuvir bind to the NS5A and NS5B proteins, respectively. Daclatasvir (BMS-790052) was designed by the extensive optimization of a leading iminothiazolidinone (Fig. 3) obtained from the high throughput phenotypic screening (Belema and Meanwell, 2014). Daclatasvir in the

Table 12
Efficacy of 90 mg ledipasvir + 400 mg sofosbuvir QD for 24 weeks, or with ribavirin for 12 weeks in phase 2 and 3 clinical trials.

Cirrhosis	Prior treatment	SVR12 rate			Study and trial phase	Ref.	
		GT-1	GT-3	GT-4			
90 mg ledipasvir + 400 mg sofosbuvir QD for 24 weeks							
No cirrhosis	Naïve	99.5% (181/182)			ION-1, phase 3	Afdhal et al., 2014a	
	PI + PegIFN α +RBV	97.2% (35/36)			ION-2, phase 3	Afdhal et al., 2014b	
	PegIFN α +RBV	100% (50/50)					
Cirrhosis	Naïve	96.9% (31/32)			ION-1, phase 3	Afdhal et al., 2014a	
	Experienced	96.1% (74/77)			SIRIUS, phase 2	Bourliere et al., 2015	
	PI + PegIFN α +RBV	100% (14/14)			ION-2, phase 3	Afdhal et al., 2014b	
	PegIFN α +RBV	100% (8/8)					
90 mg ledipasvir + 400 mg sofosbuvir QD with ribavirin for 12 weeks							
No cirrhosis	Experienced	100% (36/36)			Patients from 5 trials Phase 2	Wyles et al., 2015	
	Naïve	100% (25/25)				Gane et al., 2014a	
	Experienced	100% (9/9)			LONESTAR, phase 2	Lawitz et al., 2014b	
	Experienced	100% (10/10)				ELECTRON-1, phase 2	Gane et al., 2013b
	Experienced	100% (7/7)				ELECTRON-2, phase 2	Gane et al., 2015; Gane et al., 2013b
	Naïve		100% (20/20)		SOLAR-1, phase 2	Charlton et al., 2015b	
	Experienced	100% (19/19)	89.3% (25/28)			SOLAR-2, phase 2	Manns et al., 2016
	Naïve or experienced	96.3% (52/54)		100% (1/1)	ION-1, phase 3	Afdhal et al., 2014a	
	Naïve or experienced	93.3% (42/45)		100% (7/7)	ION-2, phase 3	Afdhal et al., 2014b	
	Naïve	100% (178/178)			SIRIUS, phase 2	Bourliere et al., 2015	
	PI + PegIFN α +RBV	100% (51/51)				LONESTAR, phase 2	Lawitz et al., 2014b
	PegIFN α +RBV	100% (38/38)			Patients from 5 trials Phase 2	Wyles et al., 2015	
	Experienced	97.4% (75/77)				SOLAR-1, phase 2	Gane et al., 2014a
	Cirrhosis	Experienced	100% (11/11)			SOLAR-1, phase 2	Charlton et al., 2015b
		Experienced	100% (14/14)				SOLAR-2, phase 2
Experienced		100% (9/9)			ELECTRON-1, phase 2	Gane et al., 2013b	
Naïve or experienced		87.3% (96/110)		100% (3/3)		ELECTRON-2, phase 2	Gane et al., 2015; Gane et al., 2013b
Naïve or experienced		91.8% (90/98)		77.8% (14/18)	ION-1, phase 3	Afdhal et al., 2014a	
Experienced		100% (27/27)				ION-2, phase 3	Afdhal et al., 2014b
Naïve		100% (20/20)	100% (6/6)		Real-world study	Cheung et al., 2016	
Experienced			72.7% (16/22)				
Naïve		100% (33/33)					
PI + PegIFN α +RBV		84.6% (11/13)					
PegIFN α +RBV		77.8% (7/9)					
Experienced		89.2% (132/148) ^a	61.4% (35/57) ^a				

^a SVR24 rates were used in the study.

Table 13
Efficacy of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir fixed-dose two pills QD plus 250 mg dasabuvir BID for 12w.

Cirrhosis	Prior treatment	SVR12 in GT-1	Study and trial phase	Ref.
No cirrhosis	Naïve	88.6% (70/79) ^a	AVIATOR, phase 2b	Kowdley et al., 2014b
	Null response	1b: 100% (32/32)	PEARL-II, phase 3	Andreone et al., 2014
	Partial response	1b: 100% (26/26)		
	Relapse	1b: 100% (33/33)	PEARL-IV, PEARL-III, phase 3	Ferenci et al., 2014
	Naïve	1a: 90.2% (185/205), 1b:99% (207/209)		
	Naïve, stage 4 or 5 chronic kidney disease	1b: 100% (7/7)		
	Naïve	1b: 97.6% (81/83)	MALACHITE-I, phase 3	Dore et al., 2016a
	Naïve	1b: 97.8% (391/400)		
	Experienced	1b: 99% (103/104)	Real-world study	Ioannou et al., 2016
	Cirrhosis	Naïve	1b: 100% (27/27)	TURQUOISE-III, phase 3b
Experienced		1b: 100% (33/33)	Real-world study	Ioannou et al., 2016
Naïve		1b: 100% (24/24)		

^a SVR24 rates were evaluated in the clinical study.

symmetric and dimeric form may bind at positions 31 and 93 of HCV NS5A, thus interfering with the protein-protein interactions at the membrane interface (Nettles et al., 2014). Moreover, the cooperative interaction between daclatasvir and other NS5A inhibitors (e.g. syn-395) suggests the possible synergistic anti-HCV activity, providing additional options for HCV combination therapy (Sun et al., 2015). In HCV replicons, the EC₅₀ values of daclatasvir achieved 0.05 ± 0.013 nM against GT1a, 0.009 ± 0.004 nM against GT1b, 0.071 ± 0.017 nM against GT2a, 0.146 ± 0.034 nM against GT3a, 0.012 ± 0.004 nM against GT4a, and 0.033 ± 0.01 nM against GT5a (Table 2). Moreover,

pharmacokinetic analyses in animal models (e.g. rat, dog, cynomolgus monkey) further supported the potent antiviral activity of daclatasvir (Belema and Meanwell, 2014). In addition to the combination of daclatasvir plus sofosbuvir, daclatasvir was previously considered to be used with asunaprevir (Poordad et al., 2015; Muir et al., 2015; Toyota et al., 2016). Although the combination of daclatasvir plus asunaprevir was declined by the FDA, this regimen was approved in Japan.

The efficacy of daclatasvir plus sofosbuvir was proved in several clinical studies (Table 17) such as A1444040 (Sulkowski et al., 2014a), ALLY-1 (Poordad et al., 2016a), ALLY-3 (Nelson et al.,

Table 14

Efficacy of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir fixed-dose two pills QD plus 250 mg dasabuvir BID plus ribavirin for 12w.

Cirrhosis	Prior treatment	SVR12 rate in GT-1			Study and trial phase	Ref.
		GT-1 1b	GT-2	GT-4		
No cirrhosis	Naïve	95% (38/40) ^a			AVIATOR, phase 2b	Kowdley et al., 2014b
	Null response	95.5% (21/22) ^a				
	Naïve	97.2% (35/36)			Phase 2	Lalezari et al., 2015
	Experienced	100% (2/2)				
	Null response	1b:93.5% (29/31)			PEARL-II, phase 3	Andreone et al., 2014
	Partial response	1b:96% (24/25)				
	Relapse	1b:100% (32/32)			PEARL-IV, PEARL-III, phase 3	Ferenci et al., 2014
	Naïve	1a: 97% (97/100), 1b:99.5% (209/210)				
	Naïve	1a: 97.1% (67/69), 1b: 98.8% (83/84)			MALACHITE-I	Dore et al., 2016a
	Null response	100% (49/49)				
	Partial response	100% (25/25)			MALACHITE-II, phase 3	
	Relapse	96.3% (26/27)				
	Naïve	1a:95.3% (307/322), 1b:98% (148/151)			SAPPHIRE-I, phase 3	Feld et al., 2014
	Null response	95.3% (82/86)				
	Partial response	100% (65/65)			SAPPHIRE-II, phase 3	Zeuzem et al., 2014c
	Relapse to PegIFN α +RBV	95.2% (139/146)				
	Naïve, stage 4/5 chronic kidney disease	1a: 84.6% (11/13)			RUBY-I, phase 3	Pockros et al., 2016
Naïve	1a:95% (689/725), 1b:98.6% (140/142)					
Experienced	1a:94.6% (212/224), 1b:97.9% (47/48)			Real-world study	Ioannou et al., 2016	
Cirrhosis	Naïve	1a: 92.2% (59/64), 1b:100% (22/22)			TURQUOISE-II, phase 3	Poordad et al., 2014
	Null response to PegIFN α /RBV	1a:80% (40/50), 1b:100% (25/25)				
	Partial response to PegIFN α /RBV	1a: 100% (11/11), 1b:85.7% (6/7)			Real-world study	Ioannou et al., 2016
	Relapse to PegIFN α /RBV	1a: 93.3% (14/15), 1b:100% (14/14)				
	Naïve	1a:95.5% (190/199), 1b:100% (133/133)				
	Experienced	1a:96.6% (56/58), 1b:100% (60/60)				

^a SVR24 rates were evaluated in the clinical study.**Table 15**

Efficacy of 12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir fixed-dose two pills QD plus 250 mg dasabuvir BID plus ribavirin for 24w.

Cirrhosis	Prior treatment	SVR12 rate in GT-1			Study and trial phase	Ref.
		GT-1 1b	GT-2	GT-4		
Cirrhosis	Naïve	1a: 92.9% (52/56), 1b:100% (18/18)			TURQUOISE-II, phase 3	Poordad et al., 2014
	Null response to PegIFN α /RBV	1a:92.9% (39/42), 1b:100% (20/20)				
	Partial response to PegIFN α /RBV	1a: 100% (10/10), 1b:100% (3/3)				
	Relapse to PegIFN α /RBV	1a: 100% (13/13), 1b:100% (10/10)				

Table 16

Efficacy of Technivie™ with or without ribavirin for 12 weeks.

Cirrhosis	Prior treatment	SVR12 rate			Study and trial phase	Ref.
		GT-1 1b	GT-2	GT-4		
12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir fixed-dose two pills QD for 12w						
No cirrhosis	Experienced	88.9% (16/18)	72.2% (13/18)		Phase 2	Chayama et al., 2015
	Experienced	90% (36/40)				
	Naïve	95.2% (40/42)		90.9% (40/44)	PEARL-I, phase 2b	Lawitz et al., 2015c
	Naïve	95.7% (198/207)			PEARL-I, phase 2b	Hezode et al., 2015
	Experienced	96.5% (110/114)			GIFT-I, phase 3	Kumada et al., 2015b
Cirrhosis	Naïve	97.9% (46/47)			PEARL-I, phase 2b	Lawitz et al., 2015c
	Experienced	96.2% (50/52)				
	Naïve	100% (9/9)			GIFT-I, phase 3	Kumada et al., 2015b
	Experienced	90.9% (30/33)				
12.5 mg ombitasvir + 75 mg paritaprevir + 50 mg ritonavir fixed-dose two pills QD plus ribavirin BID for 12w						
No cirrhosis	Naïve			100% (42/42)	PEARL-I, phase 2b	Hezode et al., 2015
	Experienced			100% (49/49)		

2015), and ALLY-3+ (Leroy et al., 2016). Here, we attempted to estimate drug efficacy by merging all clinical data regardless of the distinct nature between different clinical trials. (i) Regarding the treatment of 60 mg daclatasvir + 400 mg sofosbuvir QD for 12w, the SVR12 rates were 99% (100/101) in GT1-infected patients without cirrhosis, 88.8% (103/116) in GT1-infected patients with cirrhosis, 96.3% (105/109) in GT3-infected patients without cirrhosis, and 62.5% (20/32) in GT3-infected patients with cirrhosis (Table 17). (ii) Regarding the treatment of 60 mg daclatasvir + 400 mg sofosbuvir QD + ribavirin for 12w, the SVR12

rates were 100% (33/33) in GT1-infected patients without cirrhosis versus 92% (23/25) with cirrhosis, and 94.1% (16/17) in GT3-infected patients without cirrhosis versus 83.3% (15/18) with cirrhosis (Table 17). In a real-world study enrolling 485 patients, SVR12 rates of daclatasvir plus sofosbuvir achieved more than 90% regardless of HCV genotypes, cirrhosis, liver transplant or HIV/HCV coinfection status (Welzel et al., 2016b). The most common adverse events in clinical studies were headache and fatigue.

Table 17
Efficacy of 60 mg daclatasvir + 400 mg sofosbuvir QD with or without ribavirin for 12 weeks.

Cirrhosis	Prior treatment	SVR12 rate			Study and trial phase	Ref.
		GT-1	GT-2	GT-3		
60 mg daclatasvir + 400 mg sofosbuvir QD for 12w						
No cirrhosis	Naïve	100% (35/35)			A1444040, phase 3	Sulkowski et al., 2014a
	Naïve			97.3% (73/75)	ALLY-3, phase 3	Nelson et al., 2015
	Experienced			94.1% (32/34)		
	Experienced or naive	?		?	ANRS CO23 CUPILT	Coilly et al., 2016
	Naïve	100% (25/25)			ANRS/AFEF Hepather	Pol et al., 2017
	Experienced	97.6% (40/41)				
Cirrhosis	Naïve	100% (6/6)			A1444040, phase 3	Sulkowski et al., 2014a
	Naïve	1a: 83.3% (5/6), 1b: 100% (1/1)			ALLY-1, phase 3	Poordad et al., 2016a
	Experienced	1a: 100% (7/7), 1b: 100% (2/2)				
	Naïve			57.9% (11/19)	ALLY-3, phase 3	Nelson et al., 2015
	Experienced			69.2% (9/13)		
	Experienced or naive	?		?	ANRS CO23 CUPILT	Coilly et al., 2016
	Naïve	79.5% (31/39)			ANRS/AFEF Hepather	Pol et al., 2017
	Experienced	92.8% (51/55)				
	Experienced	66.7% (2/3) ^a		40% (2/5) ^a	Real-world study	Cheung et al., 2016
60 mg daclatasvir + 400 mg sofosbuvir QD + ribavirin for 12w						
No cirrhosis	Naïve	1a:100% (8/8), 1b:100% (1/1)		100% (5/5)	ALLY-1, phase 3	Poordad et al., 2016a
	Experienced	1a:100% (10/10), 1b:100% (5/5)		83.3% (5/6)		
	Experienced			100% (2/2)	ALLY-3+, phase 3	Leroy et al., 2016
	Naïve			100% (4/4)		
	Naïve	?/36			A1444040, phase 3	Sulkowski et al., 2014a
	Naïve	100% (1/1)			ANRS/AFEF Hepather	Pol et al., 2017
	Experienced	100% (8/8)				
	Naïve		100% (5/5)		Real-world study	Mangia et al., 2016
	Experienced		100% (3/3)			
Cirrhosis	Naïve			50% (1/2)	ALLY-3+, phase 3	Leroy et al., 2016
	Experienced			87.5% (14/16)		
	Naïve	100% (2/2)			ANRS/AFEF Hepather	Pol et al., 2017
	Experienced	91.3% (21/23)				
	Naïve	?/5			A1444040, phase 3	Sulkowski et al., 2014a
	Experienced	88.2% (30/34) ^a		69.2% (72/104) ^a	Real-world study	Cheung et al., 2016

?: Question marks indicate that the data was unavailable.

^a SVR24 rates were used in the study.

4.9. Elbasvir + grazoprevir (Zepatier™)

In January 2016, the FDA approved the fixed-dose combination of 100 mg grazoprevir plus 50 mg elbasvir with or without ribavirin against HCV genotype 1 or 4 infections (Table 1). Grazoprevir (MK-5172) and elbasvir (MK-8742) bind to HCV NS3/4A and NS5A, respectively. On the one hand, grazoprevir (Fig. 2) was discovered using a molecular modeling-derived strategy that optimized the contacts of protease inhibitors with a P2-to-P4 macrocyclic ring to the HCV NS3/4A active sites (Harper et al., 2012; Liverton et al., 2008). On the other hand, elbasvir was discovered by introducing a tetracyclic indole into the early clinical candidate MK-4882 (Fig. 3) that significantly improved its virologic profiles (Coburn et al., 2013). In HCV cell-based replicons, the EC₅₀ values of grazoprevir were 0.4 nM against GT1a, 0.5 nM against GT1b, 2.3 nM against GT2a, 2.1 nM against GT3a, 0.3 nM against GT4a, 6.6 nM against GT5a, and 0.9 nM against GT6a (Lahser et al., 2016). Meanwhile, EC₅₀ values of elbasvir achieved 0.004 nM against GT1a, 0.003 nM against GT1b, 0.003 nM against GT2a, 0.14 nM against GT3a, 0.0003 nM against GT4a, 0.001 nM against GT5a, and 0.009 nM against GT6 (Lahser et al., 2016). The combination of grazoprevir plus elbasvir not only exhibited a potent activity against HCV replication, but harbored a higher genetic barrier to drug resistance compared to individual compounds (Lahser et al., 2016). Overall, both grazoprevir and elbasvir attained potent antiviral activities in cell culture assays and animal models (Lahser et al., 2016; Harper et al., 2012; Coburn et al., 2013).

The efficacy of grazoprevir plus elbasvir was proven in many clinical trials such as C-EDGE (Zeuzem et al., 2015a), C-EDGE CO-

STAR (Dore et al., 2016b), C-EDGE Head-2-Head (Sperl et al., 2016), C-EDGE TE (Kwo et al., 2017), C-SURFER (Roth et al., 2015), C-SCAPE (Brown et al., 2015), C-WORTHY (Sulkowski et al., 2015; Lawitz et al., 2015d), and C-SALVAGE (Forns et al., 2015b). Here, we attempted to estimate drug efficacy by merging all clinical data regardless of the distinct nature between different clinical trials (Table 18). (i) Regarding the therapy of 100 mg grazoprevir QD + 50 mg elbasvir QD for 12w, the SVR12 rates were 95.5% (751/786) in GT1-infected patients without cirrhosis, 96.3% (181/188) in GT1-infected patients with cirrhosis, 94.1% (32/34) in GT4-infected patients without cirrhosis, and 63.2% (12/19) in GT6-infected patients without cirrhosis (Table 18). (ii) Regarding the treatment of 100 mg grazoprevir QD + 50 mg elbasvir QD + RBV BID for 12w, its SVR12 rates fulfilled 94.6% (142/150) in GT1-infected patients without cirrhosis, and 94.8% (73/77) in GT1-infected patients with cirrhosis. With limited number of enrolled patients described in the C-SCAPE study (Brown et al., 2015), the SVR12 rates in non-cirrhotic patients were 80% (24/30), 100% (10/10), 100% (4/4), and 75% (3/4) against GT2, GT4, GT5, or GT6 infections, respectively. Due to the enrollment of patients co-infected with HCV and HIV in the C-EDGE TE study (Kwo et al., 2017), we were unable to obtain the SVR12 data of grazoprevir + elbasvir + RBV to treat HCV mono-infections. The most common side effects of Zepatier™ were anemia and headache. Furthermore, NS5A polymorphisms at 4 amino acid positions (M28, Q30, L31, Y93) may reduce the treatment efficacy in clinical trials (Komatsu et al., 2017). Overall, the combination of grazoprevir plus elbasvir with or without ribavirin is effective and safe for patients with GT1 or GT4 infections (see Table 19).

The combination of 100 mg grazoprevir + 50 mg

Table 18
Efficacy of Zepatier™ with or without ribavirin in phase 2 and 3 clinical trials.

Cirrhosis	Prior treatment	SVR12 rate					Study and trial phase *	Ref.
		GT-1	GT-2	GT-4	GT-5	GT-6		
100 mg grazoprevir QD + 50 mg elbasvir QD for 12w								
No cirrhosis	Naïve	97.7% (43/44)					C-WORTHY, phase 2	Sulkowski et al., 2015
	Experienced	89.5% (17/19)						Lawitz et al., 2015d
	Naïve	1a: 91.7% (144/157), 1b: 97.9% (95/97)		100% (16/16)		80% (8/10)	C-EDGE, phase 3	Zeuzem et al., 2015a
	Naïve	100% (92/92)					C-SURFER, phase 3	Roth et al., 2015
	Experienced	94.4% (17/18)						
	Naïve or Experienced	?		?			C-EDGE Head-2-Head, phase 3	Sperl et al., 2016
	Naïve	1a: 94.5% (104/110), 1b: 90.9% (20/22)		87.5% (7/8)		20% (1/5)	C-EDGE CO-STAR, phase 3	Dore et al., 2016b
	Naïve			90% (9/10)	25% (1/4)	75% (3/4)	C-SCAPE, phase 2	Brown et al., 2015
Cirrhosis	Naïve	96.6% (28/29)					C-WORTHY, phase 2	Lawitz et al., 2015d
	Null response	92.9% (13/14)						
	Naïve	100% (4/4)					C-SURFER, phase 3	Roth et al., 2015
	Experienced	100% (2/2)						
	Naïve or Experienced	?		?			C-EDGE Head-2-Head, phase 3	Sperl et al., 2016
	Naïve	1a: 93.3% (28/30), 1b: 100% (6/6)		100% (4/4)			C-EDGE CO-STAR, phase 3	Dore et al., 2016b
	Naïve	1a: 94.1% (32/34), 1b: 100% (34/34)		100% (2/2)			C-EDGE, phase 3	Zeuzem et al., 2015a
100 mg grazoprevir QD + 50 mg elbasvir QD + ribavirin BID for 12w								
No cirrhosis	Experienced	95.2% (20/21)					C-WORTHY, phase 2	Lawitz et al., 2015d
	Naïve	92.9% (79/85)						Sulkowski et al., 2015
	Naïve		80% (24/30)	100% (10/10)	100% (4/4)	75% (3/4)	C-SCAPE, phase 2	Brown et al., 2015
Cirrhosis	PI-treated	97.7% (43/44)					C-SALVAGE, phase 2	Forns et al., 2015b
	PI-treated	94.1% (32/34)						
	Naïve	96.9% (31/32)					C-WORTHY, phase 2	Lawitz et al., 2015d
	Null response	90.9% (10/11)						
100 mg grazoprevir QD + 50 mg elbasvir QD + ribavirin BID for 16w								
No cirrhosis	Null or partial response	?		?			C-EDGE TE, phase 3	Kwo et al., 2017
Cirrhosis	to PegIFN α /RBV	?		?				

?: Question marks indicate that the SVR12 data was unavailable in literature.

elbasvir + 400 mg sofosbuvir was recently evaluated in the phase 2 C-SWIFT study (Lawitz et al., 2016b). After the therapy of this triple-drug combination at 6 or 8 weeks, treatment-naïve patients infected with GT1 or GT3 infections demonstrated high SVR12 rates (>80%) (Lawitz et al., 2016b). Such findings suggest that an NS3/4A inhibitor plus an NS5A inhibitor and an NS5B inhibitor may offer better SVR12 rates and short-duration therapy.

4.10. Sofosbuvir + velpatasvir (Epclusa®)

In June 2016, the FDA approved Epclusa® as the first fixed-dose combination of 400 mg sofosbuvir plus 100 mg velpatasvir (GS-5816) with or without ribavirin against HCV genotype 1 to 6 infections. Epclusa® is the first pan-genotypic therapy approved for the treatment of all HCV genotypes except genotype 7. While the global prevalence of genotype 7 is rather low (Murphy et al., 2015), a case report showed the treatment success of Epclusa® in one patient infected with HCV genotype 7 (Schreiber et al., 2016). As the key component of Epclusa®, velpatasvir (Fig. 3) was discovered as a second-generation NS5A inhibitor with potent antiviral activity in cell culture assays. EC₅₀ values of velpatasvir were 0.013 nM against GT1a, 0.015 nM against GT1b, 0.009 nM against GT2a, 0.01 nM against GT2b, 0.013 nM against GT3a, 0.009 nM against GT4, 0.059 nM against GT5, and 0.007 nM against GT6 (Cheng et al., 2013). Moreover, sofosbuvir is the best-in-class NS5B inhibitor manufactured by Gilead Sciences. Thus the fast approval of sofosbuvir plus velpatasvir marks a new era of anti-HCV therapies that

effectively cure the majority of HCV-infected patients regardless of HCV genotypes. Unlike most FDA-approved therapies which require 12- to 24-week treatment, Epclusa® also attained a promising efficacy at 8 weeks (Everson et al., 2015).

Effectiveness of sofosbuvir plus velpatasvir was demonstrated in phase 2 and 3 clinical studies such as ASTRAL-1 (Feld et al., 2015a), ASTRAL-2 (Foster et al., 2015b), ASTRAL-3 (Foster et al., 2015b), and ASTRAL-4 (Curry et al., 2015) (see summary in Table 19). Here, we attempted to collect treatment outcomes and to estimate drug efficacy with integrated data regardless of the distinct nature between different clinical trials. (i) As for the fixed-dose combination of 400 mg sofosbuvir + 100 mg velpatasvir once daily for 12w, the SVR12 rates were 98.7% (299/303) in GT1-infected patients without cirrhosis versus 93.9% (139/148) with cirrhosis, 99.5% (217/218) in GT2-infected patients without cirrhosis versus 100% (33/33) with cirrhosis, 96.8% (243/251) in GT3-infected patients without cirrhosis versus 85.8% (103/120) with cirrhosis, 98.9% (95/96) in GT4-infected patients without cirrhosis versus 100% (31/31) with cirrhosis, 96.5% (28/29) in GT5-infected patients without cirrhosis versus 100% (5/5) with cirrhosis, and 100% (40/40) in GT6-infected patients without cirrhosis versus 100% (6/6) with cirrhosis (Table 19). (ii) As for the fixed-dose combination of 400 mg sofosbuvir + 100 mg velpatasvir once daily plus ribavirin for 12w, its SVR12 scored 100% (18/18) in GT1-infected patients without cirrhosis versus 94.8% (74/78) with cirrhosis, and 100% (26/26) in GT3-infected patients without cirrhosis versus 92.3% (36/39) with cirrhosis (Table 19). The most common adverse events observed in

Table 19
Efficacy of Epclusa® in phase 2 and 3 clinical trials.

Cirrhosis	Prior treatment	SVR12 rate						Study and trial phase	Ref.
		GT-1	GT-2	GT-3	GT-4	GT-5	GT-6		
400 mg sofosbuvir + 100 mg velpatasvir fixed-dose, single pill, QD for 12w									
No cirrhosis	Experienced	100% (20/20)		100% (27/27)				GS-US-342-0109, phase 2	Pianko et al., 2015
	Naïve	100% (28/28)	100% (10/10)	92.6% (25/27)	85.7% (6/7)		100% (5/5)	Phase 2	Everson et al., 2015
	Naïve or experienced	1a: 97.5% (157/161), 1b: 100% (94/94)	100% (93/93)		100% (89/89)		96.6% (28/29)	100% (35/35)	ASTRAL-1, phase 3 Feld et al., 2015a
	Naïve		99% (99/100)	98.2% (160/163)				ASTRAL-2, ASTRAL-3, phase 3	Foster et al., 2015b
	Experienced		100% (15/15)	91.2% (31/34)				GS-US-342-0109, phase 2	Pianko et al., 2015
Cirrhosis	Experienced	100% (7/7)		88.5% (23/26)				Phase 2	
	Naïve or experienced	1a: 100% (49/49), 1b: 95.8% (23/24)	100% (10/10)		100% (27/27)		100% (5/5)	100% (6/6)	ASTRAL-1, phase 3 Feld et al., 2015a
	Naïve		100% (15/15)	93% (40/43)				ASTRAL-2, ASTRAL-3, phase 3	Foster et al., 2015b
	Experienced		100% (4/4)	89.2% (33/37)				ASTRAL-1, phase 3	
	Naïve or experienced	1a: 88% (44/50), 1b: 88.9% (16/18)	100% (4/4)	50% (7/14)	100% (4/4)			ASTRAL-4, phase 3	Curry et al., 2015
400 mg sofosbuvir + 100 mg velpatasvir fixed-dose, single pill, QD + ribavirin for 12w									
No cirrhosis	Experienced	100% (18/18)		100% (26/26)				GS-US-342-0109, phase 2	Pianko et al., 2015
Cirrhosis	Experienced	90% (9/10)		96.2% (25/26)				GS-US-342-0109, phase 2	Pianko et al., 2015
	Naïve or Experienced	1a: 94.4% (51/54), 1b: 100% (14/14)	100% (4/4)	84.6% (11/13)	100% (2/2)			ASTRAL-4, phase 3	Curry et al., 2015

clinical studies were nausea, headache, fatigue, insomnia, anemia, and diarrhea.

5. New agents under development

During the past three years, advancements of HCV drug discovery have been made to produce a large amount of new inhibitors with better potency and safety profiles. In our previous reviews, we described experimental compounds against HCV infections (De Clercq, 2015; De Clercq, 2012; De Clercq, 2014; De Clercq and Li, 2016). In this section, we update the recent progress of anti-HCV agents targeting NS3/4A (Fig. 2), NS4B (Fig. 6), NS5A (Fig. 3), NS5B (Figs. 4 and 5), E1/E2 or p7 proteins (Fig. 7). Immuno-stimulators and host targeting agents that inhibit host proteins are also summarized (see Table 20, Fig. 8).

5.1. NS3/4A inhibitors

Development of NS3/4A inhibitors has been characterized in recent reviews (De Clercq, 2014; De Clercq and Li, 2016; McCauley and Rudd, 2016). Here, we highlight the latest advancement of danoprevir, voxilaprevir, vedroprevir, MK-8831, faldaprevir, ABT-493, BMS-605339, and BMS-890068. Discontinued NS3/4A inhibitors were GS-9256 (Sheng et al., 2012a), faldaprevir, and narlaprevir.

Voxilaprevir - Voxilaprevir (GS-9857, see Fig. 2) is an NS3/4A inhibitor entering advanced stages of clinical trials. The therapy of 100 mg voxilaprevir in combination with 100 mg velpatasvir plus 400 mg sofosbuvir once daily is now examined in phase 3 clinical trials. In previous phase 2 clinical trials investigating this triple-DAA therapy at 8 weeks, SVR12 rates were 100% (36/36) in treatment-naïve GT1-infected patients without cirrhosis, and 94% (31/33) in treatment-naïve GT1-infected patients with cirrhosis (Lawitz et al., 2016c). In addition, this 12-week treatment attained an SVR12 rate of 100% in DAA-experienced patients with or without cirrhosis (Lawitz et al., 2016c). Another phase 2 study further showed its high efficacy in patients infected with genotype 1, 2, 3, 4, or 6, demonstrating that the SVR12 rates were 100%

(36/36) in treatment-experienced patients without cirrhosis versus 97% (28/29) in treatment-experienced patients with cirrhosis (Gane et al., 2016a). The third clinical trial investigated the 4w, 6w and 8w treatment of this combination therapy against genotype 1 or 3 infections, demonstrating that 8-week treatment provided high SVR12 rates in most treatment-naïve or -experienced patients with or without compensated cirrhosis (Gane et al., 2016b). In clinical trials, voxilaprevir, sofosbuvir plus velpatasvir was well-tolerated and caused mild or moderate adverse events (Lawitz et al., 2016c; Gane et al., 2016a; Gane et al., 2016b; Rodriguez-Torres et al., 2016). In October 2016, Gilead Sciences announced the latest findings from four phase 3 trials (POLARIS-1 to POLARIS-4) in which the SVR12 rates of once-daily single tablet containing voxilaprevir, sofosbuvir, and velpatasvir were more than 96% in treatment-failure patients infected with GT1 to GT6 (<http://www.gilead.com/>). On December 8, 2016, Gilead Sciences submitted the new drug application of sofosbuvir, velpatasvir plus voxilaprevir. Overall, this combination therapy offers short-duration treatment and potentially provides salvage therapy for DAA-experienced patients.

Vedroprevir - Vedroprevir (GS-9451) is a reversible noncovalent inhibitor that effectively binds to the active site of HCV NS3/4A protease (Sheng et al., 2012b). The EC₅₀ values of vedroprevir were determined to be 13 nM and 5.4 nM in GT1a and GT1b replicon cell lines, respectively (Yang et al., 2014). Moreover, the pharmacokinetic properties of vedroprevir were shown in rats, dogs, and monkeys (Sheng et al., 2012b). In a phase 2 clinical trial, the combination of vedroprevir, ledipasvir, tegobuvir plus ribavirin led to an SVR12 rate of 63% in GT1-infected patients without cirrhosis (Wyles et al., 2014). In the phase 2 TRILOGY-2 study, the combination of vedroprevir plus ledipasvir and sofosbuvir for 8 weeks reached an SVR12 rate of 95% (21/22) in GT1-infected patients with cirrhosis (Lawitz et al., 2016d). In another phase 2a trial, 100% (20/20) of GT1-infected patients without cirrhosis maintained SVR12 after the treatment of vedroprevir plus ledipasvir and sofosbuvir for 6 weeks (Kohli et al., 2015a). As of October 2016, a phase 3 trial of vedroprevir has not been established in the drug pipelines of Gilead Sciences (www.gilead.com).

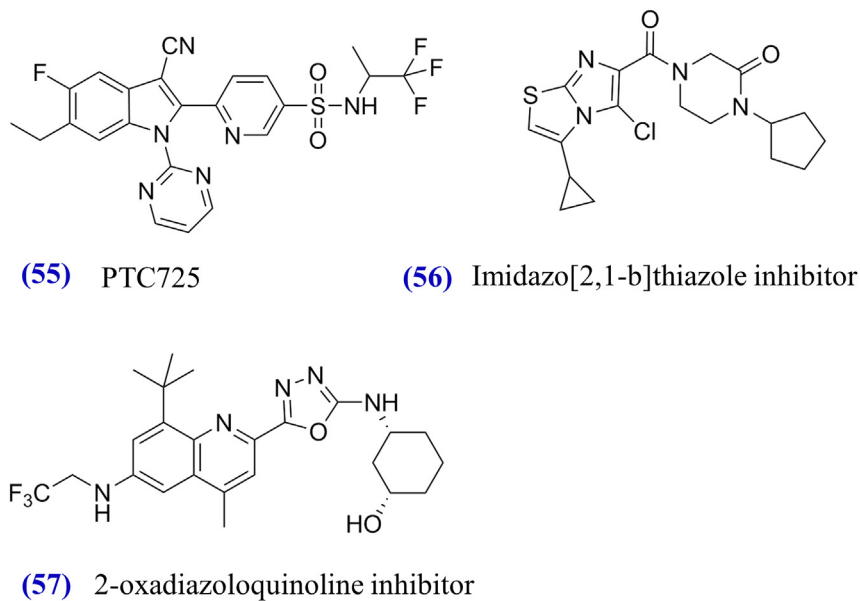


Fig. 6. Chemical formulas of experimental NS4B inhibitors from (55) to (57).

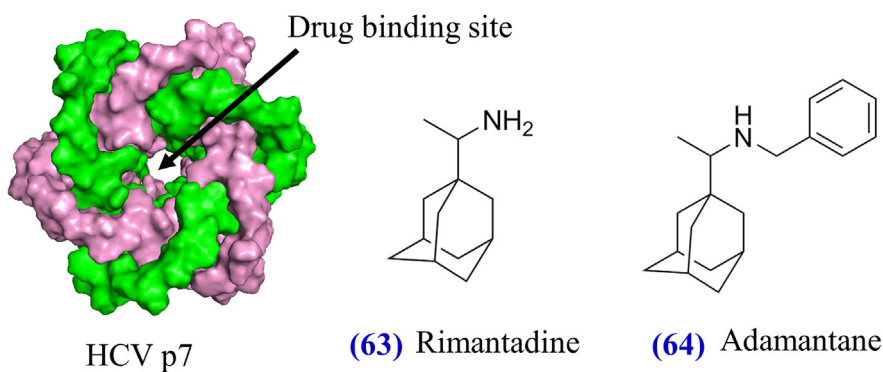
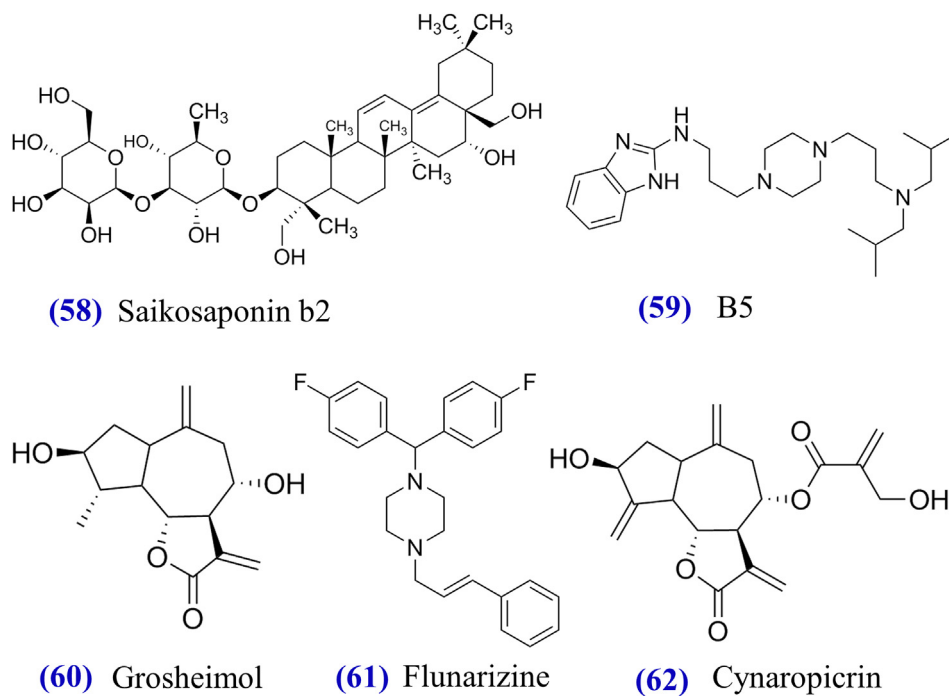


Fig. 7. Chemical formulas of experimental E1/E2 inhibitors from (58) to (62) and the tertiary structure of p7 and its experimental inhibitors from (63) to (64). Six units of the hexameric p7 channel are colored accordingly (PDB code: 2MGX). The drug binding site is located in the center of HCV p7 channel (OuYang et al., 2013).

Table 20
Summary of potent HCV inhibitors explored between 2011 and 2016.

Drug type	Drug name	Current stage
NS3/4A inhibitor	Asunaprevir (BMS-650032), boceprevir (SCH503034), telaprevir (VX-950), paritaprevir (ABT-450), grazoprevir (MK-5172), simeprevir (TMC435), vaniprevir (MK-7009)	Approved drugs
	Voxilaprevir (GS-9857), glecaprevir (ABT-493), danoprevir (ITMN-191, R7227), vedroprevir (GS-9451) ^a , faldaprevir ^a , narlaprevir (SCH900518) ^a	Clinical tests
NS4B inhibitor	BMS-605339, MK-8831, BMS-890068	Preclinical tests
NS5A inhibitor	PTC725, piperazinone derivatives, imidazo[2,1-b]thiazole agents	Preclinical tests
	Daclatasvir (BMS-790052), ledipasvir (GS-5885), ombitasvir (ABT-267), elbasvir (MK-8742), velpatasvir (GS-5816)	Approved drugs
NS5B nucleoside inhibitor	Pibrentasvir (ABT-530), ravidasvir (PPI-668), GSK2336805, ruzasvir (MK-8408), EDP-239, samatasvir (IDX719) ^a	Clinical tests
	Biphenylimidazole analogues, AV4025, disulfiram	Preclinical tests
NS5B non-nucleoside inhibitor	Sofosbuvir (GS-7977, PSI-7977)	Approved drugs
	MK-3682, mericitabine (RG7128) ^a , GS-6620 ^a , JNJ-54257099 ^a	Clinical tests
E1/E2, p7 inhibitor	Dasabuvir (ABT-333)	Approved drugs
	Beclabuvir (BMS-791325), TMC647055, GS-9669, filibuvir ^a , lomibuvir (VX-222) ^a , tegobuvir (GS-9190) ^a , deleobuvir (BI207127) ^a	Clinical tests
Immuno-stimulator and cellular protein inhibitor	6-aminoquinolone derivatives, pyrazolobenzothiazines	Preclinical tests
	Adamantane, chlorcyclizine, cynaropicrin, flunarizine, grosheimol, saikosaponin b2, rimantadine, benzimidazole derivative B5, ITX-5061, alisporivir (DEB025) ^a	Preclinical tests
Immuno-stimulator and cellular protein inhibitor	NIM258, MA026, soraphen A, isothiazolo[5,4-b]pyridines, phenyl–pyrrolidine derivatives, bis-amide derivatives	Clinical tests
		Preclinical tests

^a Discontinued compounds. See details in a recent review addressing the discontinued HCV drugs (Gentile et al., 2015).

Danoprevir - Danoprevir (ITMN-191, RG7227) was discovered based on the joint efforts of the structure-based design, the investigation of structure-activity relationship, and the optimization of drug metabolism and pharmacokinetics (Jiang et al., 2014a). This macrocyclic noncovalent reversible NS3/4A inhibitor had a slow-off rate and exhibited favorable potency across multiple HCV genotypes (Jiang et al., 2014a). The IC₅₀ values of danoprevir (Fig. 2) were 1.6 nM against GT2b, 3.5 nM against GT3a, and 0.2 nM–0.4 nM against GT1a, GT1b, GT4, GT5 or GT6 infection (Jiang et al., 2014a). Moreover, danoprevir may restore the insulin sensitivity in GT1-infected patients (Moucari et al., 2010). Early clinical studies supported the clinical use of danoprevir plus PegIFN α /RBV that offered modest potency and safety against GT1 infection (Gane et al., 2011, 2014b; Forestier et al., 2011; Marcellin et al., 2013). In the INFORM-1 trial, danoprevir plus mericitabine significantly reduced HCV RNA levels in GT1-infected patients (Gane et al., 2010). In the MATTERHORN study, the combination of danoprevir/r, mericitabine plus PegIFN α /RBV for 12 weeks was well-tolerated and yielded an SVR24 rate of 84.6% against GT1 infections (Feld et al., 2015b). In a phase 2a trial enrolling 25 GT1-infected patients without cirrhosis, the four-week treatment of danoprevir, ledipasvir plus sofosbuvir exhibited an SVR12 rate of 40% (10/25) (Kohli et al., 2015c). According to the information in ClinicalTrials.gov, a phase 3 trial of danoprevir has not been found.

Glecaprevir - Glecaprevir (ABT-493) and pibrentasvir (ABT-530) from AbbVie inhibit the activity of HCV NS3/4A (Fig. 2) and NS5A (Fig. 3), respectively. The efficacy and safety of 200 mg ABT-493 plus 120 mg ABT-530 in cirrhotic patients infected with GT1 or GT3 was demonstrated by phase 2 clinical trials (Gane et al., 2016c; Poordad et al., 2016b). The SVR12 rates succeeded by ABT-493 plus ABT-530 were 96% (26/27) of GT1-infected patients, and 96% (27/28) of GT3-infected patients (Gane et al., 2016c). Meanwhile, the SVR12 rate of ABT-493 plus ABT-530 and ribavirin reached up to 100% (27/27) among GT1-infected patients (Gane et al., 2016c). In the phase 2 MAGELLAN-I study, the SVR12 rate of 200 mg ABT-493 plus 120 mg ABT-530 reached 100% in 6 GT1-infected patients without cirrhosis (Poordad et al., 2016b). Phase 3 trials of ABT-493 and ABT-530 are ongoing.

Faldaprevir/deleobuvir - Faldaprevir (BI 201335) and deleobuvir (BI 207127) are two experimental inhibitors targeting NS3/

4A (Fig. 2) and NS5B (Fig. 5), respectively. In the phase 2 SOUND-C2 study, faldaprevir and the nonnucleoside inhibitor deleobuvir plus ribavirin offered SVR12 rates ranging from 52% to 69% in treatment-naïve GT1-infected patients (Zeuzem et al., 2013). Although the degree of liver fibrosis may not affect the efficacy of faldaprevir plus deleobuvir and ribavirin (Zeuzem et al., 2015b), the SVR12 rate of this therapy was rather lower than that of the other DAAs. NS5B variations at amino acid position 499 may reduce the treatment response (Berger et al., 2016). Further pursuit of faldaprevir plus deleobuvir was discontinued in 2014.

Narlaprevir - Narlaprevir (SCH 900518, see Fig. 2) is a second-generation NS3/4A protease inhibitor derived from boceprevir (Arasappan et al., 2010). In comparison to boceprevir, narlaprevir showed better potency, pharmacokinetic profiles, and physico-chemical characteristics (Arasappan et al., 2010). Early clinical studies suggested that narlaprevir plus PegIFN α /RBV offered an SVR12 rate up to 59.3% (19/32) in GT1-infected patients (de Bruijne et al., 2010). After a single dose of 200 mg, narlaprevir exposures were higher in cirrhotic patients than in healthy patients (Isakov et al., 2016). A phase 3 trial of narlaprevir (ClinicalTrials, gov identifier: NCT00689390) was terminated due to post-marketing commitments. Further development of narlaprevir was discontinued.

BMS-605339 - BMS-605339 (Fig. 2) was discovered as an acylsulfonamide-based tripeptide to inhibit HCV NS3/4A protease (Scola et al., 2014a). Although the potential cardiovascular liabilities in a clinical trial of BMS-605339 immediately prompted its discontinuation, small modifications in its P2* isoquinolone ring led to the discovery of asunaprevir (BMS-650032) – a compound free of cardiovascular liabilities (Scola et al., 2014b). BMS-605339 has remained in the pre-clinical stage.

MK-8831 - MK-8831 (Fig. 2) with a novel spiro-proline macrocycle is a follow-up compound of MK-5172 (Neelamkavil et al., 2016). Based on rational computational modeling and structure-guided designs, MK-8831 was found to offer robust pan-genotypic activity and good coverage of NS3/4A resistant strains (Neelamkavil et al., 2016).

BMS-890068 - The optimization of the approved NS3/4A inhibitor asunaprevir led to a potent acyclic, tripeptidic, acyl sulfonamide inhibitor called BMS-890068 (Fig. 2) that enhanced potency,

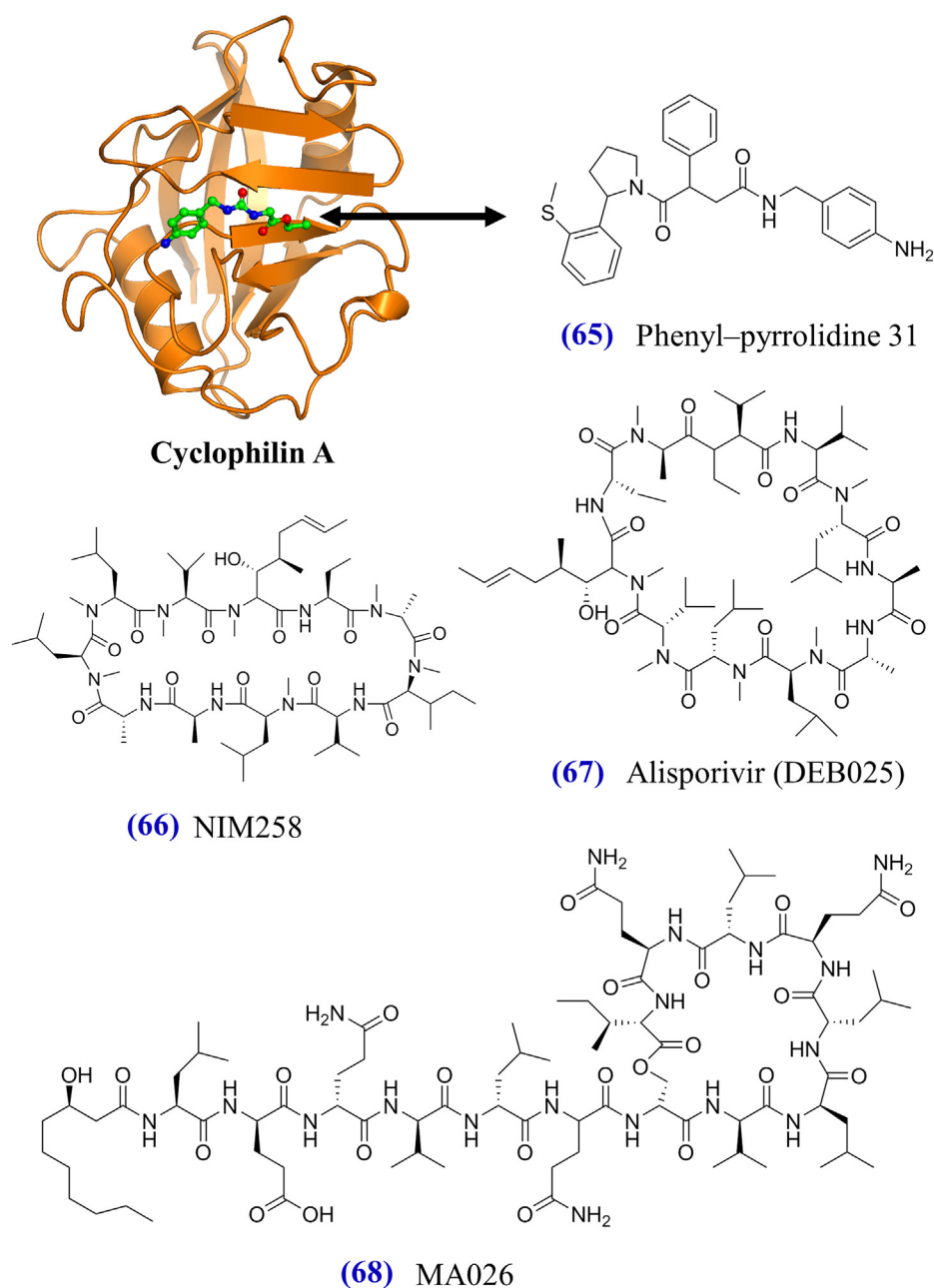


Fig. 8. Cellular protein inhibitors and immuno-stimulators from (65) to (72). Tertiary structure of host protein cyclophilin A in complex with phenyl-pyrrolidine 31 is visualized (PDB code: 3RDD). In addition, cyclophilin A inhibitors include alisporivir, bis-amide derivative 25, and NIM258 (Table 20). Other compounds target other host proteins to offer antiviral activity (see details in Section 5).

safety, metabolic stability, and pharmacokinetic profiles (Sun et al., 2016). BMS-890068 has remained in the pre-clinical stage.

5.2. NS4B inhibitors

As of today, there is no NS4B inhibitor approved by the FDA. A recent review highlighted the achievements made in the discovery of NS4B inhibitors, although most NS4B inhibitors remain in the preliminary stages (Cannalire et al., 2016).

PTC725 - NS4B inhibitor PTC725 (Fig. 6) was discovered through the optimization of 6-(indol-2-yl)pyridine-3-sulfonamides (Zhang et al., 2014). This compound exhibited potent activities against GT1 infections and offered favorable pharmacokinetic profiles in

rats, dogs, and monkeys (Zhang et al., 2014). The clinical uses of PTC725 require further investigation.

2-oxadiazoloquinoline derivative - A novel NS4B inhibitor harboring a 2-oxadiazoloquinoline scaffold showed promising inhibitory activity in vitro. The EC_{50} values of this compound were 0.08 nM against GT1a, 0.1 nM against GT1b, 3 nM against GT2a, 3.7 nM against GT3a, 0.9 nM against GT4a, and 3.1 nM against GT6a (Phillips et al., 2014). Its clinical use requires further investigation.

Imidazo[2,1-b]thiazole derivative - Experimental NS4B inhibitors with the imidazo[2,1-b]thiazole scaffold yielded synergistic responses with simeprevir, daclatasvir, and sofosbuvir so as to lower drug doses for the inhibition of HCV GT1b infections (Wang et al., 2015). Clinical uses of these new NS4B compounds require

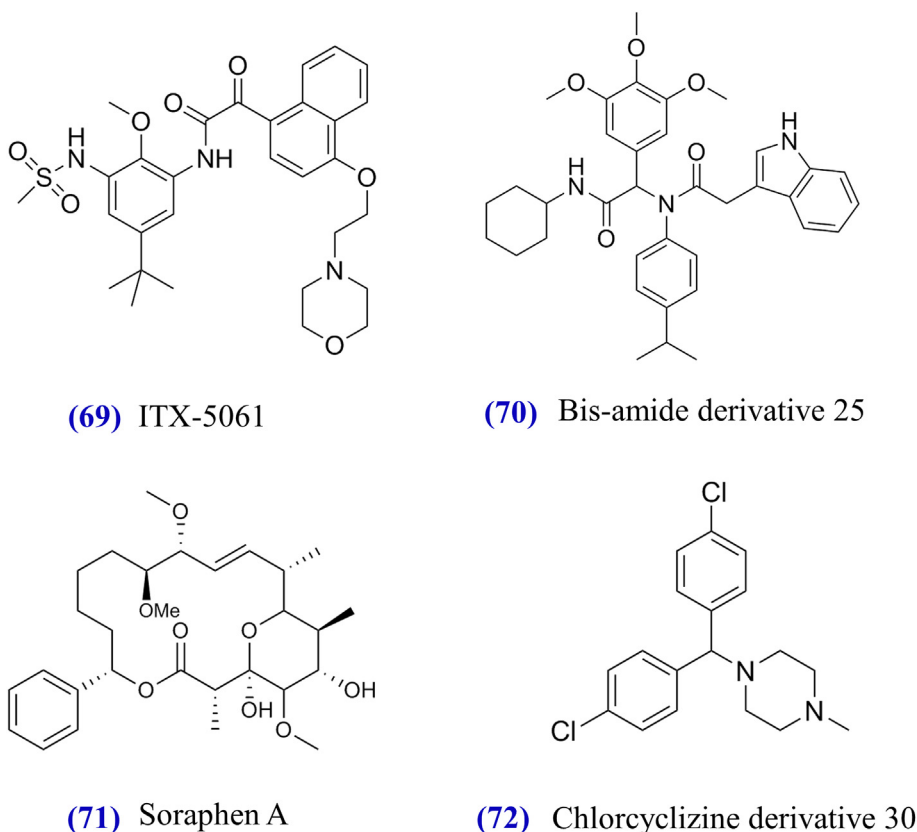


Fig. 8. (continued).

further investigation.

Piperazinone - High-throughput drug screen identified piperazinone derivatives to effectively inhibit GT1 infections through a direct binding with HCV NS4B (Kakarla et al., 2014). Nevertheless, the piperazinone class was insufficient to inhibit GT2 infections; thus, its development was discontinued (Kakarla et al., 2014).

5.3. NS5A inhibitors

A number of experimental NS5A inhibitors are currently under development (Table 20). Although most of them remain preclinical candidates, several compounds (e.g. samatasvir, ravidasvir, GSK2336805) successfully advanced into clinical trials. In this section, we provide an overview of new NS5A inhibitors, including ABT-530, ruzasvir, ravidasvir, GSK2336805, EDP-239, samatasvir, biphenylimidazole analogues, and disulfiram (Fig. 3).

Pibrentasvir - Pibrentasvir (ABT-530) was tested in a phase 2 trial enrolling 10 treatment-naïve GT3-infected patients without cirrhosis (Poordad et al., 2016c). The treatment of ABT-530, paritaprevir, ritonavir plus ribavirin cured 9 of 10 patients without severe adverse events. Moreover, ABT-530 in combination with ABT-493 showed high efficacy and safety profiles in phase 2 clinical trials (see Section 5.1). Further development of ABT-530 is therefore expected.

Ruzasvir - Ruzasvir (MK-8408) is a potent next-generation NS5A inhibitor discovered by an iterative lead optimization so that compounds were stepwise screened against a select panel of HCV replicon cells (Asante-Appiah et al., 2014). This compound maintained an $EC_{50} < 5$ pM across HCV GT1 to GT6 (Asante-Appiah et al., 2014). MK-3682 is a uridine nucleotide analogue that targets HCV NS5B. In the C-CREST 1 and 2 phase 2 clinical studies, two

triple-drug combinations (MK-3682 + grazoprevir + elbasvir, MK-3682 + grazoprevir + MK-8408) were examined in 240 treatment-naïve non-cirrhotic patients infected with GT1 to GT3 infections (Gane et al., 2016d). It has been shown that the 8-week regimen of 450 mg MK-3682, 100 mg grazoprevir plus 60 mg MK-8408 effectively gained 90.1% (55/61) of SVR24 against GT1 to GT3 infections (Gane et al., 2016d). Phase 2 clinical trials are ongoing.

Samatasvir - As a potent NS5A inhibitor, samatasvir (IDX719) selectively inhibited HCV replication (Bilello et al., 2014). Its EC_{50} values accomplished 4.1–6.2 pM against GT1a, 2.4 pM against GT1b, 21–24 pM against GT2a, 17–23 pM against GT3a, 2–6 pM against GT4a, and 18 pM against GT5a (Bilello et al., 2014). In a 3-day proof-of-concept study, the samatasvir-based monotherapy was well-tolerated and reduced HCV RNA levels substantially in 34 GT1-infected patients and 30 patients infected with GT2, GT3, or GT4 infections (Vince et al., 2014). In the absence of Merck and Janssen pipelines, samatasvir appears to be discontinued.

Ravidasvir - As an NS5A inhibitor, ravidasvir (PPI-668) is a dimeric benzimidazole–naphthylene–imidazole derivative. The EC_{50} values of this compound were up to 0.12 nM, 0.01 nM, and 1.14 nM against HCV GT1a, GT1b, and GT3a infections, respectively (Zhong et al., 2016). The efficacy and safety profiles of ravidasvir in combination with other inhibitors (e.g. faldaprevir, sofosbuvir) were demonstrated in preliminary clinical trials (Zhong et al., 2016). Phase 2 and 3 trials of ravidasvir are ongoing (ClinicalTrials.gov identifier: NCT02371408).

GSK2336805 - On the basis that ketal-based 4-spiropyrrrolidines improved potency against NS5A GT1b mutants, GSK2336805 is a spiroketal pyrrolidine harboring the 1,4-dioxo-7-azaspiro[4.4]nonane motif was designed to effectively inhibit GT1b mutants bearing L31V and Y93H in NS5A (Kazmierski et al., 2014). In

comparison to daclatasvir, GSK2336805 significantly reduced HCV RNA levels in a 20-day study (Kazmierski et al., 2014). In a phase 1 study, GSK2336805 plus PegIFN α /RBV exhibited rapid and significant antiviral activity in GT1-infected patients (Wilfret et al., 2013). Furthermore, mutations in the N-terminal region of NS5A caused decreased activity of GSK2336805 (Walker et al., 2014). Ongoing trials are currently unavailable in the database of ClinicalTrials.gov.

EDP-239 - EDP-239 is a potent and selective compound that inhibits NS5A activity and interferes with HCV replication (Owens et al., 2016a). Resistance profiles of EDP-239 in a randomized clinical study revealed drug resistant mutations in NS5A such as L31M and Q30H/R for GT1a, L31M and Y93H for GT1b (Owens et al., 2016b). Ongoing trials for EDP-239 are currently unavailable in the database of ClinicalTrials.gov.

Biarylimidazole - A novel biarylimidazole chemotype 50b with potent inhibitory activity towards GT1a and GT1b was discovered in order to remove the embedded aniline moiety of early NS5A drug candidates that caused genotoxicity in various short-term tests (Belema et al., 2014).

AV4025 - As an active NS5A inhibitor, AV4025 containing 5-[4-(4-imidazol-4-yl-phenyl)-buta-1,3-diylnyl]-1H-imidazole linkers offered potent antiviral activity ($EC_{50} = 3.4 \pm 0.2$ pM for GT1b) and favorable pharmacokinetic features in rats and dogs (Ivachtchenko et al., 2014).

Disulfiram - As an old antialcoholism drug discovered in 1920s, disulfiram was recently identified to be a novel Zn-ejector that ejected Zn²⁺ from the labile Zn-site in NS5A, thus leading to the inhibition of HCV infection (Lee et al., 2016).

5.4. NS5B inhibitors

Inspired by two FDA-approved NS5B inhibitors (sofosbuvir, dasabuvir), many experimental compounds are currently under evaluation. Several candidates have reached advanced clinical trials. This section highlights recent progress of newly discovered NS5B inhibitors (see Table 20, Figs. 4 and 5).

Beclabuvir - The introduction of a cyclopropyl moiety into the indolobenzazepine inhibitors led to the discovery of the non-nucleoside beclabuvir (BMS-791325) with favorable antiviral, safety, and pharmacokinetic properties (Gentles et al., 2014). Clinical trials were carried out to examine the combination of beclabuvir, daclatasvir plus asunaprevir with or without ribavirin (Poordad et al., 2015; Muir et al., 2015; Toyota et al., 2016; Everson et al., 2014, 2016; Hassanein et al., 2015). The SVR12 rates of 75 mg beclabuvir, 30 mg daclatasvir plus 200 mg asunaprevir were up to 100% (11/11) against GT4 in an exploratory study (Hassanein et al., 2015), 88.8% (71/80) against GT1 in a phase 2a study (Everson et al., 2016), 94% (15/16) against GT1 in another phase 2a study (Everson et al., 2014), 91.3% (379/415) against GT1 in the UNITY-1 study (Poordad et al., 2015), 90.1% (92/102) against GT1 in the UNITY-2 study (Muir et al., 2015), and 96% (208/217) against GT1 in a phase 3 study (Toyota et al., 2016). Overall, the triple-DAA regimen of beclabuvir plus daclatasvir and asunaprevir exerts promising activity against GT1 infection.

GS-9669 - Non-nucleoside analogue GS-9669 with the N-alkyl substituent and the N-acyl group was discovered through the optimization of lomibuvir and thiophene analogues (Lazerwith et al., 2014). GS-9669 binds to the thumb site II of HCV NS5B polymerase and inhibits viral replication (Gane et al., 2014a). In HCV replicon assays, the EC_{50} values of GS-9669 were less than 11 nM against GT1 and GT5, but less antiviral activity was rated against GT2 to GT4 (Fenaux et al., 2013). In the phase 2 TRILOGY-1 study, the combination of GS-9669 plus ledipasvir and sofosbuvir for 8 weeks reached SVR12 rates above 82% in the treatment of GT1-infected patients with cirrhosis (Lawitz et al., 2016d). In another

phase 2a trial, 95% (19/20) of GT1-infected patients without cirrhosis maintained SVR12 when they were treated with GS-9669 plus ledipasvir and sofosbuvir for 6 weeks (Kohli et al., 2015a). In a phase 2a trial enrolling 25 GT1-infected patients without cirrhosis, the four-week treatment of GS-9669, danoprevir, ledipasvir plus sofosbuvir had an SVR12 rate up to 20% (5/25) (Kohli et al., 2015c). Whether GS-9669 would enter phase 3 clinical trial remains unclear.

Thiophene carboxylate allosteric inhibitor - Similar to GS-9669, the improvement of lomibuvir led to the discovery of a novel thiophene carboxylate allosteric inhibitor called compound 23 (Court et al., 2016) (Fig. 5). This compound improved potency and physicochemical properties in vitro (Court et al., 2016). In HCV replicons, the EC_{50} values of compound 23 reached up to 37 nM and 23 nM against GT1a and GT1b, respectively (Court et al., 2016). This compound has remained in the pre-clinical phase.

Mericitabine - Mericitabine (RG7128) is the prodrug of cytidine nucleoside analogue RO5855 for the inhibition of HCV NS5B polymerase. Previously, mericitabine was considered to be combined with danoprevir against GT1 infection (Gane et al., 2010), or with PegIFN α plus ribavirin against GT1 or GT4 infection (Pockros et al., 2013; Wedemeyer et al., 2013). The former therapy did not exhibit any severe adverse events in the INFORM-1 trial (Gane et al., 2010). The latter therapy had its SVR24 rates up to 56.8% in the JUMP-C trial (Pockros et al., 2013) and 50.6% in the PROPEL trial (Wedemeyer et al., 2013). Moreover, substitutions L159F and L320F in NS5B conferred low-level resistance to mericitabine and cross-resistance to sofosbuvir and GS-938 (Tong et al., 2014). In 2016, Roche discontinued mericitabine due to a decreased number of participants in the clinical trials.

Deleobuvir - Based on an initial hit from high-throughput screening over a compound library, non-nucleoside inhibitor deleobuvir (BI 207127) was optimized using NMR-guided conformational restrictions and scaffold replacements (LaPlante et al., 2014). In healthy patients, deleobuvir was well-tolerated and exhibited a moderate to high clearance with favorable pharmacokinetic profiles (Chen et al., 2015). Deleobuvir was discontinued in 2013.

DAPN-PD1 - As a novel NS5B inhibitor, β -D-2'-C-methyl-2,6-diaminopurine-ribonucleotide (DAPN) phosphoramidate prodrug 1 (PD1) is metabolized intracellularly into two distinct bioactive nucleoside triphosphate (TP) analogues that effectively inhibit the NS5B-mediated RNA polymerization (Ehteshami et al., 2016). This compound presents a novel strategy that combines the intracellular delivery of two inhibitory metabolites with different incorporation profiles (Zhou et al., 2015).

TMC647055 - TMC647055 is a nonzwitterionic 17-membered-ring macrocycle that exhibits potent antiviral activity in respect of cross-genotypic coverage, virus suppression, and resistance selection (Cummings et al., 2014). As a nonnucleoside NS5B inhibitor, TMC647055 in combination with TMC435 was evaluated in phase 1 clinical trials; yet, its resistant mutations remain a concern (Wang et al., 2016). Information about the phase 2 trials of TMC647055 was not available in clinicaltrials.gov.

Filibuvir / VX-222 - Nonnucleoside inhibitors filibuvir (PF-00868554) and VX-222 (VCH-222) bind to the thumb II allosteric pocket of NS5B and prevent HCV subgenomic replicon (Yi et al., 2012). The EC_{50} values of filibuvir and VX-222 achieved up to 70 nM and 5 nM, respectively (Yi et al., 2012). Moreover, NS5B substitutions M423T and I482L could affect the antiviral activity of filibuvir and VX-222 (Yi et al., 2012). In phase 1 studies, filibuvir plus PegIFN α /RBV was well-tolerated and induced significant RNA reductions in treatment-naïve patients infected with GT1 (Wagner et al., 2011). In phase 1 and 2 clinical studies, the thiophene-2-carboxylic acid derivative VX-222 showed effective potency and significant reductions of HCV RNA in GT1-infected patients (Jiang

et al., 2014b). Development of filibuvir was discontinued in 2013, while a phase 3 trial of VX-222 is yet unavailable (clinicaltrials.gov).

GS-6620 - GS-6620 in the C-nucleoside class of NS5B inhibitors (De Clercq, 2016) was discovered during a series optimization of a 1'-cyano-2'-C-methyl 4-aza-7,9-dideaza adenosine analogue (Cho et al., 2014). C-nucleoside monophosphate prodrug GS-6620 exhibited potent activity against GT1 to GT6 infections; its EC₅₀ values were between 0.048 and 0.68 μM (Feng et al., 2014). Moreover, GS-6620 showed a high barrier to drug resistance in vitro (Feng et al., 2014). In October 2016, GS-6620 was absent in the drug pipeline of Gilead Sciences.

Tegobuvir - Tegobuvir (GS-9190) is an imidazopyridine derivative that binds to the β-hairpin in the palm site of HCV NS5B (Eltahla et al., 2014; Shih et al., 2011). The EC₅₀ values of tegobuvir were less than 16 nM, but > 100 nM for GT2 to GT6 (Wong et al., 2012). A phase 1b study showed that Y448H conferred drug resistance to tegobuvir in treatment-naïve GT1-infected patients receiving GS-9190 monotherapy. Altogether, the clinical use of tegobuvir is limited in that advanced DAAs offer pan-genotypic activity with better efficacy. Tegobuvir was discontinued in 2011.

JNJ-54257099 - As an NS5B inhibitor, JNJ-54257099 is a cyclic phosphate ester derivative in the class of 2'-deoxy-2'-spirooxetane uridine nucleotide prodrugs (Jonckers et al., 2016). This compound profoundly decreased HCV RNA levels in mouse models of HCV GT1a and GT3a infections in a dose-dependent manner (Jonckers et al., 2016). In 2016, a phase 1 trial of JNJ-54257099 was terminated (ClinicalTrials.gov identifier: NCT02510248).

6-aminoquinolone derivative - As a novel 6-aminoquinolone derivative, the 6-amino-7-[4-(2-pyridinyl)-1-piperazinyl] quinolone derivative 8 binds to the allosteric site of NS5B and exhibits a potent antiviral activity against HCV NS5B polymerase (EC₅₀ = 3.03 μM, IC₅₀ = 0.069 μM for GT1b) (Manfroni et al., 2014a). The derivative 8 has remained in the pre-clinical stages.

Pyrazolobenzothiazines - Novel pyrazolobenzothiazines were discovered to target the NS5B palm site I, exhibiting potent antiviral effect without anti-metabolic effect in HCV replicon assays (Manfroni et al., 2014b). This drug class has remained in the pre-clinical stage.

5.5. E1/E2 and p7 inhibitors

As of November 2016, no E1/E2 inhibitor has been approved by the FDA. A number of molecules (e.g. L-ficolin (Hamed et al., 2014)) can neutralize the viral glycoproteins E1 and E2, leading to the inhibition of viral attachment during the HCV viral entry. Although most E1/E2 inhibitors remain in preclinical studies, HCV entry inhibitors can be interesting because they offer an alternative strategy to clear HCV infections (Qian et al., 2016). Herein, we highlight newly discovered compounds that target HCV E1/E2 proteins (Fig. 7).

Benzimidazole derivative - In primary hepatocytes, a novel benzimidazole derivative B5 inhibited HCV infections in a dose-dependent and pan-genotypic manner (Vausselin et al., 2016). The IC₅₀ values of B5 were approximately between 0.77 μM and 2.93 μM in HCV GT1 to GT6 replicons. While the benzimidazole derivative B5 may interfere with the HCV entry, the resistance profile of B5 was attributed to a single mutation F291I in HCV E1 (Vausselin et al., 2016).

Cynaropicrin/grosheimol - Natural products cynaropicrin and grosheimol were extracted from the wild Egyptian artichoke (Elsebai et al., 2015). These two compounds in the group of sesquiterpene lactones efficiently inhibited GT1 to GT7 infections (Elsebai et al., 2015). Although their mechanisms of action remain unclear, cynaropicrin and grosheimol may interfere with HCV viral entry (Elsebai et al., 2015).

Saikosaponin b2 - Saikosaponin b2 is a naturally occurring terpenoid extracted from *Bupleurum kaoi* root that acts on HCV glycoprotein E2, leading to the inhibition of HCV infection in the early stages (Lin et al., 2015). This natural product showed pan-genotypic activity and acts at non-cytotoxic concentrations (Lin et al., 2015). Clinical uses of saikosaponin b2 require further investigation.

Chlorcyclizine derivative - A chlorcyclizine derivative called compound 30 was optimized from the antihistamine piperazine drug called chlorcyclizine HCl using the structure-activity analysis (He et al., 2016). Compound 30 may act by the interruption of viral entry into host cells (He et al., 2015). This compound increased anti-HCV activity and selectivity in HCV replicon assays (He et al., 2016).

Flunarizine - Flunarizine was identified as a submicromolar HCV inhibitor in a whole life cycle screen of a compound library including clinically approved drugs (Perin et al., 2016). This compound targeted to the E1 fusion peptide and inhibited HCV membrane fusion in a genotype-dependent manner (Perin et al., 2016). While flunarizine is a calcium antagonist that effectively combats the pathophysiology of migraine, its anti-HCV activity is preferable for genotype 2 infections (Prigozhin and Modis, 2016).

Monoclonal antibodies - Human monoclonal antibodies with breadth of neutralization and synergy were explored for the inhibition of HCV GT1, GT2 and GT3 infections (Carlsen et al., 2014). These potent neutralizing antibodies target various epitopes on HCV E1/E2 envelope proteins (Carlsen et al., 2014).

Adamantane/rimantadine - When the structure of HCV p7 was fully crystalized, in silico compound selection identified promising agents (e.g. adamantane, rimantadine) that interfered with p7 based on high-throughput screening (Foster et al., 2014). Preliminary analyses showed the rational development of potent p7 drugs (Foster et al., 2014), but further clinical trials are still required.

5.6. Immuno-stimulators and cellular protein inhibitors

In this section, we provide an overview of newly discovered agents that inhibit host proteins (e.g. cyclophilin A, cyclin G associated kinase, scavenger receptor B1).

Alisporivir - Alisporivir (DEB025, see Fig. 8) is a non-immunosuppressive compound that targets the host protein cyclophilin A to block the peptidyl-prolyl cis/trans isomerase activity (Pawlotsky et al., 2015). Additionally, alisporivir inhibits the interaction between cyclophilin A and NS5A in a dose-dependent manner (Coelmont et al., 2010). Of interest, alisporivir acts synergistically with NS5A inhibitors (e.g. daclatasvir, sofosbuvir) to achieve significant antiviral effects (Chatterji et al., 2014). Moreover, alisporivir stimulates antigen presentation and promotes antigen-specific CD8⁺ T cell activation by 40%, thereby leading to the significant anti-HCV activity (Esser-Nobis et al., 2016). In the phase 2 VITAL-1 study enrolling GT2- or GT3-infected patients, the SVR24 rate of alisporivir plus PegIFNα and ribavirin (92%, 56/61) was higher than that of alisporivir alone (72%, 13/18) (Pawlotsky et al., 2015). Alisporivir was discontinued in phase 3 studies due to severe adverse events unassociated with cyclophilin inhibition (Ahmed-Belkacem et al., 2016).

Bis-amide derivative - To pursue cyclophilin inhibitors, a novel bis-amide derivative called compound 25 was discovered using molecular modeling-based design and structure-activity exploration (Yang et al., 2015). This compound efficiently inhibited HCV replication and restored host immune responses without acute toxicity in vitro and in vivo (Yang et al., 2015).

NIM258 - NIM258 (Fig. 8) is a modified cyclosporin analogue that acts as non-immunosuppressive cyclophilin A inhibitor with promising pharmacokinetic profiles against HCV infection (Fu et al., 2014). In comparison to alisporivir, NIM258 decreased transporter

inhibition, but maintained comparable efficacy against cyclophilin A (Fu et al., 2014).

Phenyl–pyrrolidine derivative - Phenyl–pyrrolidine derivative 31 (Fig. 8) was discovered to inhibit cyclophilin A using nuclear magnetic resonance, X-ray crystallography and structure-based compound optimization (Ahmed-Belkacem et al., 2016). The EC₅₀ value of this inhibitor was $0.4 \pm 0.3 \mu\text{M}$ against HCV1b in Huh7 cells. In addition, this inhibitor could be a broad-spectrum high-barrier-to-resistance inhibitor against HIV and coronaviruses as well (Ahmed-Belkacem et al., 2016).

Isothiazolo[5,4-b]pyridine - Isothiazolo[5,4-b]pyridines were developed to target cyclin G associated kinase (GAK) which is a serine/threonine protein kinase associated with cyclin G in cell cycle regulation (Kovackova et al., 2015). It is known that GAK is essential for HCV entry and assembly (Kovackova et al., 2015). Drug mechanisms of isothiazolo[5,4-b]pyridines were thereby traced to their inhibition upon HCV entry and assembly (Kovackova et al., 2015). Lead derivatives of isothiazolo[5,4-b]pyridines showed potent anti-HCV activity with $K_d < 9 \text{ nM}$ (Kovackova et al., 2015).

ITX-5061 - ITX-5061 (Fig. 8) is an inhibitor of scavenger receptor B1 - an essential receptor for HCV entry (Syder et al., 2011). As a host factor antagonist, ITX-5061 could be combined with DAAs without conferring cross-resistance in vitro (Zhu et al., 2012). In a phase 1b clinical trial, 150 mg ITX-5061 per day for up to 28 days was safe and well tolerated (Sulkowski et al., 2014b).

MA026 - MA026 (Fig. 8) is a lipocyclodepsipeptide isolated from the fermentation broth of *Pseudomonas* sp (Shimura et al., 2013). While MA026 effectively inhibits HCV entry, the possible antiviral mechanism can be traced to the interaction between MA026 and an HCV entry receptor called claudin-1 using the phase display screening and surface plasmon resonance binding analyses (Shimura et al., 2013).

Soraphen A - As a natural product, soraphen A is a myxobacterial metabolite that interferes with the membranous web formation, leading to the inhibition of HCV replication (Koutsoudakis et al., 2015). The EC₅₀ values of soraphen A were up to 2.3 nM against HCV GT2a infection (Koutsoudakis et al., 2015).

6. Conclusions and future perspectives

In this review, we provide an overview of FDA-approved therapies and newly discovered agents against HCV GT1 to GT6 infections. Moreover, this study presents the first detailed survey of drug efficacy of all FDA-approved therapies based on more than 100 clinical trials and real-world studies. Similar to HIV cocktail therapies, HCV combination therapies containing two or three DAAs to block different stages of the viral life cycle can offer favorable inhibitory efficacy and low incidences of side effects. Unlike the first generation of anti-HCV drugs with pegylated interferons and ribavirin, many FDA-approved DAAs directly bind to HCV proteins (NS3/4A, NS5A, NS5B). Furthermore, the pursuit of next generation anti-HCV inhibitors yields a large number of investigational agents with novel mechanisms of drug actions. Most inhibitors have been synthesized to inhibit HCV proteins (E1/E2, p7, NS4B) or human proteins (e.g. cyclophilin A, cyclin G associated kinase, scavenger receptor B1). Accumulated over the past five years, a large pool of FDA-approved drugs and promising experimental inhibitors provides a strong reinforcement to cure HCV infections in most patients. Nevertheless, many challenges lie ahead to develop next generation DAAs against HCV infections in worldwide populations.

The first challenge is the development of effective therapies for difficult-to-treat populations such as HCV-infected patients with end-stage renal diseases (Morales and Fabrizi, 2015), HCV-infected patients before and after kidney transplantation (Ferenci, 2015; Gambato et al., 2014), HCV-infected patients with liver graft

infection (Felmlee et al., 2016), and HCV-infected patients with compensated and decompensated cirrhosis (Ferenci et al., 2015). It has been suggested that treating HCV-infected patients early in their disease course may prevent HCV-related cirrhosis and its complications (AASLD/IDSA HCV Guidance Panel, 2015), whilst the pre-emptive DAA treatment may benefit the prevention and treatment of liver graft infection (Felmlee et al., 2016). A recent study reported unexpected high rates of early tumor recurrence after the treatment of DAAs in 103 HCV-infected patients with prior hepatocellular carcinoma (Reig et al., 2016). Despite the high potency of DAAs, the occurrence of liver cancer cannot be reduced in HCV-infected cirrhotic patients with sustained virologic response (Conti et al., 2016). Nevertheless, a following study integrating three distinct prospective cohorts showed no increased risk of HCC recurrence in 267 patients after DAA treatments (Thecollaborative st, 2016). Whether DAA treatments increase HCC occurrence or recurrence rates will remain a subject for debate until very large patient cohorts have emerged with a proper control arm to assess this important question (Llovet and Villanueva, 2016).

The second challenge is the demanding request of pan-genotypic drugs with minimal drug resistance. On the one hand, it is crucial to apply pan-genotypic therapies for all HCV genotypes, especially when genotyping tests are unavailable in resource-limited areas. Until today, Eplusea[®] is the only therapy approved for the treatment of GT1 to GT6 infections. On the other hand, drug resistance remains a concern, and natural variations in different genotypes may confer resistance to genotype-specific drugs (Sarrazin, 2016; Pawlotsky, 2016). For instance, NS5A resistance-associated substitutions were presented in 8–16% of GT1-infected patients prior to the treatment of ledipasvir plus sofosbuvir, while their presence exerted a negative impact on treatment outcomes (Zeuzem et al., 2017). Moreover, HCV GT3 variants within the NS5A region not only reduced sensitivity to nucleotide analogues (e.g. sofosbuvir) but also increased viral fitness in cell culture systems (Ramirez et al., 2016). In addition, many NS3/4A inhibitors show potent activities against GT1 but not GT3, because of the polymorphisms between HCV genotypes in the drug binding pocket, as well as the substantial loss of intermolecular hydrogen bonding interactions and dynamic cross-correlations between NS3/4A inhibitors and the active site of NS3/4A in GT3 (Soumana et al., 2016). Lists of HCV resistance-associated substitutions are available at (Sarrazin, 2016; Pawlotsky, 2016). Altogether, development of pan-genotypic drugs remains important.

The third challenge is the optimization of simple regimens with low pill burden and shorter treatment duration. Recent attempts have been made to shorten the course of anti-HCV treatment from the recommended 48 weeks to 8 weeks or even less. For instance, findings of an open-label phase 2a trial suggested that 3 weeks of response-guided therapy (e.g. sofosbuvir + ledipasvir + asunaprevir, sofosbuvir + daclatasvir + simeprevir, sofosbuvir + daclatasvir + asunaprevir) not only accomplished an SVR12 rate of 100%, but drastically reduced the cost of therapy, improved drug adherence, and eliminated the incidences of adverse events (Lau et al., 2016). In another phase 2 study, ledipasvir plus sofosbuvir for 6 weeks also exhibited an SVR12 of 100% in 20 patients with acute GT1 mono-infection (Deterding et al., 2017). Novel HCV therapeutics may aim at shorter treatment duration with optimal efficacy.

Given the breakthroughs achieved by the current DAAs, we anticipate that in the next decade we will witness the substantial decrease of HCV infections in global populations. The ultimate eradication of HCV infections also requires universal diagnosis of HCV infections, improving access to health care, and the potential implementation of a prophylactic HCV vaccine. To supplement this review, an update of HCV drug development will be available at our

online platform (<http://www.virusface.com>).

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