Hepatitis C Virus Infection Treatment: Recent Advances and New Paradigms in the Treatment Strategies

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Abstract

The advancement in hepatitis C virus (HCV) therapeutics has been profoundly enhanced by an improved understanding of viral life cycle in host cells, development of novel direct-acting antivirals (DAAs), and exploring other emerging treatment paradigms on the horizon. The approvals of first-, second-, and next-wave direct-acting antivirals highlight the swift pace of progress in the successful development of an expanding variety of therapeutic regimens for use in patients with chronic hepatitis C virus infection. Triple or quadruple therapies based on a combination of different direct-acting antivirals with or without pegylated interferon (IFN) and ribavirin (RBV) have raised the hopes to improve the current treatment strategies for other difficult-to-treat individuals. The development of more efficacious, well-tolerated, and cost-effective interferons with a low frequency of adverse events and short treatment durations is also in the pipeline. An experimental protective vaccine against hepatitis C virus demonstrated promise in preliminary human safety trials, and a larger phase II clinical trials are under consideration to further determine the efficacy of the vaccine. This pragmatic book chapter discusses the current state of knowledge in hepatitis C virus therapeutics and provides a conceptual framework of emerging and investigational treatment strategies directed against this silent epidemic.

Keywords: HCV medications, direct-acting antivirals, NS3/4A serine protease inhibitors, NS5A inhibitors, polymerase inhibitors, antiviral resistance, all oral interferon-free antivirals, triple or quadruple therapies, interferon lambda, anti-HCV vaccine model
1. Introduction

Afflicting around 170 million people worldwide, hepatitis C virus (HCV) infection represents a disease of significant global impact. The regional prevalence for HCV varies substantially around the world, where the infection presents the state of universal coverage in East and South Asia (e.g., in Egypt, the prevalence is as high as 22%) to no access at all to others (i.e., some North American and European countries) [1, 2]. The new incidents of chronic HCV infection are increasing 3–4 million every year without previous ascertainment of HCV risk, and it seems tough to determine because many acute HCV cases are not noticed clinically [2, 3]. Analogously, acute HCV infection, a multifaceted disease is often asymptomatic or sometimes linked with nonspecific symptoms that lead to chronic hepatitis C in 80% of infected individuals [4]. Chronic HCV-infected patients may at high risk of developing HCV-associated liver diseases (fibrosis, cirrhosis, and hepatocellular carcinoma) if not treated timely, and infection persists for an extended time [5]. The morbidity and the mortality rate is rising unexpectedly in developing world and even in resource-replete countries (e.g., the United States and the United Kingdom), where more patients are now dying from HCV and associated hepatic diseases (e.g., hepatocellular carcinoma) than HIV [6].

Pegylated interferon alpha (PEG-IFNα) and weight-based nucleoside analog ribavirin (RBV) were recommended as “gold standard of care” more than a decade and still considered an integral part of some newly developed anti-HCV direct-acting antiviral therapeutic regimens [2]. The therapy is used in combination to attain a sustained virologic response rate (SVR; HCV RNA undetectable after 6-month treatment completion) in acute and chronic HCV-infected individuals [2, 7]. The SVR rates achieve up to 80% in HCV genotype 3-infected patients and not more than 50–60% of genotype 1- and 4-infected patients [2, 7]. At present, less than 10% of patients with chronic HCV have been treated successfully because of the failure of risk-based screening to identify all infected patients and the low efficacy and high rate of side effects from regimens based on IFN and RBV [2, 8]. By this token then, PEG-IFN/RBV has proven an ineffective means of managing the HCV infection burden. It is highly significant then that we stand today, at the cusp of a pharmacological revolution.

The current therapeutic approaches in the pipeline to coup HCV infection are the development of novel direct-acting antivirals (DAAs), which directly target viral genome via covalent or non-covalent interactions and disrupt HCV replication and translation [9]. The most widely studied direct-acting antivirals are protease inhibitors (PIs), NS5A inhibitors, and polymerase inhibitors which inhibit HCV translation and replication, respectively, by achieving higher sustained virologic response rates (SVR) with or without PEG-IFNα/RBV in treated patients (Table 1) [10]. Two first-generation protease inhibitors (i.e., telaprevir (TLV) and boceprevir (BOC)) were approved by the United States (US) Food and Drug Administration (FDA) in 2011 to treat chronic HCV genotype 1 infection [11]. Simeprevir classified as second-generation protease inhibitor got approval in 2013 to treat chronic HCV genotype 1 populations, and sofosbuvir (SOF) (a viral RNA-dependent RNA polymerase inhibitor) was also recommended in the same year to treat chronic HCV genotype 1-, 2-, 3 and 4-infected patients [11]. These innovative treatment regimens have revolutionized the field of HCV medicine and
| Drug name               | Drug efficacy | Drug-resistance barrier | Pan-genotype coverage | Adverse effects | Drug-drug interactions | Development phase | Target site                  |
|------------------------|---------------|-------------------------|-----------------------|-----------------|------------------------|------------------|-----------------------------|
| (1) Protease inhibitors|               |                         |                       |                 |                        |                  |                             |
| Telaprevir             | ++            | +                       | +                     | +++             | +++                    | Discontinued     | NS3/4A Serine protease      |
| Boceprevir             | ++            | +                       | +                     | ++              | +                      | Discontinued     | NS3/4A Serine protease      |
| Simeprevir             | +++           | ++                      | ++                    | +               | +                      | Approved         | NS3/4A Serine protease      |
| Simeprevir plus sofosbuvir | +++ | ++                      | ++                    | +               | +                      | Approved         | NS3/4A Serine protease/NS5B Inhibitor |
| Faldaprevir            | ++            | +++                     | ++                    | +               | +                      | Withdraw         | NS3/4A Serine protease      |
| Asunaprevir            | ++            | +++                     | +                     | ++              | +                      | Phase III clinical trials | NS3/4A Serine protease |
| Danoprevir             | ++            | ++                      | +                     | ++              | +                      | Phase III clinical trials | NS3/4A Serine protease |
| Vaniprevir             | ++            | +                       | +                     | ++              | +                      | Phase III clinical trials | NS3/4A Serine protease |
| (2) NS5A inhibitors    |               |                         |                       |                 |                        |                  |                             |
| Daclatasvir            | +++           | +                       | +                     | +               | +                      | Approved         | NS5A inhibitors            |
| Daclatasvir plus sofosbuvir | +++ | +                       | +                     | +               | +                      | Approved         | NS5A inhibitors/NS5B inhibitors |
| (3) RNA-dependent RNA polymerase inhibitors (NS5B inhibitors) | | | | | | | |
| (3.1) Nucleoside analog inhibitors (NIs) | | | | | | | |
| Sofosbuvir             | +++           | +++                     | +++                   | +               | +                      | Approved         | NS5B inhibitors            |
| Sofosbuvir plus ledipasvir | +++ | +++                     | +++                   | +               | +                      | Approved         | NS5B inhibitors/NS5A inhibitors |
| Sofosbuvir plus velpatasvir | +++ | +++                     | +++                   | +               | +                      | Approved         | NS5B inhibitors/NS5A inhibitors |
| Elbasvir plus grazoprevir | +++ | ++                     | +++                   | ++              | +                      | Approved         | NS3-4A Serine protease/NS5A Inhibitor |
| Mericitabine           | +++           | +++                     | +                     | +               | +                      | Phase III clinical trials | NS5B inhibitors |
| Paritaprevir ombitasvir-ritonavir and dasabuvir combination | +++ | +++                     | ++                   | +               | +                      | Approved         | NS3-4A Serine protease/NS5A Inhibitor/NNIs |
provided optimism that cure rates in chronically infected HCV patients have much improved with these new drugs.

From 2015, HCV therapy has achieved higher response rates, fewer contraindications, shorter durations, and greater tolerability after the approval of interferon-free antiviral therapies. All oral interferon-free therapeutic regimens directed against hepatitis C virus are shown to be highly effective in the entire spectrum of patient populations, including the previously

| Drug name      | Drug efficacy | Drug-resistance barrier | Pan-genotype coverage | Adverse effects | Drug-drug interactions | Development phase | Target site                |
|----------------|---------------|-------------------------|-----------------------|----------------|------------------------|-------------------|---------------------------|
| Tegobuvir      | ++            | +                       | ++                    | ++             | +                      | Phase II clinical   | NS5B/NNI site 1/ thumb 1   |
| Setrobuvir     | ++            | +++                     | +                     | +              | +                      | Phase II clinical   | NS5B/NNI site 1/ thumb 1   |
| Filibuvir      | ++            | ++                      | +                     | +              | +                      | Phase II clinical   | NS5B/NNI site 2/ thumb 2   |
| BMS-791325     | ++            | ++                      | +                     | +              | +                      | Phase II clinical   | NS5B/NNI site 4/ palm 1     |

(3.2) Non-nucleoside analog inhibitors (NNIs)

(4) Interferon derivatives

Consensus interferon

Interferon lambda

(5) HCV vaccines

ChronVac-C

GI-5005

TG4040

ChAd3/MVA

Table 1. The most promising direct-acting antivirals against HCV with their therapeutic activity profile, current stage of development, and targeted active sites.

a Drug efficacy profile was based on the overall SVR rates achieved in phase II/III clinical trials where SVRs > 95% = high profile, SVRs > 90% = average profile, and SVRs > 85–90% = low profile.

b Drug-resistance barrier profile is based upon the clinical data registered to clinicaltrials.gov.

c Pan-genotypic coverage was based on the fact that the DAA combination was therapeutically effective against 1–6 genotypes = high profile, two/three genotypes = average profile, and one genotype = low profile.

d Adverse event (AE) profile was accomplished on the basis of percentage occurrence of adverse effects in phase II/III clinical trials which caused treatment discontinuation in treated individuals, where 10% AEs > high profile, 10–5% AEs > average profile, and 5–0% AEs > low profile.

e Drug-drug interaction profile was established on the basis of the DAA ability to induce/inhibit hepatic cytochrome P450 system, P-glycoprotein (P-gp), and organic anion-transporting polypeptide (OATP) induction/inhibition. CYP 450, P-gp, and OATP induction/inhibition = high profile, P-gp and OATP induction/inhibition = average profile, and one or none of these CYP 450 or P-gp or OATP inductions/inhibitions = low profile. High profile = ++, average profile = +, and low profile = +.
difficult-to-treat “special” situations (e.g., HCV subtype 1a patients with resistance-associated amino acid variants (RAVs), partial or null responders to first-generation protease and PEG-IFN/RBV-based triple therapies, decompensated cirrhosis, IL28 polymorphism, chronic kidney diseases, and HCV/HIV-coinfected patients). These revolutionary drug strategies now incorporate a cocktail of agents blended to take advantage of the synergistic mechanism of action. With these patient-friendlier attributes, the demand for treatment will conceivably reach unprecedented heights, but will health services be able to match this demand with supply? HCV antiviral therapy is not cheap; the current going rate, which new therapies are likely to exceed, stands at approximately US $ 80,000–100,000 per treatment course. So with more than 170 million people living with chronic infection around the world, clearly we cannot afford at least immediately to treat everyone.

On the other hand, treatment-emergent adverse events (e.g., risk of developing hepatocellular carcinoma and adverse cardiovascular effects) in treated individuals are also posing some serious challenges to the newly developed DAAs. The developers of prophylactic or protective vaccines have faced the most difficult challenges of rapid mutation rate (10$^{-5}$ to 10$^{-4}$ nucleotides per HCV replication cycle) and remarkable genetic heterogeneity of the virus in experimental trials [12,13]. It is beyond the scope of this article to cover every anti-HCV drug studies in details, so we primarily focus on FDA-approved direct-acting antivirals whose clinical efficacies have proven against chronic HCV both in vitro and in vivo (Table 1). We also highlight some interferon derivatives and investigational HCV vaccine models which mark the recent trends and new paradigms in the treatment strategies against HCV (Table 1).

2. Potential active sites for anti-HCV agents

HCV replication and translation into the cytoplasm of host cells facilitate the direct exposure of direct-acting antivirals to their targeted active sites [14]. Moreover, the direct-acting antivirals are structure specific to their targeted active sites (Figure 1) [15]. Viral-encoded proteases (e.g., NS3/4A serine protease), HCV replication complex (NS5A), and viral RNA-dependent RNA polymerase (RdRp; NS5B) enzyme are the core targeted sites for the first-, second-, and next-wave direct-acting antivirals (Figure 1) [16]. Viral attachment and entry into the host cell, viral assembly, packaging, and virion release are the less specific anti-HCV drug targets but still important to develop novel anti-HCV compounds with promising therapeutic activity (Figure 1) [17]. NS3/4A inhibitors inhibit viral translation by disrupting the downstream polyprotein processing of HCV genome. NS5B inhibitors obstruct HCV replication by blocking the addition of further nucleotide in growing mRNA chain (Figure 1) [15]. NS5A inhibitors prevent the formation of a membranous web structure which is crucial for HCV replication [16]. Viral assembly and packaging are disturbed by host-targeting agents which target a host-encoded enzyme responsible for viral assembly [18]. Cyclophilin (nonimmune suppressive cyclosporins) inhibitors block the interaction of cyclophilin (a family of a highly conserved cellular peptidylprolyl isomerase involved in protein folding and trafficking) with other HCV proteins to prevent the formation of a functional replication complex (Figure 1) [17]. α-Glucosidase inhibitors interrupt the release of newly formed viral particles from the host cells [17]. Some immunoglobulins (i.e., monoclonal and polyclonal antibodies) prevent the viral attachment and entry into host cells, but their therapeutic benefits are not highly significant [11].
3. NS3-4A serine protease inhibitors

3.1. First-generation protease inhibitors

Telaprevir and boceprevir represent the first in class of direct-acting antivirals or more correctly the first-generation protease inhibitors which were approved by the US FDA in 2011 for the treatment of genotype 1 hepatitis C infection. Telaprevir (TLV, Incivek®) is an orally bioavailable, a peptidomimetic NS3-4A protease inhibitor, which forms a reversible covalent bond with NS3/4A serine protease and impedes downstream HCV polyprotein processing [19]. The therapy was recommended along with PEG-IFN α and RBV for treatment-naïve genotype 1 adult patients with associated liver diseases, in null responders (i.e., HCV RNA decline <2 log₁₀ at week 12 during PEG-IFN α plus RBV therapy), in poor or partial responders (HCV RNA decline ≥2 log₁₀ at week 12 but positive at week 24 during PEG-IFN α plus RBV therapy), and in the relapers (HCV RNA negative at the end of treatment but recurrence of HCV RNA during the follow-up of 6 months) of PEG-IFN α plus RBV dual therapy [20]. The therapeutic efficacy was evaluated in a series of multicenter phase II and III clinical trials named as PROVE 1, PROVE 2, PROVE 3, ADVANCE, ILLUMINATE, and REALIZE (Table 2) [20]. The overall SVR rates were achieved from 60 to 90% in treated patients. However, TLV monotherapy and suboptimal doses resulted in

Figure 1. Potential active sites for direct-acting antivirals against hepatitis C virus. NS3/4A serine protease, NS5A replication complex, and RNA-dependent RNA polymerase are the key targeted sites for anti-HCV drug development. Some other anti-HCV drug targets have also been demonstrated by the investigators including viral attachment and entry into host cells, host-targeting agents, and α-glucosidase. However, these drug targets are less specific but still significant to develop novel anti-HCV agents. Direct-acting antivirals according to their target specificity are also enlisted in and rectangular square boxes in the figure. NIs, nucleoside analog inhibitors; NNIs, non-nucleoside analog inhibitors; CLDN1, claudin 1; OCLN, occludin.
| Referenced clinical studies | Trials name/phase | ±PEG-IFN/RBV | Treatment-naïve patient | Treatment-experienced | Genotype coverage | NCT#         | Overall SVR   |
|-----------------------------|------------------|--------------|-------------------------|----------------------|------------------|--------------|--------------|
| (1) NS3-4A serine protease inhibitors |                  |              |                         |                      |                  |              |              |
| (1.1) Ketoamide derivative inhibitors |                  |              |                         |                      |                  |              |              |
| Telaprevir                  |                  |              |                         |                      |                  |              |              |
| McHutchison et al., [116]   | PROVE-1          | +            | +                       | −                    | 1                | NCT00336479  | PROVE-1 = 61 %|
| Hezode et al., [115]        | PROVE-2          | +            | +                       | −                    | 1                | NCT00372385  | PROVE-2 = 69 %|
| McHutchison et al., [117]   | PROVE-3          | +            | −                       | +                    | 1                | NCT00420784  | PROVE-3 = 53 %|
| Jacobson et al., [118]      | ADVANCE          | +            | +                       | −                    | 1                | NCT00627926  | ADVANCE = 69 %|
| Sherman et al., [119]       | ILLUMINATE       | +            | +                       | −                    | 1                | NCT00758043  | ILLUMINATE = 92 %|
| Zeuzem et al., [120]        | REALIZE          | +            | −                       | +                    | 1                | NCT00703118  | REALIZE = 66 %|
| Boceprevir                  |                  |              |                         |                      |                  |              |              |
| Kwo et al., [121]           | SPRINT-1         | +            | −                       | +                    | 1                | NCT00423670  | SPRINT-1 = 75 %|
| Poordad et al., [122]       | SPRINT-2         | +            | +                       | −                    | 1                | NCT00705432  | SPRINT-2 = 68 %|
| Bacon et al., [123]         | RESPOND-2        | +            | −                       | +                    | 1                | NCT00708500  | RESPOND-2 = 65 %|
| (1.2) Tripeptide or macrocyclic inhibitors |                  |              |                         |                      |                  |              |              |
| Simeprevir                  |                  |              |                         |                      |                  |              |              |
| Jacobson et al., [21]       | QUEST-1          | +            | +                       | −                    | 1                | NCT01289782  | QUEST-1 = 80–90 %|
| Manns et al., [124]         | QUEST-2          | +            | +                       | −                    | 1                | NCT01290679  | QUEST-2 = 67–80 %|
| Forns et al., [125]         | PROMISE          | +            | −                       | +                    | 1                | NCT01281839  | PROMISE = 79 %|
| Moreno et al., [126]        | RESTORE          | +            | +                       | +                    | 4                | NCT01567735  | RESTORE = 66 %|
| Simeprevir and sofosbuvir combination |                  |              |                         |                      |                  |              |              |
| Lawitz et al., [109]        | COSMOS           | +            | +                       | +                    | 1                | NCT01466790  | Overall SVR = 90–94 %|
| Kwo et al. [22]             | OPTIMIST-1       | +            | +                       | +                    | 1                | NCT02114177  | Overall SVR = 97 %|
| Lawitz et al. [23]          | OPTIMIST-2       | +            | +                       | +                    | 1                | NCT02114151  | Overall SVR = 83 %|
| Referenced clinical studies | Trials name/phase | ±PEG-IFN/RBV | Treatment-naïve patient | Treatment-experienced | Genotype coverage | NCT#        | Overall SVR                          |
|----------------------------|------------------|-------------|-------------------------|-----------------------|-------------------|------------|-------------------------------------|
| **(2) NS5A inhibitors**    |                  |             |                         |                       |                   |            |                                     |
| **Daclatasvir**             |                  |             |                         |                       |                   |            |                                     |
|                            | Phase III        | +           | +                       | +                     | 1-6               | –          | Overall SVRs = 59–100 %             |
|                            |                  |             |                         |                       |                   |            |                                     |
| Hezode et al. [24]          | COMMAND-1        | +           | +                       | –                     | 1/4               | NCT01125189| GT1 (20 mg) = 65 %                   |
|                            |                  |             |                         |                       |                   |            | GT1 (60 mg) = 90 %                   |
|                            |                  |             |                         |                       |                   |            | GT4 (20 mg) = 75 %                   |
|                            |                  |             |                         |                       |                   |            | GT4 (60 mg) = 100 %                  |
| Hezode et al. [24]          | COMMAND-4        | +           | +                       | –                     | 4                 | NCT01448044| COMMAND-4 = 82–86 %                 |
| **Daclatasvir and sofosbuvir combination** | | | | | | | |
| Poordad et al. [25]         | ALLY-1           | ±RBV        | +                       | +                     | 1/3               | NCT02032875| GT1 (cirrhotics) = 83 %             |
|                            |                  |             |                         |                       |                   |            | GT1 (posttransplant) = 95 %         |
|                            |                  |             |                         |                       |                   |            | GT3 = 88 %                          |
| Sulkowski et al. [110]      | A1444040         | ±RBV        | +                       | +                     | 1/3               | NCT01359644| GT3 T.N = 89 %                      |
|                            |                  |             |                         |                       |                   |            | GT2 T.N = 92 %                      |
|                            |                  |             |                         |                       |                   |            | GT1 T.N = 98 %                      |
|                            |                  |             |                         |                       |                   |            | GT1T. E = 98 %                      |
| Wyles et al. [26]           | ALLY-2           | –           | +                       | +                     | 1/4               | NCT02032888| GT1/4 T.N (12 weeks) = 97 %         |
|                            |                  |             |                         |                       |                   |            | GT1/4 T.N (8 weeks) = 76 %          |
|                            |                  |             |                         |                       |                   |            | GT1/4 T.E (12 weeks) = 98 %         |
| Nelson et al. [27]          | ALLY-3           | –           | +                       | +                     | 3                 | NCT02032901| GT3 T. N = 90 %                     |
|                            |                  |             |                         |                       |                   |            | GT3 T. E = 86 %                     |
| Leroy et al. [28]           | ALLY-3+          | ±RBV        | +                       | +                     | 3                 | –          | GT3 T. N (12 weeks) = 88 %          |
|                            |                  |             |                         |                       |                   |            | GT3 T. E (16 weeks) = 92 %          |
| **Daclatasvir asunaprevir and bedabuvir combination** | | | | | | | |
| Poordad et al. [29]         | UNITY-1          | –           | +                       | +                     | 1                 | NCT01979939| GT1 T.N = 92 %                     |
Referenced clinical studies | Trials name/phase | ±PEG-IFN/RBV | Treatment-naïve patient | Treatment-experienced | Genotype coverage | NCT# | Overall SVR |
--- | --- | --- | --- | --- | --- | --- | --- |
Muir et al. [30] | UNITY-2 | ±RBV | + | + | 1 | NCT01973049 | GT1 T.E = 89 % |
Ledipasvir and sofosbuvir combination
Afzal et al. [31] | ION-1 | ±RBV | + | – | 1 | NCT01701401 | ION-1 = 97–99 % |
Afzal et al. [31] | ION-2 | ±RBV | – | + | 1 | NCT01768286 | ION-2 = 94–96 % |
Kowdely et al., [111] | ION-3 | ±RBV | + | – | 1 | NCT01851330 | ION-3 = 93–95 % |
Naggie et al., [112] | ION-4 | – | + | + | 1/4 | NCT02073656 | ION-4 = 96 % |
(3) RNA-dependent RNA polymerase inhibitors (NS5B inhibitors)
(3.1) Nucleoside analog inhibitors (NIs)
Sofosbuvir
Lawitz et al. [32] | NEUTRINO | + | + | – | 1/4/5/6 | NCT01641640 | NEUTRINO = 90 % |
Lawitz et al. [33] | FISSION | +RBV | + | – | 2/3 | NCT01497366 | FISSION = 67 % |
Jacobson et al. [34] | POSITRON | +RBV | – | + | 2/3 | NCT01542788 | POSITRON = 78 % |
Jacobson et al. [35] | FUSION | +RBV | – | + | 2/3 | NCT01604850 | FUSION = 50–73 % |
Sofosbuvir and velpatasvir combination
Feld et al. [36] | ASTRAL-1 | – | + | + | 1/2/4/5/6 | NCT02201940 | ASTRAL-1 = 99 % |
Foster et al., [113] | ASTRAL-3 | + | + | + | 3 | NCT02201953 | ASTRAL-3 = 95 % |
Foster et al., [113] | ASTRAL-2 | – | + | + | 2 | NCT02220998 | ASTRAL-2 = 99 % |
Curry et al., [114] | ASTRAL-4 | – | + | + | 1-6 | NCT02201901 | ASTRAL-4 = 83 % |
Wyles et al., [127] | ASTRAL-5 | – | + | + | 1-6 | NCT02480712 | ASTRAL-5 = 95 % |
Elbasvir and grazoprevir combination
Dore et al. [37] | C-EDGE CO-STAR | – | + | – | 1/4/6 | NCT02105688 | Overall SVR = 95 % |
Referenced clinical studies | Trials name/phase ±PEG-IFN/RBV | Treatment-naïve patient | Treatment-experienced | Genotype coverage | NCT# | Overall SVR
--- | --- | --- | --- | --- | --- | ---
Rockstroh et al. [38] | C-EDGE coinfection | – | + | – | 1/4/6 | NCT02105662 | Overall SVR = 96%
Kwo et al. [39] | C-EDGE T. E ±RBV | – | + | – | 1/4/6 | NCT02105701 | C-EDGE + RBV(12) = 94%
 | | | | | | C-EDGE-RBV(12) = 92%
 | | | | | | C-EDGE + RBV(16) = 97%
 | | | | | | C-EDGE-RBV(16) = 92%
Zeuzem et al. [40] | C-EDGE T. N | – | + | – | 1/4/6 | NCT02105467 | C-EDGE T. N = 95%
Roth et al. [41] | C-SURFER | – | + | – | 1 | NCT02092350 | C-SURFER = 99%
Paritaprevir-ombitasvir-ritonavir combination
Feld et al. [42] | SAPPHIRE I | +RBV | + | – | 1 | NCT01716585 | Overall SVR = 96%
Zeuzem et al. [43] | SAPPHIRE II | +RBV | – | + | 1 | NCT01715415 | Overall SVR = 96%
Sulkowski et al. [44] | TURQUOISE I | +RBV | + | + | 1/HIV | NCT01704755 | Overall SVR = 91-94%
Poordad et al. [45] | TURQUOISE II | +RBV | + | + | 1 | NCT01939197 | Overall SVR = 92-96%
Andreone et al. [46] | PEARL II | ±RBV | – | + | 1b | NCT01674725 | Overall SVR = 96-100%
Ferenci et al. [47] | PEARL III | ±RBV | + | – | 1b | NCT01767116 | Overall SVR = 99-99.5%
Ferenci et al. [47] | PEARL IV | ±RBV | + | – | 1a | NCT01833533 | Overall SVR = 90-97%
Paritaprevir-ombitasvir-and ritonavir combination
Hezode et al. [24] | PEARL-I | ±RBV | + | + | 4 | NCT01685203 | Overall SVR = 91-100%
Asselah et al., [129] | AGATE-I | +RBV | + | + | 4 | NCT02265237 | Overall SVR = 97-98%
Waked et al., [128] | AGATE-II | +RBV | + | + | 4 | NCT02247401 | Overall SVR = 93-97%

* Only those clinical studies/trials were referenced which were considered by the US FDA for the approval of anti-HCV compounds and which were registered to clinicaltrials.gov. Similarly, the clinical trial data regarding the inclusion of patients, genotype coverage, and SVR rates were extracted only from FDA, NCBI, and clinicaltrials.gov websites. + = included, - = not included, ± = with or without, T.N = treatment-naïve, T.E = treatment-experienced, RBV = ribavirin, GT = genotype, SVR = sustained virologic response.

Table 2. The most promising phase II and III clinical trials for novel direct-acting antivirals with overall SVRs in HCV-infected patients.
The rapid emergence of viral escape mutants followed by viral breakthrough (HCV RNA remains lower limit of quantification but increased to ≥100 IU/ml or ≥1 log_{10} during telaprevir therapy) in some patients during therapy and significantly in all the patients after treatment completion [48]. The addition of PEG-IFN α plus RBV reduced the frequency of viral escape mutants and achieved higher SVR rates in both treatment-naive and treatment-experienced patients in phase II and III clinical trials than PEG-IFN α plus RBV therapy alone [49]. Similarly, some studies also describe that telaprevir is not equally useful for difficult-to-treat populations (i.e., patients with decompensated liver diseases, IL28B polymorphism, HCV genotype 3, 4, 5, and 6 infections) as compared with HCV genotype 1 infection [48]. For such HCV-infected patients, interferon-free antiviral combination therapies are the best treatment options. Boceprevir (BOC; Victrelis®) another first-generation protease inhibitor shares the similar pharmacokinetics and pharmacodynamics like telaprevir to confer anti-HCV activity. The clinical efficacy of boceprevir-based triple therapy (along with PEG-IFN α plus RBV) was evaluated in phase III clinical trials both in treatment-naive (SPRINT-2 clinical trial) and poor responders to PEG-IFN α and RBV therapy (RESPOND-2 clinical trial) patients (Table 2) [50–52]. The overall cure rates were almost equivalent to telaprevir-based triple therapy. However, the treatment-naive patients achieved higher SVR rates than treatment-experienced patients (Table 2). Telaprevir or boceprevir monotherapy seems effective in treatment-naive HCV genotype 1-infected patients. However, administration of telaprevir or boceprevir as monotherapy in infected individuals is not a suitable option because of the early emergence of viral escape mutants during therapy and followed by viral breakthrough after treatment completion [53]. The minor resistant populations exist at baseline in all HCV-infected individuals and are selected rapidly with telaprevir or boceprevir monotherapy (Table 3) [51]. Consequently, the first-generation DAAs/PIs still require a platform of PEG-IFN α and RBV to prevent the emergence of viral escape mutants and also to achieve significantly higher SVR rates [54]. Similarly, notable drug-drug interactions with many HIV antiretrovirals and calcineurin inhibitors also decrease the therapeutic activity of telaprevir and boceprevir monotherapy [54]. Drug toxicities and unusual adverse event profile (anemia, rashes, dysgeusia, and depression) of first-generation protease inhibitors also limit their clinical efficacy in treated patients [54]. These adverse effects result in treatment withdrawal in the majority of treated individuals, and this ratio is 10% much higher in telaprevir- or boceprevir-based triple therapy vs. PEG-IFNα and RBV therapy alone [50, 52]. The physicians and hepatologists do not recommend telaprevir or boceprevir monotherapy or even in combination with PEG-IFN/RBV due to the harsh adverse event profile and the emergence of viral escape mutants. Similarly, premature treatment discontinuation and mandatory intake of food have heightened the adherence concern related to first-generation protease inhibitors. The treatment has already discontinued in the United States and expected to be stopped from other parts of the world after the approval of interferon-free therapeutic regimens.

3.2. Second-generation protease inhibitors

Telaprevir and boceprevir were found more effective in treatment-naive HCV genotype 1 patients and had to be administered three times daily [54]. The situation demanded to
develop the regimens, which must be effective against other difficult-to-treat HCV populations with an improved dosing schedule. Simeprevir (Olysio®) was approved by the US FDA to treat chronic HCV genotype 1 infection in 2013 [55, 56]. The drug was approved along with PEG-IFN α-2a or 2b and RBV for treatment-naive HCV genotype 1-infected patients [35], for patients with compensated liver diseases (fibrosis and cirrhosis), and for those who are relapsers and nonresponders to interferon-based therapy [32,57]. Simeprevir 150 mg once daily along with PEG-IFN/RBV achieved overall SVR rates approximately 80–90 % in treatment-naïve and relapers [56] and 67–80 % of previously treated and null responders to interferon-based therapy (Table 2) (QUEST-1 and QUEST-2 clinical trials). The therapy seems to be more efficient than telaprevir/boceprevir-based triple therapies; however, there are certain disadvantages. For example, SVR rates were lower at the start of antiviral therapy in patients with advanced hepatic fibrosis as well as in patients with Q80R/K polymorphism of NS3 protein [58]. It is a naturally occurring amino acid substitution at NS3 codon 80, where glutamine is replaced to lysine. The Q80K polymorphism exists naturally in HCV genotype 1a (30–50 %)- and 1b (0.5 %)-infected patients [56]. It substantially reduces the efficacy of simeprevir-based triple therapy in HCV genotype 1a-infected patients. In QUEST-1 clinical

| Drug name       | Resistance amino acid variants                        |
|-----------------|-------------------------------------------------------|
| Telaprevir      | V36A/M; T54S/A; R155K/T/Q; A156S; B156T/V             |
| Boceprevir      | V36A/M; T54S/A; V55A; R155K/T/Q; A156S; B156T/V; V170A/T |
| Simeprevir      | Q80R/K; R155K/T/Q; B156T/V; D168A/V/T/H               |
| Faldaprevir     | R155K/T/Q; D168A/V/T/H                                |
| Danoprevir      | R155K/T/Q; D168A/V/T/H                                |
| Asunaprevir     | Q80R/K; R155K/T/Q; D168A/V/T/H                         |
| ABT-450         | R155K/T/Q; D168A/V/T/H                                |
| Daclatasvir     | M28; Q30; L31; Y93Y/H                                 |
| Sofosbuvir      | 282 T                                                 |
| Ledipasvir      | K24R, M28T/V, Q30R/H/K/L, L31M, Y93H/N                |
| Velpatasvir     | Q30R, Y93H/N, L31M                                    |
| Elbasvir        | A156T, D168A/Y, R155K                                 |
| Grazoprevir     | M28, Q30, L31, Y93                                    |
| Paritaprevir    | V36A/M/T, F43L, V55I, Y56H, Q80L, L132V, R155K, A156G |
| Ombitasvir      | K24R, M28A/T/V, Q30E/K/R, H/Q54Y, H58D/P/R, Y93C/H/N  |
| Dasabuvir       | G307R, C316Y, M414I/T, E446K/Q, A450V, Y561H          |

Table 3. Resistance amino acid variants (RAVs) associated with antiviral drug resistance to direct-acting antivirals.
studies, SVR rates were demonstrated 20% lower in HCV genotype 1a-infected individuals than 1b [21, 56]. Furthermore, approximately one-third HCV genotype 1a patients were found with Q80K NS3 protein mutation at baseline [21]. Interestingly, the mutation was infrequent, was nonexistent, and did not impact the SVR rates significantly in HCV genotype 1b patients [56, 59]. HCV genotype 1a-infected patients are strongly recommended for Q80K polymorphism screening and NS3 genotype test before the start of treatment and to avoid any complications during therapy. The mutations associated with NS3 protease inhibitors may detect by reverse transcribing HCV RNA, followed by PCR amplification and then DNA sequencing of NS3 gene [21, 60]. Some institute/laboratories in the United States have launched the tests for Q80K polymorphism (Quest Diagnostics®) and HCV drug-resistance test for NS3/4A protease inhibitors (LabCorp®). Treatment withdrawal and alternative treatment strategies may adopt for the patients found with a Q80K polymorphism in NS3 genotype analysis. Skin rashes, itching, nausea, and photosensitivity reactions are the most commonly observed adverse events with simeprevir-based triple therapies [56]. Sunscreen creams and lotions are highly recommended to use while taking the drug [61].

In 2014, simeprevir was approved in combination with sofosbuvir to treat chronic HCV genotype 1 patients with or without cirrhosis. The combination regimen (150/400 mg q.d.) without PEG-IFN/RBV was found almost equally effective in both patient arms; however, relatively better in treatment-naïve than treatment-experienced patients (OPTIMIST-1 and OPTIMIST-2 clinical trials) (Table 2) [22, 23]. Similarly, the therapeutic outcome was much significant in subtype 1a patients without Q80K polymorphism and in noncirrhotics as compared to those with cirrhosis and Q80K polymorphism (Table 2). The frequency of treatment-emergent adverse events was similar in both clinical trials, and the drug combination was well tolerated and safe even in patients with Q80K polymorphism as compared to simeprevir and PEG-IFN/RBV-based triple therapies.

4. NS5A inhibitor-based direct-acting antivirals

Daclatasvir (Daklinza®) is the first in a new class of direct-acting antivirals, which inhibit the action of NS5A, a protein essential to play a diverse role in HCV replication, assembly, and release [62]. The US FDA approved initially daclatasvir and sofosbuvir combination to treat chronic HCV genotype 3-infected patients in July 2015. However, the indications were modified and expanded to treat HCV genotype 1- and 3-infected patients, patients with decompensated cirrhosis, patients with post-liver transplantation, and HCV/HIV-coinfected patients in February 2016 [63]. Daclatasvir monotherapy along with PEG-IFN/RBV leads initially to higher SVR rates in treated patients, but viral escape mutants occur rapidly indicating its relatively lower genetic barrier to resistance (COMMAND-1 and COMMAND-4 clinical trials) (Table 2) [64]. To overcome the emergence of viral escape mutants and to achieve high SVR rates, the treatment is recommended along with sofosbuvir and with or without ribavirin. Higher SVR rates were documented in HCV genotype
1 and 3 treatment-naive patients when administered to daclatasvir and sofosbuvir combination [24]. Other multi-series clinical trials also demonstrate high clinical efficacy and well tolerability of daclatasvir-sofosbuvir with or without RBV against HCV genotype 1–6 infected patients (ALLY 1–3 clinical trial) (Table 2) [25–28]. Similarly, higher SVR rates (87–98%) were achieved in HCV genotype 1-infected patients treated with a combination of daclatasvir, asunaprevir, and beclabuvir for 12 weeks (UNITY 1–2 clinical trial) (Table 2) [29, 30]. Headache, nausea, and vomiting were the most commonly noticed adverse effects, and the adverse event profile was almost similar in all genotype-treated patients and managed during or after the treatment completion [62]. The viral-resistant mutants were found commonly at residue M28, Q30, L31, and Y93 of NS5A region in HCV genotype 1a patients (Table 3) [25, 28]. However, viral-resistant mutants were reported less frequently at position L31 and Y93 in HCV genotype 1b patients [64]. Some experimental studies demonstrate that these mutations are responsible for increasing the \( EC_{50} \) (i.e., the concentration of a drug which produces therapeutic response halfway between the baseline and maximum after a certain period of time) of daclatasvir in treated patients [64]. In contrast to NS3 protease, viral-resistant mutants against NS5A inhibitors do not impair replication fitness and do not disappear during follow-up examinations at the end of treatment. One-year follow-up studies predict the persistence of NS5A-resistance mutants, but no cross-resistance has reported between daclatasvir and other direct-acting antivirals as yet [65]. The approval of daclatasvir monotherapy and daclatasvir with other different regimens is expected soon.

5. HCV polymerase inhibitor-based therapeutic regimens

Sofosbuvir (Sovaldi®; SOF) was approved by the US FDA in 2013 to treat treatment-naïve and treatment-experienced HCV genotype 1–6-infected patients [66]. SOF is a uridine nucleotide analog inhibitor, which exerts its antiviral activity by competing with endogenous uridine triphosphate of the growing HCV mRNA chain incorporated by HCV polymerase enzyme [66,67]. After incorporation into growing mRNA chain, no further nucleotides can be added, and mRNA chain is terminated [67]. The drug is active against all HCV genotypes (1–6) as well as difficult-to-treat HCV populations [68]. The approval was momentous and based on the results of four registration studies in phase III clinical trials (FISSION, POSITRON, FUSION, and NEUTRINO clinical trials) where the therapy endpoint was to achieve a sustained virologic response rate at week 12 after stopping the active treatment (i.e., SVR12) (Table 2) [69]. Sofosbuvir in combination with ribavirin presented very promising clinical efficacy data against HCV genotype 1–4-treated patients (Table 2) [33, 34]. The relapse rate was only 9% among all the four phase III drug registration studies [34]. No major side effects or severe cardiac adverse events were reported during SOF plus RBV dual therapy. However, the adverse event profiles of sofosbuvir monotherapy and even in combination with RBV are not sufficiently addressed because of the lack of controlled trials [66]. Meaningful historical controls, as well as ribavirin monotherapy arm as a comparator, are not available due to which we may not draw conclusions about the eventual adverse effects of sofosbuvir [33, 34]. However, adverse events associated with sofosbuvir-related therapies are not frequent and
severe in nature. Antiviral resistance was only observed in a single patient after sofosbuvir monotherapy, which was successfully re-treated with SOF plus RBV dual therapy [69].

Sofosbuvir in combination with ledipasvir (Harvoni®; the first fixed-dose drug combination claims to “two firsts”) with or without RBV was approved in 2014 for HCV genotype 1 infection treatment. Later on, the approval was expanded to treat HCV genotype 4-, 5-, 6-, and HCV/HIV-coinfected patients in 2015. Recently, the US FDA has extended the treatment recommendation to liver transplant genotype 1 and 4 patients with compensated cirrhosis and in genotype 1 patients with decompensated cirrhosis (http://www.hepatitisc.uw.edu/page/treatment/drugs/ledipasvir-sofosbuvir#drug-summary) [31, 70]. Ledipasvir (90 mg) and sofosbuvir (400 mg) have formulated as a single coformulated pill [31]. However, ledipasvir (an NS5A inhibitor) monotherapy is not approved by the US FDA to treat the infection as yet. This combo drug seems very efficient to achieve SVR rates more than 90% of HCV genotype 1- and 4-treated patients without cirrhosis when administered to 8, 12, or 24 weeks (Table 2) [31, 71]. Cirrhotic patients may also achieve higher SVR rates with 12- or 24-week treatment schedule [72]. Severe adverse events are rare and manageable during or after the therapy. The inclusion of ribavirin does not affect the therapeutic efficacy and achievement of higher SVR rates. Consequently, the addition of RBV does not seem prerequisite in every all-oral DAA combinations against HCV. RBV results in hemolytic anemia and is highly teratogenic [11], and for this reason, RBV-sparing regimens are considerably advantageous and eagerly awaited in the future.

6. Treatment paradigms for difficult-to-treat populations

As the treatment success in HCV-infected patients mainly depends on HCV genotype, previous treatment history, cirrhosis or fibrosis score, and the high barrier to antiviral drug resistance, the combination of experimental drug velpatasvir and sofosbuvir might simplify the treatment strategies in “difficult-to-treat subgroups” in the future. Velpatasvir (An NS5A inhibitor) and sofosbuvir in a fixed-dose combination (100 mg/400 mg) exhibit the pan-genotypic coverage with a simple 12-week regimen in treatment-naive, treatment-experienced, as well as in cirrhotic patients as determined in multicentered clinical trials (i.e., ASTRAL 1–5) conducted at 81 sites including the United States, Canada, and Europe in 2014 (Table 2) [36]. The adverse event profile was almost similar to each clinical trials, and headache, nausea, and vomiting were the most common ones. However, overall the drug combination was well tolerated and safe in treated patients. The US FDA has approved the fixed-dose combination of velpatasvir and sofosbuvir (Epclusa®) in June 2016 to treat chronic HCV genotype 1–6-infected adult patients.

The discovery and development of elbasvir/grazoprevir fixed-dose combination have shifted the treatment paradigm for genotype 1 and 4 patients with stage 4–5 chronic kidney disease (CKD) and HCV/HIV coinfection [40, 41]. The US FDA has approved elbasvir/grazoprevir (50/100 mg q.d.) (Zepatier®) combination for HCV genotype 1 and 4 infection with chronic kidney diseases and HCV/HIV coinfection with some specific clinical requirements (i.e., viral genotype, prior treatment experience, NS5A-associated RAVs at position M28, Q30,
Similarly, the treatment regimen is prescribed with many precautions in subtype 1a patient with prior testing of NS5A-associated RAVs, because it determines the overall treatment duration and the inclusion of ribavirin to therapy (Table 3) [38, 40, 41]. The FDA approval was granted on the findings of a series of multicenter phase III clinical trials (C-EDGE and C-SURFER) in treatment-naïve, treatment-experienced, and other difficult-to-cure populations (i.e., HCV/HIV coinfection, stage 4/5 CKD including hemodialysis patients) where overall SVR rates were highly promising (i.e., 92–99 %) (Table 2) [37–41]. The adverse event profile was not serious in the treatment groups, and the renal system adverse effects were comparable without significant changes in estimated glomerular filtration rate (eGFR) value and creatinine levels [38, 40, 41]. Headache, nausea, and fatigue were the most commonly observed adverse events with an elevation in alanine aminotransferase (ALT) levels five times more than the normal one [38, 40, 41]. However, most of the adverse effects resolved at or after the treatment completion.

The first 3D regimen, “Viekira Pak®” (i.e., a combination of three direct-acting antivirals: ombitasvir, paritaprevir, and dasabuvir) along with ritonavir, to treat chronic HCV genotype 1 infection was approved by the US FDA in 2014 (http://www.hepatitisc.uw.edu/page/treatment/drugs/3d#drug-summary) [75]. The drug combination is prescribed to genotype 1-compensated cirrhotic patients, however, still contraindicated to decompensated cirrhotics [42]. The approval was based on phase II/III multicenter clinical trials (Table 2) involving more than 2300 patients with chronic HCV 1 infection, some of whom had cirrhosis [43–47, 76]. Cure rates across the various groups were ranged from 91 % to 100 % (Table 2). The therapeutic outcomes demonstrated that the drug combination was safe with no significant adverse effects in a population with compensated cirrhosis [47]; however, the drug combination is forbidden in decompensated cirrhotic patients.

A recent ongoing clinical trial conducted in HCV genotype 4 noncirrhotic patients showed the promising therapeutic activity of ombitasvir, paritaprevir, and ritonavir with or without RBV for a 12-week course (PEARL-I clinical studies) [24]. Similarly, the therapeutic regimen along with RBV was also found highly effective to treat genotype 4, cirrhotic patients, as shown by the results of AGATE-I and AGATE-II ongoing clinical studies. PEARL-I, phase 2 open-label, randomized, and multicentre clinical trials were conducted in Europe, Turkey, and the United States [24]. The treatment-naïve patients included in the study received the ombitasvir, paritaprevir, and ritonavir combination with or without RBV; however, all treatment-experienced patients (i.e., previously treated with PEG-IFN/RBV) received the active treatment with RBV. Interestingly, dasabuvir was not included in the treatment regimen as it does not show therapeutic activity against HCV genotype 4. The overall SVR rates were achieved 91 % (40/44) in treatment-naïve patients who received active treatment without RBV and 100 % (42/42) in those who took RBV along with the regimen. All treatment-experienced patients achieved SVR12 at the treatment completion (49/49, 100 %) (Table 2). Viral relapse occurred in two patients, and the virologic breakthrough was experienced in one patient. The adverse event profile was almost negligible including headache and decreased hemoglobin level (i.e., 100 g/L, anemic state), but no treatment discontinuation was attributed to active regimen except the dose modification of RBV to nullify anemic state [24].
The AGATE-I open-label phase 3 multicenter and randomized clinical trial revealed the therapeutic outcome of ombitasvir, paritaprevir, and ritonavir plus RBV in HCV genotype 4 patients with compensated cirrhosis [77]. The study was conducted in both treatment-naïve and treatment-experienced patients (PEG-IFN/RBV treated) at different locations in Europe and the United States. Overall, 120 adult patients were enrolled in the study of which 59 patients received 12-week treatment (i.e., ombitasvir, paritaprevir, and ritonavir plus RBV) and 61 patients were assigned to take 16 weeks of the same treatment regimen. The overall SVR rates were achieved 97 % (57/59) in the 12-week group and 98 % (60/61) in the 16-week group. The adverse event frequency was significantly higher, mixed in both patient arms, and noticed in more than 10 % of all patients including asthenia, fatigue, headache, anemia, pruritus, nausea, and dizziness more common ones. However, virologic breakthrough was reported in one patient, and one patient discontinued the treatment at day 1 in the 12-week treatment group, and one missed the posttreatment week 12 visit in the 16-week group [77].

The AGATE-II open label and partly randomized clinical studies were conducted in native Egyptian population infected with HCV genotype 4 [77]. Overall 182 patients both treatment-naïve and treatment-experienced (previously treated to PEG-IFN/RBV-based dual therapies) anticipated in the clinical trial of whom 160 were eligible for inclusion criteria. 100 patients had no cirrhosis and received active regimen and RBV for 12 weeks. The remaining 60 having cirrhosis were randomly assigned to 12-week active treatment (n = 31) or 24-week treatment group (n = 29). The SVR rates were achieved 94 % in the noncirrhotic 12-week patient arm (94/100), 97 % in cirrhotic patients (30/31) enrolled in the 12-week treatment group, and 93 % (27/29) in cirrhotic patients administered for 24-week active treatment (Table 2). The adverse event profile was significantly higher particularly in noncirrhotic patients including headache and fatigue the most common (i.e., 41 % and 35 %, respectively) ones than the cirrhotic ones (29–38 %, respectively). However, no treatment discontinuation was related to drug side effects or active treatment itself [77].

The findings of the ongoing PEARL-I, AGATE-I, and AGATE-II clinical trials are promising while treating HCV genotype 4 cirrhotic and noncirrhotic patients; however, no additional benefits were reported in terms of higher SVR, when treatment duration was extended for cirrhotic patients from 12 weeks to 16 weeks or even 24 weeks in native Egyptian population. Similarly, a small number of patients enrolled in the clinical trials limit the comprehensive determination of the drug side effects. Further studies are eagerly awaited in this prospect, and as a precaution, any HCV cirrhotic patients using such combination therapeutic regimens should be hepatically intact for any hepatic complications/comorbidities because the patients in clinical trials are often very carefully monitored than patients in real-world clinical practice [40].

7. Interferon derivatives

Over the years, the principal objectives to develop novel interferon formulations include the replacement of conventional interferon with the new ones, to reduce the adverse effects of current interferon and ease of administration with an improved dosing schedule [78].
The treatment success for HCV is primarily dependent on the patient adherence to therapy so that the development of unique IFN formulations with improved pharmacokinetics is a prime objective of HCV therapeutics nowadays. The main advantage of this approach may seem to maintain viral suppression across the longer dose interval, avoid of inter-dose trough, and reduce dosage frequencies (twice or even once per month as compared to once per week for the current PEG-IFN and consensus interferons). Although the development of new interferon formulations primarily focuses on HCV genotype 1 patients, their administration can also be prized for HCV genotype 2- and 3-infected individuals [79]. Similarly, the duration of treatment can also be reduced in easy-to-treat populations (HCV genotype 2- and 3-infected patients) up to 12 weeks if a rapid virologic response achieves earlier [79]. This approach may suggest a very convenient therapeutic regimen of only three injections if long-acting IFNs are used in treated patients.

7.1. Interferon lambda

Interferon lambda (IFN-λ) is still promising and may be beneficial as an adjuvant therapy to treat HCV infection in combination with other DAAs in the near future. It has also raised the hopes to replace the conventional interferons (IFN-α 2a, IFN-α 2b, PEG-IFN α) to reduce the frequency of side effects and treatment discomfort during and after the treatment completion in infected individuals. The discovery of three interferon-λ cytokines (i.e., interferons λ1, λ2, and λ3 encoded by IL29, IL28A, and IL28B, respectively) in 2003 has suggested their plausible role in suppression of HCV replication [80]. This fact was supported by the identification of common genetic variants in IL28B genome, which are highly associated with responses to PEG-IFN α/RBV treatment for chronic HCV infection [81]. Similarly, genome-wide association studies and in vitro studies also demonstrate an interactive and complementary relationship between IFN-α and IFN-λ for suppressing HCV replication [82]. IFN-λ like another type 3 interferons binds to different host cell receptors than type 1 interferons (e.g., IFN-α 2a, 2b) to trigger the JAK-STAT antiviral pathways (Figure 2). However, the downstream cell signaling pathways are largely comparable to interferon-α which upregulate several hundreds of interferon-stimulated genes to initiate antiviral activity [80]. IFN-λ binds mainly to IL28 receptors, which are positioned only to hepatocytes, plasmacytoid dendritic cells, peripheral B cells, and epithelial cells [83]. This restricted distribution of IL28 receptors for interferon-λ facilitates its targeted hepatic delivery, better tolerability, and increase safety profile than the conventional interferons [83]. IFN-λ may also escalate the subsaturating levels of IFN-α and increase its antiviral efficacy. In vitro studies reveal that IFN-α induces the expression of IFN-λ genes that upregulate a distinct pattern of signal transduction and interferon-stimulated genes than IFN-α to abort HCV replication [84]. Consequently, the combination of IFN-λ and IFN-α may provide additive therapeutic effects due to the complementary roles of two types of cytokines. Interferon-λ has been pegylated and phase I clinical trials with or without ribavirin have been completed [85]. Subsequent phase II clinical trials demonstrated that the administration of PEG-IFN λ (240 μg, 180 μg, or 120 μg once weekly) showed 10 % higher rapid virologic response (RVR; HCV RNA negative after 4 weeks of therapy) rates and 20 % higher extended rapid virologic response (eRVR; HCV RNA negative at the lower limit of detection but not the lower limit of quantification between week 4 and week 12 during PEG-IFN therapy) rates.
than PEG-IFN α-2a in treatment-naïve patients [86]. IFN-λ is associated with less adverse event profile, including less hematologic toxicity, flu-like symptoms, and muscular pain, but increased aminotransferase and bilirubin levels in treated patients [86]. Now, full phase III clinical trials of interferon-λ with other DAAs (i.e., in combination with daclatasvir and asunaprevir plus RBV) are under consideration [87].

8. HCV vaccine technology

8.1. Barriers to developing prophylactic and protective HCV vaccines

The tendency of acute HCV infection to develop into chronic infection and optimal outcomes of the current therapies in the majority of treated patients underscores an urgent need to search and develop potential anti-HCV vaccine molecules. Interestingly, the efforts to develop HCV vaccines are facing real challenges due to some reasons. First, despite the consistent efforts by the researchers, still now there is no permissive cell culture system available where HCV can replicate persistently at high enough levels to evaluate antibodies which may neutralize [88]. Second, there is no authenticated and handy animal model available which is susceptible to HCV and can tackle the candidate vaccine challenge studies [88]. Although the chimpanzee model is the first choice for the investigators in HCV replication and candidate vaccine studies, it is expensive, endangered, and difficult to handle [89]. Third, genome variations in HCV genotypes, subtypes, and quasispecies nature of HCV may require the construction of polyvalent vaccines, which protect against a significant number of closely related epitopes [90]. In fact, the genetic heterogeneity of HCV in an infected individual and immune responses...
selected for neutralization of escape mutants within the hypervariable regions (HVR1) of E1 envelope glycoprotein as well as cytotoxic T lymphocytes (CTL) escape mutants may limit the effectiveness and utility of any HCV vaccine model [91]. Fourth, it is unclear that either HCV envelope glycoproteins contain all the antigenic determinants require for effective neutralization or not [92]. Fifth, acute HCV infection persists in the majority of infected individuals, even though innate and acquired immune responses accelerate against nearly all of the HCV-encoded polyproteins [93]. T-cell responses immediately accelerate to clear HCV in acutely infected individuals; therefore, a successful anti-HCV vaccine has to elicit both CD\(^4\) and CD\(^8\) T-cell responses in infected individuals [94]. Sixth, HCV may associate with immunoglobulin or β-lipoprotein in the blood, which may “mask” the virus and reduce the efficiency of neutralization [95].

8.2. Current anti-HCV vaccine models

Due to the above-mentioned qualms, the development of prophylactic or protective HCV vaccine is a highly challenging task and fraught with barriers. Anyhow, the advancement in vaccinology has inspired the researchers to work out different models of protective HCV candidate vaccines (Table 1). In this vein, the principle goal of an anti-HCV vaccine design should be to wipe out the chronic infection in exposed individuals or remove the virus from already infected individuals by boosting the innate and acquired host immune responses, which also seems an uphill task [94]. In this context, the strategy paradigms have been shifted from the production of traditional recombinant envelope proteins to the engineering of complex viral vectors directing the expression of multiple hepatitis C viral antigens (i.e., Core, NS3, NS4, and NS5B) [94]. We briefly describe here the recent advancement in HCV vaccine technology, which may consider as the role models for the successful development of an effective preventive/protective vaccine in the future.

An HCV immunoglobulin (Civacir) has studied for its therapeutic effects on recurrent hepatitis C following liver transplantation in phase II clinical trials, but the SVR data from these trials are not yet available [96]. Similarly, the high-dose monoclonal antibodies were evaluated against HCV glycoprotein E2 in genotype 1a patients undergoing liver transplantation in phase II clinical trials [97]. Although the strategy was not effective to prevent the recurrence of HCV infection, the reappearance of viremia in liver transplant patients significantly delayed as compared to the placebo control population [97]. Now, this regimen along with a direct-acting antiviral is under consideration for further clinical trials.

Currently, a DNA vector-based vaccine (ChronVac-C) is in clinical development against chronic HCV genotype 1 infection [98]. By using DNA electroporation, the gene-encoding HCV NS3/4A protein was introduced into the patient skeletal muscle. The skeletal muscle expressed NS3/4A protein, which in turn stimulated the particular host innate and acquired immune responses against HCV. The clinical efficacy of ChronVac-C was evaluated in 12 treatment-naïve HCV genotype 1-infected patients with four different doses given monthly for four months in phase I/II clinical trials [98]. T-cell responses were detected in one patient, and viral load reduction up to 1.2 log\(_{10}\)–2.4 log\(_{10}\) was reported in two out of three patients with the highest dose [98]. Further clinical trials of this candidate vaccine are under consideration.
8.3. T-cell-based vaccines

Vaccines based on robust T-cell responses are vital and crucial because such vaccine triggers both antibodies and cytotoxic T-cell responses against an insidious virus [11]. The HCV-infected cells display the viral surface and internal particles/molecules (i.e., HCV genome) to the immune system of the body, especially to CD4+ and CD8+ killer cells, which induce the host innate and acquired immune responses against the virus surface particles as well as the HCV genome [11]. CD8+ T-cell immunity induced by CD4+ T cells is mainly responsible for HCV viral infection control in human as well as in chimpanzee challenge studies [11]. Recently researchers have evaluated heterologous T-cell vaccine (ChAd3/MVA) targeting HCV non-structural proteins in HCV genotype 1b-infected individuals [13]. The vaccine is a combination of replication-defective chimpanzee adenovirus (ChAd3) and modified vaccinia Ankara (MVA) vectors so named as ChAd3/MVA [13]. The vaccine induced higher magnitude of T-cell responses in most infected individuals against all six nonstructural (from NS2 to NS5B) antigenic pools in phase I clinical studies [13]. CD8+ memory T cells were also generated, and CD8+ T-cell polyfunctionality was also increased in vaccinated individuals [13]. There were no signs of regulatory T-cell induction which might suppress an anti-HCV immune response [13]. The viral heterogeneity and high mutation rate of HCV are always potential biological barriers to developing protective HCV vaccine. ChAd3/MVA vaccine generates cross-reactive T-cell responses between heterologous viral genotypes so may be tested in diverse HCV populations [13]. Now, this vaccine is under consideration for a larger phase II clinical trials to further determine the efficacy of the vaccine.

Another therapeutic T-cell-based vaccine (GI-5005) is in clinical development which contains a fusion of HCV structural “core” and nonstructural “NS3” protein in yeast vector. The therapeutic efficacy of the vaccine was tested in both treatment-naïve and prior null responders to PEG-IFNα/RBV treatment in phase IIb clinical trials [99, 100]. Overall, 133 HCV genotype 1-infected patients (96 treatment-naïve patients) were administered to once monthly dose of vaccine along with PEG-IFNα plus RBV vs. placebo (PEG-IFNα/RBV) for 48 weeks [100]. SVR rates were reported slightly higher in vaccinated patient’s arm than the patients in placebo (47 % vs. 35 %, respectively) [100]. However, the achieved SVR rates were not statistically significant. The same trends in SVR rates were reported for the patients who were stratified by their prior treatment status. In that case, the previous null responders of PEG-IFNα plus RBV therapy achieved SVR rates only 17 % upon vaccination as compared to control studies who received PEG-IFN/RBV (SVR 5 % only) [101]. At subtype levels, treatment-naïve patients with unfavorable IL28B TT polymorphism met SVR24 in vaccinated patient’s arm as compared to those who treated with PEG-IFNα plus RBV alone [100]. The study was further expanded to 17 additional treatment-naïve patients with IL28B TT polymorphism, and the results were compared to the original 10 IL28B TT patients from the first cohort [100]. An undetectable HCV RNA level at the end of treatment was reported in 10 out of 16 patients (63 %) who received GI-5005 as compared to 3 out of 11 patients (27 %) who were treated with PEG-IFNα plus RBV alone [100].

Another recombinant poxvirus vaccine (TG-4040) expressing HCV nonstructural proteins (NS3, NS4, and NS5B) is in phase I clinical trials and demonstrated significantly higher SVR
rates in treated patients [102]. Some other potential anti-HCV vaccine models are also in the pipeline, including T-cell-based peptide vaccines, recombinant HCV subunit vaccines, and pseudo-viral particles expressing HCV glycoproteins (E1 and E2) [103, 104]. Preclinical studies of some of these vaccine models have shown spectacular results, but a lot of further clinical studies are required to evaluate their therapeutic outcomes.

8.4. Clinical issues regarding HCV vaccine trials

No doubt, much work is being done on the development of an effective anti-HCV vaccine model, but all the efforts revolve around HCV genotype 1 infection. We know very well that more than 100 million people worldwide are infected with HCV genotype 3, 4, 5, and 6 infections, and in some countries like Egypt, the infection is almost an endemic [11]. It may indicate that the cross-reactive protective immunity induced by a proposed HCV vaccine model against one genotype would not be sufficient for selecting the best candidate HCV vaccine for the whole world. The other potential challenge is the lack of vaccine clinical trials in a high-risk population where some other factors are responsible for HCV transmission. In the United States and the Western world, where only 1–2% general population are infected with HCV, more than 100,000 infected individuals have to be enrolled in clinical trials to determine vaccine efficacy[94]. The situation is different in developing world where HCV prevalence depends on various predisposing factors including injection drug users (IDUs), blood transfusion without screening anti-HCV antibodies, unsterilized medical instruments use, piercing of ears and nose, unprotected sexual act, and healthcare workers. Such regions represent an alternative place to conduct HCV vaccine clinical trials. Similarly, in a highly endemic area (e.g., Egypt, where almost 22% of the country’s population have afflicted with HCV), preventive or protective vaccine trials may initiate. Unfortunately, the test results would be genotype specific in that area (i.e., HCV genotype 4 is the most prevalent genotype in Egypt and the Middle East) and may not apply to the other parts of the world. The lack of basic health infrastructure and intrinsic ethical and drug regulatory issues are also potential challenges in developing countries to initiate vaccine clinical trials.

Designing of vaccine clinical trials in some concrete and high-risk populations also depends on certain crucial factors including HCV prevalence, exposure frequency of the infection, viral infectivity, and infection chronicity. The infection rate should be reduced up to 50% in the appropriate population size for the acceptable vaccine efficacy. By assigning a certain value to the above mentioned parameters in a high-risk population, at least, 500–10,000 individuals are required in vaccine clinical trials [105]. Although this number can be managed easily with the high-risk population and in HCV endemic area, but the testing and handling of large candidate vaccines pose particular challenges in this context. The ultimate success and realistic goal of HCV vaccines must be to prevent the chronic infection in infected individuals. To achieve this objective, the term “chronic HCV infection” must be clearly defined and should not rely on the classical definition based on chronic HBV infection (i.e., the persistence of HCV viral replication more than 6 months detected quantitatively by polymerase chain reaction). Some studies have demonstrated that during acute HCV infection, viral RNA fluctuates markedly from undetectable limit to a higher level of quantification (10^6 IU/ml) [106]. In such acutely infected individuals, the viral clearance may not occur until 1 year later. Consequently, the clinical trials for HCV
vaccines need longer test duration (approximately 2 years). Withholding therapy is another ethical issue while treating acutely infected patients with PEG-IFNα and RBV after a close follow-up of 6–9 months [107]. Development of surrogate biomarkers and their validation to evaluate vaccine efficacy is also challenging. In all clinical trials, a standard methodology should be applied to compare the vaccine efficacy results with each other especially in high-risk populations.

One of the big concerns which is always debatable among doctors, researchers, public healthcare workers, policy makers, and patient groups is how to implement a successful vaccine program when once effective vaccines would be available in the future. In this scenario, cost-effective analysis, careful monitoring of the adverse effects, and compliance with futility rules must be extensively scrutinized before issuing public health policies regarding mass vaccination program [108]. Implementation of a universal HCV vaccination program instead of targeting high-risk populations would be more appropriate and have a profound impact in some developing countries where HCV is highly prevalent.

9. Conclusions

The development of novel direct-acting antivirals has simplified the treatment paradigm for chronically infected and difficult-to-treat HCV genotype populations around the globe. These game changer regimens have revolutionized the HCV therapeutics regarding pan-genotypic coverage, low pill burden, fewer drug-drug interactions, improved adverse event profile, and high barrier to drug resistance. However, the treatment cost and accessibility of the drugs to infected patients are major issues which must be resolved to get full therapeutic benefits of such therapeutic regimens. Interferon lambda is promising as more efficacious, well tolerated, and short treatment of duration and has been entered into phase III clinical trials with several direct-acting antivirals. It may be beneficial as an adjuvant therapy in IFN-α-intolerant patients as well as in individuals where IL28B genetic polymorphism is highly associated to lower SVR rates. The steadily improved DAAAs are playing a frontline role to surmount the burden of HCV around the globe, but the development and implementation of a successful HCV vaccine program would be mandatory to win an uphill battle against this silent epidemic. In this context, the core knowledge of intricate interplays between molecular and cellular immune responses toward HCV, viral clearance and persistence, and long-lasting immune responses would play a significant role to develop an effective protective HCV vaccine model. Adenovirus-based vector vaccines have shown promising results while generating durable, broad, sustained, and balanced innate and acquired immune response in chimpanzees and humans. A heterologous T-cell vaccine (ChAd3/MVA) has also shown very high levels of T-cell responses against multiple HCV antigens in HCV genotype 1b patients. If HCV vaccines are available in the near future, then mass vaccination program in high-risk populations would probably have a profound impact on eradicating HCV infection. The technology and scientific innovation definitely play its part, but the role of scientific community, implementation of controlled HCV healthcare policies, applications of risk prediction tools, collective will, and public health notes will galvanize the efforts to a proper ending of this silent epidemic from the world. Thus, this two-pronged attack on HCV—a variety of novel
direct-acting antivirals and the possibility of a vaccine—suggests the global eradication of 
HCV. Overall, the achievements and improvements in the field of HCV medicine predict that 
the future of HCV therapeutics is bright and becoming brighter every day.

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**References**

[1] Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW: Aging of hepatitis C virus 
(HCV)-infected persons in the United States: a multiple cohort model of HCV preva 
lence and disease progression. *Gastroenterology* 2010, 138(2):513-521, 521 e511-516.

[2] Innes H, Goldberg D, Dillon J, Hutchinson SJ: Strategies for the treatment of Hepatitis 
C in an era of interferon-free therapies: what public health outcomes do we value most? 
*Gut* 2015, 64(11):1800-1809.

[3] Shahid I, Gull S, Ijaz B, Ahmad W, Ansar M, Asad S, Kausar H, Sarwar MT, Khan MK, 
Hassan S: Stable Huh-7 cell lines expressing non-structural proteins of genotype 1a of 
hepatitis C virus. *J Virol Methods* 2013, 189(1):65-69.

[4] Attaullah S, Khan S, Ali I: Hepatitis C virus genotypes in Pakistan: a systemic review. 
*Virol J* 2011, 8:433.

[5] Ahmad W, Ijaz B, Gull S, Asad S, Khaliq S, Jahan S, Sarwar MT, Kausar H, Sumrin A, 
Shahid I et al: A brief review on molecular, genetic and imaging techniques for HCV 
fibrosis evaluation. *Virol J* 2011, 8:53.

[6] Kabiri M, Jazwinski AB, Roberts MS, Schaefer AJ, Chhatwal J: The changing burden of 
hepatitis C virus infection in the United States: model-based predictions. *Ann Intern Med* 
2014, 161(3):170-180.
[7] Ascione A, De Luca M, Tartaglione MT, Lampasi F, Di Costanzo GG, Lanza AG, Picciotto FP, Marino-Marsilia G, Fontanella L, Leandro G: Peginterferon alfa-2a plus ribavirin is more effective than peginterferon alfa-2b plus ribavirin for treating chronic hepatitis C virus infection. *Gastroenterology* 2010, **138**(1):116-122.

[8] Manns M, Zeuzem S, Sood A, Lurie Y, Cornberg M, Klinker H, Buggisch P, Rossle M, Hinrichsen H, Merican I *et al*.: Reduced dose and duration of peginterferon alfa-2b and weight-based ribavirin in patients with genotype 2 and 3 chronic hepatitis C. *J Hepatol* 2011, **55**(3):554-563.

[9] Yuan XL, Li MY, Zhang DZ: Efficacy and safety of a protease inhibitor with pegylated interferon and ribavirin in patients with untreated chronic hepatitis C: a meta analysis. *Zhonghua Gan Zang Bing Za Zhi* 2012, **20**(5):357-362.

[10] Summa V, Ludmerer SW, McCauley JA, Fandozzi C, Burlein C, Claudio G, Coleman PJ, Dimuzio JM, Ferrara M, Di Filippo M *et al*.: MK-5172, a selective inhibitor of hepatitis C virus NS3/4a protease with broad activity across genotypes and resistant variants. *Antimicrob Agents Chemother* 2012, **56**(8):4161-4167.

[11] Shahid I, ALMaki WH, Hafeez MH, Hassan S: Hepatitis C virus infection treatment: An era of game changer direct acting antivirals and novel treatment strategies. *Crit Rev Microbiol* 2014, **42**(4):1-13.

[12] Schaefer M, Capuron L, Friebe A, Diez-Quevedo C, Robaesys G, Neri S, Foster GR, Kautz A, Forton D, Pariante CM: Hepatitis C infection, antiviral treatment and mental health: a European expert consensus statement. *J Hepatol* 2012, **57**(6):1379-1390.

[13] Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, Halliday J, Kelly C, Bowen D, Fergusson J *et al*.: A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Sci Transl Med* 2014, **6**(261):261ra153.

[14] Tran TT: A review of standard and newer treatment strategies in hepatitis C. *Am J Manag Care* 2012, **18**(14 Suppl):S340-349.

[15] Wahid A, Dubuisson J: Virus-neutralizing antibodies to hepatitis C virus. *J Viral Hepat* 2013, **20**(6):369-376.

[16] Gutierrez JA, Lawitz EJ, Poordad F: Interferon-free, direct-acting antiviral therapy for chronic hepatitis C. *J Viral Hepat* 2015, **22**(11):861-870.

[17] Ascher DB, Wielens J, Nero TL, Doughty L, Morton CJ, Parker MW: Potent hepatitis C inhibitors bind directly to NS5A and reduce its affinity for RNA. *Sci Rep* 2014, **4**:4765.

[18] Gaudieri S, Rauch A, Pfafferott K, Barnes E, Cheng W, McCaughan G, Shackel N, Jeffrey GP, Mollison L, Baker R *et al*.: Hepatitis C virus drug resistance and immune-driven adaptations: relevance to new antiviral therapy. *Hepatology* 2009, **49**(4):1069-1082.

[19] Perry CM: Telaprevir: a review of its use in the management of genotype 1 chronic hepatitis C. *Drugs* 2012, **72**(5):619-641.
[20] Kong Y, Wang X, Shang Y, Schroder PM, Liang W, Ling X, Guo Z, He X: Efficacy and tolerability of telaprevir for chronic hepatitis virus C genotype 1 infection: a meta-analysis. *PLoS One* 2012, 7(12):e52158.

[21] Jacobson IM, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O *et al*.: Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014, 384(9941):403-413.

[22] Kwo P, Gitlin N, Nahass R, Bernstein D, Etzkorn K, Rojter S, Schiff E, Davis M, Ruane P, Younes Z *et al*.: Simeprevir Plus Sofosbuvir (12 and 8 Weeks) in HCV genotype 1-infected patients without cirrhosis: OPTIMIST-1, a phase 3, randomized study. *Hepatology* 2016.

[23] Lawitz E, Matusow G, DeJesus E, Yoshida EM, Felizarta F, Ghalib R, Godofsky E, Herring RW, Poleynard G, Sheikh A *et al*.: Simeprevir plus sofosbuvir in patients with chronic hepatitis C virus genotype 1 infection and cirrhosis: A Phase 3 study (OPTIMIST-2). *Hepatology* 2015.

[24] Hezode C, Asselah T, Reddy KR, Hassanein T, Berenguer M, Fleischer-Stepniewska K, Marcellin P, Hall C, Schnell G, Pilot-Matias T *et al*.: Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naïve and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): a randomised, open-label trial. *Lancet* 2015, 385(9986):2502-2509.

[25] Poordad F, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes EA, Noviello S, Swenson ES. Daclatasvir with sofosbuvir and ribavirin for HCV infection with advanced cirrhosis or post-liver transplant recurrence. *Hepatology*. 2016, doi:10.1002/hep.28446.

[26] Wyles DL, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, Sherman KE, Dretler R, Fishbein D, Gathe JC, Jr., Henn S, Hinestrosa F, Huynh C, McDonald C, Mills A, Overton ET, Ramgopal M, Rashbaum B, Ray G, Scarsella A, Yozviak J, McPhee F, Liu Z, Hughes E, Yin PD, Noviello S, Ackerman P, Investigators A. Daclatasvir plus sofosbuvir for HCV in patients coinfected with HIV-1. *N Engl J Med* 2015, 373:714-725.

[27] Nelson DR, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA, Team A-S. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*. 2015, 61:1127-1135.

[28] Leroy V, Angus P, Bronowicki JP, Dore GJ, Hezode C, Pianko S, Pol S, Stuart K, Tse E, McPhee F, Bhore R, Jesus Jimenez-Exposito M, Thompson AJ. Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and Advanced liver disease: a randomized phase III study (ALLY-3+). *Hepatology*. 2016, doi:10.1002/hep.28473.
[29] Poordad F, Sievert W, Mollison L, Bennett M, Tse E, Brau N, Levin J, Sepe T, Lee SS, Angus P, Conway B, Pol S, Boyer N, Bronowicki JP, Jacobson I, Muir AJ, Reddy KR, Tam E, Ortiz-Lasanta G, de Ledinghen V, Sulkowski M, Boparai N, McPhee F, Hughes E, Swenson ES, Yin PD, Group U-S. Fixed-dose combination therapy with daclatasvir, asunaprevir, and beclabuvir for noncirrhotic patients with HCV genotype 1 infection. JAMA. 2015, 313:1728-1735.

[30] Muir AJ, Poordad F, Lalezari J, Everson G, Dore GJ, Herring R, Sheikh A, Kwo P, Hezode C, Pockros PJ, Tran A, Yozviak J, Reau N, Ramji A, Stuart K, Thompson AJ, Vierling J, Freilich B, Cooper J, Ghersi W, Yang R, McPhee F, Hughes EA, Swenson ES, Yin PD. Daclatasvir in combination with asunaprevir and beclabuvir for hepatitis C virus genotype 1 infection with compensated cirrhosis. JAMA. 2015, 313:1736-1744.

[31] Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P et al: Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014, 370(20):1889-1898.

[32] Lawitz E FX, Zeuzem S, et al.: Simeprevir (TMC435) with Peginterferon/Ribavirin for Treatment of Chronic HCV Genotype 1 Infection in Patients who Relapsed After Previous Interferon-Based Therapy: Results from PROMISE, a Phase III trial. In. Edited by Week PaDD. 18–21 May; 2013.

[33] Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR et al: Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med 2013, 368(20):1878-1887.

[34] Jacobson IM, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M et al: Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med 2013, 368(20):1867-1877.

[35] Jacobson I DG, Foster GR, et al.: Simeprevir (TMC435) with peginterferon/ribavirin for chronic HCV genotype 1 infection in treatment-nave patients: results from QUEST-1, a phase III trial. In. Edited by International Liver Congress. Amsterdam; 2013.

[36] Feld JJ, Jacobson IM, Hezode C, Asselah T, Ruane PJ, Gruener N, Abergel A, Mangia A, Lai CL, Chan HL et al: Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. N Engl J Med 2015, 373(27):2599-2607.

[37] Dore G, Altice F, Litwin AH, et al. C-EDGE CO-STAR: efficacy of grazoprevir and elbasvir in persons who inject drugs (PWID) receiving opioid agonist therapy. Presented at the 2015 Annual Meeting of the American Association for the Study of Liver Diseases, San Francisco; Abstract 40. 2015.

[38] Rockstroh JK, Nelson M, Katlama C, Lalezari J, Mallolas J, Bloch M, Matthews GV, Saag MS, Zamor PJ, Orkin C et al.: Efficacy and safety of grazoprevir (MK-5172) and elbasvir (MK-8742) in patients with hepatitis C virus and HIV co-infection (C-EDGE CO-INFECTION): a non-randomised, open-label trial. Lancet HIV 2015, 2(8):319-327.
[39] Kwo P, Gane E, Peng CY, et al. Efficacy and safety of grazoprevir/elbasvir+/− RBV for 12 or 16 weeks in patients with HCV G1, G4, or G6 infection who previously failed peginterferon/RBV: C-EDGE treatment experienced. Presented at the 50th Annual meeting of the European Association for the Study of the Liver, Vienna; Abstract P0886. 2015

[40] Zeuzem S, Ghalib R, Reddy KR, Pockros PJ, Ben Ari Z, Zhao Y, Brown DD, Wan S, DiNubile MJ, Nguyen BY et al: Grazoprevir-elbasvir combination therapy for treatment-naive cirrhotic and noncirrhotic patients with chronic hepatitis C virus genotype 1, 4, or 6 infection: a randomized trial. Ann Intern Med 2015, 163(1):1-13.

[41] Roth D, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H, Jr., Martin P, Pol S, Londono MC, Hassanein T et al: Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. Lancet 2015, 386(10003):1537-1545.

[42] Feld JJ, Jacobson IM, Jensen DM, Foster GR, Pol S, Tam E, Jablkowski M, Berak H, Vierling JM, Yoshida EM et al.: Randomized study of danoprevir/ritonavir-based therapy for HCV genotype 1 patients with prior partial or null responses to peginterferon/ribavirin. J Hepatol 2014, 62(2):294-302.

[43] Zeuzem S, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourliere M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P et al: Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med 2014, 370(17):1604-1614.

[44] Sulkowski MS, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ et al: Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. JAMA 2015, 313(12):1223-1231.

[45] Poordad F, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM et al.: ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. N Engl J Med 2014, 370(21):1973-1982.

[46] Andreone P, Colombo MG, Enejosa JV, Koksal I, Ferenci P, Maieron A, Mullhaupt B, Horsmans Y, Weiland O, Reesink HW et al: ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. Gastroenterology 2014, 147(2):359-365 e351.

[47] Ferenci P, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A et al: ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. N Engl J Med 2014, 370(21):1983-1992.

[48] Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H: Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. J Viral Hepat 2012, 19(2):e134-142.
[61] Vermehren J, Sarrazin C: The role of resistance in HCV treatment. *Best Pract Res Clin Gastroenterol* 2012, 26(4):487-503.

[62] Hezode C, Hirschfield GM, Ghesquiere W, Sievert W, Rodriguez-Torres M, Shafran SD, Thuluvath PJ, Tatum HA, Waked I, Esmat G et al: Daclatasvir plus peginterferon alfa and ribavirin for treatment-naive chronic hepatitis C genotype 1 or 4 infection: a randomised study. *Gut* 2014.

[63] Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, Serrano-Wu MH, Langley DR, Sun JH, O’Boyle DR, 2nd et al: Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010, 465(7294):96-100.

[64] Fridell RA, Wang C, Sun JH, O’Boyle DR, 2nd, Nower P, Valera L, Qiu D, Roberts S, Huang X, Kienzle B et al: Genotypic and phenotypic analysis of variants resistant to hepatitis C virus nonstructural protein 5A replication complex inhibitor BMS-790052 in humans: in vitro and in vivo correlations. *Hepatology* 2011, 54(6):1924-1935.

[65] Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib R, Reindollar R, Rustgi V, McPhee F, Wind-Rotolo M et al: Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012, 366(3):216-224.

[66] Gane EJ, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Symonds WT, Hindes RG, Berrey MM: Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013, 368(1):34-44.

[67] Sofia MJ, Bao D, Chang W, Du J, Nagarathnam D, Rachakonda S, Reddy PG, Ross BS, Wang P, Zhang HR et al: Discovery of a beta-d-2’-deoxy-2’-alpha-fluoro-2’-beta-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J Med Chem* 2010, 53(19):7202-7218.

[68] Lam AM, Espiritu C, Bansal S, Micolochick Steuer HM, Niu C, Zennou V, Keilman M, Zhu Y, Lan S, Otto MJ et al: Genotype and subtype profiling of PSI-7977 as a nucleotide inhibitor of hepatitis C virus. *Antimicrob Agents Chemother* 2012, 56(6):3359-3368.

[69] Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R et al: Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2013, 368(1):804-811.

[70] http://www.hepatitisc.uw.edu/page/treatment/drugs/ledipasvir-sofosbuvir#drug-summary.Pdf [Accessed: 2016-06-10].

[71] Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R et al: Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014, 370(16):1483-1493.

[72] Gane EJ, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Subramanian GM, Symonds WT, McHutchison JG, Pang PS: Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 2014, 146(3):736-743 e731.
[73] http://www.hepatitisc.uw.edu/page/treatment/dugs/elbasvir-grazoprevir#drug-summary.Pdf [Accessed: 2016-06-12].

[74] http://www.hepatitisc.uw.edu/page/treatment/dugs/elbasvir-grazoprevir#drug-summary.Pdf [Accessed: 2016-06-14].

[75] http://www.hepatitisc.uw.edu/page/treatment/drugs/3d#drug-summary.Pdf [Accessed: 2016-06-16].

[76] Feld JJ, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T et al: Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med 2014, 370(17):1594-1603.

[77] Keating GM: Ombitasvir/paritaprevir/ritonavir: a review in chronic HCV genotype 4 infection. Drugs 2016, 76(12):1203-1211.

[78] Clark V, Nelson DR: Novel interferons for treatment of hepatitis C virus. Clin Liver Dis 2009, 13(3):351-363.

[79] Ramos EL: Preclinical and clinical development of pegylated interferon-lambda 1 in chronic hepatitis C. J Interferon Cytokine Res 2010, 30(8):591-595.

[80] Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP: IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 2003, 4(1):69-77.

[81] Grebely J, Petoumenos K, Hellard M, Matthews GV, Suppiah V, Applegate T, Yeung B, Marks P, Rawlinson W, Lloyd AR et al: Potential role for interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. Hepatology 2010, 52(4):1216-1224.

[82] Marcello T, Grakoui A, Barba-Spaeth G, Machlin ES, Kotенко SV, MacDonald MR, Rice CM: Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology 2006, 131(6):1887-1898.

[83] Dickensheets H, Sheikh F, Park O, Gao B, Donnelly RP: Interferon-lambda (IFN-lambda) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes. J Leukoc Biol 2013, 93(3):377-385.

[84] Freeman J, Friborg J, Kraft Z, Gray T, Hill M, McPhee F, Hillson J, Lopez-Talavera JC, Wind-Rotolo M: Pegylated interferons Lambda-1a and alfa-2a display different gene induction and cytokine and chemokine release profiles in whole blood, human hepatocytes and peripheral blood mononuclear cells. J Viral Hepat 2014, 21(6):e1-9.

[85] Muir AJ, Shiffman ML, Zaman A, Yoffe B, de la Torre A, Flamm S, Gordon SC, Marotta P, Vierling JM, Lopez-Talavera JC et al: Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. Hepatology 2010, 52(3):822-832.
[91] Weiner AL, Braun M, Reimann KH, Tung J, Crawford K, Bonino F, Saracco G, Choo QL, Hoaghton M et al. Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the persistent hepatitis C virus infection. Proc Natl Acad Sci U S A 1992; 89(2):834-848.

[92] Weiner AL, Geysen HM, Christopherson C, Hall JE, Mason TJ, Saracco G, Bonino F, Crawford K, Marion CD, Crawford KA et al. Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: potential role in chronic HCV infections. Proc Natl Acad Sci U S A 1991; 88(3):369-373.

[93] Chien DY, Choo QL, Kuo C, Ralston R, Speere R, Tong M, Hoaghton M, Kuo C. Persistence of HCV in vivo and immune reactivity to envelope glycoproteins. J Virol 1993; 67(2):842-849.

[94] Liang TJ. Current progress in development of hepatitis C virus vaccines. Nat Med 2013; 19(7):869-878.

[95] Walker CM. Adaptive immunity to the hepatitis C virus. Adv Virus Res 2010; 78:43-86.

[96] Thomassen R, Bok S, Thele A. Density heterogeneities of hepatitis C virus in human sera due to the binding of beta-lipoproteins and immunoglobulins. Med Microbiol Immunol 1993; 182(6):329-334.

[97] Davis GL, Nelson DR, Terrault NA, Piantadosi JA, Curry MP, Schiano TD, Emre S, Corey K, Merkunnov E, Herd M, et al. Human monoclonal antibody MBL-HCV delays HCV viral rebound following liver transplantation: a randomized controlled study. Liver Transpl 2013; 19(1):104-105.

[98] Friborg J, Levine S, Chen C, Sheaffer AK, Chanteau S, Voss S, Lema J, McGhee E, Combinations of lambda interferon with direct-acting antiviral agents are highly efficent in suppressing hepatitis C virus replication. Antimicrob Agents Chemother 2013; 57(3):132-132.

[99] Asad S, Ijaz B, Ahmad W, Kausar H, Sarwar MT, Gull S, Shahid I, Khan MK, Hassan S. Development of persistent HCV genotype 3a infection cell culture model in huh-7 cell. Virol J 2012; 9(1):1294-1298.

[100] Choo QL, Kuo G, Ralston R, Spaete R, Tong M, Houghton M, Kuo C. Persistence of HCV despite antibodies to both putative envelope glycoproteins. Lancet 1993; 342:933.
[98] Sallberg M, Frelin L, Diepolder H, et al. A first clinical trial of therapeutic vaccination using naked DNA delivered by in vivo electroporation shows antiviral effects in patients with chronic hepatitis C. Presented at the 44th Annual Meeting of the European Association for the Study of the Liver. Copenhagen, Denmark; abstract #43. 2009.

[99] Pockros P, Jacobson I, Boyer TD, et al.: GI-5005 therapeutic vaccine plus PEG-IFN/ribavirin improves sustained virologic response versus PEG-IFN/ribavirin in piror non-responders with genotype 1 chronic HCV infection. Hepatology; 2010, 52:404A.

[100] Shiffman ML MA, Vierling JM, et al.: GI-5005 therapeutic vaccine enhances virologic clearance by Peg-IFN/ribavirin in naïve HCV genotype 1 patients with IL28B genotype T/T. Presented at the 63rd Annual Meeting of the American Association for the Study of Liver Diseases. In: Boston, MA; 2012.

[101] Shiffman ML: Treatment of hepatitis C in 2011: what can we expect? Curr Gastroenterol Rep 2010, 12(1):70-75.

[102] Habersetzer F, Honnet G, Bain C, Maynard-Muet M, Leroy V, Zarski JP, Feray C, Baumert TF, Bronowicki JP, Doffoel M et al.: A poxvirus vaccine is safe, induces T-cell responses, and decreases viral load in patients with chronic hepatitis C. Gastroenterology 2011, 141(3):890-899 e891-894.

[103] Folgori A, Capone S, Ruggeri L, Meola A, Sporeno E, Ercole BB, Pezzanera M, Tafi R, Arcuri M, Fattori E et al.: A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. Nat Med 2006, 12(2):190-197.

[104] Elmowalid GA, Qiao M, Jeong SH, Borg BB, Baumert TF, Sapp RK, Hu Z, Murthy K, Liang TJ: Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. Proc Natl Acad Sci U S A 2007, 104(20):8427-8432.

[105] Strickland GT, El-Kamary SS, Klenerman P, Nicosia A: Hepatitis C vaccine: supply and demand. Lancet Infect Dis 2008, 8(6):379-386.

[106] Heller T, Rehermann B: Acute hepatitis C: a multifaceted disease. Semin Liver Dis 2005, 25(1):7-17.

[107] Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver D: Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009, 49(4):1335-1374.

[108] Krahn MD, John-Baptiste A, Yi Q, Doria A, Remis RS, Ritvo P, Friedman S: Potential cost-effectiveness of a preventive hepatitis C vaccine in high risk and average risk populations in Canada. Vaccine 2005, 23(13):1549-1558.

[109] Lawitz E, Sulikowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N et al.: Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. Lancet 2014, 384(9956):1756-1765.
Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath P J et al: Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014, 370(3):211-221.

Kowdley KV, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M et al: Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. N Engl J Med 2014, 370(20):1879-1888.

Naggie S, Cooper C, Saag M, Workowski K, Ruane P, Towner WJ, Marks K, Luetkemeyer A, Baden RP, Sax PE et al: Ledipasvir and Sofosbuvir for HCV in Patients Cointfected with HIV-1. N Engl J Med 2015, 373(8):705-713.

Foster GR, Afdhal N, Roberts SK, Brau N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C et al: Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. N Engl J Med 2015, 373(27):2608-2617.

Curry MP, O’Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, Reddy KR, Lawitz E, Flamm SL, Schiano T et al: Sofosbuvir and Velpatasvir for HCV in Patients with Decompensated Cirrhosis. N Engl J Med 2015, 373(27):2618-2628.

Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourliere M, Gharakhanian S, Bengtsson L et al: Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009, 360(18):1839-1850.

McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ, Team PS: Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009, 360(18):1827-1838.

McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J et al: Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010, 362(14):1292-1303.

Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A et al: Telaprevir for retreatment of HCV infection. N Engl J Med 2011, 364(25):2417-2428.

Kwo PY, Lawitz EJ, Mccone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N et al: Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. Lancet 2010, 376(9742):705-716.
[122] Poordad F, McCone J, Jr., Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N et al: Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011, 364(13):1195-1206.

[123] Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N et al: Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011, 364(13):1207-1217.

[124] Manns M, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M et al: Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 2014, 384(9941):414-426.

[125] Forns X, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, Horban A, Brown A, Peeters M, Lenz O et al: Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. Gastroenterology 2014, 146(7):1669-1679 e1663.

[126] Moreno C, Hezode C, Marcellin P, Bourgeois S, Francque S, Samuel D, Zoulim F, Grange JD, Shukla U, Lenz O et al: Efficacy and safety of simeprevir with PegIFN/ribavirin in naive or experienced patients infected with chronic HCV genotype 4. J Hepatol 2015, 62(5):1047-1055.

[127] Wyles D, Brau N, Kottilil S, et al. Sofosbuvir/velpatasvir fixed dose combination for 12 weeks in patients co-infected with HCV and HIV-1: the phase 3 ASTRAL-5 study. Presented at the 51st Annual Meeting of the European Association for the Study of the Liver, Barcelona, April 13-17, 2016. Abstract PS104.

[128] Waked I, Shiha G, Qaqish BR, Esmat G, Yosry A, Hassany M, Soliman R, Mohey AM, Allam N, Zayed N et al: Ombitasvir, paritaprevir, and ritonavir plus ribavirin for chronic hepatitis C virus genotype 4 infection in Egyptian patients with or without compensated cirrhosis (AGATE-II): a multicentre, phase 3, partly randomised open-label trial. Lancet 2016, S2468-1253(16)30002-4.

[129] Asselah T, Hézode C, Qaqish BR, ElKhashab M, Hassanein T, Papatheodoridis G, Feld JJ, Moreno C, Zeuzem S, Ferenci P et al: Ombitasvir, paritaprevir, and ritonavir plus ribavirin in adults with hepatitis C virus genotype 4 infection and cirrhosis (AGATE-I): a multicentre, phase 3, randomised open-label trial. Lancet 2016, S2468-1253(16)30001-2.
