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Status of Direct-acting Antiviral Therapy for HCV Infection and Remaining Challenges

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Abstract

Chronic infection with hepatitis C virus (HCV) is a major cause of liver disease and hepatocellular carcinoma worldwide. Following the discovery of HCV 3 decades ago, the identification of the structure of the viral proteins, combined with high-throughput replicon models, enabled the discovery and development of direct-acting antivirals. These agents have revolutionized care of patients, with cure rates of more than 90%. We review the status of direct-acting antiviral therapies for HCV infection and discuss remaining challenges. We highlight licensed compounds, discuss the potential to shorten therapy even further, and review different options for treatment failure and resistance. We also provide an overview on clinical experience with generic agents and evidence for their efficacy. Finally, we discuss the need for new drugs and outline promising targets for future therapies.

Keywords

hepatitis C; direct acting antivirals; resistance; treatment failure

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Rapid advances in therapy with oral direct-acting antivirals (DAAs) have resulted in significant improvement in safety and efficacy—7 all-oral regimens have been approved by the Food and Drug Administration (FDA) for treatment of chronic hepatitis C virus (HCV) infection. These produce rates of sustained virologic response (SVR) that exceed 95% [1]. DAAs target various points of the HCV replication cycle, binding directly to components of the replicase complex or initiating RNA chain termination. The rapid virologic response observed with potent DAA combinations have permitted progressively shorter durations of treatment with equivalent rates of SVR—although only patients without factors associated with lack of response, such as cirrhosis and prior treatment failure. Guidelines include regimens as short as 8 weeks for selected patients (Table 1), and registration trials and real-world cohorts have confirmed the efficacy of these regimens. However, it would be beneficial to shorten treatment durations even further, to reduce cost for patients and payors, decrease adverse effects, and optimize treatment adherence.

Three pan-genotypic combination regimens (sofosbuvir and velpatasvir; sofosbuvir, velpatasvir, and voxilaprevir; and glecaprevir and gibrentasvir) are effective in treatment of all HCV genotypes, as well as patients with cirrhosis or HIV coinfection. These pan-genotypic DAAs could simplify care and facilitate treatment expansion worldwide. We review key remaining challenges and opportunities in the treatment of chronic HCV infection, including further truncation of treatment duration with ultrashort regimens, incorporation of generic formulations of DAA regimens, roles and interpretations of testing for resistance-associated substitutions (RAS), evidence-based approaches to treatment of DAA failures, and the remaining need and prospect for future HCV therapies.

How short can therapy be?

Two oral DAA regimens have been approved for 8-week treatment duration in selected patients with chronic HCV infection, including sofosbuvir and ledipasvir and glecaprevir and pibrentasvir (G/P). The combination of sofosbuvir and ledipasvir can be used in patients infected with HCV genotype 1 who are treatment-naïve, without cirrhosis, non-black, and HIV-negative. In the phase 3 ION-3 protocol, 647 treatment-naïve patients without cirrhosis infected with HCV genotype 1 were randomly assigned to groups given 8 or 12 weeks of sofosbuvir and ledipasvir. No significant difference was observed between groups in proportions of patients with an SVR (93% vs 95%), although rates of virologic relapse were higher in the 8-week group [2]. This finding was confirmed in several large real-world cohorts, which reported similar levels of efficacy for the 8- and 12-week regimens [3–5]. In contrast to sofosbuvir and ledipasvir, the 8-week regimen of glecaprevir and pibrentasvir has been approved by the FDA for treatment-naïve, patients without cirrhosis infected with HCV genotypes 1–6. This recommendation is supported by the results of 4 clinical trials, including the ENDURANCE-1 protocol, in which 703 DAA-nai’ve patients without cirrhosis infected with HCV genotype 1 were randomly assigned to groups given 8 or 12 weeks of glecaprevir and pibrentasvir. In this study, 99.1% (348/351) of patients in the 8-week group and 99.7% (351/352) of patients in the 12-week group achieved an SVR (ref [6]). In the ENDURANCE-3 protocol, 390 patients without cirrhosis infected with HCV genotype 3 who were naïve to DAA therapy were randomly assigned to receive 8 or 12

weeks of glecaprevir and pibrentasvir. In this study, 95% (149/157) of patients in the 8-week group and 95% (222/233) of patients in the 12-week group achieved SVRs. In the SURVEYOR-II, Part 4, and ENDURANCE-4 protocols, 266 DAA-naïve patients without cirrhosis and infected with HCV genotypes 2, 4, 5, or 6 were given glecaprevir and pibrentasvir for 8 or 12 weeks. There were no significant differences in SVRs between 8- and 12-week groups of patients with HCV genotype 2 infection (98% vs 99.5%) or genotype 4–6 infections (93% vs 99%) [7]. Preliminary data from 2 real-world cohorts (from Italy and Germany) confirmed the efficacy of the 8-week regimen [8,9].

In summary, short (8-week) treatment with oral DAA regimens showed high levels of efficacy in clinical trial and real-world cohorts of patients infected with HCV genotypes 1–6. These are recommended as equivalent to 12-week regimens within AASLD/IDSA, EASL, and APASL guidance documents, but should not be used in patients with cirrhosis or prior exposure to DAA regimens. Whereas the 8-week regimen of sofosbuvir and ledipasvir has been approved by the FDA for select treatment-naïve patients without cirrhosis with HCV genotype 1 infection, the 8-week glecaprevir and pibrentasvir regimen has been approved for all treatment-naïve patients and those previously treated with interferon and ribavirin, without cirrhosis, infected with HCV genotypes 1, 2, 4, 5, or 6, and all treatment-naïve patients without cirrhosis with HCV genotype 3 infection. Short-course regimens under 12 weeks are not recommended for other DAA regimens such as sofosbuvir and simeprevir; paritaprevir, ritonavir, and ombitasvir with dasabuvir; grazoprevir and elbasvir; sofosbuvir and velpatasvir; or sofosbuvir, velpatasvir, and voxilaprevir.

Ultrashort DAA regimens

Due to the efficacy of 8-week regimens, multiple ultrashort regimens, combining 3 or 4 DAAs for treatment durations less than 8 weeks, have been studied. Despite early optimism from a trial by Kohli et al, which reported 95%–100% SVR in 60 treatment-naïve patients with HCV genotype 1 treated with sofosbuvir and ledipasvir or sofosbuvir and ledipasvir plus either GS-9669 or GS-9451[10], several subsequent trials have confirmed low rates of SVR with 4- and 6-week regimens[11,12], particularly among individuals with advanced liver disease. Advanced liver disease is associated with lack of SVR, which may be related to factors such as altered immune signaling pathways and impaired drug delivery, uptake, and metabolism due to venous shunting, liver fibrosis, and impaired liver function [13]. In the LEPTON protocol, Gane et al evaluated the efficacy of 4-, 6-, or 8-week regimens of a 3 DAA combination of sofosbuvir, velpatasvir, and GS-9857 in 161 treatment-naïve or previously treated patients with HCV genotype 1 or 3 infections, with or without compensated cirrhosis. The 4-week regimen led to an SVR in 27% (4/15) of treatment-naïve patients with HCV genotype 1 infection without cirrhosis, whereas the 6-week regimen led to an SVR in 79% (62/78) of patients [14]. The C-SWIFT trial was a singlecenter study of treatment-naïve patients without cirrhosis infected with HCV genotypes 1 or 3 given sofosbuvir, grazoprevir, and elbasvir for 4–12 weeks. SVRs were achieved by 32% (10/31) and 87% (26/30) of patients treated for 4 and 6 weeks, respectively [15]. The FOURward study was an open-label trial of 28 treatment-naïve, patients without cirrhosis infected with HCV genotype 1 who received daclatasvir, asunaprevir, and beclubavir for 4 or 6 weeks. SVRs were achieved by 23% and 57% of patients treated for 4 and 6 weeks, respectively [16].

The low rates of SVR in patients receiving 4 or 6 weeks of treatment reveal the limitations of ultrashort regimens, despite the high potency 3- or 4-DAA combinations—particularly in patients with 1 or more factor associated with lack of SVR.

Industry benchmarks for SVR (>95%) are unlikely to be achieved with ultrashort regimens with 3 or 4 DAAs available in clinical practice—particularly in real-world settings, in which many patients have factors associated with lack of SVR. Approved regimens of 8–12 weeks are associated with excellent safety and efficacy, but ultrashort or response-guided regimens are not recommended [17-21].

How Effective Are Generic Agents?

The World Health Organization (WHO) released a global strategy on viral hepatitis which calls for the elimination of HCV infection as a public health threat by 2030, defined by an 80% reduction in new HCV infections, and a 65% reduction in HCV-associated mortality [27], which is estimated to result in an absolute decrease in annual global HCV-associated deaths from 1.4 million to fewer than 0.5 million [28]. However, this will require diagnosing 90% of people living with HCV and treating 80% of diagnosed people with DAAs. Despite major advances in therapy, ongoing deficits in the care cascade for chronic HCV persist [29,30], although small steps of progress are being achieved in screening and diagnosis [31], linkage to care [32], and treatment, most notably within the Veterans Health Administration [33].

However, in the US and worldwide, access to treatment is a main barrier to eliminating HCV, due to the high costs of drugs [34–36]. Within high-income countries and upper middle-income countries, drug costs remain prohibitively high; the nominal price of sofosbuvir for 12 weeks across 26 Organization for Economic Cooperation and Development countries range from US $37,729 in Japan to $64,680 in the US, with a median of US $42,017 [37]. Within low- and middle-income countries (LMIC), which have an estimated 72% of the infected individuals worldwide, a combination of tiered pricing by originator companies, voluntary and compulsatory licenses to generic manufacturers, unlicensed manufacture of generic medications within countries lacking patent protection, and import of generic medications, have permitted significant expansion of treatment through lower cost medications [38]. According to the March 2018 WHO Progress Report on Access to Hepatitis C Treatment [39], voluntary license agreements for manufacturing of generic DAAs have been signed by 2 originator companies. Gilead has licensed 3 of products (sofosbuvir, sofosbuvir and ledipasvir, and sofosbuvir and velpatasvir) to 11 generic manufacturers in India; as of August 2017, the agreement permitted sale and marketing of generic formulations to 105 LMIC. Bristol-Myers Squibb signed a voluntary license for the generic manufacture of daclatasvir, which permits sale and marketing in 112 LMIC through sublicensing agreements with 10 generic companies. Bioequivalent pharmacokinetics for generic sofosbuvir and daclatasvir in comparison to originator versions have been established [40], and minimum manufacturing costs for 12-week courses of combination DAAs range from US $122 for sofosbuvir and daclatasvir to US $192 for sofosbuvir and ledipasvir [41]. The range of publicly reported prices for a 28-day supply of generic DAA formulations range from US $7.50-$143 for daclatasvir, US $20-$728 for sofosbuvir, US $75-$364 for fixed-dose combination sofosbuvir and ledipasvir, US $450 for fixed-dose combination sofosbuvir and

daclatasvir, and US $125-$130 for the fixed-dose combination of sofosbuvir and velpatasvir. Even at these lower prices for generic DAAs, and the decreasing prices of originator and generic DAAs over time, cost remains a barrier to access in many LMIC.

Although the availability of generic DAA formulations at prices far below US market prices has transformed the capacity to expand HCV treatment in LMIC, there are concerns about the safety, reliability, and efficacy of the generic supply chain. Early data from real-world studies indicate that SVR rates achieved with generic DAAs are similar to those reported with originator drugs in clinical trials and real-world settings (Table 2). Multiple observational studies have reported rates of SVR in patients with genotypes 1–4 HCV ranging from 92% to 98% with generic sofosbuvir and ribavirin, sofosbuvir and ledipasvir, or sofosbuvir and DAC regimens from India [42], China [43], Iran [44], Egypt [45], and Argentina [46], in addition to high rates of SVR in patients with chronic kidney disease [47], post-renal transplant [48], and thalassemia [48]. Only 1 observational study reported on the efficacy of generic sofosbuvir and velpatasvir in 228 individuals (69 coinfected with HCV and HIV and 159 infected with only HCV) treated with sofosbuvir, with or without ribavirin, for 12 weeks. In this population, 97.1% individuals with HCV and HIV coinfection and 98.1% of individuals with only HCV infection achieved SVRs[49].

Several large international cohorts have evaluated the efficacy of generic DAAs in real-world clinical practice. Freeman et al reported the results from the REDEMPTION-1 study, which evaluated 448 patients infected with HCV genotypes 1–6 receiving treatment with a generic DAA (sofosbuvir and ribavirin, sofosbuvir and ledipasvir ± ribavirin, or sofosbuvir and daclatasvir ± ribavirin). In this group, 28% of patients had cirrhosis and 43% had prior treatment. SVRs were achieved by 91% (275/301) of patients with HCV genotype 1 infection and 90% (403/448) of patients overall [50]. Hill et al studied the efficacy of generic sofosbuvir /LDV ± ribavirin or sofosbuvir daclatasvir, with or without ribavirin, in 616 patients with HCV genotype 1–6 infections who obtained their drugs through online buyers' clubs in Australia, Southeast Asia, or Eastern Europe; of this group, 11% had cirrhosis. Overall, 99% of subjects had SVRs (247/250), with no significant differences among genotypes or treatment regimens [51]. Omar et al reported preliminary results from patients treated with generic DAAs in a national HCV treatment program in Egypt. During the first 2 months of the program 18,378 patients with HCV genotype 4 infection began treatment with generic sofosbuvir and daclatasvir ± ribavirin, and 95.1% of patients overall had an SVR; premature treatment discontinuation was reported for only 1.5% of patients [52]. An estimated 330,000 individuals were treated in this program from October 2014 through March 2016—continued reports of safety and efficacy are anticipated [53].

Early findings from analyses of regional and international cohorts indicate the efficacy of generic formulations of DAAs, with rates of SVR similar to those reported from clinical trials and real-world cohorts. However, caution is needed in the interpretation of these studies, which have been limited by predominantly observational study designs without control groups (no randomized controlled trials), relative paucity of safety, adherence, and follow-up data, variable sourcing of generic DAAs, reporting of per-protocol without intent to treat rates of SVR, and selection bias could not be excluded. Importantly, researchers recognize concerns about counterfeit DAAs and the reliability of DAAs produced with or

without licenses from originator companies, so these analyses were conducted almost exclusively in context of generic sourcing from licensed manufacturers. Further studies are needed to determine whether similar findings will be observed for a broader range of generic sources.

In light of significant heterogeneity in study population, treatment infrastructure, and sources of generic DAAs within existing datasets, the observed SVR >90% is remarkably high and provides reassurance that similar efficacy may be expected as with originator DAAs. Nevertheless, strengthening trust in the manufacturing supply chain is essential to permit selection of quality-assured generic DAA products, particularly for procurement within large national treatment programs. Although additional investigation is warranted to confirm the safety and efficacy of low-cost generic DAAs in larger representative cohorts, generic DAAs will help facilitate broad expansion of the mass treatment programs necessary to achieve the WHO's ambitious goal of eliminating HCV infection by 2030.

Management of DAA failures

Treatment with DAAs leads to selection for drug-resistant HCV variants. HCV infection is a master among viruses at acquisition of resistance-associated substitutions (RAS), which arise in patients receiving suboptimal antiviral regimens. Risk of treatment failure is low in patients receiving 2 different classes of highly active DAAs [54,55]. However, even if we assume a low rate of treatment failure (less than 2% in previously untreated patients with compensated liver disease who adhered to an optimal first-line regimen), the number of patients who will need retreatment becomes significant given the enormous number of patients treated globally for HCV [56]. It is not known whether broader use of generic DAAs in low- and middle-income countries is associated with the increase in treatment failure, which could lead to a worldwide increase in DAA RAS. Thorough and optimized management of DAA failures is required to prevent HCV-associated disease progression in individuals as well as to prevent transmission and further spread, worldwide, of HCV with RAS—especially in high-risk populations [57]. For example, the longterm persistence of a highly fit strain of HCV, with substitutions associated with resistance to sofosbuvir and that is difficult to treat, was recently described in a population of men who have sex with men and are coinfected with HIV and HCV. This observation raises concerns of an international spread of difficult to treat virus variants [58].

Association of Failed DAA Regimens With RAS

In most patients who fail DAA therapy, strains of HCV emerge with variants that mediate resistance to the drug the patient received. The risk for selection of RAS is higher among patients with virologic breakthrough compared to patients with relapse. In 2 large multicenter studies (1 from European collaborators and 1 from Italy), the prevalence of drug-specific RAS after DAA failure ranged from 66% to 77% after a relapse, and 86% to 97% after nonresponse or on-treatment virologic breakthrough, depending on the DAA regimen used [59,60]. The proportion of patients with RAS varied among HCV subtypes—it was higher in patients infected with HCV subtype 1a (85.7%) or genotype 3 (92.3%) compared to other genotypes (73.9%). These findings support previously reported HCV

subtype-specific differences in the baseline prevalence of DAA-specific RAS observed in treatment-naive patients [61].

Duration of DAA treatment also matters—most patients with treatment failure after longterm DAA administration relapse with RAS, whereas after short-term treatment, the wild-type virus usually reappears. In a review of data from all phase 2 and 3 trails of ledipasvir and sofosbuvir, the percentages of patients with NS5A RASs after failure of ledipasvir and sofosbuvir were 37.5%, 66.7%, 94.7% and 100% in patients treated for 6, 8, 12, and 24 weeks, respectively [62]. If patients failed by 12 weeks treatment with sofosbuvir plus an NS5A inhibitor do not carry a strain of HCV with DAA-specific RAS, the presence of a viral variant that is resistant to the NS5A inhibitor can be suspected. Certain HCV subtypes (such as 1l and 4r) contain naturally occurring polymorphisms that could contribute to primary resistance to NS5A inhibitors—especially against ledipasvir [67,68]. Although these HCV subtypes are rare in Western countries, they seem to have a broader distribution in Central African countries [67]. These findings indicate the importance of collection of HCV sequence data from well-defined large cohorts in low- and middle-income countries [69].

Analyses from the European HCV Resistance Study Group and the Italian network VIRONET-C, which evaluated 1094 patients failed by different DAA-based regimens (mostly daclatasvir or ledipasvir plus sofosbuvir, simeprevir plus sofosbuvir, or the 2D or 3D regimens), showed that a higher proportion of patients failed by an NS5A inhibitor-based regimen developed HCV RASs than patients failed by NS3/4 protease inhibitor-based regimens (91.9% vs 66.9%). This could be due to the shorter half-life of the NS3/4-RAS inhibitors compared to NS5B inhibitors. [60]. Among patients failed by an NS5A inhibitor regimen, more than 20% and up to 66% carry HCV strains with 2 or more NS5A RAS [62]. Development of multiclass resistance is the biggest challenge to second-line treatment—it was observed in 44% of 282 patients previously treated with 2 or more classes of DAAs (26.6% of patients had RAS to NS3 and NS5A inhibitors and 11.3% of patients had RAS to NS3, NS5A, and NS5B inhibitors) [60]. In contrast, selection of the RAS S282T—the only substitution shown to confer to resistance to the NSB5 inhibitor sofosbuvir in cultured cells, is rarely found in patients failed by sofosbuvir-containing regimens (<4%). However, RAS S282T is more frequently found in HCV genotype 4 compared to types 1 or 3 [59,63,64]. The significance of other RASs in patients failed by sofosbuvir-based regimens, like the L159F and C316N RASs is unclear. These RAS seem to increase the half maximal effective concentration (EC50) of sofosbuvir in co-existence with the RAS S282T[65,66].

Testing for Baseline RAS

A genotype (analysis of RAS) to phenotype (susceptibility to DAAs) personalized approach to treatment (selecting the optimal combination from the available 3 different DAA classes, based on baseline RAS) seems logical. So why don’t guidelines support baseline tests for resistance— especially when facing second-line treatments for patients failed by a previous all-oral DAA regimen? [1,70]. The positive predictive value for treatment failure associated with certain preexisting baseline RAS is low [71]. Although RAS are part of the equation if treatment fails, RAS are not the only determinant—other features of patients, such as stage of liver disease, genetic factors, immune response, and sex, as well as level of HCV

replication and HCV subtype, all affect response to treatment [59,60,72,73]. Cirrhosis, previous treatment and emergence of RAS for NS5A inhibitors (or multiclass RAS), and infection with HCV genotypes 1a or 3 are associated with DAA failure [54].

The gap between HCV genotypic and susceptibility to DAA treatment might involve time to restoration of HCV-specific immune response with DAA-mediated decrease HCV replication [74,75]. The baseline innate immune response contributes to the efficacy of DAA therapy. The innate immune response can prevent emergence and further selection of RAS [76]. Part of the confusion about tests for RAS arise from variations in methods to detect RAS and assess levels of resistance (usually by measuring fold change in EC50). However, there is also confusion because different studies evaluate drug-specific or DAA class-specific RASs in different HCV genotypes or subtypes [77]. There is consensus that a drug-specific RAS must present in at least 15% of the HCV population to reduce odds for an SVR. However, a systematic review revealed limitations in methods of HCV sequencing; these must be overcome for development, validation, and sharing of standardized methods for all genotypes and subtypes [78].

The clinical implications of testing for RAS to select second-line treatments, especially for patients failed by NS5A inhibitor-based treatment, are limited (see AASLD/ISDA and EASL guidelines; Table 3). Selection of antiviral regimen based on baseline analyses of RAS and identification of DAA combinations most likely to be effective has been shown to be possible in real-world cohorts, and result in rates of SVR of approximately 90% [79]. Testing for RAS after DAA failure might be of value for patients with limited access to the single-tablet triple regimen (such as voxilaprevir, velpatasvir, and sofosbuvir) or in areas where only regimens that require optimization based on pre-treatment resistance testing are available [70]. A systematic review of how the effects of RAS to first- and second-line treatments vary among DAAs and HCV genotypes and subtypes could help guide selection of DAA combinations for clinical practice. This area of study is complex, and we must better understand how levels of resistance conferred by specific substitutions vary among HCV subtypes and for different DAAs (see Table 4) [80].

Treatment of Patients Failed by DAAs

Second-line treatment strategies should involve combinations of DAAs that target different viral proteins and have non-overlapping resistance profiles. Sofosbuvir has become the backbone of most of the recommended regimens for patients failed by DAAs, due to its pangenotypic activity and a high barrier to development of resistant virus [81]. This nucleoside NS5B inhibitor is effective in nearly all patients, regardless of treatment history, except for patients with HCV strains that acquire the sofosbuvir-specific RAS S282T [63,65].

Failure of first-generation protease inhibitors

Patients failed by first-generation protease inhibitors (such as boceprevir and telaprevir) or suboptimal regimens of sofosbuvir plus ribavirin ± peg-interferon can be effectively treated with the combination of an NS5A inhibitor and sofosbuvir. These combinations produce SVRs in 94%–100% of patients, depending on cirrhosis, treatment duration, and addition of ribavirin [82]. However, neither the role of treatment duration nor ribavirin has been fully

explored—the addition of ribavirin and/or extension of treatment to 24 weeks might be required. Regimens containing second-generation PI and NS5A inhibitors with high barriers to resistance, such as glecaprevir and pibrentasvir, and to a lesser extent elbasvir and grazoprevir, are also options for these patients [83–85]. The AASLD/IDSA recommendations for treating patients after DAA failure, depending on the regimen that failed, are summarized in Table 5.

Failure of regimens that do not contain NS5A inhibitors

A second-line regimen that contains an NS5A inhibitor plus sofosbuvir was tested in a phase 3 study of patients failed by DAA regimens that did not contain an NS5A inhibitor; this study excluded patients who received a DAA with peginterferon-based therapy [86]. For comparison, some patients received a triple combination regimen containing all DAA classes (voxilaprevir, velpatasvir, and sofosbuvir). The triple regimen led to SVRs in 98% of patients compared to 90% of patients who received the dual regimen. The higher rate of relapse observed after dual therapy (9% vs 1% for the triple regimen) was due to relapse in patients with cirrhosis or infected with HCV subtypes 1a or 3. Consequently, the approved triple regimen is recommended for patients with HCV type 1a or 3 infections failed by regimens without peg-interferon or an NS5A inhibitor (see Table 3).

Failure of regimens containing NS5A inhibitors

Management of patients after failure of a regimen that contains an NS5A inhibitor is a challenge, because NS5A inhibitors are part of all treatment regimens, and the RAS that arise in NS5A tend to persist and can increase replication fitness [87,88]. However, 96% of patients retreated with all 3 classes of DAAs in a single tablet (voxilaprevir, velpatasvir, and sofosbuvir) achieve an SVR (100% of patients with subtype 1b infection, 96% of patients with subtype 1a infection, 95% of patients with type 3 infections, and 91% of patients with type 4 infections) [86]. The triple therapy regimen has therefore become the treatment of choice for patients failed by regimens containing a NS5A inhibitor.

A 3-class regimen could be the ultimate treatment for patients failed by an NS5A inhibitor, with or without an NS3/4 inhibitor. The regimen is effective with non-approved triple combinations such as sofosbuvir, daclatasvir, simeprevir plus ribavirin or sofosbuvir with grazoprevir and elbasvir plus ribavirin. These regimens may be useful in regions in which triple therapy is not available [89–91]. Moreover, preliminary data from a phase 3b study evaluating glecaprevir and pibrentasvir treatment of patients with HCV genotype 1 infection failed by an NS5A inhibitor and sofosbuvir (with or without ribavirin) indicated that 95% of patients maintained an SVR for 4 weeks after treatment. However, even the 3-class regimen can fail, and then there is a high risk of multiclass resistance. Pibrentasvir has the highest level of efficacy against RAS in NS5A, of all the available NS5A inhibitors, so the triple combination of sofosbuvir, glecaprevir, and pibrentasvir (with or without ribavirin) might become an interesting rescue approach for the remaining non-responders [92].

Are New Drugs Needed?

Given the unprecedented revolution in treatment paradigm in the treatment of chronic HCV infection by the licensing of DAAs, are the licensed and next-generation DAAs sufficient to eradicate HCV? Or, will new drugs and new targets still be needed? Observational studies from the real world indicate that more than 90% of patients with chronic HCV infection are cured by DAA-based regimens. Nevertheless, several challenges remain [93]. Probably the most important challenge is the limited access to DAA regimens in low-resource countries but also in special populations in high resource countries such as patients with limited health care coverage or drug abuse [94]. Indeed overall access to DAA has been estimated to be less than 10% of the HCV-infected patients on a global level [95]. Another challenge is limited screening of HCV infection. The absent access and screening for most patients translates into very limited effect on the global burden of HCV-associated diseases such as HCC [96]. One reason for limited access are the high costs of FDA-approved DAAs. Furthermore, management of special populations, or difficult to treat patients, require adapted treatment regimens and DAA resistance or failure can occur in a small minority of patients.

An emerging challenge is the growing number of HCV infections associated with the opioid epidemic [97]. Compliance and access to DAA is limited in patients who inject drugs and curing HCV infection does not prevent re-infection, so a protective vaccine is needed, to decrease HCV prevalence, particularly in this growing population. Another clinical challenge is the persistent risk of HCC in patients cured of HCV infection but with advanced fibrosis or comorbidities such as diabetes. Although the risk of de novo HCC is reduced after an SVR to therapy, HCC can develop even more than 10 years after HCV clearance [101]. Meta-analyses have shown that there are no differences in HCC risk following DAA cure or interferon-based treatment regimens. Post-SVR HCC development and recurrence is more frequent in some groups of patients that have undergone HCC resection [101]. Given the increasing incidence of HCC and the challenges to prevention and early detection, we need alternative or commentary strategies to reduce HCC risk after HCV clearance for patients with advanced fibrosis. Despite the unprecedented success of DAAs, new targets and compounds can still provide opportunities to improve patient care.

What are the Most Promising Targets?

In addition to developing DAAs, researchers are developing strategies to stimulate the anti-HCV immune response. For example, therapeutic vaccines might be used to boost T-cell responses to the virus, or broadly neutralizing antiviral antibodies to prevent HCV infection of grafts. In clinical trials, therapeutic vaccines have had only limited success compared to DAAs—most likely due to their inability to restore functional T-cell responses in patients with chronic infection and virus immune evasion[102]. Broadly neutralizing monoclonal antibodies have been successful in preventing HCV infection—monoclonal antibodies against the HCV envelope glycoprotein E2 were shown to prevent liver graft infection, alone or in combination with DAAs [103,104]. strategies to boost the anti-virus immune response might be used to reduce risk of HCV infection and provide useful information for the development of a preventive HCV vaccine.

Other agents in development target human factors required for viral infection. Studies of models of HCV infection have increased our understanding of the virus life cycle, and led to discovery of compounds that target hepatocyte factors required for the HCV life cycle, called host-targeting agents (HTA) [105]. Studies in cell culture, animal models, and clinical trials show that HTA have broad antiviral activity and a high genetic barrier to drug resistance, most likely due to the low rate of mutation in human cells[105]. HTAs have synergistic effects with DAAs and are efficient against DAA-resistant strains of HCV. Several HTAs have shown effects in animal models and are being evaluated in phase 2 and 3 trials.

Host molecules have been identified that are important for all steps of the virus life cycle including cell entry, replication, assembly, and egress (Fig. 1). Factors required for HCV cell entry include scavenger receptor class B member 1 (SRBI), the tetraspanin CD81, and the tight-junction proteins claudin 1 and occludin [106]. The small molecule ITX-5061 interferes with binding of HCV to SRBI and was the first HCV entry inhibitor investigated in clinical trials. Although ITX-5061 had limited efficacy in patients with chronic HCV infection, it significantly limited virus evolution and delayed infection in patients undergoing liver transplantation [107]. The limited potency of the compound combined with virus escape may have been the reasons for incomplete protection.

Monoclonal antibodies can also inhibit virus entry, by blocking the extracellular domains of cell receptors. Monoclonal antibodies against HCV entry prevent HCV infection in animal models[104]. Mice with humanized livers given antibodies against claudin were cured of chronic HCV infection, so entry inhibitors might be used to treat patients with chronic HCV infection [108]. Furthermore, several natural compounds that interfere with HCV entry of cells have been explored, [109] although their clinical positions are unclear. It is important to note that combinations of entry inhibitors and DAAs have synergistic antiviral effects[110]. Entry inhibitors are of particular interest for preventing HCV infection in patients undergoing liver transplantation, including those receiving HCV-infected organs. This is because prevention of infection provides advantages compared to post-transplant treatment, when virus-induced disease may already have been established [104,111]. Clinical trials are required to determine whether entry inhibitors might be effective during organ transplantation, compared to or in combination with DAAs.

Cellular miRNA122 is required for HCV replication, and has been explored as antiviral target in clinical trials. The stability of HCV RNA and its replication require microRNA-122 (MIR122)[112]. In cells, miravirsen/SPC3649, a MIR122 antisense locked nucleic acid, inhibits HCV replication. MIR122 binding sites are highly conserved among HCV genotypes, so miravirsen has pan-genotype antiviral effects [113]. In a clinical trial, administration of miravirsen resulted in a prolonged dose-dependent reduction in HCV RNA without serious adverse effects [114115]. A single dose of RG-101, a hepatocyte-targeted N-acetylgalactosamine-conjugated oligonucleotide that antagonizes MIR122, produced a significant decrease in HCV RNA in patients with chronic infection with different genotypes. Virus rebound occurred after administration of each compound, likely due to substitutions in MIR122 binding sites [115116].

Safety is an important issue to address for HTAs. Although short-term administration of MIR122 inhibitors appears to be safe, long-term studies are needed, since suppression of MIR122 has been associated with liver disease progression and HCC in animal models[117]. Intravenous administration of an antagomir could be an option for patients who cannot comply with an 8–12-week oral course of therapy. This strategy may also be useful in patients who only present sporadically to health care providers. Furthermore, it may provide an opportunity to shorten DAA regimens, if included in combination therapy.

Cyclophilins are cell proteins that interact with the NS5A and have been explored as therapeutic targets[118]. Although these agents had robust antiviral efficacy in clinical trials [124,125], safety limitations delayed or halted their development. Moreover, several other cell factors involved in HCV assembly and egress, trafficking, and lipid metabolism have been explored (see Figure 1)[105]. However, clinical proof of concept is pending or was characterized by either limited efficacy or adverse effects precluding further development. Finally, repurposing of FDA-approved drugs has been proposed as a low-cost approach for treatment of HCV infection. A well characterized example is the use of the antihistamine chlorcyclizine and related compounds for treatment of HCV infection[127].

HTAs could complement DAAs in treatment of HCV infection [128]. Entry inhibitors provide a complementary strategy for prevention of HCV infection in patients undergoing organ transplantation. Furthermore, HTAs that target virus replication could reduce resistance to DAAs and shorten therapy duration. Trials are needed to determine how HTAs might be used in combination with DAAs. One challenge is the general lack of funding for research and development of new treatments, including HTAs. Increased funds from the public or foundations might provide opportunities to address future unexpected unmet medical needs such as DAA multi-resistance or not yet discovered long-term safety issues.

Future Directions

Thirty years after the discovery of HCV, the development of DAAs revolutionized the care of patients with chronic infection. Licensed DAAs achieve cure more than 90% of patients in the real world. Combinations have reduced the duration of therapy, although there appears to be a minimum length of treatment, and truncated regimens are not optimal for all patient populations. Difficult to treat patients, such as those with advanced liver disease, renal failure, or HCV genotype 3 infection, might benefit from the next generation of DAAs. However, most infected individuals live in low resource countries, so DAAs have a limited impact on the global burden of liver cirrhosis and HCC. Furthermore, the opioid epidemic is increasing the incidence of HCV infection in high resource countries. Although effective strategies have been developed to address DAA resistance and treatment failure, efforts must be continued to prevent and treat multi-resistant strains. DAA therapy decreases the overall progression of liver disease, yet a significant increase in risk of HCC persists following cure of HCV infection in patients with advanced fibrosis and/or co-morbidities. Further research efforts are needed to address these challenges and complement the therapeutic arsenal of DAAs.

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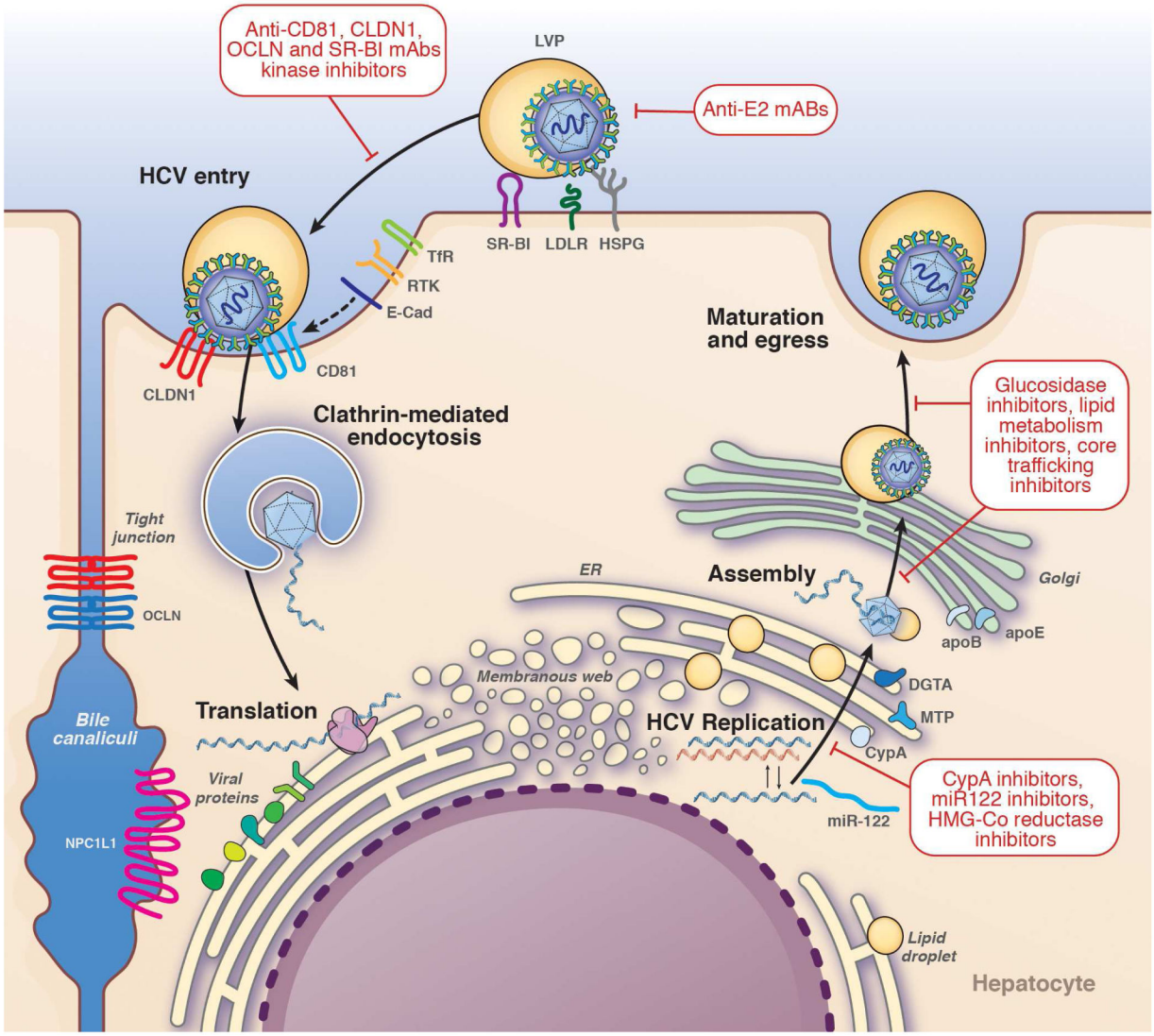

Figure 1. Hepatocyte Targets and the HCV viral life cycle.

In a simplified model of the HCV cycle human hepatocytes, agents inhibit virus cell entry, replication, assembly, and release. HCV lipoviroparticles (LVPs) circulating in the blood bind to hepatocyte cell surface receptors such as heparan sulfate proteoglycans, low density lipoproteins (LDL), and SR-B1. HCV is transferred to the claudin 1 and CD81 co-receptor complex and occludin. Following clathrin-mediated endocytosis and uncoating, the positive strand virus RNA is translated into a single polyprotein that is processed into at least 10 proteins, which are anchored in the endoplasmic reticulum. Virus replication occurs in membranes derived from the endoplasmic reticulum (ER), called the membranous web, and requires cyclophilins and MIR122. The assembly process is induced by core protein trafficking to lipid droplets (LD). Morphogenesis of the virus is associated with synthesis of ver low densty lipoproteins (VLDL). New virions are transported and maturated through the Golgi before being released as LVPs. Assembly and egress requires factors such as

apolipoproteins (APOB and APOE), diglyceride acyltransferase (DGAT), and microsomal triglyceride transfer protein (MTTP). Entry and replication inhibitors have been tested in clinical trials. (figure modified from ref [105].).

Table 1.

AASLD/IDSA Guidance for 8-week Oral DAA Regimens for Chronic HCV Infection

Genotype	Regimen	Populations Eligible for 8 Week Treatment
1a	glecaprevir/pibrentasvir sofosbuvir/ledipasvir	treatment-naïve without cirrhosis peg–interfereon and ribavirin-experienced without cirrhosis treatment-naïve without cirrhosis (non-black, HIV-uninfected)
1b	glecaprevir/pibrentasvir sofosbuvir/ledipasvir	treatment-naïve without cirrhosis peg–interfereon and ribavirin-experienced without cirrhosis treatment-naïve without cirrhosis (non-black, HIV-uninfected)
2	glecaprevir/pibrentasvir	treatment-naïve without cirrhosis peg–interfereon and ribavirin-experienced without cirrhosis
3	glecaprevir/pibrentasvir	treatment-naïve without cirrhosis
4	glecaprevir/pibrentasvir	treatment-naïve without cirrhosis peg–interfereon and ribavirin-experienced without cirrhosis
5 or 6	glecaprevir/pibrentasvir	treatment-naïve without cirrhosis peg–interfereon and ribavirin-experienced without cirrhosis

Table 2.

Efficacy of Generic Oral DAA Regimens for Chronic HCV Infection in Real-world Cohorts

Author/year	Country	Regimen	Population	N	SVR 12
Premkumar (2017)	India	sofosbuvir/daclatasvir/ribavirin	GT1-4	11,105	93%
Goel (2017)	India	sofosbuvir/daclatasvir +/− ribavirin	GT3	100	91.9%
Zeng (2017)	China	sofosbuvir/ledipasvir +/− ribavirin	GT1b	192	96.9%
Freeman (2016)	International	sofosbuvir/ledipasvir or sofosbuvir/daclatasvir	GT1-6	448	90-100%
Hajarizadeh (2017)	Iran	sofosbuvir/daclatasvir	GT1 and 3 Cirrhosis or Post-Transplant	104	98%
Yakoot (2016)	Egypt	sofosbuvir/ribavirin	GT4	48	95.8%
Sharma (2018)	India	sofosbuvir/ledipasvir or sofosbuvir/daclatasvir	GT1 and 3 CKD	71	100%
Liu (2018)	Taiwan	Sofosbuvir/velpatasvir	HCV and HCV/HIV	228	97.1-98.1%
Gupta (2018)	India	sofosbuvir/ribavirin,ledipasvir, daclatasvir	GT1 and 3	499	95.9%
Omar (2018)	Egypt	sofosbuvir/daclatasvir +/− ribavirin	GT4	18,378	95.1%
Hill (2017)	International	sofosbuvir/ledipasvir or sofosbuvir/daclatasvir	GT1-6	616	99%
Nagral (2017)	India	sofosbuvir/ledipasvir or sofosbuvir/daclatasvir	GT1 and 3 Thalassemia	29	100%
Marciano (2018)	Argentina	sofosbuvir/daclatasvir; sofosbuvir/daclatasvir/ribavirin; or sofosbuvir/ribavirin	GT1	321	90%

Table 3.

Treatment Options for Patients Failed by DAA-containing Regimens

Previous DAA experience	Genotype or subtype	Presence of cirrhosis	AASLD/IDSA recommendation (rating)	Treatment duration	Alternative regimens according to AASLD/IDSA
1. No NS5A inhibitor exposure					
Boceprevir, telaprevir, or simeprevir					
	1 (+4) *	no cirrhosis	sofosbuvir/ledipasvir (A) sofosbuvir/velp atasvir (A)	12 weeks	Elbasvir/grazoprevir for 12-16 weeks depending on subtype and baseline NS5A RAS
			glecaprevir/pibr entasvir (B)		
		with cirrhosis	sofosbuvir/velp atasvir (A) glecaprevir/pibr entasvir (B)		sofosbuvir/ledipasvir + RBV 12-16 weeks depending on HCV subtype and baseline NS5A RAS
Sofosbuvir-containing regimen					
	1a	no cirrhosis	sofosbuvir/velp atasvir/voxilapr evir, and (A) glecaprevir/ pibr entasvir (B)	12 weeks	sofosbuvir/ledipasvir + ribavirin 12 weeks (except simpepravir failures)
	1b	no cirrhosis	glecaprevir/pibr entasvir (B) sofosbuvir/velp atasvir(B)	12 weeks	sofosbuvir/ledipasvir + RBV 12 weeks (except simeprevir failures)
	1a	with cirrhosis	sofosbuvir/velp atasvir/voxilapr evir (A) glecaprevir/pibr entasvir (B)	12 weeks	NA
	1b	with cirrhosis	glecaprevir/pibr entasvir (B) sofosbuvir/velpatasvir (B)	12 weeks	NA
	2	± cirrhosis	sofosbuvir/velpatasvir (B) glecaprevir/pibr entasvir (B)	12 weeks	NA
	3, 4, 5, and 6	± cirrhosis	sofosbuvir/velp atasvir/voxilapr evir (A)	12 weeks	NA
2. NS5A inhibitor experience					
Any regimen containing an NS5A inhibitor	All genotypes (1, 2, 3, 4, 5, and 6)	± cirrhosis	sofosbuvir/velp atasvir/voxilapr evir (A) For genotype 3 and cirrhosis adding ribavirin is recommended (C)	12 weeks	Only for type 1: glecaprevir/pibrentasvir for 16 weeks if no NS3/4 PI pretreatment (B) ** Other triple regimens with early data under evaluation (see text)

Note: according to AASLD /IDSA guideline 2018

* HCV Type 4 is not specifically mentioned in the AASLD/ISDA guideline;

** These regimens were not mentioned in the AASLD/ISDA guidelines

Table 4.

Fold Change in EC50s With RAS Y93H for Approved NS5A Inhibitors

NS5A Inhibitor	Fold-change in vitro[1]				
	GT1a	GT1b	GT2	GT3	GT4
Daclatasvir	1400–5432	12–145	749–1750	2154–3733	45–169
Elbasvir	220–600	12-67	ND	157	7.5
Ledipasvir	1677–3309	1319–1807	ND	30	1000
Ombitasvir	41,383	77	4710	6728	20–100
Pibrentasvir	7	0.6	ND	2.5	ND
Velpatasvir	609	3	46	724	3

Note: data are according to ref 80

[1]Y93H fold change value in comparison with wild-type strains; maximum and minimum values are reported.

For first-generation NS5A-inhibitors, RASs with more than 100-fold changes are shown in red (resistance likely); RASs with 20–100-fold changes are reported in blue (resistance possible); RASs with 3–20-fold changes are reported in green (likely susceptible). For second-generation NS5A-inhibitors, such as elbasvir and velpatasvir, RASs with more than 10-fold chnages are reported in red (resistance likely); RASs with 3–9-fold changes are reported in blue (resistance possible); RASs with less than 2.5-fold changes are reported in green (likely susceptible);

GT, genotype; ND, no data available; EC50, effective concentration for 50% inhibition