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Oral Combination Therapies for Hepatitis C Virus Infection: Successes, Challenges, and Unmet Needs

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Keywords

HCV, direct-acting antiviral, HIV/HCV coinfection, decompensated cirrhosis, liver transplant, kidney transplant, end-stage liver disease

Abstract

The current standard of care for the treatment of hepatitis C virus (HCV) consists of interferon-free direct-acting antiviral (DAA) regimens, including combinations of DAAs and fixed-dose combination pills. DAAs for HCV are likely to be heralded as one of medicine's greatest advancements. Viral eradication rates are pushing 100% for many HCV-infected populations, including patients with HIV/HCV coinfection, decompensated cirrhosis, liver and kidney transplants, and end-stage liver disease. We highlight the greatest successes of combination DAA therapies, discuss the ongoing challenges, and identify the remaining patient subgroups with unmet medical needs.

INTRODUCTION

DAA: direct-acting antiviral

SVR: sustained virologic response

Hepatitis C virus (HCV) represents a significant health burden worldwide, with an estimated 185 million people chronically infected (1). A leading cause of liver transplantation (2), HCV infection can result in severe liver disease, including cirrhosis and hepatocellular carcinoma, and is now reported to have the highest mortality of any nationally notifiable infectious disease in the United States (3). Cure of HCV infection results in substantial decreases in liver-related morbidity and mortality (4). Interferon-based HCV therapies offered only 40% cure for the most difficult to treat genotype-1 infection, required 48 weeks of therapy with an injectable interferon, and were accompanied by significant adverse events (5). Cure rates were even lower for HIV-positive or African-American patients, and were poorly tolerated or contraindicated for others, including patients with mental illness, chronic kidney or end-stage renal disease, and cirrhosis, particularly those with decompensated liver disease (6–8). The current standard of care for the treatment of HCV comprises interferon-free direct-acting antiviral (DAA) regimens, including combinations of DAAs and fixed-dose combination pills (**Table 1**). Sustained virologic response (SVR), which is defined as an undetectable level of HCV RNA 12 weeks after the end of therapy and is the

Table 1 Direct-acting antiviral (DAA) mechanisms and sustained virologic response (SVR): approved treatment regimens for chronic hepatitis C virus (HCV) infection

DAA approved as part of an antiviral combination	Mechanism of action	FDA status	SVR12 for treatment-naïve HCV (reference)
Boceprevir + P/R	NS3/4A protease inhibitor	withdrawn 2014	GT 1–63% (9)
Telaprevir + P/R	NS3/4A protease inhibitor	withdrawn 2015	GT 1–75% (10)
Simeprevir[a]	NS3/4A protease inhibitor	approved 2013	GT 1–97% (11)
Sofosbuvir + P/R	NS5B polymerase inhibitor	approved 2013	GT 1–89% (12)
Ledipasvir/sofosbuvir	NS5A inhibitor and NS5B polymerase inhibitor	approved 2014	GT 1–99% (13) GT 4–94% GT 5–93% GT 6–96%
Paritaprevir/r/ombitasvir + dasabuvir	NS3/4A protease inhibitor, NS5A inhibitor, and NS5B non-nucleoside polymerase inhibitor	approved 2014	GT 1–96% (14)
Paritaprevir/r/ombitasvir + ribavirin	NS3/4A protease inhibitor and NS5A inhibitor	approved 2015	GT 4–100% (15)
Daclatasvir[a]	NS5A inhibitor	approved 2015	GT 1–96% (16) GT 3–90%
Elbasvir/grazoprevir	NS5A inhibitor and NS3/4A protease inhibitor	approved 2016	GT 1–95% (17) GT 4–97%
Sofosbuvir/velpatasvir	NS5B polymerase inhibitor and NS5A inhibitor	approved 2016	GT 1–99% (18) GT 2–100% GT 3–97% GT 4–100% GT 5–97% GT 6–100%

Abbreviations: FDA, US Food and Drug Administration; GT, genotype; P/R, pegylated interferon/ribavirin; r, ritonavir boosting; SVR12, sustained virologic response 12 weeks after end of treatment.
[a]Response rates provided are for the oral combination of the DAA + sofosbuvir as per the label.

Table 2 Populations with and without effective interferon-free regimens

Effective interferon-free regimens	Limited interferon-free regimens
Genotypes 1–6	genotype 3 cirrhosis
HIV-HCV	children and adolescents
Decompensated cirrhosis	pregnant women
Post liver transplant/post kidney transplant	breastfeeding mothers
Severe renal impairment/end-stage renal disease	direct-acting antiviral failures

virologic surrogate for clinical cure, has improved to >95% for most populations across all HCV genotypes,and the safety of these regimens is comparable to placebo (9–18).

Although DAAs have revolutionized HCV treatment for all patients, the greatest advancement and the potential for greatest impact have been seen in the special patient populations (**Table 2**) who historically suffered disparities in HCV treatment safety and/or efficacy compared to the general population infected with HCV. Although many of these specific populations stand to benefit from current DAAs, there remain a few for whom safety and/or efficacy remain unaddressed and in whom future studies are desperately needed.

With all great medical advances there come expected and unexpected challenges, many of which are the focus of ongoing research and implementation projects. This article highlights the greatest successes of combination DAA therapies, discusses the ongoing challenges, and identifies the remaining patient subgroups with unmet medical needs in 2016.

TARGETS AND MECHANISMS OF ANTIVIRALS FOR HEPATITIS C VIRUS

HCV is a positive-stranded RNA virus. Its 9.6-kb genome is translated into a polyprotein that is processed into structural and nonstructural (NS) proteins, including NS3, NS4A, NS4B, NS5A, and NS5B (**Figure 1**) (19). The NS proteins are the targets for the current approved DAAs, including NS3/4A protease inhibitors (PIs), NS5A inhibitors, and NS5B nucleotide/nucleoside analogues (NA) and non-nucleoside analogues (NNA) (**Figure 1** and **Table 1**). A major challenge to the design and implementation of DAAs for HCV is the incredible genetic diversity of HCV. HCV contains six major clinical genotypes, as defined by phylogenetic and sequence analysis of the viral genome. These genotypes vary by 30–35% at the nucleotide level and contain nearly 70 subtypes (19). Initially, with first- and second-wave HCV regimens, this genetic diversity translated into different regimens based on genotype and even subtype (1a versus 1b), with the first DAAs approved only for genotype-1 infections.

The ideal HCV DAA regimen would have pan-genotype efficacy, simplifying approaches to therapy and negating the need for genotype testing, which is not available in many parts of the world. However, because of the virus's genetic diversity and the DAAs' mechanisms of action, this has been difficult to achieve. Although there are regimens with in vitro activity for all HCV genotypes, there has been great variation in the clinical efficacy of these regimens across genotypes and/or a lack of supporting clinical data for genotypes less prevalent in the high-income countries where most registration trials are conducted.

The first all-oral regimen of sofosbuvir, a first-in-class NA, and ribavirin was approved by the US Food and Drug Administration (FDA) for genotypes 1–4, with in vivo evidence to support efficacy in genotypes 5 and 6 (12). With approval, the regimen became the standard of care for genotypes 2 and 3, but its efficacy was suboptimal in the most common HCV genotype in the United States—genotype 1. Furthermore, the need for ribavirin in this regimen has

NS: nonstructural
PI: protease inhibitor
NA: nucleotide/nucleoside analogue
NNA: non-nucleoside analogue

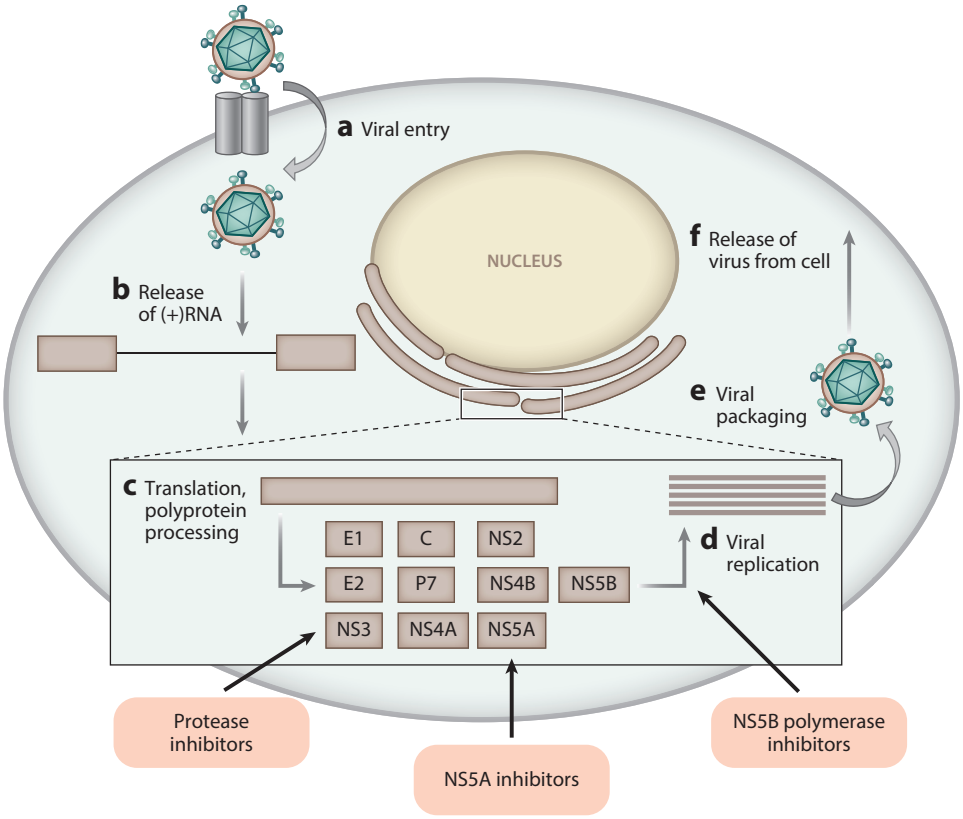

Figure 1

Life cycle of hepatitis C viral infection and targets for mechanism of action for direct-acting antivirals. (*a*) Virus particle–receptor binding and endocytosis; (*b*) cytoplasmic release and uncoating; (*c*) translation and polyprotein processing with structural and nonstructural proteins shown at the endoplasmic reticulum—the site for the mechanism of action of NS3/4 protease inhibitors; (*d*) RNA replication occurring in the membranous web—the site for the mechanism of action of the NS5A inhibitors and NS5B polymerase inhibitors; (*e*) virion packaging and assembly; and (*f*) virion maturation and release.

been a limitation owing to its renal clearance, teratogenic risk, and adverse-event profile that includes hemolytic anemia. Regimens with ribavirin will be more challenging for expanded use in low- and middle-income countries, where the greatest burden of disease exists. Daclatasvir, a pan-genotypic NS5A inhibitor, when combined with sofosbuvir, provided the first DAA combination pan-genotypic regimen and was approved in Europe for genotypes 1–4. Daclatasvir has good activity against genotype 3 and maintains activity against genotype 2 polymorphisms (16). Unfortunately, the efficacy of this regimen in cirrhotic patients was low, particularly in those with genotype-3 infection (20), and the cost of the combined regimen, as well as a lack of sufficient data in patients with compensated cirrhosis, left a clinical need for either more data or more regimens.

The FDA approval of sofosbuvir/velpatasvir (an NS5A inhibitor) brought the first daily, fixed-dose, combination, pan-genotypic pill to the clinic (18). The combination of NS5A and NS5B polymerase inhibitors offers exceptional SVR across all genotypes (**Table 3**). This regimen comes the closest to providing a streamlined approach across all genotypes and baseline patient characteristics, although there remain a few clinical examples where the addition of ribavirin optimizes

Table 3 Sustained virologic response (SVR) to the combination of NS5B/NS5A, sofosbuvir/velpatasvir (18)

Population	% SVR (number of responders/N)
Genotype 1a	98% (206/210)
Genotype 1b	99% (117/118)
Genotype 2	99% (237/238)
Genotype 3	95% (264/277)
Genotype 4	100% (116/116)
Genotype 5	97% (34/35)
Genotype 6	100% (41/41)
Patients with cirrhosis	96% (212/220)
Patients who previously failed interferon-containing regimens	97% (283/291)

treatment outcome. Yet this is another step closer to a single simplified approach to therapy, which is believed by many to be critical to achieve universal access to cure.

SUCCESSES

The advancement of the field of HCV therapeutics as described above is truly transformational. The benefit of these revolutionary therapies can be best highlighted by describing the impact they have had on some of the historically most difficult-to-treat patient populations.

HIV/HCV Coinfection

Advanced liver disease is a leading cause of HIV-related morbidity and mortality, accounting for 13% of all deaths in the large prospective multinational D:A:D (Data collection on Adverse events of Anti-HIV Drugs) observational cohort (21, 22). In the United States, chronic HCV infection is the leading cause of liver disease and related mortality in HIV-infected individuals and has a clear synergistic effect on liver disease pathogenesis (21). A large, well-designed study in the Veterans Health Administration compared HCV monoinfected patients with HIV/HCV coinfected patients and found that the latter have a higher rate of hepatic decompensation [hazard ratio (HR) 1.56, confidence interval (CI) 1.31–1.86], and that this risk occurs despite maintaining excellent HIV control (23). Owing to this accelerated disease pathogenesis and a poorer response to interferon-based therapies, patients with HIV/HCV coinfection have been identified as a special population with an unmet medical need. Prior to DAAs, uptake of HIV/HCV coinfected patients into HCV therapy was poor (24). This was related to competing medical priorities, the complexity of comorbid disease, bias of both providers and patients as to the benefit of interferon-based therapies, and intolerance of such therapies.

The first evidence that DAAs might level the playing field for HIV-infected patients came with the first wave of therapies in which DAAs were combined with pegylated interferon and ribavirin. Phase III trials of pegylated interferon and ribavirin in HIV/HCV genotype-1 coinfected patients reported SVR of 27–29% (6, 25). The addition of a DAA to pegylated interferon and ribavirin resulted in SVR of 79–91% in HIV/HCV cohorts, with rates very similar to those described with the same regimens in patients with HCV monoinfection (26, 27). Multiple phase III studies of DAA combination therapies without interferon or ribavirin support the high safety and efficacy of these regimens in patient with HIV/HCV coinfection. Their findings include SVR rates of 96% with daclatasvir plus sofosbuvir, elbasvir/grazoprevir, or ledipasvir/sofosbuvir, and 95% with sofosbuvir/velpatasvir (23, 28–30).

Although there remain complexities in treating patients with HIV/HCV coinfection, in large part due to the potential for drug interactions between the two types of antivirals, the perception of the treatment of these patients and of their potential for benefit has been dramatically changed. HIV/HCV coinfected patients had been previously identified by the FDA as a "specific" population with an unmet medical need. The recent FDA document for DAA Development states, "The SVR rates in HIV-1/HCV coinfected patients receiving all oral antiviral drugs are similar to HCV mono-infected patients. As a result, both HIV-1/HCV coinfected patients and HCV mono-infected patients can enroll into the same clinical trial" (31, p. 15). The FDA also noted that it no longer considers HIV/HCV coinfected patients as having an unmet medical need.

Hepatic Impairment and Liver Transplantation

Patients with advanced liver disease, especially those with decompensated cirrhosis, have historically had a very poor prognosis and limited treatment options owing to the risk of decompensation and/or death with interferon-containing regimens (8, 32). Without liver transplantation, these patients are unlikely to survive their liver disease. In patients with chronic HCV infection at the time of transplantation, recurrent HCV infection is universal, and for a significant minority the course of fibrosis post-transplant is accelerated, with increased morbidity, mortality, and graft loss (33–35).

Sofosbuvir with ribavirin was evaluated as part of a compassionate-use program for post-transplant patients with severe recurrent hepatitis C and achieved 59% (54/92) SVR (36). There are now multiple studies attesting to the safety and efficacy of all-oral DAA combination therapies in patients with moderate (Child-Pugh class B) and severe (Child-Pugh class C) hepatic impairment. The SOLAR studies investigated ledipasvir/sofosbuvir with ribavirin 600 mg daily (low dose) for 12 versus 24 weeks; ALLY-1 investigated daclatasvir plus sofosbuvir with low-dose ribavirin for 12 weeks; and ASTRAL-4 investigated sofosbuvir/velapatasvir with or without ribavirin for 12 weeks and without ribavirin for 24 weeks (37–40). Overall, the studies report 83–96% SVR for patients with moderate impairment, with the best response rates achieved by the 12-week regimens that included ribavirin. Patients with severe impairment did not fare as well with these regimens, achieving SVRs of 56–87%, but the possibility of >50% cure in this most difficult-to-treat population with no previous treatment options is quite phenomenal and is another sign of the potency of these regimens. Some DAAs are contraindicated in patients with decompensated liver disease (Child-Pugh class B and C), including simeprevir, elbasvir/grazoprevir, and paritaprevir/ritonavir/ombitasvir, mainly because of the increased exposure of the NS3/4A PI components of these regimens (11, 14, 15, 17).

Of equal importance is the safety of these regimens in this very high-risk patient population. Across the large studies noted above, the premature discontinuation rate was 2–4%, and the vast majority of discontinuations were not likely to be treatment related but instead related to the severity of liver disease in the study population (37–40). Patients with decompensated liver disease have had few options other than transplantation. A pivotal yet unanswered question is predicting the ability of the liver to recover following virologic cure once portal hypertension has developed. Treatment studies have demonstrated improvement in some markers of liver function 12 to 24 weeks out from treatment (37, 38). Clinicians are currently struggling with recommendations to patients with advanced liver disease. For patients with compensated cirrhosis and mild portal hypertension with MELD (Model for End-Stage Liver Disease) scores up to 15, antiviral treatment is recommended, with the goal of avoiding the complications of portal hypertension and the need for liver transplantation. Patients with Child-Pugh class C disease are generally felt to not benefit from antiviral cure in terms of recovery of liver function and portal hypertension

and are advised to proceed to transplantation. The clinical challenges arise with the mid-range patients, those with MELD scores in the 20s or with Child-Pugh class B cirrhosis. We do not know if their portal hypertension will improve to the point of reasonable quality of life. Antiviral treatment could have negative consequences if virologic cure stabilizes liver function and leaves the patient with the morbidity of portal hypertension complications but prevents progression to a MELD score at which the patient is likely to receive an offer for transplantation. Cohort studies are enrolling patients in these scenarios to understand the best approach, but clinicians will need to guide patients for the next several years without definitive data.

The ability to cure HCV after liver transplantation is another factor that impacts decisions to allow patients to proceed to transplantation with advanced disease. Prior to the DAA era, antiviral treatment with interferon-based regimens was poorly tolerated with low response rates. All patients develop recurrent HCV in the allograft, and the natural history of HCV is accelerated post-transplantation. Studies have reported rates of 10–30% progression to cirrhosis within five years of transplantation and >40% at ten years (41). However, clearance of HCV prior to transplantation does significantly decrease the risk of recurrence of HCV infection in the graft (42), and rates of viral clearance post-transplantation associates primarily with the severity of liver disease and not the transplant status (37, 38). In two studies assessing safety and efficacy of ledipasvir/sofosbuvir pre- and post-transplantation, post-transplantation patients without cirrhosis or with compensated cirrhosis achieved SVR 96–98%, the same rates as those reported in patients without cirrhosis or with compensated cirrhosis without a history of transplantation (43, 44). Effective antiviral treatment after liver transplantation is expected to improve long-term transplantation outcomes and has provided greater confidence in the use of HCV-positive organs for patients requiring liver transplantation.

Renal Impairment Including End-Stage Renal Disease and Kidney Transplantation

The interaction of HCV and the kidney is multifaceted. HCV infection is associated with the development of numerous extrahepatic manifestations, one of the most severe being essential mixed cryoglobulinemia (45). One clinical manifestation of cryoglobulinemia is glomerulonephritis. Furthermore, HCV infection can accelerate the decline in renal function in patients with chronic kidney disease (46). Patients on hemodialysis are at higher risk for acquiring HCV infection and, once infected with HCV, have a higher all-cause mortality rate than do noninfected hemodialysis patients (47). Moreover, studies among kidney transplant recipients show infection with HCV decreases graft survival and increases overall mortality (48). For these reasons, the FDA has long recognized patients with advanced chronic kidney disease as a group with an unmet medical need. There would be a definite perceived benefit to clearance of HCV in this patient population. However, until recently, these patients had few options for safe and effective HCV therapy. The kidneys play a role in the catabolism and filtration of both interferon and ribavirin (49). Of particular concern is that ribavirin clearance is impaired in patients with advanced chronic kidney disease [glomerular filtration rate (GFR) <50 ml/min], and ribavirin is not removed by hemodialysis, resulting in an increased severity of hemolytic anemia among persons in whom anemia is already a common comorbidity (49). Even with current DAAs, several regimens and populations require ribavirin. Furthermore, until recently, the majority of the ribavirin-free DAA regimens included sofosbuvir, which is not recommended for use in patients with GFR <30 ml/min.

The first DAA regimen approved by the FDA in patients with severe chronic kidney disease and end-stage renal disease was elbasvir/grazoprevir, a ribavirin-free, once-daily, fixed-dose combination pill approved in January 2016 (17). Elbasvir is a NS5A inhibitor and grazoprevir is a

next-generation NS3/4A PI. Neither drug is renally excreted (<1%), so no change in dosage is required in patients with renal dysfunction. The only registration trial of DAAs in patients with renal impairment to date reported an intention-to-treat (ITT) SVR of 94% with a 12-week course (50). No subject in the treatment arm discontinued therapy for an adverse event, and tolerance and safety were the same as in the comparator placebo arm. This study was completed with an intermediate versus delayed treatment arm, with the delayed arm initially receiving placebo for 12 weeks and then rolling over into treatment. The delayed treatment arm also achieved a high ITT SVR of 95% (17). Overall only three patients out of 215 in the study suffered relapse. The approval of elbasvir/grazoprevir has provided great hope for many patients with severe renal impairment for whom cure of HCV was not thought to be readily achievable.

The availability of a safe and efficacious regimen in patients with severe renal impairment has initiated the conversation of when to treat HCV in the renal transplant candidate. Among patients planning to receive a living donor kidney, eradicating HCV prior to transplantation makes good sense. However, for patients awaiting cadaveric donor organs, there may be benefit to accepting HCV-positive donor organs and postponing curative DAA therapy until after transplantation. This approach has been reported to decrease wait-list times significantly (51) and could also increase the pool of kidneys available from HCV-positive donors, as these organs are frequently discarded or not even harvested (51). In the past, patients with advanced liver fibrosis and end-stage renal disease were not offered kidney transplantation alone and were instead considered for liver–kidney transplantation. Kidney transplant recipients were not treated with interferon-based regimens owing to risk of allograft rejection, and the concern was that complications of HCV cirrhosis would soon develop in the setting of immunosuppression. DAA regimens that are safe and effective in patients with severe renal impairment have allowed more patients with advanced fibrosis and compensated cirrhosis to proceed to kidney transplant alone following eradication of HCV prior to transplantation. The first large study of HCV therapy following renal transplantation was presented at the 2016 International Liver Congress in Barcelona. This study of ledipasvir/sofosbuvir assessed 12 versus 24 weeks of the fixed-dose combination pill in patients who had functioning renal grafts and were a median of 10–12 years from transplantation (52). The study reported that all 114 patients achieved SVR12, regardless of length of therapy, presence of cirrhosis, or prior treatment history. Needless to say, DAAs have significantly changed the conversation for patients with renal impairment, and undeniably for the better. In fact, as a testament to the impact of DAA therapies for the renal transplant population, the first study is under way that will explore renal transplantation using HCV-infected donors for HCV-negative recipients (NCT02781649).

CHALLENGES

Although the advances of combination DAA therapies have elevated most patients to a >95% eradication rate, there are still select patient groups with multiple negative baseline predictors who suffer unacceptable failure rates. In addition, baseline and treatment-emergent resistance is a risk factor for treatment failure.

Genotype 3

As noted above, HCV has incredible genetic diversity, with six major genotypes. These genotypes vary by 30–35% at the nucleotide level and contain nearly 70 subtypes (19). Clinically, this genetic diversity has translated into different regimens based on genotype and even subtype (1a versus 1b). In the interferon era genotype-1 infection was the most difficult to treat, but genotype 3 has

taken its place in the combination DAA era. Even with the most recently approved pan-genotypic regimen of sofosbuvir/velpatasvir, the highest rate of relapse in patients with compensated liver disease was in patients with genotype-3 infection (53). Globally, genotype-3 infection is reported to account for ~20% of HCV infections (54).

Genotype-3 HCV has been singled out as a more fibrogenic and metabolically active virus (55). HCV genotype-3 infection has increased prevalence of steatosis and higher rates of cirrhosis and hepatocellular carcinoma (56). Steatosis in genotype-3 infection appears at least in part directly related to viral cytopathic effects, as magnitude of steatosis correlates with viral load and is independent of other steatogenic factors such as PNPLA3 (56). Genotype-3 infection selectively interferes with the late cholesterol synthesis pathway, and this interaction resolves with clearance of the viral infection (57). Although steatosis and insulin resistance were predictors of treatment failure with interferon-containing therapies, there is no evidence this remains true with oral combination DAA therapies. Why genotype-3-infected patients have higher relapse rates with DAA therapies is unclear. There is certainly potential it is related to virally mediated steatosis, insulin resistance, and severe liver disease. It is also true that the original replicon system used to understand the viral life cycle of HCV was based on genotype-1b infection, and it was not until additional genotypes were studied via more advanced replicon systems and the genotype-2a isolate JFH1 emerged (which in 2005 enabled the first cell-culture virus production system) that the true differences between genotypes could be appreciated (58). In vitro potencies of DAA regimens to date have shown significant variation by genotype and in particular lower potency for genotype-3 replicons. Next-wave and next-generation agents are demonstrating improved in vitro potency, now in the picomolar range for genotype-3 infection (59, 60). Sofosbuvir/velpatasvir (a picomolar NS5A) offers 98% SVR12 for treatment-naïve patients without cirrhosis (53). However, for treatment-experienced patients without cirrhosis and patients with cirrhosis regardless of treatment experience, SVR is lower (89–93%). A next-generation program now in phase III study, includes a NS3/4A PI, ABT-493, and a NS5A inhibitor, ABT-530, in combination. ABT-493 is a pan-genotypic NS3/4A PI with nanomolar potency in genotype-3 replicons. ABT-530 is a pan-genotypic NS5A inhibitor with picomolar potency in genotype-3 replicons. Early phase II studies of this regimen in genotype-3-infected patients reported 97–100% SVR with only eight weeks of therapy in patients with ($N = 24$) and without ($N = 29$) cirrhosis (59, 60). There is great hope that this investigational combination will further streamline the approach to HCV treatment, although there is likely to be role for optimization of therapy for particularly difficult-to-treat subgroups.

DAA Failures and Resistance

The issue of drug resistance frequently arises when using mechanistically targeted therapies, especially in virology. Owing to the lack of integration of HCV into the host genome, there is no archive of viral populations, and many people used this to argue that resistance would not have a role in oral DAA therapies. We now know this was not correct, although the impact of preexisting baseline resistance mutations and treatment-emergent resistance mutations is variable and depends on the potency of the DAA regimen and its collective barrier to resistance. Other baseline patient characteristics also play a role in the impact of these resistance mutations, including presence of cirrhosis and prior treatment exposure, among others.

The ability of HCV to develop de novo resistance to antiviral drugs is quite high. HCV replicates as a quasispecies; therefore, resistance-associated variants (RAVs) can preexist within the viral population at baseline and emerge as the dominant species during treatment. The high mutability of HCV has to do with the highly error-prone nature of the HCV RNA-dependent

RNA polymerase and large viral populations (61). In fact, it has been predicted that in a single day, HCV can generate genomes with all possible single and double nucleotide changes, and as long as these genomes maintain fitness, they could confer antiviral resistance (61). Each class of DAAs can select for RAVs, but the genetic barriers and fitness of these RAVs vary. NAs inherently have high barriers to resistance because they directly target the conserved polymerase active site, and resistant variants have low fitness (61). On the other hand, NS5A inhibitors, PIs, and NNAs all have low barriers to resistance, with single amino-acid substitutions conferring high-level resistance. In the minority of patients who suffer virologic failure during DAA combination therapy, dual and triple RAVs are reported (11–18).

What happens to RAVs after the cessation of treatment? This depends on the fitness of those variants. For first-generation PIs, a recent report of long-term follow-up of patients treated with boceprevir (first-generation NS3-NS4A PI) found that after three years, 27% of patients still had RAVs, and that the median time for all RAVs to become undetectable was 1.11 years (62). This carries important clinical implications for retreatment decisions in a patient with RAVs. For example, NS5A RAVs exhibit more replicative fitness and appear to persist for >2 years (63).

Viral sequences with preexisting polymorphisms can present a therapeutic challenge, and with current regimens, this is most clinically relevant with NS5A RAVs. Baseline NS5A polymorphisms that confer resistance are significantly more common than those identified in NS3/4A or NS5B genes. In a pooled resistance analysis of the ledipasvir/sofosbuvir phase III programs, 16% of patients overall had NS5A RAVs at baseline, and significantly more patients suffering virologic failure harbored these RAVs (43%) at baseline (64). There are now several patient subgroups where baseline NS5A RAVs appear to lower SVR, including genotype-1a patients receiving elbasvir/grazoprevir, genotype-3 patients with cirrhosis receiving daclatasvir plus sofosbuvir and sofosbuvir/velpatasvir, and treatment-experienced patients with cirrhosis receiving ledipasvir/sofosbuvir (17, 20, 53, 64). Currently, the FDA recommends NS5A testing prior to initiation of therapy only with the elabasvir/grazoprevir regimen and only in GT1a infection. However, the AASLD/IDSA HCV Guidance Panel also recommends NS5A testing for several patient subgroups with genotype-3 infection who are prescribed either daclatasvir plus sofosbuvir or sofosbuvir/velpatasvir and considers NS5A testing for treatment-experienced genotype-1-infected patients with cirrhosis who are prescribed ledipasvir/sofosbuvir (65).

Treatment-emergent polymorphisms also increase the risk of relapse with retreatment approaches. Patients enrolled in the ledipasvir/sofosbuvir registration program who failed therapy were offered options for retreatment roll-over studies. Several approaches were attempted, including treatment with ledipasvir/sofosbuvir for 24 weeks with or without ribavirin. Patients with treatment-emergent NS5A RAVs receiving 24 weeks of ribavirin-free therapy achieved 60% SVR, compared to 100% of those without NS5A RAVs (66). Meanwhile, in a different study assessing rescue therapy with 24 weeks of ledipasvir/sofosbuvir with ribavirin reported 86% SVR in patients with treatment-emergent NS5A RAVs (67). Ultimately, retreatment with triple or quadruple DAA regimens or awaiting next-generation regimens will be the best options for rescuing the rare patients who fail potent combination DAA therapies.

UNMET MEDICAL NEEDS

Amazing strides have been made in expanding the benefit of DAA therapies to the HCV-infected populations with the greatest unmet medical needs. Yet, for several core patient populations, there are still limited or no data and a great opportunity for benefit. Children, pregnant women, and nursing mothers remain poorly represented groups with an unmet medical need. To date, there are no published studies of pregnant women or nursing mothers on DAA regimens. A single phase

I study that has not yet opened to enrollment will investigate the safety and pharmacokinetics of antenatal ledipasvir/sofosbuvir treatment for 12 weeks during the second and third trimesters (NCT02683005). More strides have been made for children and adolescents. The first study of any DAA therapy in children and adolescents was reported at the 2016 International Liver Congress in Barcelona. One hundred adolescents aged 12–17 years received 12 weeks of standard-dosing ledipasvir/sofosbuvir and achieved 97% SVR without any virologic failures (68). Additional studies are ongoing, including an evaluation of paritaprevir/ritonavir/ombitasvir and dasabuvir with or without ribavirin in genotype 1 (NCT02486406) and sofosbuvir with ribavirin in genotypes 2 and 3 (NCT02175758).

CONCLUSION

Direct-acting antivirals (DAAs) for HCV are likely to be heralded as one of medicine's greatest advancements. The possibility of eradicating HCV from the globe seems within reach. With SVR pushing 100% for many HCV-infected populations, including patients with HIV/HCV coinfection, decompensated cirrhosis, liver or kidney transplants, and end-stage liver disease, there is much reason for hope. The exceptional response rates reported in clinical trials have been reproduced in real-world cohorts. The beginning of the pan-genotypic era could be the basis for diagnostic and treatment algorithms safely offered in primary-care clinics, methadone programs, or prisons in any country of the world. Expansion of treatment programs away from tertiary-care centers and subspecialists will be critical if we are in fact going to eradicate this infection. Yet the unfortunate reality is that because of the high cost of these medications, many patients will be denied coverage and the opportunity of cure. In high-income countries, many payers are providing coverage only to those patients with the highest stages of fibrosis. For many low- and middle-income countries, the cost has created a great disparity in access reminiscent of HIV antiretrovirals. Medical discovery has given us the tools to eradicate HCV, but do we have the will to follow through?

DISCLOSURE STATEMENT

The authors report the following relationships (dating back three years) that might be perceived as affecting the objectivity of this review: Both A.J.M. and S.N. have served as scientific advisors for AbbVie, Bristol Meyers Squibb, Merck Inc., and Gilead Sciences. In addition, A.J.M. has served as a scientific advisor for Inovio Pharmaceuticals, Intercept, Portola Pharmaceuticals, and Shire Pharmaceuticals. Both A.J.M. and S.N. have received research grants from AbbVie, Bristol Meyers Squibb, Gilead Sciences, Merck Inc., and Janssen Pharmaceuticals. In addition, S.N. has received research grants from Tacere, and A.J.M. has received research grants from Hologic, Intercept, NGM Biopharm, and Roche.

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