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Review article: HCV genotype 3 – the new treatment challenge

J. Ampuero*,†, M. Romero-Gómez* & K. R. Reddy†

*Unit for the Clinical Management of Digestive Diseases and CIBERehd, Valme University Hospital, University of Seville, Seville, Spain.
†Division of Gastroenterology and Hepatology, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Correspondence to:
Prof. K. R. Reddy, Director of Hepatology, University of Pennsylvania, 2 Dulles, 3400 Spruce Street, HUP Philadelphia, PA 19104, USA.
E-mail: rajender.reddy@uphs.upenn.edu

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SUMMARY

Background
Over the past several years, hepatitis C therapy has been pegylated interferon and ribavirin based. Although protease inhibitor-based therapy has enhanced response rates in genotype 1, the recent advances in therapy have demonstrated a challenge in genotype 3, a highly prevalent infection globally.

Aim
To provide a comprehensive summary of the literature evaluating the unique characteristics and evolving therapies in genotype 3.

Methods
A structured search in PubMed, the Cochrane Library and EMBASE was performed using defined key words, including only full text papers and abstracts in English.

Results
HCV genotype 3 is more prevalent in Asia and among intra-venous drug users. Furthermore, it interferes with lipid and glucose metabolism, and the natural history involves a more rapid progression of liver disease and a higher incidence of hepatocellular carcinoma (HCC). New therapies with protease inhibitors have focused on genotype 1 largely and have demonstrated enhanced responses, but have limited activity against genotype 3. Thus far, in clinical trials, NS5B and NS5A inhibitors have performed more poorly in genotype 3, while a cyclophilin inhibitor, alisporivir, has shown promise.

Conclusions
As treatments for HCV have evolved, genotype 3 has become the most difficult to treat. Furthermore, genotype 3 has special characteristics, such as insulin resistance and alterations in lipid metabolism, which may partly explain the lower treatment responses. A great deal of emphasis on advancing therapy is needed in this population that appears to have a more rapid progression of liver disease and a higher incidence of HCC.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem that affects 170 million people worldwide, and approximately 55% (95 million) of the infected population is in South East Asia and Western Pacific countries.[1] Chronic hepatitis C is associated with significant morbidity and mortality, which result mainly from the progression towards cirrhosis and hepatocellular carcinoma (HCC).[2] Factors associated with rapid progression include: (i) host factors (i.e. older age at infection, male gender, Afro-American race, alanine aminotransferase level, liver fibrosis, genetic factors, metabolic factors); (ii) viral factors (i.e. genotype, viral load, viral kinetics); (iii) co-infections (i.e. hepatitis B virus or human immunodeficiency virus); (iv) exposure to toxic agents (i.e. alcohol, tobacco or cannabis).[3] Fibrosis progression in hepatitis C genotype 3 seems to be more rapid than in genotype 1 and is probably related to a higher degree of steatosis.

Hepatitis C virus does not integrate into the human genome. Thus, a sustained virological response (SVR), a feature strongly linked to reduced likelihood of progressive liver disease and mortality, is key to achieving cure.[4] Traditionally, genotypes 2 and 3 have been placed in the same group when making a decision on the dose of ribavirin or treatment duration. More recently, genotype 2, unlike genotype 3, has been found to be sensitive to various direct-acting anti-virals (DAAs), thus resulting in differences in SVR rates.[5] Therefore, genotype 2 and genotype 3 should not be grouped together for analyses of SVR rate or for therapeutic strategies. Given the recent approval of the DAAs for genotype 1 and with new imminent therapies, it is important that we recognise that genotype 3 is now the more difficult genotype to treat.[6] With the unique features and challenges in treatment in mind, we reviewed the literature and provide a perspective on chronic hepatitis C genotype 3.

EPIDEMIOLOGY

The hepatitis C virus, resulting from high-error rates of RNA-dependent RNA polymerase during the replication of the HCV genome, comprises six genotypes and several subtypes.[7] HCV genotype 1 (both 1a and 1b) is dominant in the US, Western Europe and Australia, representing up to 60% of global HCV infections. HCV-1b predominates in Japan, China and Russia.[8] Genotype 4a is the most common subtype in Egypt,[9] whereas other subtypes of HCV-4 are found in Central Africa.[10] Genotype 5a accounts for 50% of infections in South Africa, while HCV-3 and HCV-6 are common in Southeast Asia. Specifically, in populous countries such as India and Pakistan, HCV-3 is the predominant genotype.[11, 12] In addition, in certain European countries, such as Greece,[13] Poland[14] and the Netherlands,[15] HCV-3 can be found in up to 30% of all cases.

There has been an interesting and intriguing association between the mode of HCV transmission and HCV genotypes. Although HCV-1b has been encountered more often in patients who acquired HCV through blood transfusion[16] (the prevalence of HCV-1b has decreased concurrently with the implementation of screening of blood and blood products for HCV), HCV-3a has been associated with intravenous drug use (IVDU),[17] tattooing and piercing.[18] A unique worldwide epidemic among drug abuser communities has been proposed because nonstructural protein (NS) 5B of HCV-3a showed similar sequences in intravenous drug users across different countries,[19] suggesting a common origin (apparently emerged and diversified in Asia)[20] of the outbreak. Indeed, molecular evolutionary analysis suggests that a common ancestor of HCV subtype 3a strains existed approximately 200 years ago, and a Bayesian skyline plot suggested a spread to Thailand during the mid-1970s and early 1980s, partially overlapping with the Vietnam War (1955–1975), where the widespread use of injection drug use pervaded the US Army.[21] High rates of HCV transmission and prevalence among IVDUs are related to unsafe injecting practices, such as drug preparation materials, needles or syringe sharing, together with the social context of drug abuse.

HCV GENOTYPE 3 AND STEATOSIS

HCV infection shares pathological features with metabolic syndrome, especially liver steatosis, due to HCV's effects on lipid and glucose metabolism,[22] and thus presents an increased cardiovascular risk.[23] The magnitude of HCV-3 infection-related steatosis correlates with the level of viral replication,[24] and it disappears after successful anti-viral therapy,[25] suggesting a cause and effect relationship between the virus and the genotype. HCV-3 genotype is associated with the highest rates of steatosis among all HCV genotypes, reaching up to 70%.[26] Rubbia-Brandt *et al.* assessed 101 HCV patients and demonstrated a significant correlation between steatosis and HCV RNA level in patients infected with HCV-3, but not in those infected with HCV-1, providing evidence that steatosis is the morphological expression of a viral cytopathic effect of HCV-3.[27] Furthermore, in patients

with HCV cirrhosis, genotype 3 has been independently associated with an increased risk of HCC[28] (Figure 1).

Insulin resistance

Several studies have noted a direct role of HCV in altering glucose metabolism, leading to insulin resistance and diabetes.[29, 30] Of interest is the observation that achieving SVR with therapy has resulted in improvement in insulin resistance and a reduced incidence of diabetes mellitus.[31] An epidemiological overlap between HCV and glucose metabolism impairment could explain the impact of metabolic abnormalities on SVR,[32] independent of HCV genotype[33, 34] or IL28B genotype.[35]

HCV core protein promotes degradation of insulin receptor substrates 1 and 2 [by over-expressed tumour necrosis factor a (TNFa) and suppression of cytokine signalling-3 (SOCS)],[36] leading to defective downstream PI3K and Akt phosphorylation. As the PI3K/Akt pathway is critical for the inhibition of gluconeogenesis in the liver, this process could lead to increased glucose production.[37] HCV-3a core protein induces the expression of SOCS-7, which is partially involved in the development of insulin resistance, and downregulation of peroxisome proliferator-activated receptor γ expression.[38] The ability of insulin to decrease the plasma glucose level in HCV transgenic mice provides direct experimental evidence for the role of HCV in the development of insulin resistance in human HCV infection.[39]

Lipid metabolism

Low-density lipoprotein receptor facilitates entry of HCV into the hepatocyte.[40] Then, HCV core protein and NS5A interact with lipid droplets, which in turn may play a role in the pathogenesis of lipid metabolism and contribution to hepatic steatosis.[41] Clark *et al.* observed that HCV-3, but not HCV-2, selectively interfered with the late cholesterol synthesis pathway, which then resolved after achieving SVR.[42] Cram *et al.* observed that HCV-3a and HCV-1b core proteins up-regulated the fatty acid synthase promoter. However, HCV-3a core protein expression induced significantly higher fatty acid synthase promoter activity than HCV-1b core. Thus, it was concluded that the stronger effect of HCV-3a core protein was a plausible reason for the higher prevalence and severity of steatosis in HCV-3a infection.[43]

CURRENT TREATMENT IN HCV-3

Peginterferon (PEG-IFN) α-2b (1.5 mg/kg/week) plus ribavirin (RBV) (800–1400 mg/day) or PEG-IFN α-2a (180 mg/week) plus RBV (800 mg/day) for 24 weeks have been the established standard of care regimens for patients with HCV-3.[44] However, the optimal administration of PEG-IFN and RBV, especially the duration, has still not been clearly established. Manns *et al.*[45] and Fried *et al.*[46] carried out two randomised controlled trials that established a duration of 48 weeks of combination therapy with PEG-IFN and RBV as the standard of care for chronic hepatitis C. Mangia *et al.* observed that

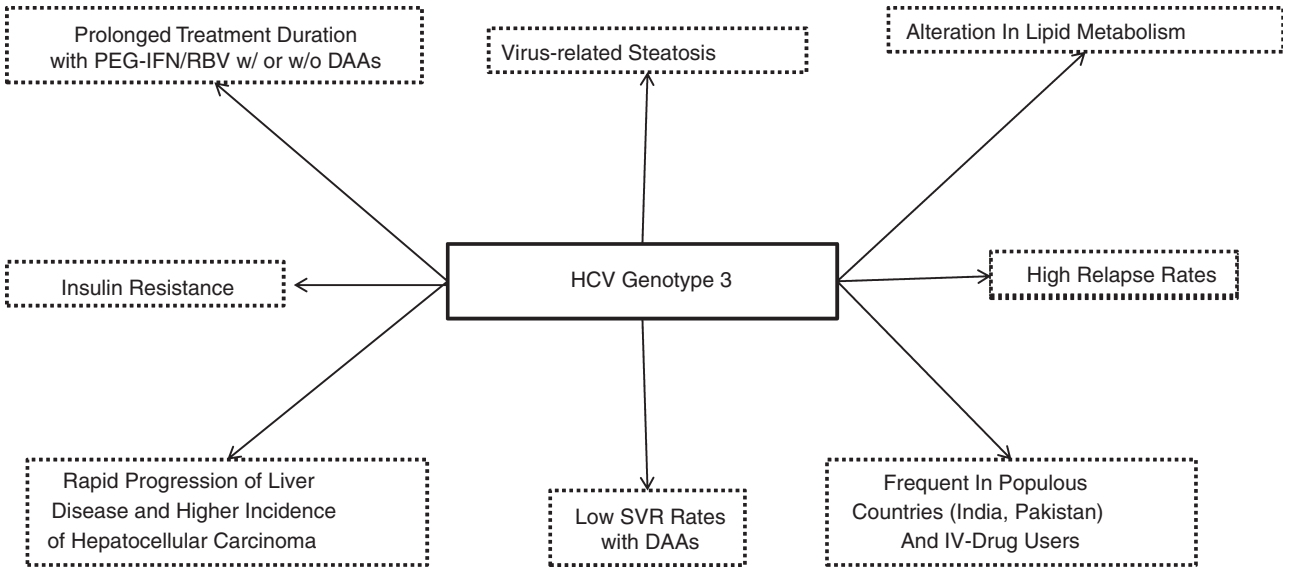

Figure 1 | Special issues related to HCV genotype 3.

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a shorter course of therapy over 12 weeks with PEG-IFN and RBV was as effective as a 24-week course for patients with HCV-3 who responded to treatment at 4 weeks (RVR).[47] Patients achieving RVR had similar SVR rates to HCV-3 patients treated for either 12 or 24 weeks (86.4% vs. 83.7%). However, in those without RVR, 36 weeks of therapy resulted in a higher rate of SVR compared with 24 weeks (72.5% vs. 63.0%). As such, it has been suggested that HCV-3 patients with RVR could be treated for 12 weeks, whereas in those without RVR, it was felt preferable to extend treatment to 36 weeks.[48] Therefore, virological response at week 4 of dual PEG-IFN and RBV therapy could be crucial in differentiating easy-to-treat from difficult-to-treat genotype 3 patients.[49] In contrast, the NORDynamIC trial observed that treatment for 12 weeks in HCV-3 was generally inferior to 24 weeks (SVR 58% after 12 weeks, 78% after 24 weeks).[50] Similarly, the N-CORE study showed that extended duration of treatment in non-RVR patients provided benefits in HCV-3 patients adherent to study protocol with SVR24 of 73% following 48 weeks of treatment and 54% after 24-week treatment duration. This was largely driven by differences in relapse rates (22% vs. 41%).[51] On the other hand, Hadziyannis *et al.* observed that 800 mg/d of RBV plus PEG-IFN for 24 weeks was enough to cure the majority of patients with HCV-3.[52]

In contrast, Zeuzem *et al.* enrolled 224 HCV-2 and HCV-3 patients, and evaluated PEG-IFN plus RBV administered for 24 weeks and noted higher rates of SVR in HCV-2 patients (93%) than in those with HCV-3 (79%).[53] These results were confirmed in a subsequent meta-analysis: 74% in HCV-2 in comparison with 69% in HCV-3.[54] The presence of steatohepatitis and more advanced fibrosis in HCV-3 could contribute to poorer therapeutic response in these patients.[55, 56] Shah *et al.* observed higher relapse rates in patients with steatosis (17.4% and 20.9% for low and high baseline levels of HCV RNA respectively) than in those without steatosis (2.5% and 8.8%).[57] Lastly, factors predictive of higher relapse rates in genotype 3 have included male gender (16% vs. 7%), age >55 years old (27% vs. 12%), high viral load (20% vs. 7%) and advanced fibrosis (20% vs. 6%).[58]

Factors reducing SVR in HCV-3

PEG-IFN and RBV achieve 70%–80% SVR among patients infected with genotype 3.[59] Prolongation of treatment duration in HCV-3 genotype has been controversial in recent years, so it is essential to identify difficult-to-cure patients to optimise disease management and prevent relapses. HCV-3 cirrhotic patients with high viral load (HVL) and those without RVR are the most difficult to treat.[60] Bridging fibrosis or cirrhosis was similarly predictive of reduced SVR for the standard 24-week treatment (76% and 60%) and for the short 16-week treatment (67% and 48%) in a *post hoc* analysis of the ACCELERATE study.[61] On the other hand, Dalgard *et al.* aimed to determine the efficacy of 14 weeks of treatment in HCV-3 patients who achieved early virological response, and observed that low viral load (LVL) (<600 000 IU/mL) was a strong predictor of SVR (98% vs. 79%).[62] Following 24 weeks of therapy, the same group achieved 80–90% SVR24 in HCV-3 patients with RVR compared to 56% in the absence of RVR.[63] Similarly, Diago *et al.* observed high SVR rates in those with LVL (<400 000 IU/mL) regardless of duration of treatment of 16 or 24 weeks (91% and 95%, respectively), while patients with HVL achieved SVR of 79% and 89% respectively.[64] Von Wagner *et al.* encountered a significantly lower SVR rate (59% vs. 85%) in HCV-3 patients with HVL (>800 000 IU/mL) than in those with LVL.[65] Ferenci *et al.* demonstrated that viral kinetics, primarily in genotype 1 patients, as noted by the decline in HCV level on treatment, correlated with the probability of SVR. In those with ≤ 2 $\log_{10}$ suppression of virus at week 12, the probability of SVR was approximately 10%, thus leading to the concept of a 'stopping rule' in such null responders.[66]

Taking into account RVR, baseline viral load, cirrhosis, metabolic abnormalities and 12-week virological response, EASL guidelines[67] recommend that in HCV-3: (i) in patients with RVR and baseline low viral load (<400 000–800 000 IU/mL) treatment duration could be shortened to 16 weeks at the expense of a slightly higher chance of post-treatment relapse; (ii) in the presence of advanced fibrosis, cirrhosis or cofactors affecting response (insulin resistance, metabolic syndrome, nonviral steatosis), the treatment duration should not be shortened to 16 weeks even if the patient has baseline low viral load and achieves RVR, and at least 24 weeks of therapy should be administered; (iii) patients with early or delayed virological response should be treated for 48 or 72 weeks, provided their HCV RNA is undetectable at week 24; (iv) patients with non-RVR and ≤ 2 $\log_{10}$ drop or positive RNA at 24 week should discontinue therapy (Figure 2).

The addition of a protease inhibitor (telaprevir[68] and boceprevir[69]) to the standard of care has substantially improved the treatment response in HCV-1 patients, but not in HCV-3. Telaprevir as monotherapy had minimal activity against HCV-3, as noted by a mild decrease in

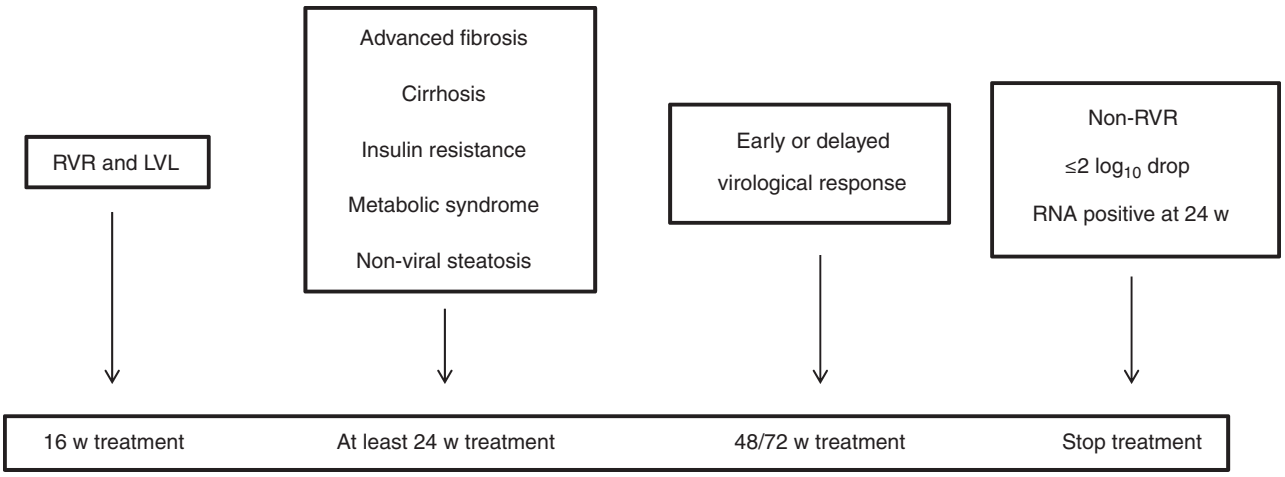

Figure 2 | Peginterferon plus ribavirin treatment in HCV-3 genotype.

HCV RNA levels (-0.54 $\log_{10}$ IU/L at day 15),[70] while boceprevir has been found to be slightly more active than telaprevir against HCV-3 in cell-based assays, and to decrease HCV RNA levels comparable to those observed with the same dose in HCV-1 treatment-experienced patients in a phase 1 study.[71] As we have advanced in HCV therapy, even second wave protease inhibitors simeprevir, asunaprevir, faldaprevir and danoprevir have demonstrated limited activity against HCV-3.[72]

ADVANCES IN THERAPY

The main mechanism of action of most DAAs is the inhibition of an enzyme (protease or polymerase),[73] although some inhibit the assembly of the replication complex (NS5A inhibitors) or target the host factors that the virus uses (cyclophilin inhibitors)[74] (Table 1). Such all-oral therapy regimens appear to be very well tolerated and achieve high response rates even without the backbone of pegylated interferon.

IFN-based regimens (Table 2)

NS3/4A protease inhibitors. NS3/4A is a serine protease essential for viral replication. Inhibitors of NS3/4A have a high potency but a low barrier to resistance, and are not effective against all HCV genotypes (i.e. telaprevir and boceprevir are only effective for HCV-1). Apart from telaprevir and boceprevir, other NS3/4A protease inhibitors have been developed to overcome the current

Table 1 | Characteristics of HCV DAAs and host targeting anti-virals

Characteristics	Protease inhibitors	Protease inhibitors	Polymerase inhibitors	Polymerase inhibitors	NS5A inhibitors	Cyclophilin inhibitors
	First generation	Second generation	Nucleoside analogues	Non-nucleoside analogues		
Potency	Variable among HCV genotypes	Variable among HCV genotypes	Consistent across genotypes	Variable among HCV genotypes	Multiple HCV genotypes	Multiple HCV genotypes
Barrier to resistance	Low	Low	High	Very low	Low	High
Drugs	Telaprevir Boceprevir	Simeprevir Asunaprevir Faldaprevir Vaniprevir Danoprevir ABT/r†	Sofosbuvir Mericitabine	BMS-791325 Tegobuvir ABT-333†	Daclatasvir MK-8742 Ledipasvir* GS-5816 ABT-267†	Alisporivir
Efficacy in HCV-3	No	No	Yes	No	Yes	Yes

* Ledipasvir has low activity against genotype 3.

† No data available.

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Table 2 | Summary of DAA studies in HCV-genotype 3 patients

Author	Year	Drug	Patients characteristics	Study design	Outcome	Comments
Dore[81]	2013	Daclatasvir	80 HCV-3 patients All treatment-naïve 22.5% cirrhotics	DCV 60 mg/day and PEG-IFN/RBV 12 weeks DCV 60 mg/day and PEG-IFN/RBV 16 weeks placebo and PEG-IFN/RBV 24 weeks	SVR24 rates a) 85% b) 82% c) 69%	HCV-3 patients showed higher relapse rates than HCV-2
Yeh[82]	2013	MK-8742	48 HCV-1/HCV-3 Noncirrhotics	50 mg 5-day monotherapy 100 mg 5-day monotherapy	HCV RNA levels were reduced -3.4 $\log_{10}$ IU/mL in HCV-3	More sustained virological suppression in the 100 mg group
Lawitz[83]	2013	GS-5816	20 HCV-3 patients Treatment naïve Noncirrhotics	25 mg 3-day monotherapy 50 mg 3-day monotherapy 150 mg 3-day monotherapy	HCV RNA levels were reduced -3.25 $\log_{10}$ IU/mL -3.12 $\log_{10}$ IU/mL -3–14 $\log_{10}$ IU/mL	
Lawitz[86]	2013	Sofosbuvir	25 HCV-2/HCV-3 Treatment naïve Noncirrhotics	SOF 400 mg/day plus PEG-IFN and RBV for 12w	SVR12 rate: 92%	
Lawitz[87]	2013	Sofosbuvir	24 HCV-3 patients Treatment failure 55% cirrhotics	SOF 400 mg/day plus PEG-IFN and RBV for 12w	SVR12 rates: 83% in noncirrhotics and cirrhotics	HCV-2 achieved higher SVR12 rates (100% and 92%, respectively)
Gane[88]	2013	Sofosbuvir	40 HCV-2/HCV-3 Treatment naïve Noncirrhotics	SOF 400 mg/day plus RBV 12w SOF 400 mg/day plus RBV 12w and PEG-IFN 4w SOF 400 mg/day plus RBV 12w and PEG-IFN 8w SOF 400 mg/day plus RBV 12w and PEG-IFN 12w SOF 400 mg/day plus PEG-IFN/RBV for 8w SOF 400 mg/day 12w	SVR24 rates 100% 100% 100% 100% 100% 60%	
Lawitz[89]	2013	Sofosbuvir	359 HCV-3 patients Treatment naïve 20% cirrhotics	SOF 400 mg/day plus RBV for 12w PEG-IFN/RBV for 24w	SVR12 rate in SOF 400 mg/day plus RBV for 12w was 67%	PEG-IFN/RBV for 24w achieved also 67% SVR12
Jacobson[93]	2013	Sofosbuvir	135 HCV-3 patients for whom treatment with PEG-IFN was not an option 20% cirrhotics	SOF 400 mg/day plus RBV for 12w Placebo	SVR12 rates Overall SVR 61% (68% noncirrhotics, 21% cirrhotics)	Overall SVR12 rate in HCV-2: 93% (92% noncirrhotics, 94% cirrhotics)
Jacobson[93]	2013	Sofosbuvir	127 HCV-3 patients Treatment failure 30% cirrhotics	SOF 400 mg/day plus RBV for 12w SOF 400 mg/day plus RBV for 24w	SVR12 rates 62% 30%	SVR12 was achieved in 19% HCV-3 cirrhotics In HCV-2, SVR12 was 86% after 12 weeks and 94% after 16 weeks

Table 2 | (Continued)

Author	Year	Drug	Patients characteristics	Study design	Outcome	Comments
Zeuzem[94]	2013	Sofosbuvir	250 HCV-3 patients 58% treatment failure 21% cirrhotics	SOF 400 mg/day plus RBV for 24w Placebo	Overall SVR12 rate with sofosbuvir was 85%	SVR12 rates 94% treatment-naive patients without cirrhosis 92% treatment-naive cirrhotics 87% treatment-experienced noncirrhotics 60% treatment-experienced cirrhotics
Gane[90]	2010	Mericitabine	25 HCV-2/HCV-3 Treatment failure Noncirrhotics	Mericitabine 1500 mg/12 h plus PEG-IFN/RBV 24w Mericitabine 1500 mg/12 h plus PEG-IFN/RBV 48w Placebo plus PEG-IFN/RBV	SVR12 rates: 67% 90% 60%	SVR12 rates did not differ between HCV-2 and HCV-3 patients (63% and 67% respectively)
Fisiak[91]	2008	Alisporivir	7 HIV/HCV-3-coinfected patients Noncirrhotics	Alisporivir 1200 mg 14 days Placebo 14 days	HCV RNA levels were reduced -3.63 $\log_{10}$ IU/mL -0.73 $\log_{10}$ IU/mL	
Fisiak[92]	2009	Alisporivir	HCV-1/2/3/4 patients Treatment naïve Noncirrhotics	ALV 200 mg/day plus PEG-IFN for 4w ALV 600 mg/day plus PEG-IFN for 4w ALV 1000 mg/day plus PEG-IFN for 4w ALV 1000 mg monotherapy for 4w PEG-IFN monotherapy for 4w	The 600- and 1000-mg combinations reduced HCV RNA levels HCV-1: -4.61 ± 1.88 HCV-2: -5.91 ± 1.11 HCV-3: -5.89 ± 0.43 HCV-4: -4.75 ± 2.19	
Pawlotsky[95]	2012	Alisporivir (ALV)	194 HCV-3 patients Treatment-naïve Noncirrhotics	ALV 1000 mg/day for 24w ALV 600 mg/day plus RBV for 24w ALV 800 mg/day plus RBV for 24w ALV 600 mg/day plus PEG-IFN for 24w PEG-IFN/RBV for 24w	SVR24 rates 90% in ALV plus RBV 72% ALV 1000 mg 70% PEG-IFN/RBV	Late relapse was not observed after ALV plus RBV in contrast to PEG-IFN/RBV or ALV monotherapy
Sulkowski[97]	2012	Daclatasvir plus Sofosbuvir	18 HCV-3 patients Treatment naïve Noncirrhotics	SOF 400 mg/day for 7 days then add DCV 60 mg/day for 24w DCV 60 mg/day plus SOF 400 mg/day for 24w DCV 60 mg/day plus SOF 400 mg/day plus RBV for 24w	SVR4 rates 88% 100% 86%	Virological response did not vary by IL28B status or with the use of RBV

limitations of first-generation drugs, with regard to the pharmacokinetic profile, barrier to resistance, adverse events and activity among other genotypes.[75] Simeprevir (TMC-435), the next line protease inhibitor, demonstrates good tolerability even with the infrequent mild and asymptomatic hyperbilirubinaemia (reversible at the end of the therapy).[76, 77] It is effective against HCV-1, but also against HCV-2, HCV-5 and HCV-6. However, it has shown limited efficacy against HCV-3.[78]

NS5A inhibitors. NS5A is a zinc-binding phosphoprotein that plays an important but currently unclear role in HCV replication. Daclatasvir (BMS-790052) is an HCV NS5A inhibitor that has been demonstrated to have anti-viral activity across all genotypes.[79] Daclatasvir-resistant variants have been shown to remain sensitive to interferon and other HCV protease and non-nucleoside polymerase inhibitors. Consequently, the addition of interferon or other DAAs to daclatasvir would enhance response to therapy, while minimising the risk of emergence of viral resistance.[80] The COMMAND study included 80 treatment-naïve HCV-3 patients, who were treated with daclatasvir and PEG-IFN/RBV (12 or 16 weeks) vs. placebo and PEG-IFN/RBV (24 weeks). SVR24 was achieved by 85%, 82% and 69% in HCV-3 patients in the 12-week, 16-week and placebo groups, respectively.[81]

In a phase 1b, randomised, placebo-controlled study, MK-8742, an NS5A inhibitor, was administered as 5-day monotherapy in 48 HCV-1 and HCV-3 patients without cirrhosis. Plasma HCV RNA declined rapidly from baseline by 3.4 $\log_{10}$ IU/mL in HCV-3 patients, and mean viral load reductions were similar in 50- and 100-mg dose groups with more sustained virological suppression after cessation of dosing in the 100-mg group.[82] On the other hand, GS-5816, a second-generation NS5A inhibitor, with anti-viral activity against all HCV genotypes, is well tolerated and has demonstrated potent anti-viral activity against HCV genotypes 1-4.[83]

NS5B polymerase inhibitors. NS5B is an HCV RNA-dependent RNA polymerase that plays a crucial role in HCV replication. Nucleotide inhibitors have been found to be pan-genotypic and possess high potency and high barrier to resistance, as the active site of NS5B is highly conserved across all HCV genotypes. Non-nucleoside inhibitors allosterically target the NS5B region, and inhibit the initiation stage of RNA synthesis. This class of inhibitors displays a low barrier to resistance, mild potency and limited effectiveness across all HCV genotypes.

Sofosbuvir (GS-7977) is a pyrimidine nucleotide analogue with high anti-viral activity against all genotypes and shows a high genetic barrier to resistance.[84] Regardless of HCV genotype, although in relatively small cohorts of genotype 3, sofosbuvir-based triple therapy resulted in SVR rates of 83–100%.[85] In the PROTON study, a total of 121 patients with HCV-1 were randomised to three cohorts: PEG-IFN/RBV plus sofosbuvir 200 mg, sofosbuvir 400 mg or a placebo once daily for 12 weeks; in addition, a fourth arm was included with HCV-2 and HCV-3 patients who received sofosbuvir 400 mg plus PEG-IFN/RBV for 12 weeks. The combination of sofosbuvir (200 mg or 400 mg once daily) and PEG-IFN/RBV for 12 weeks demonstrated efficacy, reaching SVR12 of 91% in HCV-1 and 92% in HCV-2/HCV-3, while it was 58% in placebo plus PEG-IFN/RBV group.[86] Recently, the LONESTAR-2 study assessed the combination of sofosbuvir plus PEG-IFN/RBV in 47 previous treatment-failure HCV-2 and HCV-3 patients (55% with compensated cirrhosis) treated for 12 weeks. HCV-2 achieved 100% and 92% of SVR12 in those without and with cirrhosis, respectively, while the response rates were lower and at 83% in both groups of HCV-3 patients.[87] Additional studies evaluated the safety and efficacy of sofosbuvir and RBV in various IFN-based and IFN-free regimens in HCV-1, HCV-2 and HCV-3 patients, but in small cohorts. In HCV-2 and HCV-3 treatment-naïve patients, 100% of patients treated with sofosbuvir and PEG-IFN/RBV achieved SVR (30/30), as well as the all-oral regimen of sofosbuvir and RBV (10/10), vs. 60% (6/10) with sofosbuvir monotherapy. On the other hand, only 68% of all treatment-experienced patients with HCV-2 and HCV-3 receiving a combination of sofosbuvir and RBV achieved SVR12.[88] The FISSION trial, a randomised study of 12 weeks of sofosbuvir plus RBV vs. PEG-IFN/RBV during 24 weeks in untreated HCV-3 patients achieved an identical SVR12 rate of 67% in HCV-3 patients with both regimens.[89]

Mericitabine (RG7128) is a nucleoside polymerase inhibitor, with anti-viral activity demonstrated *in vitro* against all HCV genotypes. In HCV-2 and HCV-3 treatment-failure patients, mericitabine-treated patients (plus PEG-IFN/RBV) achieved a higher RVR rate of 95% vs. 60% in the PEG-IFN/RBV arm, as well as higher SVR when treated with mericitabine for 48 weeks (90%) than in those treated for 24 weeks (67%). Overall, SVR rates did not differ between HCV-2 and HCV-3 patients (63% and 67% respectively).[90]

Cyclophilin inhibitors. Alisporivir (DEB025) is able to inhibit HCV viral replication by interfering with the interaction between cyclophilin A and NS5. A proof of concept study was done with alisporivir monotherapy in HIV/HCV-coinfected patients. The conclusion was that alisporivir at a dose of 1200 mg twice daily for 15 days had a significant viral inhibitory effect against HCV-1, HCV-3 and HCV-4.[91] In another study in treatment-naïve patients, alisporivir had potent activity against the four most prevalent genotypes of HCV (HCV-1: -4.61 ± 1.88; HCV-2: -5.91 ± 1.11; HCV-3: -5.89 ± 0.43; HCV-4: -4.75 ± 2.19 $\log_{10}$ IU/mL at week 4), when it was combined with PEG-IFN.[92]

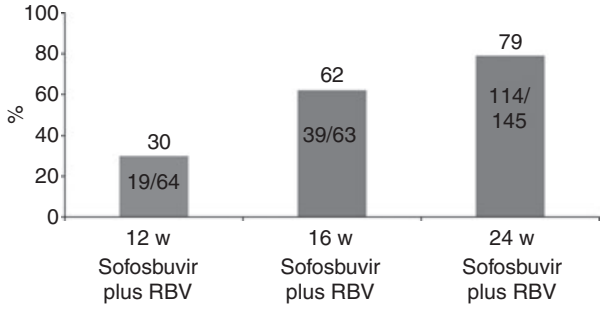

Figure 4 | Response rates, in treatment-experienced patients, with all-oral DAAs in HCV-3.

IFN-free regimens

Combination of DAAs with RBV (Table 2 and Figures 3 and 4). POSITRON and FUSION trials evaluated all-oral therapy regimens of sofosbuvir and RBV in genotypes 2 and 3 IFN ineligible, IFN intolerant, and prior-pegylated interferon and RBV failures for 12 or 16 weeks with the longer duration being in prior failures. The POSITRON trial observed that sofosbuvir and RBV resulted in 78% overall SVR12, with 93% in HCV-2 and 61% in HCV-3 of patients for whom IFN treatment was not an option. Those with genotype 3 and cirrhosis fared poorly, with an SVR12 rate of only 21%. The FUSION trial compared sofosbuvir 400 mg plus RBV for 12 or 16 weeks, and there were better SVR12 rates after 16 weeks (73% vs. 50%). There were differences between genotypes: SVR12 was achieved in 86% after 12 weeks and 94% after 16 weeks in HCV-2, while SVR12 was 30% and 62% after 12 and 16 weeks in HCV-3. Again, patients with HCV-3 cirrhosis achieved SVR12 in only 19% of cases.[93] The VALENCE trial was conducted in Europe, and assessed the safety and efficacy of sofosbuvir plus RBV, administered for 12 or 24 weeks in treatment-naïve or treatment-experienced patients infected with HCV-3. Eighty-five per cent (n = 212/250) of treatment-naïve or treatment-experienced patients with HCV-3 who received a 24-week regimen achieved SVR12. However, it was noted that only 60% of prior treatment-experienced patients with cirrhosis achieved SVR with sofosbuvir plus RBV for 24 weeks.[94]

The VITAL-1 study evaluated alisporivir, randomising 340 treatment-naïve patients infected with HCV-2 and HCV-3 to five arms (ALV 1000 mg; ALV 600 mg plus RBV; ALV 800 mg plus RBV; ALV 600mg plus PEG-IFN; PEG-IFN/RBV). Combination of alisporivir and RBV achieved higher SVR24 rates (90%) in comparison with patients receiving alisporivir monotherapy (72%) and those receiving PEG-IFN and RBV (70%). Late relapse was not observed after alisporivir and RBV treatment in contrast to PEG-IFN treatment or

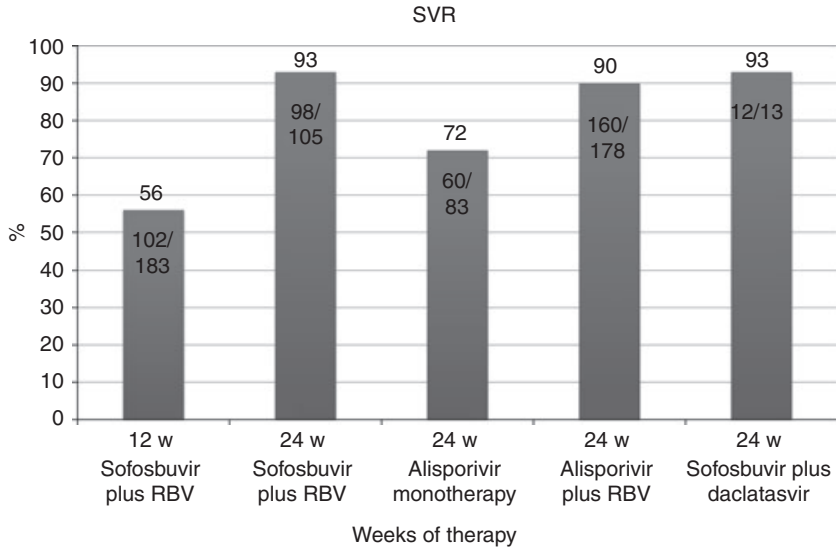

Figure 3 | Response rates, in treatment-naïve patients, with all oral DAAs in HCV-3.

alisporivir monotherapy.[95] In a *post hoc* analysis, IFN--free alisporivir treatment was well tolerated compared to IFN-based treatment.[96]

Combination of DAAs without RBV. Combination of daclatasvir and sofosbuvir, with or without RBV, has been assessed in HCV-1, HCV-2 and HCV-3 patients and such therapy, for 24 weeks, achieved SVR in more than 95% of the overall cohort: the SVR rate was 100% in HCV-1 patients, while it was 91% in both HCV-3 and HCV-2 patients. The data demonstrated that virological response did not vary by IL28B status, genotype, HCV genotype 1 subtype or with the administration of RBV.[97] Garcia-Rivera investigated if alisporivir showed synergistic, additive or antagonist effects with other DAAs in the human hepatoma cell line Huh 7.5. They found that combining alisporivir and boceprevir had an additive effect in inhibiting HCV replication, while the combinations of alisporivir and NS5B inhibitors (mericitabine or sofosbuvir) and NS5A inhibitors (daclatasvir) exhibited greater synergistic effects in HCV-3.[98]

FUTURE DIRECTIONS

The landscape of therapy for hepatitis C virus infection is changing rapidly. Currently, the standard of care for HCV infection is a combination of a protease inhibitor (telaprevir or boceprevir) plus PEG-IFN and RBV for HCV-1, and only PEG-IFN and RBV for HCV-2/6. Recently, newer DAAs (simeprevir and sofosbuvir) in combination with PEG-IFN and RBV have become available for genotype 1 patients.[76, 77, 99, 100] The advent of protease inhibitors has improved the likelihood of cure, but with a number of inherent limitations: (i) they do not have anti-viral activity in HCV genotypes other than HCV-1; (ii) they need to be administered with PEG-IFN and RBV, which have extensive and well-established side-effect profiles that are currently aggravated by the addition of telaprevir or boceprevir.

On the other hand, a multitude of DAAs are being developed in clinical trials with or without PEG-IFN and RBV.[101] The tremendous improvement in SVR rates in genotype 1 and genotype 2 has rendered genotype 3 HCV the major challenge, as it continues to globally afflict a large population of patients. Based on SVR rates with these new drugs, HCV-3 has become the more difficult genotype to treat. Special characteristics of HCV-3, such as insulin resistance or disturbances in lipid metabolism, could be closely related to these suboptimal responses. A better mechanistic understanding of the reasons for suboptimal response, which appears to be mediated by a higher relapse rate, at least partly, needs to be explored. In addition, the clear association between HCV-3 and liver progression, as well as increased incidence of HCC, should be the focus of attention in this genotype. The low incidence of side effects, the relatively short duration of treatment and the pan-genotypic properties of new drugs are compelling reasons to opt for these regimens, while effective new therapies are developed without PEG-IFN.

Sofosbuvir and ribavirin is the first all-oral therapy regimen that has been approved in the US by the FDA for use in Genotype 2 and 3 patients, while it is also a consideration in select genotype 1 patients.[99] However, these regimens have not yet been approved outside of the US. Thus, globally, as it stands now, pegylated interferon and RBV continue to be the mainstay of therapy. With the rapid development of additional DAA strategies, through combination of multiple drugs such as a pangenotypic NS5A inhibitor and nucleotide NS5B inhibitor, along with other combinations, it may be reasonable to wait for these in those with relatively mild disease severity. Patients with more advanced fibrosis and cirrhosis have an urgent need and for those who are treatment naïve, either pegylated interferon and ribavirin for a variable duration (Figure 2), or sofosbuvir and RBV for 24 weeks are good options; in prior-pegylated interferon and ribavirin failures, sofosbuvir and ribavirin for 24 weeks is the only treatment option. Although studied in small cohorts,[86–88] pegylated interferon, ribavirin and sofosbuvir for 12 weeks, primarily for reasons of cost, is a viable consideration as well.

AUTHORSHIP

Guarantor of article: K. Rajender Reddy.

Author contributions: Javier Ampuero, Manuel Romero-Gómez and K. Rajender Reddy wrote the paper and contributed to the design of the review. All authors approved the final version of the manuscript.

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