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THERAPY IN PRACTICE

Genotype 3 Infection: The Last Stand of Hepatitis C Virus

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Abstract Hepatitis C virus (HCV) represents a significant global disease burden, with an estimated 130–150 million people worldwide living with chronic HCV infection. Within the six major clinical HCV genotypes, genotype 3 represents 22–30% of all infection and is described as a unique entity with higher rates of steatosis, faster progression to cirrhosis, and higher rates of hepatocellular carcinoma. Hepatic steatosis in the setting of hepatitis C genotype 3 (HCV-3) is driven by viral influence on three major pathways: microsomal triglyceride transfer protein, sterol regulatory element-binding protein-1c, and peroxisome proliferator-associated receptor-α. Historically with direct-acting antivirals, the rates of cure for HCV-3 therapies lagged behind the other genotypes. As current therapies for HCV-3 continue to close this gap, it is important to be cognizant of common drug interactions such as acid-suppressing medication and amiodarone. In this review, we discuss the rates of steatosis in HCV-3, the mechanisms behind HCV-3-specific steatosis, and current and future therapies.

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Key Points

Hepatitis C genotype 3 (HCV-3) infection represents a unique entity, with genotype-specific molecular pathways for higher rates of steatosis.

Although rates of cure had lagged behind for HCV-3 in the modern direct-acting antiviral era, new therapies are being developed that have closed this treatment gap.

1 Introduction

Hepatitis C virus (HCV) represents a significant global burden of disease, with an estimated 130–150 million people worldwide living with chronic HCV (CHC) infection [1, 2]. There are six major clinical HCV genotypes and over 50 subtypes; however, genotype 3 infection represents a unique entity, with higher rates of steatosis and more rapid fibrosis progression [3]. In the direct-acting antiviral (DAA) era, cure rates for genotype 3 infection have lagged behind the other genotypes until the approval of daclatasvir (DCV) and sofosbuvir (SOF) in 2015 and, more recently, the approval of the fixed-dose combination SOF and velpatasvir (VEL) [4, 5]. This review will discuss the pathogenesis of accelerated fibrosis and current treatment options for HCV genotype 3 infection.

2 Epidemiology

Globally, HCV genotype 3 (HCV-3) infection accounts for 22–30% of all HCV infection, second only to HCV genotype 1 (HCV-1) infection (Fig. 1) [6, 7]. The highest global

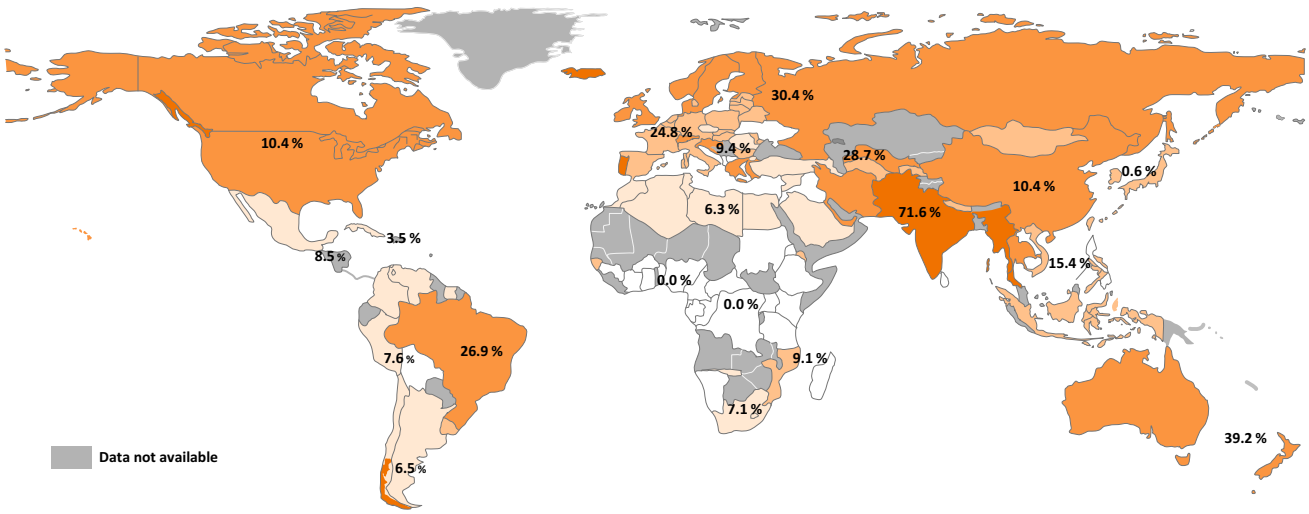

Fig. 1 Global prevalence of HCV genotype 3

prevalence is in South and Central Asia, where it represents 71.6% of all HCV infection. In Western Europe, the overall prevalence of HCV-3 is 24.8%, with Norway (50%), England (47%), Finland (46%), and Denmark (43%) among those countries with the highest prevalence [6]. South America is the next geographic region of highest HCV-3 prevalence at 26.9%, and with rates of up to 30% in Brazil. There is a significantly lower prevalence in Africa [7–9], while North America is split, with HCV-3 representing only 10–12% of all CHC infection in the US, and accounting for 22% in Canada.

3 Fibrogenesis in Genotype 3 Infection

Fibrosis is a wound-healing response that occurs in the setting of chronic liver injury. In CHC infection, multiple factors are thought to play a role in the rate of fibrosis progression, including age, sex, coinfection with HIV or HBV, alcohol intake, and, potentially, HCV genotype. Early studies suggested a potential association with the HCV-3 genotype and greater severity of fibrosis, but these retrospective studies were limited by small cohorts, variability in patient characteristics such as insulin resistance or body mass index (BMI) or HCV genotype distribution, and inconsistencies in methodology, particularly with respect to the grading of steatosis [13, 14]. One large retrospective analysis of the Swiss Hepatitis C Study Cohort of 1189 patients indicated that HCV-3 was independently associated with fibrosis across multiple different estimates of progression rates [3]. A meta-analysis of eight single biopsy studies representing 2349 patients with CHC confirmed an association between fibrosis and genotype 3, with an odds ratio of 1.52 for an accelerated fibrosis progression rate [10]. The same meta-analysis included eight paired biopsy studies that were underpowered and did not reveal a similar association [10]. The primary limitation of these studies reporting an association between HCV-3 and fibrosis was that they did not account for steatosis. Meanwhile, a large meta-analysis of 3068 CHC patients from North America, Europe and Australia reported that HCV-3 was associated with steatosis, not fibrosis, and a multivariate analysis of fibrosis identified steatosis and level of inflammatory activity on histopathology as independent predictors of disease, not HCV-3 [11]. Multiple studies support the association of higher grades of steatosis with higher rates of fibrosis progression [12]. In addition, other studies support the concept that liver fibrosis is predominantly associated with steatosis in HCV-3 infection [13–15]. Thus, the burden of data does not support pathogenic evidence for enhanced direct viral-mediated hepatic fibrogenesis for HCV-3 compared with other genotypes, and while the pathogenesis of disease progression in HCV-3 remains unclear, it is at least in part related to the higher rates of steatosis and hepatic inflammation reported in HCV-3 infection.

4 Hepatitis C Virus Genotype 3 Infection and Steatosis

Hepatic steatosis is a common histological feature of patients with CHC infection and is multifactorial in etiology [16]. Observational data from several sources have indicated that steatosis is an independent variable that is associated with both severity and progression of fibrosis in CHC patients, and increases the risk of hepatocellular carcinoma (HCC) [17, 18]. In non-alcoholic fatty liver disease (NAFLD), steatosis is considered to be the initial histologic manifestation of the metabolic syndrome and is

associated with risk factors such as obesity, type 2 diabetes mellitus, and dyslipidemia [19]. In CHC infection, specifically in genotypes 1 and 4, hepatic steatosis is associated with insulin resistance and appears to be associated with more historical host factors [20].

Hepatic steatosis in the setting of HCV-3 is a unique entity in CHC, and while it is likely to be both viral- and host-mediated, viral factors appear more central in HCV-3 compared with other genotypes. HCV-3 infection is typically associated with moderate-to-severe steatosis, and a significant association between viral load and grade of steatosis has been observed [15, 21–24]. Further support for direct viral-mediated steatosis in HCV-3 was obtained from early clinical studies that demonstrated a significant improvement in biopsy-proven steatosis in most HCV-3-infected patients following sustained virologic response (SVR), which was independent of changes in BMI [21, 22, 25]. Steatosis may also be seen in non-obese genotype 3a patients[14], further supporting the concept of direct viral-mediated steatosis in genotype-3-infected patients. In contrast, HCV RNA levels do not correlate with the degree of steatosis in non-genotype 3 infection [12, 14]. In these patients, steatosis appears to be associated with host metabolic factors such as BMI and visceral obesity, and there is no significant improvement in steatosis when patients achieve viral clearance.

HCV-3 infection is associated with higher rates of steatosis, more rapid progression to liver disease, and higher risk for HCC [26–29]. Studies that were unable to control for the role of steatosis have suggested the higher risk of HCC is related to the virus [28, 29]. However, similar to fibrosis, it is likely that the steatosis is a critical confounder in this reported association between HCV-3 and HCC. Even after SVR12, HCV-3 infection is associated with higher rates of HCC, likely as a result of more advanced liver disease, which has previously been shown to be related to the steatosis, not the virus [30]. With viral-mediated steatosis, a central driver of pathogenesis in HCV-3 infection, it is important to note that steatosis itself does not induce a proinflammatory state but likely reflects the presence of other lipogenic pathways, such as lipid peroxidation and insulin resistance, which result in enhanced profibrogenic stimuli. Lipids play an important role in several key aspects of the HCV lifecycle, including formation of the virion structure, cell receptor recognition, membrane fusion, viral replication, assembly, and export [31]. Although the precise pathogenic mechanisms of HCV-3-mediated steatosis are still unknown, HCV-3 modulates host lipid metabolism and appears to influence unique mechanisms of fat metabolism and transportation within the liver, including microsomal triglyceride transfer protein (MTTP), sterol regulatory element-binding protein 1c (SREBP-1c), and peroxisome proliferator-associated receptor-α (PPAR-α) [20].

The next section will explore the specific mechanisms by which HCV-3 is able to modulate host lipid metabolic pathways, resulting in increased fatty acid accumulation and disease progression.

4.1 Microsomal Triglyceride Transfer Protein (MTTP) Inhibition

HCV-3 is associated with lower levels of low-density lipoprotein (LDL), hypobetalipoproteinemia, and steatosis due to viral-mediated inhibition of MTTP, which plays an important role in triglyceride secretion from the liver [32]. MTTP is primarily responsible for the assembly of lipid molecules with apolipoprotein B (ApoB), which forms very-low-density lipoprotein (VLDL) that exports triglycerides into the bloodstream [32]. Mutations in ApoB produce a disease state called ApoB lipoproteinemia, which is characterized by low levels of circulating ApoB in addition to hepatic steatosis [33]. This pathway was initially implicated in HCV disease by Rubbia-Brandt et al., who described decreased levels of circulating ApoB in patients with HCV-3 infection and hepatic steatosis [23]. In a transgenic mouse model, Perlemuter et al. noted that HCV core protein overexpression inhibited the ability of MTTP to transfer lipid molecules to ApoB, thus resulting in increased ApoB degradation and decreased production of VLDL [34]. Although all genotypes had some capability to inhibit the function of MTTP, Mirandola et al. noted that this effect was greatest with HCV-3a core proteins [35]. Cells transfected with HCV demonstrate colocalization of HCV core protein [36] and HCV non-structural protein 5A (NS5A) [37] to intracytoplasmic triglyceride-rich lipid droplets. Analysis of the primary sequence of the HCV core protein has revealed a unique domain necessary for association of the core protein with lipid droplets [38]. Our group has previously shown that specific HCV core protein polymorphisms are associated with intrahepatic lipid accumulation in HCV-3a, providing further evidence for viral- and genotype-specific steatosis [39]. In addition, HCV-3 appears to selectively disrupt *de novo* lipogenesis in the distal cholesterol biosynthesis pathway [40], with restoration of distal lipid metabolites following successful DAA therapy [41]. This is in keeping with observed restoration in total cholesterol and ApoB levels following viral clearance [42]. In summary, viral inhibition of MTTP, reduced ApoB levels, and selective disturbance in sterol synthesis may result in overall decreased hepatocyte lipid export and may represent a pathway to hepatic steatosis in HCV-3 infection (Fig. 2).

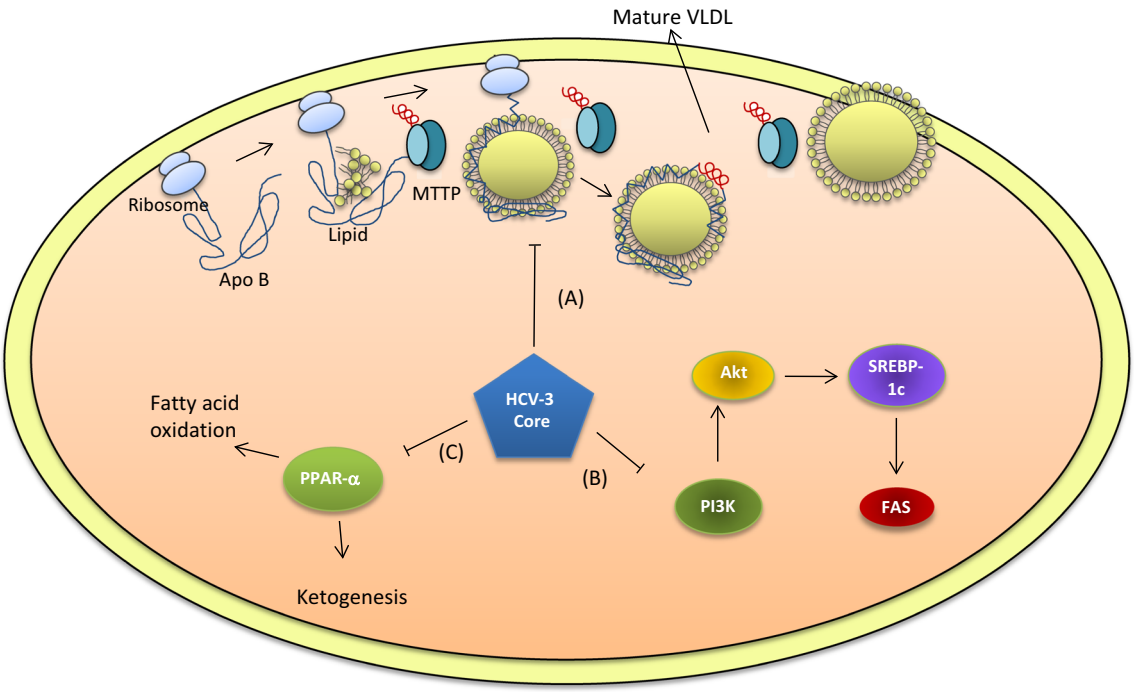

Fig. 2 HCV-3 viral-mediated mechanisms of steatosis. *a* HCV-3 core inhibits MTTP, affecting assembly of ApoB and lipid to VLDL. *b* HCV-3 core induces the PI3K-Akt pathway, increasing activity of SREBP-1c and increasing FAS. *c* HCV core increases levels of PPAR-α, leading to hepatic lipid accumulation. *HCV-3* hepatitis C genotype 3, *VLDL* very-low-density lipoprotein, *ApoB* apolipoprotein B, *MTTP* microsomal triglyceride transfer protein, *PPAR* peroxisome proliferator-associated receptor-α, *AKT* protein kinase B, *SREBP-1c* sterol regulatory element-binding protein 1c, *FAS* fatty acid synthase, *PI3K* phosphoinositide-3-kinase

4.2 Sterol Regulatory Element-Binding Protein 1c (SREBP-1) Activation

SREBP-1c is a transcription factor that regulates lipogenic pathways, including fatty acid synthesis, and which has been investigated for associations with HCV infection [43]. SREBP-1c controls both cholesterol and fatty acid synthesis and serves as a transcription factor for multiple downstream enzymes such as fatty acid synthase (FAS) [44]. FAS plays an important role in lipid synthesis and triglyceride accumulation in hepatocytes by catalyzing the reaction of acetyl coenzyme A (CoA) and malonyl-CoA in the synthesis pathway for triglycerides. With respect to HCV-3, Jackel-Cram et al. demonstrated that SREBP-1c activity and subsequent FAS promoter upregulation was increased in the presence of HCV-3a core protein (Fig. 2) [45]. This finding would indicate that HCV-3a core protein was capable of increasing triglyceride production within the liver. Further studies noted that SREBP-1c activity was likely driven by upstream factors phosphoinositide-3-kinase (PI3K) and protein kinase B, also known as AKT. Directly downstream of the insulin receptor, the PI3K-AKT pathway is a highly conserved master regulatory pathway involved in cell proliferation, genetic stability, and apoptosis. Therefore, other than upregulation of FAS, it is possible that there are pathways that are altered in the setting of HCV-3a core protein. Other literature suggests that viral proteins NS5A and NS4B are also capable of SREBP-1c activation, however it is unclear if this is unique to HCV-3 or also occurs in other genotypes [46]. The exact mechanism of HCV-3a core protein-mediated SREBP-1c activation remains unknown, but several pathways have been implicated, including insulin receptor signaling [45].

4.3 Peroxisome Proliferator-Associated Receptor-α (PPAR-α) Inhibition

Another contributor to HCV-3 steatogenesis is inhibition of the PPAR-α pathway involved in metabolic regulation. PPAR-α is a transcription factor that induces hepatic fatty acid oxidation and ketogenesis, while also upregulating hepatic glucose production, the primary adaptive response to fasting [47]. PPAR-α is the pharmacologic target for the fibrate class of cholesterol-lowering medications, such as gemfibrozil and fenofibrate, which function as PPAR-α agonists. Other PPAR agonists are also under current evaluation for cardiometabolic disease and NAFLD [48]. Conversely, inhibition of PPAR-α results in decreased triglyceride breakdown and accumulation of intrahepatic fatty acids. HCV-3a core protein has been demonstrated to function as an inhibitor of PPAR-α activity in vitro (Fig. 2) [49]. De Gottardi et al. compared the expression of PPAR-

α in HCV-1b versus HCV-3a infection using liver biopsies of infected patients and in vitro models. This study noted that levels of PPAR-α messenger RNA (mRNA) were significantly decreased in HCV-3a compared with HCV-1b, independent of the extent of liver steatosis; however, inhibition of PPAR-α does not appear to be limited to HCV-3. Initial associations of PPAR-α inhibition were noted with HCV-1b core protein, although the effect appears less potent compared with that observed with HCV-3a [50, 51].

4.4 Interleukin-28B and Patatin-Like Phospholipase Domain-Containing Protein 3 (PNPLA3) in HCV-3-Related Steatosis

Interleukin (IL)-28B and patatin-like phospholipase domain-containing protein 3 (PNPLA3) genetic polymorphisms have been identified as important prognostic factors for the progression of steatosis and treatment response in CHC [52, 53]. *IL28B* is a gene that codes for interferon (IFN)-λ, and the favorable polymorphism rs12979860 CC has been associated with improved responsiveness to IFN-containing therapies and greater rates of natural clearance of HCV infection [54]. This polymorphism has also been associated with a decreased prevalence of steatosis in the setting of CHC compared with the unfavorable wild-type TT or heterozygote CT genotypes [53, 55, 56]. The PNPLA3 gene codes for a hydrolase against triglycerides and retinyl esters in hepatic stellate cells. The polymorphism rs738409 GG was initially identified as a risk factor for NAFLD [57] and was associated with an increased risk of steatosis and fibrosis in the setting of CHC [58, 59]. Unfortunately, studies assessing genetic polymorphisms and CHC have included few HCV-3 patients, and the effect of IL28 CC or PNPLA3 GG on genotype-specific steatosis remains uncertain in HCV-3.

5 Treatment of HCV Genotype 3 Infection

The treatment of HCV-3 infection has generally paralleled the advances in HCV therapy as a whole, although with some key distinctions. In the era of peginterferon (PEG) and ribavirin (RBV), HCV-3 had higher rates of SVR compared with HCV-1 and 4, with rates of SVR ranging from 66 to 80% [60–62]. In addition, the treatment course for HCV-3 was shorter than HCV-1 and -4, i.e. usually 6 months. Unfortunately, while demonstrating in vitro activity against HCV-3, the first-generation DAA agents telaprevir and boceprevir failed to demonstrate any significant additional clinical benefit, and PEG/RBV remained the standard of care until 2013 [63, 64].

5.1 Sofosbuvir

Sofosbuvir (SOF), an NS5B polymerase inhibitor, represented the first major advance in HCV-3 therapy [65]. Four large trials assessed the efficacy of SOF with weight-based RBV (SOF/RBV) for HCV-3: FISSION, FUSION, POSITRON, and VALENCE (Table 1). These studies included both HCV-2 and HCV-3 patients.

- FISSION enrolled a total of 527 patients, including 359 treatment-naive patients with HCV-3 infection who were all randomized to PEG/RBV for 24 weeks or

Table 1 Summary of HCV-3 trials and rates of SVR12

Trial name	Regimen	Treatment duration (weeks)	Total no. of HCV-3 patients	SVR12 for treatment-naive, non-cirrhotic patients [*n* (%)]	SVR12 for treatment-naive, cirrhotic patients [*n* (%)]	SVR12 for treatment-experienced, non-cirrhotic patients [*n* (%)]	SVR12 for treatment-experienced, cirrhotic patients [*n* (%)]
FISSION	SOF + RBV	12	359	102/183 (56)		–	–
FUSION	SOF + RBV	12	127	14/38 (37)	5/26 (19)	–	–
	SOF + RBV	16		25/40 (63)	14/23 (61)	–	–
POSITRON	SOF + RBV	12	98	57/84 (68)	3/14 (21)	–	–
VALENCE	SOF + RBV	24	261	87/92 (95)	12/13 (92)	85/98 (87)	29/47 (62)
BOSON	SOF + RBV	16	196	58/70 (83)	12/21 (57)	41/54 (76)	17/36 (47)
		24	199	67/72 (90)	18/22 (82)	44/54 (81)	6/34 (76)
ALLY-3	DCV + SOF	12	152	73/75 (90)	11/19 (58)	32/34 (94)	9/13 (69)
ALLY-3+	DCV + SOF + RBV	12	24	–	7/8 (88)	–	14/15 (93)
		16	26	–	12/12 (100)	–	12/14 (86)
ASTRAL-3	SOF + VEL	12	558	160/163 (98)	40/43 (91)	31/34 (93)	34/37 (89)

HCV-3 hepatitis C genotype 3, *SVR12* sustained viral response at 12 weeks of treatment, *SOF* sofosbuvir, *RBV* ribavirin, *DCV* daclatasvir, *VEL* velpatasvir

SOF/RBV for 12 weeks. SVR rates for the SOF/RBV arm were 56% compared with 63% for PEG/RBV [66]. Predictors of treatment failure included HCV-3 and the presence of cirrhosis.

- The POSITRON and FUSION trials further characterized the efficacy of SOF/RBV as a salvage regimen for patients who either had a contraindication or intolerance to IFN, or who were treatment-experienced with PEG/RBV, respectively [67]. The POSITRON trial was a placebo-controlled evaluation of SOF/RBV for 12 weeks, and FUSION was a randomized controlled trial of SOF/RBV for 12 weeks versus 16 weeks. POSITRON reported an SVR12 of 68% ($n = 57/84$) after 12 weeks of SOF/RBV for HCV-3 subjects without cirrhosis, and 21% ($n = 3/14$) SVR12 for those with cirrhosis, confirming an overall low rate of SVR12 in HCV-3 with 12 weeks of therapy and an unacceptable relapse rate in patients with cirrhosis in particular. FUSION was the first study to assess an extension of therapy in more difficult-to-treat patients, and noted that 16 weeks of SOF/RBV had higher overall rates of SVR12 (62 vs. 30%) compared with 12 weeks in HCV-3 infection [67]. Again, HCV-3 infection and cirrhosis were predictors of treatment failure.
- VALENCE was initially designed as a placebo-controlled, multicenter, phase III trial of SOF/RBV for 12 weeks versus placebo in HCV-2 and -3 infection [68]. The study included both treatment-experienced patients and patients with cirrhosis. Results of the FUSION trial, suggesting HCV-3 response rates were higher with extension of therapy to 16 weeks, were published while the VALENCE trial was ongoing. Based on these results, the study was unblinded, the placebo group was terminated, and all HCV-3-infected patients were extended to 24 weeks of SOF/RBV. Patients with HCV-3 who received 24 weeks of SOF/RBV achieved an overall SVR12 rate of 85% ($n = 213/250$), the highest reported with this regimen in HCV-3 infection. VALENCE also provided insight into the impact of prior treatment failure and cirrhosis on SVR. Treatment-naive patients achieved an SVR12 of 92% with cirrhosis and 95% without cirrhosis, while treatment-experienced patients achieved an SVR12 of 62% with cirrhosis and 87% without cirrhosis [68].

Recognizing the limitations of the SOF/RBV regimen in particularly hard-to-treat HCV-3-infected patients, the BOSON study set out to find an optimized regimen. BOSON was a randomized phase III, open-label trial that included treatment-experienced and -naive patients, randomizing them to SOF/RBV for 16 or 24 weeks, and SOF/PEG/RBV for 12 weeks [69]. The overall SVR12 rate in HCV-3-infected patients was 71% in the 16-week arm, 84% in the 24-week arm, and 93% for those who received SOF/PEG/RBV for 12 weeks. The differences between all groups were statistically significant, suggesting the most efficacious regimen for HCV-3 infection was SOF/PEG/RBV [69]. The higher SVR of the SOF/PEG/RBV regimen held true regardless of treatment experience and presence of cirrhosis, with all subgroups achieving SVR12 >90%, with one exception: treatment-experienced patients with cirrhosis achieved an SVR12 of 86% (30/35), although still superior to the other regimen. Both SOF-containing regimens remain recommended as per the current European Association for the Study of Liver Disease (EASL) HCV treatment guidelines, but were recently removed from the American Association for the Study of Liver Disease/Infectious Diseases Society of America (AASLD/IDSA) HCV treatment guidance due to the availability of several DAA combination therapies [70, 71].

5.2 Daclatasvir

In 2013, the approval of daclatasvir (DCV), a pangenotypic NS5A inhibitor, in combination with SOF for the treatment of HCV-3 infection represented the beginning of a new era for HCV-3 therapy [5]. Although IFN and RBV-free DAA combination therapies had already been approved for genotype 1 infection, these regimens did not extend to the HCV-3-infected population.

- The ALLY-3 study was an open-label, single-arm study of DCV (60 mg daily) in combination with SOF (400 mg daily) for 12 weeks (DCV + SOF) in all patients, including those with cirrhosis and who had not responded to prior treatment with IFN-based therapies [72]. The overall SVR12 rate was 90% in treatment-naive patients and 86% in treatment-experienced patients with HCV-3 infection. However, as in the prior SOF/RBV studies, the rates of SVR12 in patients with cirrhosis lagged behind, with an SVR12 of 58% (11/19) in treatment-naive patients with cirrhosis and 69% (9/13) for treatment-experienced patients with cirrhosis (Table 1). This was the first study that suggested a role for baseline NS5A resistance mutations in predicting treatment failure. Although the numbers were small, two resistance-associated substitutions (RASs) were associated with lower SVR12: (i) 14 patients had evidence of the A30 polymorphism at baseline, with an SVR12 of 100% (9/9) in patients without cirrhosis and 20% (1/5) in patients with cirrhosis; (ii) 13 patients had evidence of the Y93H polymorphism at baseline, with an SVR12 of 67% (6/9) in patients without cirrhosis and 25% (1/4) in patients with cirrhosis. These 10 failures accounted for more than half of all treatment failures, although the two RASs were only detected in 17% of patients.

- ALLY-3+ was a small, randomized controlled trial of DCV+SOF, with the primary objective of investigating the impact of weight-based RBV and treatment extension (12 vs. 16 weeks) on response rates in HCV-3 infection [73]. The study included treatment-naive and -experienced patients with severe fibrosis ($N = 14$) and compensated cirrhosis ($N = 36$). The overall SVR12 was 88% (21/24) in the 12-week arm and 92% (24/26) in the 16-week arm (Table 1). Specifically, in patients with cirrhosis, the SVR12 was 83% (15/18) and 89% (16/18), respectively. Only four relapses were noted in the study, two in each arm. The numbers were small, but, overall, RBV seemed to decrease relapse compared with ALLY-3, and extension of therapy did not significantly improve outcome. The study was too small to sufficiently investigate the impact of NS5A RASs on treatment outcome.

Thus, while DCV + SOF provided a highly effective treatment option for HCV-3 patients without cirrhosis, the management of those patients with cirrhosis remained a challenge. The addition of RBV appeared to play a role in decreasing relapse, but the lack of a randomized study made it difficult to know how great that impact was. Furthermore, the potential for benefit by extending to 24 weeks, as was seen in the SOF/RBV studies, resulted in a knowledge gap created by piecemeal registration studies, although there is increasing real-world data suggesting good effectiveness with this extended course of therapy [74]. This regimen is recommended for the treatment of HCV-3 infection in both the EASL and AASLD/IDSA HCV treatment guidelines, both of which recommend extending to 24 weeks of therapy, with the addition of RBV, when possible, in patients with cirrhosis, and a recommendation for NS5A resistance testing in specific subgroups (Table 2).

5.3 Velpatasvir (VEL)

In June 2016, the approval of velpatasvir (VEL), a pangenotypic NS5A inhibitor, in combination with SOF, ushered in the first fixed-dose pan-genotypic regimen (SOF/VEL) for the treatment of hepatitis C infection [75]. This regimen was the first combination DAA therapy approved for the treatment of all HCV clinical genotypes 1–6.

- ASTRAL-3 was an open-label, randomized trial comparing SOF/VEL (100 mg daily) for 12 weeks with SOF/RBV for 24 weeks in treatment-naive and -experienced HCV-3-infected patients [76]. Overall, SVR12 was 95% versus 80%, respectively, confirming superiority of the combination DAA therapy (Table 1). However, a similar trend emerged: SVR12 was 91% in patients with cirrhosis versus 97% in those without cirrhosis, and 89% in the most difficult treatment-experienced patients with cirrhosis. Furthermore, of the 25 patients with the Y93H NS5A RAS at baseline, 84% (21/25) achieved SVR12, compared with 97% (225/231) of patients without NS5A RASs.

Thus, while SOF/VEL brings great hope for the majority of HCV-3-infected patients, the higher relapse rate in HCV-3 infection ($N = 11/277$) versus all other genotypes ($N = 3/758$) in the HCV mono-infected registration program of patients without cirrhosis, or with compensated cirrhosis, suggests there is room for improvement, particularly for patients with cirrhosis. It is likely that multiple baseline predictors add up to increase the risk of treatment failure, including prior treatment failure, presence of cirrhosis, and presence of high fold NS5A RASs such as the Y93H variant. For this reason, the AALSD/IDSA HCV Treatment Guidance Panel recommends NS5A testing in treatment-experienced patients without cirrhosis and

Table 2 Indications for RAS testing in HCV-3

Treatment regimen	Presence of cirrhosis	Prior treatment	NS5a RAS testing indication
DCV+SOF	−	−	−
	+	−	+
	−	+	+
	+	+	−[a]
SOF+VEL	−	−	−
	+	−	+
	–	+	+
	+	+	−[b]

RAS resistance-associated substitutions, *HCV-3* hepatitis C genotype 3, *NS5a* non-structural protein 5a, *DCV* daclatasvir, *SOF* sofosbuvir, *VEL* velpatasvir

[a] Add weight-based ribavirin and treat for 24 weeks regardless of the presence of NS5a RAS

[b] Add weight-based ribavirin to the 12 week therapy regardless of the presence of NS5a RAS

treatment-naive patients with cirrhosis, and adding weight-based RBV when the Y93H RAS is detected (Table 2). Due to the presence of two of these three negative predictors, patients who are both prior treatment failures and have evidence of cirrhosis are recommended to receive weight-based RBV regardless of NS5A testing results. An active study (NCT02781558) is expected to provide more data on the impact of RBV on treatment response to SOF/VEL in HCV-3-infected patients with cirrhosis.

5.4 Elbasvir/Grazoprevir

Although not approved in the US, the fixed-dose combination of elbasvir (EBR), an NS5A inhibitor, and grazoprevir (GZR), a next-generation NS3/4 protease inhibitor, in combination with SOF for 12 weeks has demonstrated efficacy for the treatment of HCV-3 infection in treatment-naive patients [77].

- C-SWIFT was a randomized trial of combined DAA regimens EBR/GZR + SOF for 8 ($N = 15$) and 12 weeks ($N = 26$) in treatment-naive HCV-3-infected patients with and without cirrhosis [78]. Overall SVR12 was achieved in 93% (14/15) of patients treated for 8 weeks, 100% (14/14) of patients without cirrhosis treated for 12 weeks, and 83% (10/12) of patients with cirrhosis treated for 12 weeks.
- C-ISLE was a randomized, open-label, clinical trial of EBR/GZR + SOF in HCV-3-infected patients with cirrhosis ($N = 100$) and evaluated durations of 8–16 weeks [79]. Treatment-naive patients were randomized to 8 weeks of the triple DAA regimen with RBV versus the triple DAA regimen alone for 12 weeks. SVR12 for these two arms was 91% (21/23) and 96% (22/23), respectively. Both failures in the 8-week arm were relapses, while there was no virologic failure in the 12-week arm. Patients who previously failed PEG/RBV were randomized to one of three arms; (i) triple DAA alone for 12 weeks; (ii) triple DAA with RBV for 12 weeks; or (3) triple DAA regimen alone for 16 weeks. SVR12 for the three arms was 100% (17/17), 94% (17/18), and 94% (17/18), respectively. No treatment-experienced patients (all of whom received 12–16 weeks of therapy) suffered relapse.

5.5 Future Direct-Acting Antiviral Therapies

Now that IFN- and RBV-free treatment regimens for all genotypes have been established, future HCV regimens have pivoted towards the potential to shorten treatment duration and optimize treatment outcomes for the most difficult-to-treat populations, including combination DAA failure with multidrug resistance and HCV-3-infected patients with cirrhosis. There are several new regimens in human studies that share these objectives.

- SURVEYOR-2 was a phase II trial investigating the safety and efficacy of the pangenotypic dual combination of glecaprevir (formerly ABT-493)/pibrentasvir (formerly ABT-530) ± RBV. Glecaprevir is an NS3/4A protease inhibitor, while pibrentasvir is an NS5a inhibitor, both representing next-generation DAA agents in their respective classes [80].Overall SVR12 was 97% ($n = 28/29$) for treatment-naive HCV-3-infected patients without cirrhosis who received 8 weeks of therapy without RBV and 100% (48/48) in treatment-naive HCV-3-infected patients with cirrhosis who received 12 weeks of therapy with ($N = 24$) and without RBV ($N = 24$) [81].
- SURVEYOR-2 Part III expanded on these initial findings and further supported the efficacy of glecaprevir/pibrentasvir in HCV-3. Treatment-experienced patients without cirrhosis were randomized to 12 versus 16 weeks of therapy, while treatment-naive patients with cirrhosis received 12 weeks of therapy and treatment-experienced patients with cirrhosis received 16 weeks of therapy. Cure rates of 91% (20/22) and 96% (21/22) were reported for treatment-experienced, non-cirrhotic patients in the 12- and 16-week arms, respectively. For treatment-naive cirrhotic patients receiving 12 weeks of therapy, an SVR12 was achieved in 98% (39/40) of patients, while an SVR12 of 96% (45/47) was reported in the treatment-experienced, cirrhotic arm. Of note, 4/5 patients who had virologic relapse were noted to have multiple NS5A RASs, with the remaining patient who had a relapse having one NS5a RAS [82].
- C-CREST 2 was a phase II trial investigating the safety and efficacy of the triple combination therapy of MK-3682/GZR/MK-8408. MK-3682 is an NS5B inhibitor and ruzasvir (formerly MK-8408) is a second-generation NS5A inhibitor [83]. Patients were randomized to four therapy arms investigating the dosing of MK-3682 and EBR versus the new MK-8408. The treatment duration for all regimens was 8 weeks. The overall rate of SVR12 in HCV-3-infected, treatment-naive patients without cirrhosis was 86–95%, suggesting 8 weeks will not be the optimal therapy with this regimen for this patient population.
- Part B of C-CREST 2 investigated the safety and efficacy of 8, 12, or 16 weeks of the triple DAA regimen (MK-3682/GZR/MK-8408) with and without RBV in HCV-2 and HCV-3. For HCV-3 patients, the overall SVR12 was 96%. For the 8-week arm, SVR12 was achieved in 94% (50/53) of patients not taking RBV versus 98% (48/49) in those taking RBV. For the

most challenging subgroup of treatment-experienced patients with cirrhosis, only one relapse in 74 patients was observed, regardless of therapy duration (12 vs. 16 weeks) or the addition or RBV. This lone relapse occurred in the 16-week + RBV group. Population-based RAS sequencing revealed the Y93H RAS was present in 5% (11/206) of the HCV-3 study population, accounting for four of the seven treatment failures in the 8- and 12-week groups [84].

- LEPTON was a phase II trial investigating the safety and efficacy of a pangenotype triple DAA therapy including SOF/VEL and voxilaprevir (VOX; formerly GS-9857), a next-generation NS3/4A protease inhibitor [85]. Three HCV-3-infected groups of patients were included: treatment-naive with compensated cirrhosis, PEG + RBV failures with cirrhosis, and DAA failures with and without cirrhosis. The treatment-naive group received 6 weeks of triple therapy, while the two treatment-experienced groups received 8 weeks of triple therapy. SVR12 was 83% (15/18) in the treatment-naive group and 100% (23/23) in the treatment-experienced groups, including four patients who did not respond to prior DAA therapy.
- POLARIS-3 recruited 219 HCV-3-infected, treatment-naive and treatment-experienced patients with cirrhosis who were randomized to SOF/VEL for 12 weeks or SOF/VEL + VOX for 8 weeks. For SOF/VEL/VOX, an overall SVR12 of 96% (106/110) was reported, including one patient who withdrew consent and one death during the study unrelated to study medication. Treatment-naive patients with cirrhosis had an SVR12 of 96% (72/75) in the SOF/VEL/VOX arm, while treatment-experienced patients with cirrhosis had an SVR12 of 97% (34/35). Only six patients in the SOF/VEL/VOX arm had the Y93H RAS present and all achieved SVR12 [86].

5.6 Resistance-Associated Substitutions

RASs, also referred to as resistance-associated variants, are point mutations that are associated with drug resistance in vitro. However, the genotypic presence of a RAS does not necessarily translate to a phenotypic treatment failure. Like advanced cirrhosis or prior treatment experience, the presence of RASs represent an important factor in overall treatment outcomes, and when combined with other negative predictors may result in treatment failure. In many cases, RASs can be overcome by potent combination DAA therapies, extension of therapy, and/or the addition of RBV. The two primary techniques for genotype sequencing include next-generation (clonal) sequencing, which can detect down to a frequency of 0.5–1% of the viral variants [87], and population sequencing, which can detect a frequency of approximately 20% of the viral variants [88]. Based on the current literature, population-level sequencing is the most clinically relevant [89].

With only one exception, the clinical relevance of resistance testing has been limited to RASs in the NS5A gene. Two RASs in particular, Y93H and A30K, have emerged as the most clinically relevant polymorphisms in HCV-3 with the currently approved regimens, and are present at baseline in up to 8.3 and 6.3% of all HCV-3-infected patients, respectively [90–92]. The ALLY-3 trial of DCV + SOF in HCV-3-infected patients reported that both Y93H and A30K polymorphisms were associated with higher rates of treatment failure, especially in those patients with cirrhosis [72]. Similarly, the ASTRAL-3 trial of SOF/VEL reported a lower SVR12 in those patients with the Y93H polymorphism at baseline (84%) compared with those without the polymorphism (97%) [76]. As a result, the current recommendation from the AASLD/IDSA guideline panel, when a provider is planning to treat HCV-3 infection with SOF/VEL or DCV + SOF, is to perform population level genotyping in patients who are either treatment-naive with cirrhosis or treatment-experienced without cirrhosis (Table 2). For those patients who are treatment-experienced with cirrhosis, RBV should be added regardless of the results of resistance testing [70]. SURVEYOR-2 Part III, investigating the combination DAA glecaprevir/pibrentasvir, reported that of the five patients with virologic relapse, all had at least Y93H present. Additionally, four of the five patients had either A30K or L31F present on population-based sequencing [82]. C-CREST-2 Part B, investigating the efficacy of MK-3682/GZR+ruzasvir, reported an SVR12 of 64% (7/11) for those patients with Y93H present [84]. Finally, POLARIS-3, investigating SOF/VEL vs. SOF/VEL/VOX, reported 100% SVR12 for the six patients with Y93H present and 100% SVR12 for all NS5A RASs for the SOF/VEL/VOX group [86]. With the possible exception of SOF/VEL/VOX, RAS represent a significant risk factor for treatment failure, even in the next-generation, pan-genotype regimens.

6 Clinically Relevant Drug Interactions

Although the overall safety profile of the DAA agents is excellent, it is important to recognize that there are drug interactions that can either impact the antiviral potency of the DAA regimen or potentiate an adverse effect of either the DAA or the concomitant medication. For example, DCV is a substrate of CYP3A4 and must therefore be dose-

adjusted when administered with either inhibitors or inducers of the enzyme [5]. The University of Liverpool hosts a comprehensive, easy-to-use, and up-to-date website that contains all relevant drug–drug interactions for the different DAA regimens (http://www.hep-druginteractions.org) [93]. It is important to review all potential drug interactions prior to starting any DAA therapy. In this section, we will review the most common and severe drug interactions that one may encounter in the treatment of HCV-3.

6.1 SOF-Associated Bradycardia

Both of the currently recommended DAA regimens (SOF/VEL and DCV+SOF) for the treatment of HCV-3 infection are well tolerated with minimal side effects and an overall favorable drug interaction profile. However, there are some notable interactions and toxicities that have recently emerged since approval by both the US FDA and European Medicines Agency (EMA). In the spring of 2015, the FDA and EMA warned that bradycardia could occur when amiodarone was coadministered with SOF as part of a DAA combination regimen [94, 95]. SOF now has a package insert warning, strongly cautioning against use in combination with amiodarone [96]. A case series published by Renet et al. describes two patients who developed symptomatic bradycardia following administration of SOF and amiodarone [94]. The first patient was a 61-year-old female with compensated cirrhosis (CP-A6) who was receiving DCV + SOF for HCV-1b disease, and the second patient was a 50-year-old male with decompensated cirrhosis (CP-B9) who was receiving DCV + SOF for HCV-1b. This case series and FDA guidance was followed by a third case series by Fontaine et al. that describes three patients who developed symptomatic bradycardia while taking SOF [97]. Of note, one of these patients was not taking amiodarone and was only receiving propranolol, while another was not receiving atrioventricular nodal agents of any kind. In search of a potential mechanism of action for this toxicity, Liu et al. used an in vitro model to describe decreased AV nodal conduction in the setting of multiple different DAA agents, with the most profound AV nodal blockade occurring with SOF [98]. In addition, they noted that the effect of nodal blockade was more than additive for infusions of SOF and amiodarone [98]. These findings were further expanded upon by Regan et al. who was able to recreate the SOF + amiodarone-induced bradycardia in animal models using guinea pigs and rhesus monkeys [99]. These data strongly suggest that SOF has an independent mechanism of AV nodal blockade and that coadministration with amiodarone can potentiate a life-threatening bradycardia.

6.2 VEL and Acid-Suppressing Medications

VEL relies on an acidic environment for absorption. Initial pharmacokinetic studies described decreases in both maximum concentration (C_{max}) and area under the curve (AUC) for VEL serum drug levels when coadministered with acid-suppressing medications such as famotidine and omeprazole [100, 101]. This drop in serum drug concentration is particularly pronounced for coadministration with omeprazole regardless of whether they are administered separately or together. If H2-blocking agents are to be administered with SOF/VEL, the package insert recommends dosing simultaneously or 12 h apart, with doses not to exceed an equivalent of famotidine 40 mg [75]. For proton pump inhibitors (PPI) and SOF/VEL, the package insert recommends against coadministration, but notes that if it is necessary it should be taken with food, 4 h prior to a maximum dose of omeprazole 20 mg [75]. No data on the coadministration of SOF/VEL and acid-suppressing medications in patients with CHC are currently available, and any inferences on the subject must be drawn from data on ledipasvir (LDV) in genotype 1. Like VEL, LDV is a first-generation NS5A inhibitor that relies on stomach acid for absorption. Similar to the registration program for VEL, the program for LDV excluded the concomitant use of acid-suppressing medications. Initial data from the real-world Target-C Cohort suggested that patients who were taking any dose of PPI at the start of LDV + SOF therapy had a significantly lower SVR12 (93 vs. 98%) [102]. These findings were expanded on by Tapper et al. who noted that, in another real-world cohort, patients did not have any difference in SVR12 rates if they were taking any PPI or were taking a PPI once daily at higher than the recommended dose [103]. However, they reported decreased rates of SVR12 in patients who were taking PPIs twice daily. These findings have been confirmed in an additional study conducted through the veterans affairs (VA) pharmacy database [104]. The impact of twice-daily administration of PPIs was notable regardless of the presence of cirrhosis, but did have the greatest impact in patients with cirrhosis taking PPIs twice daily, with a reported 20% decrease in SVR12 (76.9 vs. 96.3%) [103]. Given that VEL had greater pharmacokinetic variability with acid-suppressing medication, coadministration with PPIs should be avoided until further data in patients with CHC are available [75].

7 Conclusions

Genotype 3 represents a unique entity within HCV treatment. It is associated with genotype-specific mechanisms of steatosis in addition to accelerated development of

fibrosis and higher rates of HCC. These findings underscore the need for effective therapy for this group of patients. Although DCV + SOF and SOF/VEL has finally brought HCV-3 into the modern DAA era with cure rates comparable to the other genotypes, room for improvement remains, particularly for patients with cirrhosis and NS5A RASs. These are issues that need to be addressed by the next generation of dual and triple pangenotypic regimens. Furthermore, how HCV eradication by current and future DAA regimens impacts the natural history of liver disease with this infection remains unclear and follow-up studies of steatosis resolution and fibrosis regression are needed. The DAA era has truly revolutionized HCV therapy, but we must still work to ensure that no subgroup, regardless of genotype, cirrhosis, or treatment experience, is left by the wayside.

Compliance with Ethical Standards

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