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REVIEW

Resistance detection and re-treatment options in hepatitis C virus-related chronic liver diseases after DAA-treatment failure

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Abstract

Background Introduced in 2013–2014, the second- and third-wave directly acting antivirals (DAAs) have strongly enhanced the efficacy and tolerability of anti-HCV treatment, with a sustained virological response (SVR) in 90–95% of cases treated. The aim of this paper was to focus on the type and prevalence of viral strains with a reduced sensitivity to DAAs and on treatment choices for DAA-experienced patients.

Methods The Medline was searched for "HCV infection", "HCV treatment", "Directly acting antivirals","HCV resistance".

Results Most patients who did not achieve an SVR have been found to be infected with HCV mutant strains with a reduced susceptibility to these drugs. These mutants occur frequently in the NS5A region, with a moderate frequency in the NS3/4A regions and rarely in the NS5B region. Treatment-induced mutants resistant to NS5A DAAs persist for years after treatment discontinuation, whereas those resistant to the NS3 DAAs have a shorter duration.

Conclusions Patients who have failed HCV treatment with DAA agents have several re-treatment options, but re-treatment selection may be intricate and resistance testing is recommended to optimize this choice. It is, therefore, important to bear in mind that the correct determination of HCV genotype and subtype and the identification of RASs are essential elements for choosing the optimal re-treatment. It is supposed that it is useful to give readers some other suggestions regarding therapeutic reprocessing.

Keywords HCV infection · HCV treatment · Directly acting antivirals · HCV resistance

Introduction

Hepatitis C virus (HCV) causes a symptomless acute hepatitis that progresses to chronicity in nearly 70% of cases. HCV-related chronic hepatitis is characterized by a long-term persistence of liver necro-inflammation and fibrosis, leading to liver cirrhosis in nearly 20% of the cases in 20 years; furthermore, HCV-cirrhotic patients develop hepatocellular carcinoma (HCC) with a 3–4% yearly rate [1, 2]. Several factors may speed this progression, such as old age at the time of infection, a concomitant metabolic disorder, an impaired host immune response, alcohol abuse and coinfection with HBV or HIV [2–4].

Up to 3% of the world's population live with HCV infection [5, 6], with more than 300,000 deaths and 3–4 million new cases per year [5–7]. A high prevalence (> 3.5%) of subjects with the antibody to HCV (anti-HCV) have been reported in some countries in Eastern Asia, Africa and Middle East regions [8], whereas lower values have been observed in Western countries, from 1.8% in the USA to 0.6% in Germany.

Currently, intravenous drug use, exposure to medical procedures (e.g., surgery, dialysis, dental treatment) and unsafe sex (e.g., sex with multiple partners, anal sex, sex with trauma, sex in the presence of genital lesions) are factors associated with a high risk of acquiring HCV infection [9–13]. Vertical transmission to newborn babies accounts only for 5% of the cases (from 5.8 to 10.8% if mothers are coinfected with HIV) [14]. Needlestick injury occurs

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frequently in health care workers, but its epidemiologic role is limited [15].

HCV virology

HCV is a positive sense, single-stranded RNA virus of the Flaviviridae family, with a genome of 9.6 kb containing a single open reading frame (ORF) flanked by 5-and 3-untranslated regions [16].

The ORF codes for a single polyprotein of approximately 3000 residues, cleaved into four structural proteins (core, E1, E2 and P7) and seven non-structural (NS) genes by cellular and viral proteases. Three NS genes, targeted by the directly acting antivirals (DAA) in clinical practice, play an essential role for viral replication [17]: NS3, NS5A and NS5B.

The NS3 is dedicated to the cleavage at the NS2/NS3 site, which occurs rapidly by a conformation-dependent autocatalytic mechanism [18]. The NS5A is a membrane-associated phosphoprotein of unknown structure and function, found in a basally phosphorylated form of 56 kDa and in a hyper phosphorylated form of 58 kDa. The function of NS5A in the HCV replication cycle is unknown, but adaptive mutations cluster in the central region of NS5A in the replicon system [19], suggesting its involvement in viral replication, either directly and/or by interaction with cellular proteins and pathways. This, together with the modulation of NS5A hyper-phosphorylation by the nonstructural proteins 3, 4A, and 4B [20] supports the view that NS5A is an essential component of the HCV replication complex [21]. NS5B is an RNA-dependent RNA polymerase, structurally organized in a characteristic "right hand motif" containing palm and thumb domains. The error-prone nature of the HCV NS5B polymerase and the accumulation of mutations in a small hyper-variable region in the envelope-encoding genes generate a high level of variability. This variability translates into the existence of seven major HCV genotypes (with 30–35% variation at the nucleotide level), 67 subtypes (with less than 15% difference at the nucleotide level) each composed of a myriad of viral quasi-species, and nine recombinant forms (e.g., the most frequently reported genotype 2 k/1b, which has multiple isolates) [22]. Each genotype exhibits a different degree of variability: 7 subtypes in genotype 1, 11 in genotype 2, 6 in genotype 3, 17 in genotype 4, 24 in genotype 6 and only 1 in genotypes 5 and 7 [23]. There are several consequences related to this enormous viral heterogeneity, such as the possibility of re-infections with a different viral strain because of the limited cross-antigenicity, the emergence of immune-escape mutants accounting for the high rate of patients who progress to chronicity, the genotype- and subtype-specific response to treatments and the spontaneous or drug-induced selection of viral-resistant strains.

Treatment of chronic HCV infection

For more than a decade and until 2013, the combination of pegylated interferon-alpha (peg-IFN) and ribavirin (RBV) was the treatment of choice for HCV infection. This combination therapy provided a sustained clearance of circulating HCV (sustained viral response—SVR) in half of the patients infected with HCV genotype 1, in about 70% of those with genotype 2 and in about 60% of those with HCV genotype 3. Besides an insufficient response rate, this combination therapy was poorly tolerated and produced serious adverse reactions [24–30].

Treatment of HCV infection has been revolutionized by the recent availability of the well-tolerated, potent directly acting antivirals (DAAs), which, used in combination, allow excellent chances for an HCV clearance, prevention of disease progression and reduction of both liver-related and overall mortality [31–34]. Several IFN-free DAA regimens allow up to 95% HCV clearance after a 12-week treatment [34, 35] in DAA-naïve patients: different treatment schedules have been recommended, depending on the HCV genotype and subtype and on the presence or absence of cirrhosis (Table 1).

Around 5% of patients fail to eradicate HCV infection, mostly due to the presence of cirrhosis, HCV genotype 3 or resistance-associated substitutions (RASs). The high error rate of the HCV polymerase and the fast virion production result in a mixture of naïve viral genetic populations, termed quasi-species, existing even before treatment. In an Italian study, a pre-treatment presence of NS5A RASs was detected in 14 (23.0%) of 61 patients with HCV genotype 1b and in 3 (4.9%) of the 61 with HCV genotype 1a; the pangenotypic RAS Y93H was detected in 1 (1.6%) patient with genotype 1a and in 4 (6.6%) with genotype 1b [36].

RAS distribution in the three HCV regions targeted by DAAs

The detection of RASs may be made by population sequencing or next-generation sequencing (NGS). Population sequencing can detect only variants representing > 15–20% of the quasi-species, NGS can reach those with a 0.1–1% prevalence. Although NGS has a better sensitivity, it is noted that a 15% cutoff for minor variant frequency better predicts DAA-treatment failure and that drug-specific RASs identified at a lower frequency do not reduce SVR rates with currently available DAA regimens [37–41].

The emergence of RASs, the main cause of failure to DAA therapy, depends on the HCV genotype and the DAA used (Tables 2, 3, 4).

Table 1 Therapeutic options in patients with HCV infection naïve for DAA regimens

Genotype	Liver diseases stage	Recommended DAA regimens	Duration, **weeks**	References
1a	Chronic hepatitis	Glecaprevir/pibrentasvir	8	[37, 106]
		Elbasvir/grazoprevir	12[a]	
		Elbasvir/grazoprevir + RBV	16[b]	
		Ledipasvir/sofosbuvir	12	
		Sofosbuvir/velpatasvir	12	
		Paritaprevir/ritonavir/ombitasvir with dasabuvir + RBV	24	
1a	Cirrhosis			[37, 106]
	Child–Pugh A	Glecaprevir/pibrentasvir	12	
		Elbasvir/grazoprevir Elbasvir/grazoprevir + RBV	12[a]	
		Ledipasvir/sofosbuvir	16[b]	
		Sofosbuvir/velpatasvir	12	
	Child–Pugh B	Sofosbuvir/velpatasvir + RBV	12	
1b	Chronic hepatitis	Glecaprevir/pibrentasvir	8	[37, 106]
		Elbasvir/grazoprevir	12	
		Ledipasvir/sofosbuvir	12	
		Sofosbuvir/velpatasvir	12	
		Paritaprevir/ritonavir/ombitasvir with dasabuvir	12	
1b	Cirrhosis			[37, 106]
	Child–Pugh A	Glecaprevir/pibrentasvir	12	
		Elbasvir/grazoprevir	12	
		Ledipasvir/sofosbuvir	12	
		Sofosbuvir/velpatasvir	12	
		Sofosbuvir/velpatasvir + RBV	12	
	Child–Pugh B	Sofosbuvir/velpatasvir + RBV	12	
2	Chronic hepatitis	Glecaprevir/pibrentasvir	8	[37, 106]
	Cirrhosis			
	Child–Pugh A	Sofosbuvir/velpatasvir	12	
		Glecaprevir/pibrentasvir	12	
	Child–Pugh B	Sofosbuvir/velpatasvir + RBV	12	
		Sofosbuvir/velpatasvir + RBV	12	
3	Chronic hepatitis	Glecaprevir/pibrentasvir	8	[37, 107]
		Sofosbuvir/velpatasvir	12	
	Cirrhosis			
	Child–Pugh A	Sofosbuvir/velpatasvir	16	
	Child–Pugh B	Sofosbuvir/velpatasvir + RBV	12	
4	Chronic hepatitis	Glecaprevir/pibrentasvir	8	[37, 106]
		Elbasvir/grazoprevir	12[a]	
		Elbasvir/grazoprevir + RBV	16[b]	
		Ledipasvir/sofosbuvir	12	
		Sofosbuvir/velpatasvir	12	
		Paritaprevir/ritonavir/ombitasvir with dasabuvir + RBV	12	
	Cirrhosis			
	Child–Pugh A	Glecaprevir/pibrentasvir	12	
		Sofosbuvir/velpatasvir	12	
	Child–Pugh B	Sofosbuvir/velpatasvir + RBV	12	
5, 6	Chronic hepatitis	Glecaprevir/pibrentasvir	8	[37, 106]
		Sofosbuvir/velpatasvir	12	
		Ledipasvir/sofosbuvir	12	
	Cirrhosis	Glecaprevir/pibrentasvir	12	
		Sofosbuvir/velpatasvir	12	
		Ledipasvir/sofosbuvir	12	

Table 1 (continued)

[a]Without baseline NS5A RASs and/or HCV RNA ≤ 800.000 UI/mL

[b]With baseline NS5A RASs and/or HCV RNA > 800.000 UI/mL

NS3/4A protease inhibitors and corresponding RASs (Table 2)

The first-generation PIs boceprevir and telaprevir act only against HCV genotype 1, have a low genetic barrier to resistance and considerable cross-resistances involving amino acids at positions V36, T54, R155 and A156. Instead, the second- and third-generation PIs (simeprevir, asunaprevir, paritaprevir, grazoprevir, vaniprevir, glecaprevir, danoprevir, voxilaprevir, sovaprevir and vedroprevir) have a higher genetic barrier and an enhanced antiviral activity against the different HCV genotypes (GT) [42].

Among patients with HCV GT 1, the NS3/4A mutations are detected more frequently in those with GT 1a than in those with GT 1b [43].

Boceprevir is used to treat patients with GT 1 HCV infection. The resistance mutations most frequently selected by boceprevir are V36M, T54S and R155K in GT1a-infected patients and T54A/S, V55A, A156S and V170A in those infected with GT1b [44].

Telaprevir is used to treat GT1 HCV infection; the most frequent resistance mutations selected by this drug are V36M and R155K in GT1a-infected patients and V36A, T54A and A156S in the GT1b-infected [45].

Simeprevir (SIM), a drug approved to treat HCV GT1 infection, most frequently selects R155K and D168E/V resistance mutations in GT1a-infected patients and Q80R and D168E/V in the GT1b-infected. The presence of the Q80K polymorphism substantially reduces the rate of SVR in GT 1a patients with cirrhosis, an effect not observed in those without cirrhosis; in patients with Q80K at baseline who did not achieve an SVR, an additional resistance-associated substitution (mainly R155K) may be selected during treatment failure [46].

Asunaprevir is used to treat GT1 and GT4 HCV infections. The most frequent resistance mutations selected by asunaprevir are R155K and D168E in GT1a-infected patients and D168E/V/Y in GT1b-infected patients [47, 48].

Paritaprevir, a drug approved to treat GT1 and GT4 HCV infection, may select D168A/V/Y in GT1a-infected patients, Y56H and D168V in the GT1-infected and D168V in the GT4-infected [49].

Grazoprevir is used to treat HCV GT1 infection; the most frequent resistance mutations selected by this drug are Q80K and D168A in GT1a-infected patients and T54S and V170I in the GT1b-infected [50].

Vaniprevir has been developed to treat GT1 HCV infection. The most frequent resistance mutations selected by this drug are R155K and D168T/V/Y in GT1a-infected patients and D168H/T/V in the GT1b-infected [51, 52].

Glecaprevir has a pangenotypic activity; the most frequent resistance mutations selected are V36M and D168A in GT1a-infected patients and Y56H and D/Q168K/R in the GT1b-infected [53, 54].

Voxilaprevir (VOX) has a wide pangenotypic activity. The most frequent resistance mutations selected by this drug are R155W and A156T/V in GT1a-infected patients, A156V in the GT1b-infected and R155G in the GT3-infected [55].

NS5A inhibitors and corresponding RASs (Table 3)

The NS5A inhibitors are characterized by a broad genotypic coverage and by a relatively low barrier to resistance. The NS5A mutations may be detected as natural baseline polymorphisms, and in this case the degree of resistance may have clinical relevance, as occurs for Y93H.

Daclatasvir has a pangenotypic activity and may select M28T, Q30E/H/R, L31M, H58D in GT1a-infected patients, L31M/V and Y93H in the GT1b-infected, M28I, Q30K and Y93H in those with GT3 infection and Q30H/S in those with GT4 infection [56–58].

Ledipasvir (LDV) is an NS5A inhibitor used to treat GT1 HCV infection. The most frequent resistance mutations selected by ledipasvir are Q30E/R, L31M and Y93C/H/N in the GT1a-infected and Y93H in the GT1b-infected [59, 60].

Ombitasvir has a pangenotypic activity and most frequently selects M28T and Q30R resistance mutations in GT1a-infected patients, Y93H in GT1b-infected and L28V in the GT4-infected [61].

Elbasvir is an NS5A inhibitor with pangenotypic activity that most frequently selects the resistance mutations M28T, Q30R, L31V and Y93H in GT1a-infected patients, L31V and Y93H in GT1b-infected patients and A30K, L31F and Y93H in those with GT3 infection [62].

Velpatasvir (VEL) is an NS5A inhibitor with pangenotypic activity. The most frequent resistance mutations selected by this drug are M28T, Q30R/H, L31M/V and Y93H/C/N in GT1a-infected patients, Q30R, L31M/V and Y93H/C/N in the GT1b-infected, L31I/M/V and Y93H in patients with GT2 infection and E92K and Y93H in the GT3-infected [63, 64].

Pibrentasvir is an NS5A inhibitor with pangenotypic activity. The most frequent resistance mutations selected by this drug are M/L/F28G, Q30R, L31M and Y93H in GT1a-infected patients and K24F, M/L/F28K, Q30K, L31F/I and Y93H in the GT3-infected [49, 65].

Table 2 RASs in NS3 region with fold-change compared to wild-type replicon according to HCV genotype

Mutation	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
V36M/A/G/L	Paritaprevir Asunaprevir Simeprevir Grazoprevir Vaniprevir Glecaprevir Voxilaprevir	1a-1b-6	Paritaprevir L, 2(1a), M3(1a), A3(1a) Asunaprevir L, 2(1b) Simeprevir A,3(1a), M3(1a), L(1a) Grazoprevir L,(1a), M(1a), A2(6) Vaniprevir M, 4(1a), M(1b) Glecaprevir M(1a) Voxilaprevir G, 3(1a), L2(1a), M(1a), A4(1b), M2.5(1b)	[95, 97–113]
Q41R/K	Simeprevir Vaniprevir Voxilaprevir	1a-1b-3-4-6	Simeprevir R, 12(1b) Vaniprevir R, 5(1b) Voxilaprevir R, 2.5(1a), R,3(3), R,2.5(4), R,2.5(6), K,2.5(3)	[114, 115]
V55A	Paritaprevir Asunaprevir	1a-1b	Asunaprevir A, 1(1b)	[95, 97, 101, 113, 116, 117]
Y56H/F/L	Asunaprevir Paritaprevir Grazoprevir Glecaprevir Voxilaprevir Sovaprevir	1a-1b-3-4-6	Paritaprevir H,11(1b), H,3,(1a),H,8(4) Grazoprevir H, 18(1a), H,13,(1b) Voxilaprevir H, 2.5,(6)	[95, 104, 105, 117–119]
Q80K/L/R/G, H/	Simeprevir Asunaprevir Paritaprevir Grazoprevir Vaniprevir Voxilaprevir	1a-1b-3	Simeprevir K, 11(1a), K,9(1b), G,7,(1b), H,7 (1b), L,2,(1b), R,15,(1a), R,15,(1b) Asunaprevir K, 7,(1b), R,4,(1b) Paritaprevir K, 3,(1a), K,2;(1a), R,2,(1a) Grazoprevir K, 2,(1a) Vaniprevir K, 7(1a), K,8,(1b) Voxilaprevir K, 5,(1a), K2.5(3), R,1,(3)	[99, 101, 106, 112, 113, 115, 120, 121]
S122G/R/A/I/T/D/N	Simeprevir Asunaprevir	1b-1a	Simeprevir G, 0.5(1b) Simeprevir R, 21(1b), simeprevir R, 20(1a) Simeprevir N, 8 (1a)	[99, 103, 104, 113, 122]
I132V	Simeprevir	1a		[104, 123]
R155K/T/Q/G/W/C/ I/S/L	Simeprevir Asunaprevir Paritaprevir Vaniprevir Grazoprevir Voxilaprevir	1a-1b-4	Simeprevir K, 95(1a), K,33(1b), K163(4), G,20(1b), T, 100(1a) T, 25(1b), Q, 3(1a), Q 28,(1b),W, 100(1b) Asunaprevir K, 21(1b) Paritaprevir G, 14(1a), K,95(1a), K,40(1b), K,45(4), C,59(4), Q,30,(1a), S,7(1a),T,7(1b), T,7,(1a), W,11(1a) Vaniprevir G, 182,(1a), K>1000,(1a) K,350(1b), N,40(1a), Q,819,(1a), Q132(1b), S,73(1a), T 35,(1a), T,200(1b) Grazoprevir G, 28(1b), K,6(1a), K,2(1b). K,6(4), Q,35(1a), T,10(1a), T,208(1b) Voxilaprevir G, 5(1a), K 3(1a), W,56 (1a), W,96 (1b)	[99, 100, 104, 109, 113, 116, 124–132]
A156S/G/T/A/E/F/H/ N/V/Y/K/Y	Asunaprevir Simeprevir Paritaprevir Grazoprevir Vaniprevir Voxilaprevir	1a-1b-3-4-5-6	Asunaprevir S, 7(1b), T6(1b),V, 20(1b) Simeprevir G, 27(1a), G,19(1b), G,26(4) T,100(1a), T,60(1b), V,100(1a),V,196,(1b) Paritaprevir G, 4(1a), S,2,(4), H,323(4), S,9(1a) S,8(4), T,17(1a), T,7(1b), T,40(4), V,4(1b).V,323(4) Vaniprevir G, 182(1a), G,16,(1b), S,16(1a), S,10(1b), T,997(1a) T,118(1b), V,150(1a), V,725(1b) Glecaprevir T, 1400(1a), T,630(1b), V,1800(1b), G,1500(3) V,131(1a), V,6(1b) Voxilaprevir L:>2.5(1a), T,581(1a), S,>2.5(1b) T,553(1b), T>2.5(3), T>2.5(4), T>2.5(5), T>2.5(6)	[51, 104, 106, 129, 132–135]

Table 2 (continued)

Mutation	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
D168A/C/E/G/H/Q/T/V/Y/N/F/I/K/S/R/L	Asunaprevir Simeprevir Paritaprevir Grazoprevir Vaniprevir Voxilaprevir	1a-1b-2a/2b-3-4-5-6	Asunaprevir A, 127(1b), C,65(1b), E78(1b), G,16(1b), H98(1b) V,280(1b)Y,238(1b) Simeprevir A, 79(1a), A948(1b);E,49(1a), E,90(1b), G,4(1b) H,801(1a), H,401(1b),I,1800(1b),N,7(1b), Q,<700(1b), Y,666(1b) T,334(1b) V1610(1a), V,3100(1b) Paritaprevir A, 951(1a), A76(1b), E,14(1a), E,4(1b,F219(1a) H,323(4)H,62,(1a), H,159(1b), K,882(1b), N,13(1a), N,100(1b) T,49(1b),V,115(1a), V,257(1b), V,313(4), Y,219,(1a), Y337(1b) Grazoprevir A, 273(1a), A,59(1b), E,16(1a), E,3(1b), E,6(6), G,34(1a), G,11(1b), G 31(6), H: 52(1b), K,120(1b), N,3(1a), N,4(6); T,88(1a); V,153(1a), V,14(1b), V,13(6), Y,27(1a),8(1b) Vaniprevir A, 21739(1a), A,200(1b), E,40(1b), E,57(1a), F,8473(1a), F,1120(1b), G,55(1a), H,132(1a), H,408(1b), N,20(1a), N 30(1b), T,588(1a), T,722(1b), V,588(1a), V,725(1b), Y,156(1a), Y430(1b) Voxilaprevir A >2.5(1a), A,3(1b),A>2.5(5), A>2.5(6), E,3(1b), E>2.5(4), F>2.5(1a), H,>2.5(5),H>2.5(6), I,>2.5(1a), K,>2.5(1a), K>2.5(5), L,>2.5(1a), R:>2.5(1a), R,>2.5(5), T,>2.5(1a), T>2.5 (2a, 2b), T>2.5(4), V>2.5(1a), V,3(1b), V,>2.5 (2a, 2b), V>2.5(4),Y.>2.5(1b), V>2.5(5)	[51, 97, 99, 106, 115, 117, 120, 121, 125, 128, 131]
I/V170A/T/M	Asunaprevir Simeprevir Danoprevir Voxilaprevir	1b	Simeprevir T15(1a), T,5(1b), V,8(1a) Danoprevir T, 3(1a) Voxilaprevir A, 6(1b)	[101, 104, 110, 124, 137]
V36A/M+R155K/T	Asunaprevir Simeprevir Paritaprevir Vaniprevir Grazoprevir	1a-1b	Asunaprevir 36M + 155K, 72(1b) Simeprevir 36M + 155K, 72(1a),60(1b) Paritaprevir 36M + 155K, 79(1a) Vaniprevir 36M + 155K, 1223 (1a), 440(1b) Grazoprevir 36M + 155K, 12(1a), 4.5(1b)	[51, 110, 135, 136]
F43S + D168E	Simeprevir	1b	Simeprevir 43S+168E, 694(1b)	[72, 124]
F43S+A156G	Simeprevir	1b	Simeprevir 43S+156G, 50(1b)	[72, 124]
F43S+Q80R	Simeprevir	1b	Simeprevir 43S+80R, 286(1b)	[72, 124]
Q80H/K/L+D168E/V/Y	Asunaprevir Simeprevir	1b	Asunaprevir 80K/L+168V/Y/E, 281 (1b) Simeprevir 80H+168E, 163(1b)	[102, 103]
Q80K/R+R155K	Simeprevir Paritaprevir	1a-1b	Simeprevir 80K/R+155K 2000(1a), 364(1b) Paritaprevir 80K+155K, 19(1a)	[104, 106]
Q80K/R/L+D168V/Y/E	Asunaprevir Simeprevir	1a-1b	Asunaprevir 80K/L+168V/Y/E, 280 (1b) Simeprevir 80K/R+168E 589 (1a) 412 (1b)	[102, 103]
Q80K+V170T	Simeprevir	1a-1b	Simeprevir 80K+170T, 120 (1a), 41(1b)	[102, 103]
Q80L/R/K+R155K/W	Simeprevir	1a-1b	Simeprevir 80L+155K, 128 (1b); 80R+155W, 213 (1b) Paritaprevir 80K+155K, 19 (1a)	[102–104, 106]
Q80R+A156V	Simeprevir	1b	Simeprevir 80R+156V,400 (1b)	[102]
S122G/R+R155K	Simeprevir	1a-1b	Simeprevir 122G+155K, 16(1a), 11(1b) 122R+155K, 750(1a), 194 (1b)	[136]

Table 2 (continued)

Mutation	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
R155K/Q/W+D168/A/N/V/H/E	Simeprevir Grazoprevir Vaniprevir	1a-1b	Simeprevir 155K+168A, 552 (1b) 155K+168E, 439 (1a), 162 (1b) Grazoprevir 155K+168A, 6 (1a) Vaniprevir 155Q+168N, 654 (1b)	[51, 102]
R155K+I/V170T/A	Simeprevir Asunaprevir	1a-1b	Simeprevir 155K+I/V170T, 92(1a), 8(1b) Asunaprevir 155K+V170A, 21(1b)	[47, 102]
A156V+D168V	Simeprevir Glecaprevir	1b	Simeprevir 156V+168V, 900(1b) Glecaprevir 156V+168V, 3700(1b)	[115]
Q80K/R+R155K+D168E	Simeprevir	1a-1b	Simeprevir 80K+155K+168E, 768 (1a), 183 (1b) 80R+155K+168E, 575 (1a), 1410 (1b)	[102, 103, 106, 124]
Y56H+D/Q168A/V/Y/N/R	Paritaprevir Grazoprevir Glecaprevir	1a-1b-3a-4a/d	Paritaprevir 56H+D168A/V/Y, 561(1a), 4118 (1b), 12533 (4a/d) Grazoprevir 56H+D168A, 1890(1a), 606(1b) 56H+D168N, 61(1a) Glecaprevir 56H+Q168R, >1000(3a); 56H+D168V 1100(3a)	[104, 134]
P89L/R+A156V/T	Glecaprevir	1a-1b	Glecaprevir 89L+156V, 4500 (1b) 89R+156T, 3700 (1a)	[134, 137, 138]

NS5B inhibitors and corresponding RASs (Table 4)

Given their different mechanisms and sites of interaction, no cross-resistance exists across nucleotide and non-nucleoside polymerase inhibitors.

Sofosbuvir (SOF) is a nucleoside NS5B polymerase inhibitor with pangenotypic activity whose most frequent resistance mutations are L159F and C316N in GT1a-infected patients, S282G/T and C316N/H/F in the GT1b-infected, L159F and S282T in those with GT2 infection, L159F and V321A in the GT3-infected and E237G, S282T and V321A in those with GT4 infection [69–71].

Dasabuvir has been developed to treat HCV GT1 infection; the most frequent resistance mutations selected by this drug are M414T and S556G in GT1a-infected patients and S556G in the GT1b-infected [72].

Therapeutic options for patients who failed a DAA regimen

Despite the excellent efficacy shown by the licensed DAA-based regimens, virological failures occur in around 5–10% of patients treated, mostly associated with RASs selected by the drugs used [73–80]. An example of this is shown by the data from an Italian study where 139 (3.6%) patients out of the 3,830 DAA-treated for advanced fibrosis (F3) or cirrhosis did not achieve an SVR. The failure rate was 7.6% for patients treated with a suboptimal, i.e., no longer recommended (sofosbuvir + ribavirin or simeprevir + sofosbuvir ± ribavirin) and 1.4% for those treated with a combination of sofosbuvir/daclatasvir, sofosbuvir/ledipasvir or other effective DAA treatments. Of these 139, 72 patients were re-treated with a second DAA regimen (38 with sofosbuvir + daclatasvir, 27 with sofosbuvir + ledipasvir, and 7 with other DAAs ± ribavirin), with an SVR12 in 96% of cases [81].

As regards RASs and the therapeutic options for re-treatment, it is useful to remember that the NS5A inhibitors have a low barrier to resistance and that the variants they select frequently confer cross-resistance across the drug class [87, 88], persist longer than those selected by NS3 and NS5B inhibitors [89–93] and represent a challenge for DAA-based therapy [50, 83–85].

The HCV resistance test may identify the clinically relevant RASs, but current guidelines do not offer a clear indication to their use. In fact, the American Association for the Study of the Liver recommends a resistance test for RASs only for patients who failed a DAA regimen including an NS5A inhibitor and, in positive cases, to test also for NS3 RASs, whereas a panel of Italian experts proposed resistance testing for all three genes (NS3, NS5A and NS5B) [86]. In our view, the analysis of the presence of RASs in all three HCV regions is needed to choose the best re-treatment option in patients with DAA failure. In truth, only fragmentary data come from limited clinical trials and real-life studies and currently re-treatment approaches should be based on expert opinions. We must also point out that the AASLD recommends deferring re-treatment unless it is an urgent priority.

Hezode et al. described 16 patients who had failed an NS5A-based treatment with daclatasvir plus pegylated interferon (Peg-IFN) and ribavirin (RBV), with or without

Table 3 RASs in NS5A region with fold-change compared to wild-type replicon according to HCV genotype

Mutation	Reduced sensibility to	genotype	Mean fold-change in Resistance Compared to Wild-Type Replicon [Drug, substituted aa, fold (HCV genotype)]	References
K/Q/S24R/H/G/N/Q/K	Daclatasvir Ledipasvir Ombitasvir Velpatasvir Pibrentasvir	1a-1b-3-4	Daclatasvir K, 41(6) Ledipasvir G, <100(1a), Q,<100(1a), R,3.7(1a) Velpatasvir K, 1b(<100)	[109–113, 117, 124]
M/L/F/28/A/T/V/G/M/C/I/V/F/S	Daclatasvir Ledipasvir Ombitasvir Elbasvir Velpatasvir Pibrentasvir	1a-1b-2-4	Daclatasvir A, 4591(1a),C,400(2),M,2(1b), M,10(4),T,695(1a),T,1b(25) S,9133(2), V,1(1a) Ledipasvir A,1000(1a), G,1000(1a), M,2(1b), M100,(4) Ombitasvir I, 8(4a), M,2(1b), T,8965(1a), T,661(1b), V,58(1a), V,23(4a), V,310(4d) Elbasvir A, 91(1a), G,71429(1a),M,3(1b), T,22(1a) Velpatasvir A, <100(1a), F,>2.5 (2b), G,>100(1a), M, >2.5(6), S, >2.5 (2a), T,8(1a), T, >2.5(4), V,>2.5(6) Pibrentasvir T, 2(1a), V,28(1a)	[57, 63, 95, 104, 139–148]
P29S/DELETION	Daclatasvir	1b		[104]
Q/R/K/A/L30 D/E/G/H/K/L/N/R/T/Y/A/S	Daclatasvir Ledipasvir Ombitasvir Elbasvir Velpatasvir Pibrentasvir	1a-1b-3-4	Daclatasvir A, >10(4), D,333000(1a),E,25205(1a), E,7(1b) G,8500(1a), G,2017(4), H,1475(1a), H,1215(4), H,20(1b), K,24545, K,44(3), L,4(1a), N,>500(1a), Q,10(4), R,1233(1a),R,10(4) T,4(1a), S,157(4), Y,>1000(1a) Ledipasvir C,10(4), E,5458(1a), G,1000(1a), H,183(1a), K,1000(1a), L,<100(1a), N,>100(1a), R,632(1a),R,50(4), T,<100(1a), S,100(4), Y,>1000(1a) Ombitasvir E,1326(1a), K,183(1a), R,800(1a), Y,172(1a) Elbasvir D, 1433(1a), E,56(1a), G,84(1a), H,8(1a), Q,3(1b),R,125(1a) Velpatasvir S, <100(1b), E,18(1a) G,>2.5(1a), H,2(1a), H,>2.5(3) L,1(1a), K,10(1a), K,50(3), R,2(1a) K,50(3), R,2(1a), R,2(4), V1.5(3) Pibrentasvir D, 95(1a),E,2(1a) K,1(3)	[63, 67, 74, 95, 104, 108, 109, 117, 120, 134, 135, 138, 143–147, 149–153]
L31M/I/V/F	Daclatasvir Ledipasvir Elbasvir	1a-1b-3a	Daclatasvir,105(g1a)100 (L31V -1b)15(L31V -1b) Ledipasvir, 554 (g1a) Daclatasvir, 3 (g1b)	[66, 93, 95, 101, 104, 109, 135, 147, 149]
P32L/S/A	Daclatasvir Ledipasvir Ombitasvir Velpatasvir	1a-1b-6	Daclatasvir L 140(1a),17(1b),5000(6) S 382(6) Ledipasvir L, >100(1a),<100(1b) Ombitasvir L, 44(1a) Velpatasvir A, >2.5(6) L, 28(1a),>2.5(6) Q, >2.5(6) R, > 2.5(6)	[49, 63, 68, 117, 135, 141, 142, 149, 152, 159–162]

Table 3 (continued)

Mutation	Reduced sensibility to	genotype	Mean fold-change in Resistance Compared to Wild-Type Replicon [Drug, substituted aa, fold (HCV genotype)]	References
P/H/T58D/A/L/S/N/R/T/G/H/D/P	Daclatasvir Ledipasvir Ombitasvir Elbasvir Velpatasvir	1a-1b-6-4a-3a	Daclatasvir A, 47(6) d, 500(1a) n, 532 (6) s,48 (6) Ledipasvir D 1127 (1a),1000 (1b) Ombitasvir D 243(1a), 1 2.9(4a) Elbasvir d 10 (1a) Velpatasvir A, > 2.5 (2b) D, 7 (1a) G, > 2.5(3a)>2.5 (6) H, > 2.5 (6) R, <100 (1b) T, <100 (1b)>	[48, 57, 58, 61, 63, 65, 117, 119, 133, 140, 141, 144–146, 151, 155, 161–164, 166]
A/C/E92K/R/T/S	Daclatasvir Ledipasvir Ombitasvir Velpatasvir	2a/b/c-1a-6-1b	Daclatasvir R, 133 (2a/b/c) Ledipasvir K, 1000(1b) T, <100(1a) Ombitasvir T (1b) Velpatasvir K, >100(1a)>100(1b) 1(3a) R, >2.5 (2a) S, >2.5 (2b) T, >2.5 (2b)>2.5 (6)	[63, 95, 104, 120, 135, 143, 158, 161, 163–165]
T/Y93C/F/H/N/S/I/R/W/T/L/A	Daclatasvir Ledipasvir Ombitasvir Elbasvir Velpatasvir Pibrentasvir	1a- 1b-2a/b/c-3a -4a/d-6-	Daclatasvir,C, 1864(1a) 4(1b) F, 10(1a) H, 5432(1a) 145(1b) 1750(2a/b/c) 2154(3a) 169(4a/d) N, 50(1b) 47477(1a) S, >500(1a) R, 454(4a/d) W, 1750(4a/d) Ledipasvir C, 1602(1a) 10(4a/d) F, <100(1a) H, 3309(1a) 1319 (1b) 1000(4a/d) N, >14706(1a) S, 1000(1a)>2.5 (1b) 1000(4a/d) T, 10(4a/d) W, 10000 (4a/d) Ombitasvir C, 1675(1a) F, 27(1a) H, 41383(1a) 77(1b) L, 3006(1a) N, 66740(1a) 202(1b) S, 7790(1a) 12 (1b) Elbasvir C, 50(1a) H, 600(1a) 67(1b) N, 1333 (1a) Velpatasvir C, 4(1a) 1(1b)>2.5(6) F, >2.5 (2b) H, 609(1a) 3(1b) 46 (2a, 2b) 724(3a) 3(4a/d)>2.5(6) L, <100(1a) N, 2758(1a) 3(1b)>2.5 (2a, 2b) 13(4a/d)>2.5(6) R, 497(1a)>2.5(1b) S, 64(1a) 1(1b)>2.5(4a/d)>2.5(6) T, >2.5(1a)<100(1b)>2.5(6) W, 999(1a) Pibrentasvir C, 1(1a) H, 7(1a)<2(1b) 3(3a) N, 7(1a)<2(1b)	[49, 54, 57, 60, 63, 64, 72, 95, 117, 132, 135, 142, 145, 146, 155, 157–159, 162, 165]

NS5A RAS pattern mutations	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
M/L28A/M/T/V+Q/L30R/A/H/T/V/K/S	Daclatasvir Ledipasvir Elbasvir	1a-4a/d	Daclatasvir 28A+30R, 287283(1a)28M+30A, 2450 (4a/d)28M+30H, 64(4a/d) 28M+30R, 350(4a/d)28M+30T, 200(4a/d)28M+30V, 100(4a/d)28T+30H, 103767(1a) 28T+30K, 336591(1a)28V+30R, 350(1a) Ledipasvir 28M+30H, 10000 (4a/d)28M+30S, 5000(4a/d) 28V+30R, 100(4a/d) Elbasvir M28T+Q30R, 2286(1a)	[143, 160, 168, 169]
L/F28M/V+L/T31M/F/S	Daclatasvir Ombitasvir	2a/b/c-1b-4a/d	Daclatasvir F28L+L31M, 650(2a/b/c) Ombitasvir 28M+31F, 569(1b) 28V+31F, 2170(1b) 28V+58S, 760(4a/d)>	[170]

Table 3 (continued)

NS5A RAS pattern mutations	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
F/M28+Y93H	Daclatasvir	2a/b/c-1a-3a	Daclatasvir 28L+93H, 1000(2a/b/c)28T+93H, 16623(1a)28V+93H, 279 (3a) 28L+93H, 1000(2a/b/c)	[163, 168]
R/L/Q/A30R/Q/H+M/L31F/V/M	Daclatasvir Ledipasvir Velpatasvir Elbasvir	1a-1b4a/d	Daclatasvir 30Q+31F, 90 (1b) 30Q+31M, 22(1b) 30H+31M, 330(1b) 30R+31V, >33333(1a) 15(4a/d) 30R+31M, 3300000(1a) 30K+31M, 7000(3a) Ledipasvir 30H+31V, 2000(4a/d) Velpatasvir 30H+31M, 106(1a) 30R+31M, 198(1a) Elbasvir 30R+31M, 1196(1a) 30H+31V, 1429(1a) 30R+31V, 7143(1a)	[170]
Q30R+H58D	Daclatasvir Elbasvir	1a	Daclatasvir 30R+58D, 42318(1a) Elbasvir 30R+58D 5882(1a)	[171]
Q30R+H58D	Daclatasvir	1a	Daclatasvir 30R+58D 424318(1a)	[168]
R/A/L/Q30Q/L/H/I/A/V/R/S/D/K+Y93H/R/T/S/C/N	Daclatasvir Ledipasvir Ombitasvir Elbasvir Velpatasvir Pibrentasvir	1a-4a/d-3a-1b	Daclatasvir 30H+93H, 93136(1a) 30E+93H, 67267(1a) 30I+93R,9100(4a/d) 30A+93T, 17500 (4a/d) 30V+93H, 8(3a) 30R+93H, 56817(1a) Ledipasvir 30R+93C, 1046(1a) 30S+93H, 900000(4a/d) Ombitasvir 30Q+93H, 284(1b) 30L+93H, 416(1a) 30L+93S, 218(1a) Elbasvir 30H+93H, 1235(1a) 30D+93N, 42950(1a) Velpatasvir 30H+93H, 2835 (1a) 30R+93H, >419(1a) Pibrentasvir 30K+93H, 70(3a)	[57, 60, 63, 64, 160, 162, 172]
L31F/V/P/H58L/P/S	Daclatasvir Ombitasvir		Daclatasvir 31V+58S, 162(1b) 31V+58P, 12312(1a) Ombitasvir 31F+58L 78 (1b)	[74, 160, 169]

Table 3 (continued)

NS5A RAS pattern mutations	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
L31F/M/V/I+Y93H/N	Ombitasvir Daclatasvir Elbasvir Pibrentasvir	1b-1a	Ombitasvir 31F+93H 10,272(1b) 31M+93H12323(1b) 31V+93H12328(1b) Daclatasvir L31F+Y93H 5721(1b) 31I+93H,2526(1b) 31M+93H,7105(1b) 31V+93H,166667(1a) 14789(1b) 31I+93H,2526 Elbasvir 31M+93H, 7568(1a); 5000(1b) 31V+93H, 53571(1a) Pibrentasvir 31M+93H, 195(1a)	[67, 74, 104, 133, 147, 166, 168, 172]
P/H58S/P/A/L+Y93H/D	Daclatasvir Ombitasvir Pibrentasvir	3a-1a-1b	Daclatasvir P58S+Y93H 3430(3a) H58P+Y93H 1206(1a) Ombitasvir 8A+93H, 1226(1b) 58L+93H,135(1b) 58S+93H,1401(1b) Pibrentasvir 58D+93H, 2000(1a)	[74, 134, 154, 171]
A/S62T+Y93H	Daclatasvir	3a	Daclatasvir 62T+93H, 2783(3a)	[171]
F28L+L31M+C92S	Daclatasvir	2a/b/c	Daclatasvir 28L+31M+92S, 9300(2a/b/c)	[170]
L28M+L/R30H/Q/S+Y93W/S/H	Daclatasvir Ombitasvir	4a/d-1b	Daclatasvir >28M+30H+93W, 1229500(4a/d) 28M+30S+93S, 1218000(4a/d) Ombitasvir 28M+30Q+93H, 981(1b)	[153]
L/M28M/T/V+Q/L30S/H+M/R31V	Daclatasvir Elbasvir	4a/d-1a	Daclatasvir 28M+30S+31V, 110500(4a/d) Elbasvir 28T+30H+31V, 56000(1a) 28T+30R+31V, 89143(1a) 28V+30H+31V, 2429(1a)	[143, 172]
A/R30K/Q+L31M+Y93H	Daclatasvir Pibrentasvir	1b-3a	Daclatasvir 30Q+31M+93H 37,000(1b) Pibrentasvir 30K+31I+93H>1000(3a)	[66, 163]
L31V+Q54H+Y93H	Daclatasvir	1b	Daclatasvir 31V+54H+93H, 19000(1b)	[163]
L31M+P58L+Y93H	Daclatasvir	1b	Daclatasvir 31M+58L+93H, 8300(1b)	[140]
L28I/V+P/T58L/S	Ombitasvir	4a-4d	Ombitasvir 28I+58L, 3795 (4a) 28V+58S, 760 (4d)	[82]
K24Q+T58P+Y93H	Ombitasvir	4d	Ombitasvir 24Q+58P+93H, 1098 (4d)	[167]
K/S24F/T+M28T/K+Q/A30R/K	Elbasvir Pibrentasvir	1a-3a	Elbasvir 24T+28T+30R, 7000(1a) Pibrentasvir 24F+28K+30K, >5000(3a)	[65, 172]
M28T+Q30R+L31V+Y93H	Elbasvir	1a	Elbasvir 28T+30R+31V+93H, 714286(1a)	[172]
S24F+M28K	Pibrentasvir	3a	Pibrentasvir 24F+28K, 271(3a)	[138]

Table 4 RASs in NS5B region with fold-change compared to wild-type replicon according to HCV genotype

Mutation	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
L159F	Sofosbuvir	1b-1a-2a/b/c-3a	Sofosbuvir F, 1(1b)F,2(1a) F,2(2a/b/c) 1(3a)	[104, 108, 116, 117, 124]
E237G	Sofosbuvir	1a-3a-4a/d	Sofosbuvir G, (1a) G, (3a) G, (4a/d)	[95]
S282 R/T/G	Sofosbuvir	1a-1b-2a-2b-3a-4a/d-5-6	Sofosbuvir T, 13(1a),T10(1b) T,8(2a),T,16(2b) T,4(3a) T,6(4a/d) T,18(5) T,9(6)	[70, 83, 95, 104, 156, 173–177]
C316F/H/N/Y	Sofosbuvir Dasabuvir	1a-1b	Sofosbuvir F, 2(1a) F,1(1b) H, 1(1b) N, 1(1b) Dasabuvir H, 229(1b) N, 5(1b) Y, 1472(1a),Y1569(1b)	[95, 104, 117, 150]
L320F	Sofosbuvir	1a	Sofosbuvir F, 3(1a)	[104, 182]
V321A/I	Sofosbuvir	1a-3a	Sofosbuvir A, 1(1a) 1(3a)	[104]
S368T	Dasabuvir	1b	Dasabuvir T, 139(1b)	[183]
A395G	Dasabuvir	1a	Dasabuvir G, 10(1a)	[183]
M414I/T/V	Dasabuvir	1b-1a	Dasabuvir I, 8(1a)I,17(1b) T, 32(1a) T47(1b),V, 5(1a) V18(1b)	[117, 162, 181]
C445F	Dasabuvir	1b	Dasabuvir F, 16 (1b)	[180]
E446K/Q	Dasabuvir	1a	Dasabuvir K, 54 (1a)	[180]
Y448C/H/R	Dasabuvir	1b-1a	Dasabuvir C, 940(1a),C414(1b) H, 975(1a),H,46(1b)	[180]
C451S	Dasabuvir	1b	Dasabuvir S, 16(1b)	[180]
A553T/V	Dasabuvir	1b-1a	Dasabuvir T, 152(1a) V, 120 (1b)	[95, 117, 180]
G554S/D	Dasabuvir	1a	Dasabuvir S, 198 (1a)	[74, 117]
S556G/N/R	Dasabuvir	1b-1a	Dasabuvir G, 30(1a) 11(1b) N, 29(1a) R, 261 (1a)	[44, 72, 74, 117, 150, 170, 181, 183]
G558R	Dasabuvir	1a	Dasabuvir R (1a)	[74]
D559G	Dasabuvir	1b	Dasabuvir G, 110 (1b)	[74, 117, 162]
Y561H	Dasabuvir	1a	Dasabuvir H, 21 (1a)	[162]
S565F	Dasabuvir	1a	Dasabuvir F, 17 (1a)	[179]

NS5B RASs patterns Mutations	Reduced sensibility to	Genotype	Mean fold-change in resistance compared to wild-type replicon [Drug, substituted aa, fold (HCV genotype)]	References
L159F+C316N	Sofosbuvir	1a-1b-2a/b/c-3a	Sofosbuvir 159F+316N, 2(1a) 2(1b) 1(2a/b/c) 1(3a)	[70, 83, 178]
L159F+L320F	Sofosbuvir	1a-1b-3a	Sofosbuvir 159F+320F, 7(1a) 3(1b) 2 (3a)	[178]
L159F+S282T	Sofosbuvir	1a-1b	Sofosbuvir 159F+282T, 30(1a) 15 (1b)	[178]
L320F+S282T	Sofosbuvir	1a	Sofosbuvir 320F+282T, 41 (1a)	[178]
L159F+S282T+L320F	Sofosbuvir	1b	Sofosbuvir 159F+282T+320F, 25 (1b)	[178]

asunaprevir; all 16 patients were re-treated with SOF plus SIM for 12 weeks and an SVR12 was achieved by 14 of them [87].

Feld et al. in the ASTRAL 1 TRIAL evaluated treatment-naive or treatment-experienced patients infected with HCV genotype 1, 2, 4, 5, or 6 treated with SOF/velpatasvir for 12 weeks. In this study, all 48 participants with a previous failure to a protease inhibitor plus peginterferon/ribavirin achieved an SVR12 [64]; similar results were obtained for 27 patients in a prior phase 2 open-label trial [88].

Afdhal et al. evaluated the safety and efficacy of the combination LDV/SOV in genotype 1-infected patients who had failed a prior treatment with an HCV protease inhibitor (telaprevir or boceprevir) plus peginterferon/ribavirin; a 94 and 98% SVR12 rate was obtained, respectively, with a 12- or 24-week LDV/SOF regimen. The presence of cirrhosis and/or a baseline NS5A RAS were the major reasons for relapse in the 12-week re-treatment arm [60].

Reddy et al. [89] performed a post hoc integrated safety and efficacy analysis of the LDV/SOF combination on seven trials with 240 cirrhotic patients, naïve or experienced for a combination regimen including a first-generation PI. Overall, a 96% SVR12 rate was observed; this rate was significantly lower (90%) in patients re-treated for 12 weeks, suggesting that the extension of treatment to 24 weeks or the addition of RBV might have been beneficial in this subset of patients; in the same study, the addition of ribavirin significantly improved the rate of SVR in patients with a baseline NS5A resistance re-treated with LDV/SOF for 12 (from 88 to 94%) or 24 weeks (from 85 to 100%).

The POLARIS-4 trial evaluated the efficacy of the combination SOF/VEL/VOX in re-treating GT1-4 patients who had failed a prior non-NS5A DAA regimen; patients exposed only to an NS3/4A inhibitor were not included. Among patients with a prior DAA failure, 69% had been exposed to SOF plus RBV with or without peg-IFN and 11% to SOF plus SMV. Patients re-treated with SOF/VEL/VOX for 12 weeks showed a higher SVR12 rate (98%) than those in the SOF/VEL arm (90%) [90].

The placebo-control phase 3 POLARIS-1 trial evaluated the efficacy of a 12-week course of SOF/VEL/VOX combination in patients with HCV GT 1–6 infection who had failed a previous DAA regimen containing an NS5A inhibitor. The SVR rate was 96% in patients infected with genotype 1a, 100% in those infected with genotype 1b, 100% with genotype 2, 95% with genotype 3, 91% with genotype 4 and 100% with HCV genotype 6; the only patient infected with HCV genotype 5 achieved an SVR. Overall, the SVR rate was 93% in patients with cirrhosis and 99% in those without.

The MAGELLAN-1 Part 2 randomized, open-label, phase 3 study evaluated the efficacy and safety of the combination glecaprevir/pibrentasvir (G/P) in patients with chronic HCV infection who experienced a virological failure to an NS3/4A protease- and/or an NS5A inhibitor-containing therapy. Patients with compensated liver disease, with or without cirrhosis, and HCV genotype 1, 4, 5, or 6 were 1:1 randomized to receive 12 or 16 weeks G/P treatment. An SVR was achieved by 89% (39 of 44) and 91% (43 of 47) of patients who, respectively, received 12 or 16 weeks G/P treatment; a virological relapse occurred in 9% (4 of 44) of patients treated for 12 weeks and in none of those treated for 16 weeks [101–103].

A study from de Lédinghen reported an SVR12 in 25 of 26 patients with HCV genotype 1 or 4 re-treated with SOF/grazoprevir/elbasvir + ribavirin for 16 or 24 weeks after they had developed a RAS at a prior failure to an NS5A- or NS3-inhibitor-based regimen [94].

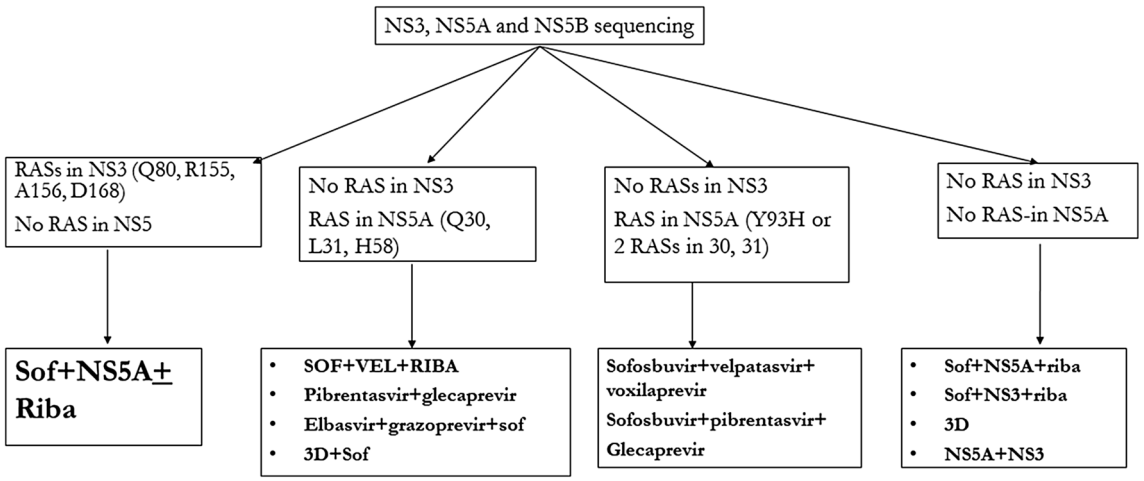

Fig. 1 Re-treatment options in the HCV genotype 1a patients with DAA failure

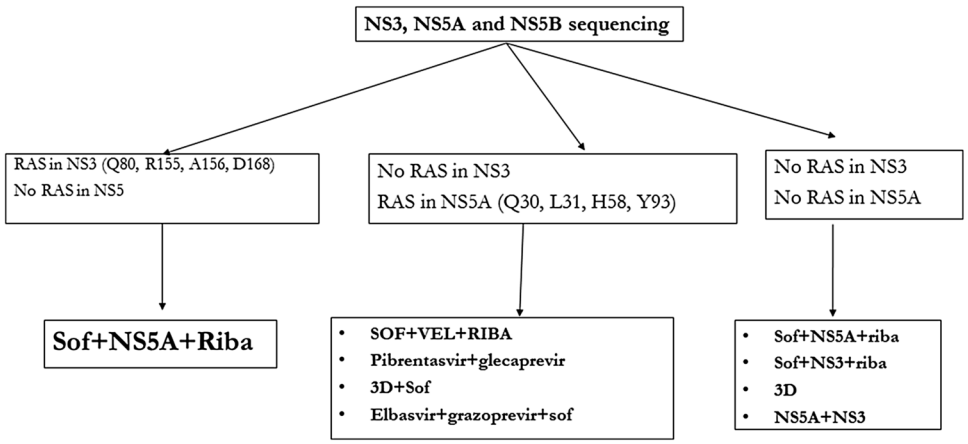

Fig. 2 Re-treatment options in the HCV genotype 1b patients with DAA failure

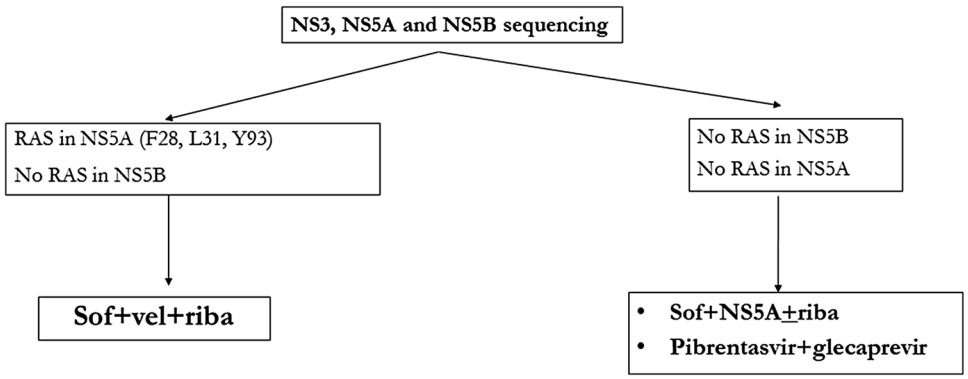

Fig. 3 Re-treatment options in the HCV genotype 2 patients with DAA failure

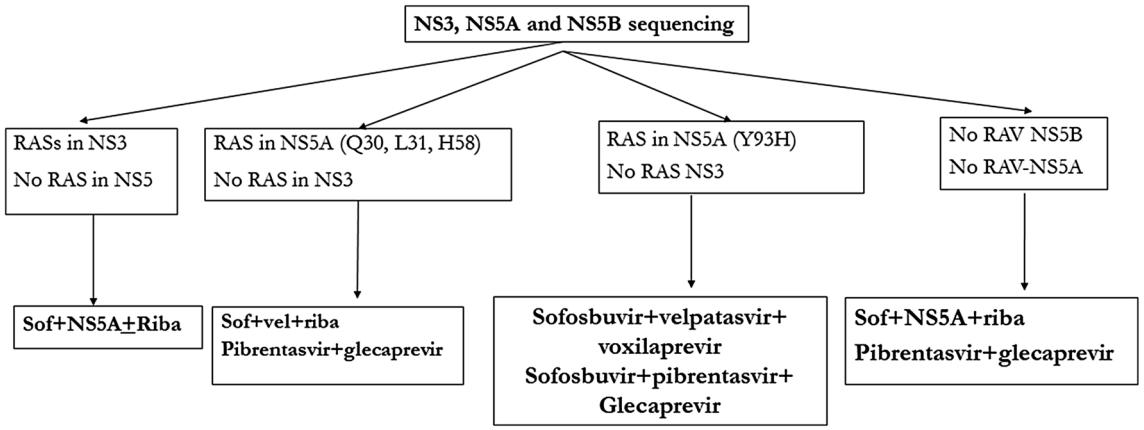

Fig. 4 Re-treatment options in the HCV genotype 3 patients with DAA failure

Conclusions

Most patients who have failed an HCV treatment with DAA agents have several re-treatment options, but re-treatment selection may be intricate and resistance testing is recommended to optimize this choice. Before resistance testing is performed, it is useful to make sure that the classification of HCV genotype and subtype has been made with a reliably sensitive test; in case of doubt, the repetition of the test is indicated. In fact, Di Maio et al. reported that HCV genotype had been misclassified in 6 (3.0%) of 197 patients who failed DAA treatment [95]. Similarly, Starace et al. [79] found HCV genotype misclassification in 13 (14.9%) of 87 patients who had failed a DAA regimen. It is therefore important to bear in mind that the correct determination of

HCV genotype and subtype and the identification of RASs are essential elements for choosing the optimal re-treatment.

We think it is useful to give readers some other suggestions regarding therapeutic reprocessing (Figs. 1, 2, 3, 4):

- in patients with HCV genotype 1a, the RASs in position 93, 30 and 31 have a high fold-change in resistance compared to the wild-type replicon for all NS5A inhibitors. In the absence of these RASs we can use sofosbuvir + velpatasvir + ribavirin, or pibrentasvir + glecaprevir or an unconventional combination (paritaprevir–ritonavir/ombitasvir/dasabuvir + sofosbuvir or elbasvir + grazoprevir + sofosbuvir), while, in the presence of these RASs, re-treatment with sofosbuvir + velpatasvir + voxilaprevir is the only available option (Fig. 1);
- in patients with HCV genotype 1b or 2, sofosbuvir + velpatasvir + ribavirin is the best re-treatment DAA option regardless of the presence or absence of NS5A RASs; in fact, the RASs in NS5A show a low fold-change in resistance compared to the wild-type replicon for new NS5A inhibitors (Figs. 2, 3);
- in patients with HCV genotype 3, Y93H has a very high fold-change in resistance compared to the wild-type replicon for all NS5A inhibitors; therefore, in the absence of Y93H we can use sofosbuvir + velpatasvir + ribavirin or pibrentasvir + glecaprevir, whereas sofosbuvir + velpatasvir + voxilaprevir is the only re-treatment option available for those showing Y93H (Fig. 4);
- few data are currently available for patients with HCV genotypes 4–6 with DAA failure and consequently it is preferable not to give indications for reprocessing these patients.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest. No financial support.

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