Research Article
Viral Hepatitis

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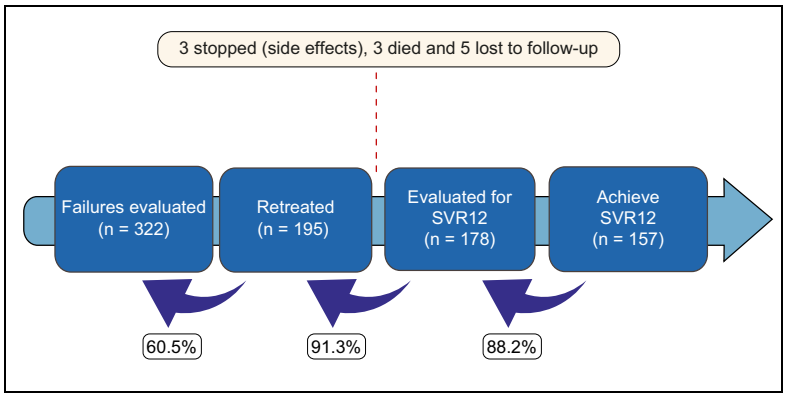
High efficacy of resistance-guided retreatment of HCV patients failing NS5A inhibitors in the real world

Graphical abstract



Highlights

- We provide recommendations on how to use resistance data and achieve 90% sustained virological response.
- If no NS5A resistance-associated substitution is found at failure, choose SOF+NS5A inhibitor with ribavirin.
- If genotype 3 and only Y93H, choose SOF+velpatasvir+ribavirin for 24 weeks.
- If both NS5A and NS3 resistance-associated substitutions, re-treat with a SOF-based 3-drug regimen+ribavirin.
- Our data may be relevant for countries with limited access to new direct-acting antiviral combinations.

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Lay summary

Hepatitis C infection can be cured with currently available antiviral agents. Only a small proportion of patients experience treatment failure, however, in absolute numbers, a high number of patients may require retreatment. Highly effective combinations of antivirals are also available for retreatment. However, these antivirals might not be available in resource-limited settings. Herein, we show how, by analyzing the cause of resistance, retreatment efficacy with old drugs can get very close to the efficacy of new drug combinations.

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High efficacy of resistance-guided retreatment of HCV patients failing NS5A inhibitors in the real world

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Background & Aims: Most hepatitis C virus (HCV)-infected patients failing NS5A inhibitors develop resistance-associated substitutions (RASs). Here we report the use of resistance-guided retreatment of patients who failed prior NS5A inhibitor-containing regimens in the GEHEP-004 cohort. This is the largest direct-acting antiviral (DAA)-resistance cohort study conducted in Spain. We aim to provide indications on how to use resistance information in settings where sofosbuvir/velpatasvir/voxilaprevir may not be available.
Methods: GEHEP-004 is a prospective multicenter cohort enrolling HCV-infected patients treated with interferon (IFN)-free DAA regimens. Prior to retreatment, population-based sequencing of HCV NS3, NS5A and NS5B genes was performed. After receiving a comprehensive resistance interpretation report, the retreatment regimen was chosen and the sustained virological response (SVR) at 12 weeks after treatment completion (SVR12) was recorded.
Results: A total of 342 patients experiencing virological failure after treatment with sofosbuvir/ledipasvir±ribavirin (54%), sofosbuvir/daclatasvir±ribavirin (23%), or paritaprevir-ritonavir/ombitasvir±dasabuvir±ribavirin (20%) were studied. After a resistance report, 186 patients were retreated. An SVR12 was achieved for 88.1% of the patients who failed after sofosbuvir/ledipasvir±ribavirin, 83.3% of the patients who failed after sofosbuvir/daclatasvir±ribavirin, 93.7% of the patients who failed after paritaprevir-ritonavir+ombitasvir±dasabuvir±ribavirin.
Conclusions: In our study, we show how resistance-guided retreatment in conjunction with an interpreted report allows patients to achieve SVR rates close to 90%. We hypothesize that SVR rates may even be improved if resistance data are discussed between experienced virologists and treating clinicians. We believe that our data may be relevant for countries where the access to new DAA combination regimens is limited.
Lay summary: Hepatitis C infection can be cured with currently available antiviral agents. Only a small proportion of patients experience treatment failure, however, in absolute numbers, a high number of patients may require retreatment. Highly effective combinations of antivirals are also available for retreatment. However, these antivirals might not be available in resource-limited settings. Herein, we show how, by analyzing the cause of resistance, retreatment efficacy with old drugs can get very close to the efficacy of new drug combinations.

Introduction

According to the World Health Organization, there are approximately 71 million people infected with hepatitis C virus (HCV) worldwide and 1.75 million people are diagnosed each year.[1] In the absence of antiviral treatment, HCV leads to cirrhosis, hepatocellular carcinoma, liver failure and death.[2] Treatment with direct-acting antivirals (DAA) is highly efficacious and it has limited side effects.[3] Current DAA combinations that are recommended as first-line treatment of HCV-infected patients by the AASLD-IDSA[4] and EASL guidelines[5] enable patients to achieve sustained virological response (SVR) rates >90% for all HCV genotypes.

Despite the high efficacy of current DAAs, 2–5% of the patients starting their first interferon (IFN)-free regimen fail to achieve HCV cure (SVR) in clinical trials and the real world for virological reasons. DAA failure is an unfortunate event that can occur with all HCV genotypes. DAA failure is frequently, but not always, associated with the presence of HCV resistance-associated substitutions (RASs).[4–7] In general, RASs detected at failure are selected during treatment, though, in some patients they may pre-exist as naturally occurring variants before treatment, impairing the efficacy of certain DAA combinations in patients infected with genotypes 1a and 3.[5,8–10]

Until the newer pangenotypic regimens, with high genetic barrier-to-resistance and antiviral potency, become extensively available in all countries, preliminary data suggest that retreatment can be optimized based on RAS testing after a DAA failure (5), particularly for tailoring personalized treatments.[11–13] According to the 2018 EASL guidelines, if resistance testing is performed, then retreatment may be guided by probabilities of response according to the resistance profile observed and the treating team's experience.

Patients that have failed their first IFN-free regimen based on sofosbuvir plus a NS3 inhibitor are easy to retreat.[5] In fact, these patients are naïve to NS5A inhibitors, that is, they have never been treated with NS5A inhibitors for their HCV infection. However, sofosbuvir is an NS5B inhibitor with a very high genetic barrier-to-resistance; hence, the retreatment of these patients with a NS5A inhibitor may be considered as another "first-line" treatment. Patients who failed a prior regimen based on sofosbuvir and a first generation NS5A inhibitor that are going to be retreated with a NS5A inhibitor face a different scenario. Although there are some important reports on how patients fail DAA regimens in real world,[14–17] there is limited evidence on how RAS-guided retreatment of NS5A failures impacts on the efficacy of retreatment.[18]

Herein, we aim to characterize virological failures of patients that did not achieve SVR in the GEHEP-004 cohort, a real-world cohort of patients who failed their first IFN-free DAA regimen in Spain. More importantly, we describe how these patients have been retreated based on the findings of the resistance test and we aim to provide recommendations concerning the selection of a retreatment regimen.

Patients and methods

The GEHEP-004 cohort

GEHEP-004 cohort is a prospective multicenter cohort including HCV-infected patients treated with IFN-free DAA regimens who attended 57 different Spanish centers. Up to November 2017, when glecaprevir/pibrentasvir and sofosbuvir/velpatasvir/voxilaprevir were approved in Spain, the cohort included 412 patients. Plasma samples of the patients were collected and submitted to the University Hospital San Cecilio for drug resistance evaluation. A total of 342 out of 412 patients failed to respond to a NS5A inhibitor-based regimen, most of them failed after sofosbuvir/ledipasvir±ribavirin (n = 185, 54.1%), 79 patients (23.1%) failed after sofosbuvir/daclatasvir±ribavirin, 68 patients (19.9%) failed after paritaprevir-ritonavir/ombitasvir±dasabuvir±ribavirin (PrOD/PrO±ribavirin), and 10 patients (2.9%) failed after simeprevir/daclatasvir.

Virological characterization

Baseline genotyping was performed as part of a routine clinical care. A commercial test available at each of the participating centers was used (Versant HCV Genotype 2.0 LiPA assay was used for the majority of the samples, but also the Abbott Real Time HCV Genotype II assay and Trugene HCV Genotyping Kit were used).

We used Sanger sequencing of the NS5B, NS5A and NS3 regions for resistance analysis. Briefly, after RNA extraction using the Magnapure compact system (Roche), we performed a random primed cDNA synthesis (ThermoScientific). cDNA was used for a primer specific or a pangenotypic amplification depending on the HCV gene and the geno/subtype, and sequenced on an ABI Prism 3500 analyzer. A detailed description of the primers, amplification, and sequencing reactions have previously been reported.[19] The HCV genotype and subtype of the samples were also determined from the NS5B sequence by manual phylogenetic analysis and the use of the COMET and Oxford subtyping tools.

All 3 HCV genes were investigated in patients failing therapy who were infected with genotypes 1 and 4. No NS3 inhibitor had been approved as treatment for patients infected with genotype 3 during the study period; therefore, only the NS5A and NS5B genes were investigated in these patients. The major sofosbuvir RAS found in the NS5B gene, S282T, was investigated in patients treated with sofosbuvir studying a 388 base-pair fragment (including changes in positions 220 to 360). In contrast, all the substitutions of interest (including changes in positions 220 to 570) found in the NS5B gene were evaluated in patients treated with dasabuvir (infected with genotypes 1a and 1b). We sequenced amplicons including positions 17 to 95 and 10 to 181 for NS5A and NS3, respectively. We used the geno2pheno HCV server for sequence alignment (https://hcv.geno2pheno.org) and RAS identification. All RASs detected were transformed into a comprehensive report for clinicians: our report included a list of mutations found in each HCV region (NS3, NS5A and NS5B). The impact of these mutations on the activity of the approved drugs was also included. We followed the recommendations given in the consensus statement by Lontok *et al.* for the translation of RASs into HCV drug activity.[20] However, the individual clinicians participating in the present study chose the retreatment regimen.

For our final analyses, we have classified the RASs according to their level resistance. For first generation DAAs (ledipasvir, daclatasvir, ombitasvir, simeprevir, paritaprevir, dasabuvir), we have considered high-level resistance (HLR) when the fold-change was >100× and intermediate-level resistance (ILR) when the fold-change was 20–100×. For second generation DAAs (elbasvir, velpatasvir, grazoprevir), HLR was considered when the fold-change >10× and ILR when the fold-change was 2.6–9×.

Ethics

The study was approved by the Clinical Research Ethics Committee of the University Hospital San Cecilio (Granada, Spain).

Results

Baseline characteristics

Patients who failed on a NS5A inhibitor in the GEHEP004 cohort were mainly men (85.7%). The median age of these patients was 53 years (interquartile range [IQR] 48–58). Their median viral load at failure was 5.82 $\log_{10}$ HCV RNA IU/ml (IQR 5.34–6.42). A total of 137 patients out of 281 (48.8%) were cirrhotic (>12.5 KPa). A total of 125 patients out of 261 (47.9%) had been previously exposed to IFN-containing regimens. A total of 119 patients out of 287 (41.5) were HIV-coinfected.

We used the NS5B sequence of the 342 samples received at failure to study the HCV genotype: 126 (36.8%) patients were infected with genotype 1a, 78 (22.8%) with genotype 1b, 83 (24.3%) with genotype 3a, 10 (2.9%) with genotype 4a, 44 (12.9%) with genotype 4d and 1 (0.3%) with genotype 4t. Table 1 shows the demographic, clinical and virological characteristics of the population we have studied.

Table 1. Demographic, clinical and virological characteristics of DAA failures in the GEHEP-004 cohort.

Demographic characteristics	
Study population (N)	**342**
Sex (male), n (%)	281/328 (85.7%)
Age (years), n (IQR)	53 (48–58)
Clinical characteristics	
Viral load (log), median (IQR)	5.82 (5.34–6.42)
Genotype, n (%)	
Genotype 1a	126 (36.8)
Genotype 1b	78 (22.8)
Genotype 3a	83 (24.3)
Genotype 4a	10 (2.9)
Genotype 4d	44 (12.9)
Genotype 4t	1 (0.3)
IFN-exposed, n/N (%)	125/261 (47.9)
Cirrhosis (>12.5 kPa), n/N (%)	137/281 (48.8)
HIV-coinfected, n/N (%)	119/287 (41.5)
Regimen failed*, n (%)	
SOF-LDV	118 (36.7)
SOF-LDV+RBV	56 (17.4)
SOF-DCV	52 (16.1)
SOF-DCV+RBV	25 (7.8)
PrO/PrOD	33 (10.2)
PrO/PrOD+RBV	29 (9.0)
Other regimens	9 (2.8)

*Twenty cases have been excluded because of a change in the reported genotype from baseline to the NS5B genotype at failure. The study of paired baseline and failure samples confirmed reinfection in 5 out of these 20 cases. No baseline samples were available for the other 15 cases to rule out a genotyping error at baseline or a reinfection.

DAA, direct-acting antiviral; DCV, daclatasvir; IQR, interquartile range; IFN, interferon; LDV, ledipasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; RBV, ribavirin; SOF, sofosbuvir.

Sofosbuvir-ledipasvir failures

Most of the patients (n = 174, 54.0%) in the cohort had failed sofosbuvir/ledipasvir with or without ribavirin. More than half of these patients were infected with genotype 1 (34.5% GT1a; 29.3% GT 1b). Whereas only 13.2% of them were infected with HCV genotype 3a. Genotype 3 was the less prone to develop RASs in NS5A (only 17.4%). Almost all patients infected with genotype 1b that failed sofosbuvir/ledipasvir developed RASs (94.1%). Only patients with genotype 1a showed NS3 RASs at failure (5% alone and 11.6% with NS5A RASs, respectively). Interestingly, S282T in NS5B was only selected in 3 patients (1.7%); all these 3 patients were infected with genotype 4. These findings are summarized in Table 2.

By December 2017, when sofosbuvir/velpatasvir/voxilaprevir was approved for retreatment in Spain, 107 patients (61.5%) were retreated with conventional regimens (52.2% of them were cirrhotic); 4 patients have been lost to follow-up, 101 patients have been evaluated for SVR at 12 weeks after treatment completion (SVR12) and 89 patients have cleared HCV infection. On a modified intention-to-treat approach, which excludes all patients that were not evaluable at SVR12 for various reasons, the efficacy of resistance-guided retreatment of sofosbuvir/ledipasvir±ribavirin failures with conventional regimens was 88.1%. These findings are shown in Fig. 1.

Six patients infected with HCV genotype 1a did not achieve SVR12 after resistance-guided retreatment. All of them had ≥1

Table 2. Prevalence of RASs in NS3, NS5A and NS5B according to the regimen failed and to the HCV genotype.

SOF/LDV ± RBV		
Genotype (n, %)	**% RASs (overall)**	**% RASs**
1a (60; 34.5%)	73.3%	5.0% NS3 56.7% NS5A 11.6% NS5A + NS3
1b (51, 29.3%)	94.1%	94.1% NS5A
3a (23, 13.2%)	17.4%	17.4% NS5A (Y93H)
4 (40, 23.0%)	32.5%	25.0% NS5A 7.5% NS5B(S282T) + NS5A
SOF/DCV ± RBV		
Genotype (n, %)	**% RASs (overall)**	**% RASs**
1a (16, 20.8%)	87.5%	62.5% NS5A 25.0% NS5A + NS3
1b (9, 11.7%)	100.0%	88.9% NS5A 11.1% NS5A + NS3
3a (51, 66.2%)	70.6%	70.6% NS5A (Y93H)
4 (1, 1.3%)	100.0%	100.0% NS5A
PrO ± D ± RBV		
Genotype (n, %)	**% RASs (overall)**	**% RASs**
1a (37, 59.7%)	86.5%	2.7% NS3 35.1% NS5A 2.7% NS5B 2.7% NS5B + NS3 10.8% NS5B + NS5A 13.5% NS5A + NS3 18.9% NS5B + NS5A + NS3
1b (16, 25.8%)	75.0%	6.2% NS5B 31.2% NS5A 12.6% NS3 12.6% NS5B + NS5A 6.2% NS5B + NS3 6.2% NS5A + NS3
3a (1, 1.6%)	100.0%	100.0% NS5A (Y93H)
4 (8, 12.9%)	50.0%	50.0% NS5A

DAA, direct-acting antiviral; DCV, daclatasvir; LDV, ledipasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; RBV, ribavirin; RAS, resistance-associated substitution; SOF, sofosbuvir.
A complete list of references to RASs is provided in the supplementary information.

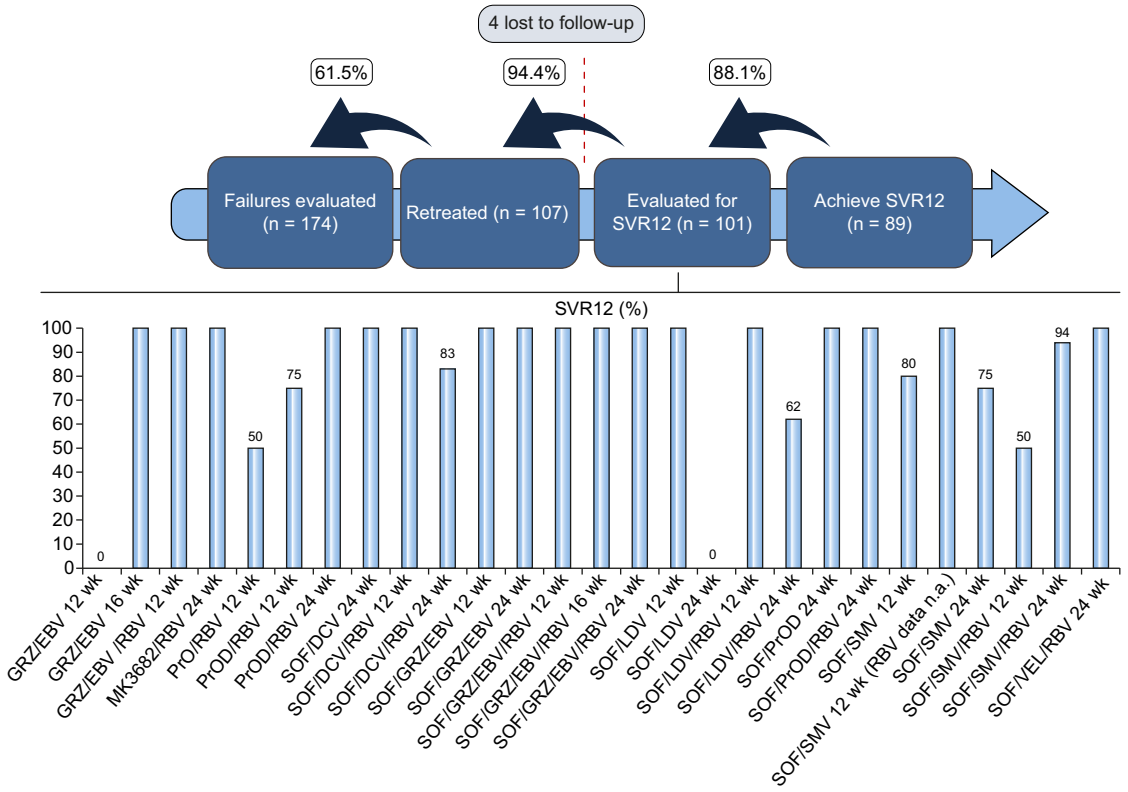

Fig. 1. Failures to sofosbuvir/ledipasvir±ribavirin: Efficacy of resistance-guided retreatment. DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; GT, genotype; LDV, ledipasvir; OMB, ombitasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; PTV, paritaprevir; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir.

Table 3. RASs detected at failure of SOF/LDV ± RBV, *in vitro* impact on the activity of DAAs, the regimen used for retreatment, its adequacy and the efficacy of retreatment (SVR12).

3A. Genotype 1a (n = 38)

RAS NS5A (n)	RAS NS3 (n)	RAS NS5B (n)	Retreatment regimen (n)	Adequacy to resistance	SVR12
WT (13)	WT (11)	WT (13)	SOF/SMV 12 wk (1)	Yes	Yes
			SOF/SMV/RBV 12 wk (1)	**Yes**	**No[1]**
			SOF/SMV/RBV 24 wk (2)	Yes	Yes
			SOF/LDV/RBV 12 wk(1)	Yes	Yes
			SOF/LDV 24 wk(1)	**Yes**	**No[2]**
			SOF/LDV/RBV 24 wk(3)	Yes	Yes
			SOF/GRZ/EBV/RBV 12 wk(1)	Yes	Yes
			MK3682/RBV 24 wk(1)	Yes	Yes
	S122G (1)		PrOD/RBV 12 wk(1)	Yes	Yes
	D168A (1)		PrOD/RBV 24 wk(1)	No	Yes
	[HLR to PTV, GRZ **ILR** to SMV]				
M28ATV + Q30R (3) **[HLR** to DCV, LDV, OMB, EBV-28AT, VEL-28A-; **ILR** to VEL-28T-]	WT (3)	WT(3)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
			GRZ/EBV 12 wk(1)	**No**	**No[3]**
			SOF/GRZ/EBV 24 wk(1)	Yes[#]	Yes
Q30HR (12) **[HLR** to DCV, LDV, OMB-30R-, EBV-30R-; **ILR** to EBV-30H-]	WT (9)	WT (12)	SOF/LDV/RBV 12 wk(1)	No	Yes
			SOF/LDV/RBV 24 wk(2)	No	Yes
				No	**No[4]**
			SOF/SMV/RBV 24 wk (4)	**Yes**	**No (1)[5]**
				Yes (3)	Yes (3)
			SOF/PrOD/RBV 24 wk(1)	Yes[#]	Yes
			SOF/GRZ/EBV/RBV 24 wk(1)	Yes[#]	Yes
	V55I (1)		GRZ/EBV 16 wk(1)	Yes	Yes
	S122G (1)		SOF/GRZ/EBV/RBV 24 wk(1)	Yes[#]	Yes
	R155T (1)		SOF/SMV/RBV 24 wk(1)	No	Yes
	[HLR to GRZ; **ILR** to SMV]				
L31IM (9) **[HLR** to DCV-31M-, LDV, EBV-31M-, VEL-31M-; **ILR** to DCV & VEL- 31I-]	WT (9)	WT (9)	SOF/SMV 24 wk(1)	Yes	Yes
			SOF/SMV/RBV 24 wk(3)	Yes	Yes
			PrOD/RBV 24 wk(1)	Yes	Yes
			SOF/GRZ/EBV/RBV 16 wk(1)	Yes[#]	Yes
			SOF/GRZ/EBV 24 wk(1)	Yes[#]	Yes
			SOF/GRZ/EBV/RBV 24 wk(2)	Yes[#]	Yes
L31F (1) **[HLR** to EBV, VEL; **ILR** to DCV]	WT (1)	WT(1)	**SOF/LDV/RBV 24 wk(1)**	**Yes**	**No[4]**

3B. Genotype 1b (n = 32)

RAS NS5A (n)	RAS NS3 (n)	RAS NS5B (n)	Retreatment regimen (n)	Adequacy to resistance	SVR12
WT (2)	WT (2)	WT (2)	**SOF/SMV/RBV 24 wk(1)**	**Yes**	**No**[1]
			PrOD/RBV 24 wk(1)	Yes	Yes
L28M + Y93H (1) **[HLR** to DCV, LDV, OMB, EBV; **ILR** to VEL]	WT (1)	WT (1)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
R30Q + Y93H (1) **[HLR** to DCV, LDV, OMB, EBV; **ILR** to VEL]	WT (1)	WT (1)	SOF/SMV 12 wk(1)	Yes	Yes
L31M (3)	WT (3)	WT (1)	PrOD/RBV 12 wk(1)	Yes	Yes
		C316N (2)	SOF/SMV 12 wk(1)	Yes	Yes
		[DSV RAS *in vitro*]	SOF/SMV ± RBV 12 wk(1)	Yes	Yes
L31IMV + Y93H (13) **[HLR** to DCV, LDV, OMB, EBV, VEL]	WT (11)	WT (4)	SOF/SMV 24 wk(1)	Yes	Yes
			SOF/SMV/RBV 24 wk(2)	Yes	Yes
			SOF/GRZ/EBV/RBV 12 wk(1)	Yes[#]	Yes
		C316N (9)	SOF/SMV/RBV 24 wk(5)	Yes	Yes
		[DSV RAS *in vitro*]	SOF/PrOD 24 wk(1)	Yes[#]	Yes
			SOF/PrOD/RBV 24 wk(1)	Yes[#]	Yes
	S122T (2)		SOF/SMV 12 wk(1)	Yes	Yes
			SOF/SMV/RBV 24 wk(1)	Yes	Yes

3C. Genotype 3 (n = 10) and Genotype 4 (n = 21)

RAS NS5A (n)	RAS NS3 (n)	RAS NS5B (n)	Retreatment regimen (n)	Adequacy to resistance	SVR12
Genotype 3 (n = 10)					
WT (10)		WT (10)	SOF/DCV/RBV 12 wk(1)	Yes	Yes
			SOF/DCV 24 wk(1)	Yes	Yes
			SOF/DCV/RBV 24 wk(5)	**Yes**	**No (1)**[1]
				Yes (4)	Yes (4)
			SOF/LDV/RBV 24 wk(1)	Yes	Yes
			SOF/GRZ/EBV/RBV 12 wk(2)	Yes	Yes
Genotype 4 (n = 21)					
WT (10)	WT (10)	WT (10)	SOF/SMV 12 wk(1)	**Yes**	**No**[2]
			SOF/SMV/RBV 12 wk(1)	Yes	Yes
			SOF/SMV/RBV 24 wk(1)	Yes	Yes
			SOF/LDV 12 wk(1)	Yes	Yes
			PrO/RBV 12 wk(2)	**Yes**	**No**[3]
				Yes	Yes
			SOF/DCV/RBV 24 wk(1)	Yes	Yes
			GRZ/EBV/RBV 12 wk(1)	Yes	Yes
			SOF/GRZ/EBV/RBV 12 wk(1)	Yes	Yes
			SOF/GRZ/EBV/RBV 24 wk(1)	Yes	Yes
L28MV (4) [**HLR** to OMB for GT-4d; **ILR** to LDV, OMB for GT-4a]	WT (3)	WT (2)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
			SOF/PrO/RBV 24 wk(1)	Yes[#]	Yes
		S282T (1)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
		[SOF RASs *in vitro*]			
	D168E (1)	WT (1)	SOF/GRZ/EBV 12 wk(1)	Yes	Yes
L30H (1) **[HLR** to DCV for GT-4a]	WT (1)	WT (1)	SOF/LDV/RBV 24 wk(1)	**Yes**	**No**[4]
Y93C (4) [*In vitro* RAS to DCV, LDV, OMB, EBV, VEL]	WT (4)	WT (4)	SOF/SMV/RBV 24 wk(3)	Yes	Yes
			SOF/GRZ/EBV/RBV 12 wk(1)	Yes	Yes
Y93H (2) **[HLR** to DCV, LDV **ILR** to EBV, VEL for GT-4a)	WT (2)	WT (1)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
		S282T (1)	SOF/VEL/RBV 24 wk(1)	Yes	Yes
		[SOF RASs *in vitro*]			

(*continued on next page*)

Table 3 (*continued*)

3C. Genotype 3 (n = 10) and Genotype 4 (n = 21)

RAS NS5A (n)	RAS NS3 (n)	RAS NS5B (n)	Retreatment regimen (n)	Adequacy to resistance	SVR12
Y93H (12) **[HLR** to DCV, LDV, OMB, EBV **ILR** to VEL]	WT (12)	WT (3)	SOF/SMV/RBV 24 wk(3)	Yes	Yes
		C316N (9)	**SOF/SMV 24 wk(2)**	**Yes**	**No[1]**
		[DSV RAS *in vitro*]		Yes	Yes
			SOF/SMV/RBV 24 wk(5)	Yes	Yes
			SOF/PrOD/RBV 24 wk(1)	Yes[#]	Yes
			SOF/GRZ/EBV 24 wk(1)	Yes[#]	Yes

WT, no RASs. [1]HIV-coinfected; [2]HIV-coinfected and IFN-exposed; [3]cirrhotic and IFN-exposed; [4]cirrhotic, HIV-coinfected and IFN-exposed; [5]cirrhotic and ribavirin suspended prematurely due to adverse effect in the first-line DAA regimen. For first generation DAAs (LDV, DCV, OMB, SMV, PTV, DSV): HLR, RASs with fold-change >100×; ILR, RASs with fold-change 20–100×. For second generation DAAs (EBV, VEL, GRZ): HLR, RASs with fold-change >10×; ILR, RASs with fold-change 2.6–9×. [#]3/4-drug regimen: resistance only to 1 of the components of the regimen and/or no further options at the time of retreatment. Patients that failed to achieve SVR12 are highlighted in bold.
DAAs, direct-acting antivirals; DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; HLR, high-level resistance; IFN, interferon; ILR, intermediate-level resistance; LDV, ledipasvir; MK3682, uprifosbuvir; OMB, ombitasvir; PrO, paritaprevir$_{(ritonavir)}$/ombitasvir; PrOD, paritaprevir$_{(ritonavir)}$/ombitasvir/dasabuvir; PTV, paritaprevir; RASs, resistance-associated substitutions; RBV, ribavirin; SOF, sofosbuvir; SMV, simeprevir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir; WT, wild-type.

WT, no RASs. [1]Cirrhotic and IFN-exposed. For first generation DAAs (LDV, DCV, OMB, SMV, PTV, DSV): HLR, RASs with fold-change >100×; ILR, RASs with fold-change 20–100×. For second generation DAAs (EBV, VEL, GRZ): HLR, RASs with fold-change >10×; ILR, RASs with fold-change 2.6–9×. [#]3/4-drug regimen: resistance only to 1 of the components of the regimen and/or no further options at the time of retreatment. Patients that failed to achieve SVR12 are highlighted in bold.
DAAs, direct-acting antivirals; DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; HLR, high-level resistance; IFN, interferon; ILR, intermediate-level resistance; LDV, ledipasvir; OMB, ombitasvir; PTV, paritaprevir; RASs, resistance-associated substitutions; RBV, ribavirin; PrO: paritaprevir$_{(ritonavir)}$/ombitasvir; PrOD, paritaprevir$_{(ritonavir)}$/ombitasvir/dasabuvir; SMV, simeprevir; SOF, sofosbuvir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir; WT, wild-type.

WT, no RASs. [1]Cirrhotic, [2]cirrhotic and IFN-exposed; [3]HIV-coinfected and IFN-exposed; [4]HIV-coinfected, cirrhotic and IFN-exposed. For first generation DAAs (LDV, DCV, OMB, SMV, PTV, DSV): HLR, RASs with fold-change >100×; ILR, RASs with fold-change 20–100×. For second generation DAAs (EBV, VEL, GRZ): HLR, RASs with fold-change >10×; ILR, RASs with fold-change 2.6–9×. [#]3/4-drug regimen: resistance only to 1 of the components of the regimen and/or no further options at the time of retreatment. Patients that failed to achieve SVR12 are highlighted in bold.
DAAs, direct-acting antivirals; DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; GT, genotype; HLR, high-level resistance; IFN, interferon; ILR, intermediate-level resistance; LDV, ledipasvir; OMB, ombitasvir; PrO, paritaprevir$_{(ritonavir)}$/ombitasvir; PrOD, paritaprevir$_{(ritonavir)}$/ombitasvir/dasabuvir; PTV, paritaprevir; RASs, resistance-associated substitutions; RBV, ribavirin; SOF, sofosbuvir; SMV, simeprevir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir; WT, wild-type.

prognostic factors for lower response to conventional DAA regimens. Two patients had no mutations in NS5A, NS3 or NS5B at failure, and both were HIV-coinfected; 1 of them failed a sofosbuvir/simeprevir/ribavirin regimen with suboptimal 12-week duration, while the other failed to achieve SVR12 on a sofosbuvir/ledipasvir 24-week regimen without ribavirin. The other 4 patients were cirrhotic, they had NS5A RASs and they were retreated with either resistance-inadequate ledipasvir-based regimens (they carried Q30R or L31F RASs), a resistance-inadequate grazoprevir+elbasvir regimen (carrying M28T+Q30R RASs), or with a suboptimal simeprevir-based regimen. For the patient with L31F RAS, ledipasvir was reported as susceptible in our initial report, based on the information available at that time in the Lontok *et al.* consensus.[20] L31F was pointed out by Sorbo *et al.* in the 2018 update as a ledipasvir RAS, with an uncertain impact on the activity of ledipasvir.[13] Two patients infected with genotype 1b and on a sofosbuvir/ledipasvir regimen failed to achieve SVR12 with a simeprevir-based regimen; both patients were cirrhotic, HIV-coinfected and IFN-exposed. One patient with genotype 3 and 3 patients with genotype 4 on sofosbuvir/ledipasvir treatment also failed to achieve SVR12 with the retreatment regimen: 3 patients showed no RASs in NS5A, NS3 or NS5B, and only 1 patient (cirrhotic) was on a suboptimal 12-week regimen of simeprevir. The fourth patient failed the retreatment therapy on a resistance-guided non-adequate ledipasvir-based regimen.

Table 3 provides a detailed description of the RASs detected at failure, their *in vitro* impact on the activity of DAAs, the regimen used for retreatment, its adequacy in relation to the resistance-guided report and the efficacy of retreatment.

Sofosbuvir-daclatasvir failures

A total of 77 patients (23.9%) failed sofosbuvir/daclatasvir±ribavirin, and almost two-thirds of the patients were infected with genotype 3. Again patients with genotype 3 infection were the least prone to developing RASs in NS5A (70.6%). In contrast, all patients infected with genotype 1b developed RASs. For genotypes 1a and 1b, RASs in NS3 were detected at failure in 25.0% and 11.1% of the patients, respectively. These findings are summarized in Table 2.

Only 44 patients (57.1%) were retreated with conventional regimens, (71% cirrhotic); 3 patients stopped treatment prematurely due to side effects and 2 died while on treatment. One patient was lost to follow-up, 36 patients have been evaluated for SVR12, and HCV clearance was found in 30 patients. On a modified intention-to-treat approach, the efficacy of resistance-guided retreatment of sofosbuvir/daclatasvir±ribavirin failures with conventional regimens was 83.3%. A detailed description of these findings is shown in Fig. 2.

After failing a daclatasvir-based regimen, 6 patients did not achieve SVR12 after resistance-guided retreatment. Two patients were infected with genotype 1a. The first patient was cirrhotic, HIV-coinfected and IFN-exposed. This first patient had a complex RAS pattern in NS5A (M28T+Q30H). This patient was retreated with sofosbuvir/simeprevir/ribavirin for 24 weeks. According to Hezode *et al.*,[21] simeprevir-containing regimens, even including ribavirin and with a 24-week duration, may be suboptimal in patients with several factors lowering the SVR rate. The second patient infected with genotype 1a was a cirrhotic patient harbouring L31V in NS5A and retreated with sofosbuvir/ledipasvir/ribavirin for 24 weeks. Ledipasvir was

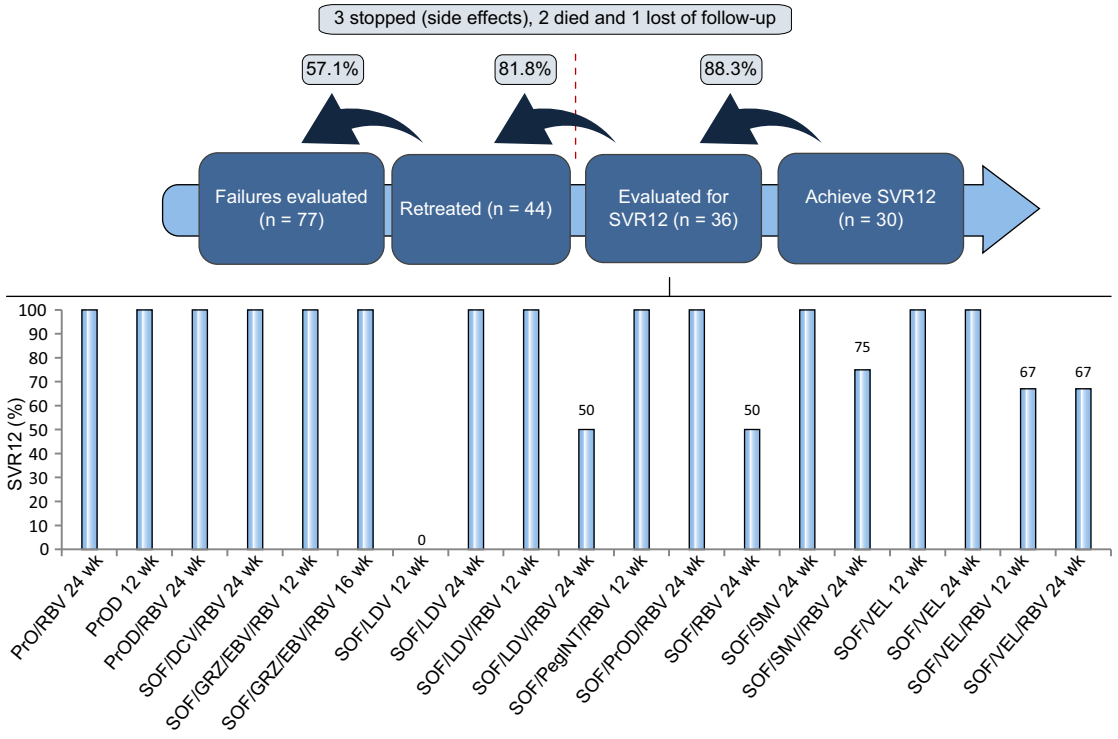

Fig. 2. Failures to sofosbuvir/daclatasvir±ribavirin: Efficacy of resistance-guided retreatment. DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; GT, genotype; LDV, ledipasvir; OMB, ombitasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; PTV, paritaprevir; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir.

Table 4. RASs detected at failure of SOF/DCV±RBV, *in vitro* impact on the activity of DAAs, the regimen used for retreatment, its adequacy and the efficacy of retreatment (SVR12).

RAS NS5A (n)	RAS NS3 (n)	RAS NS5B (n)	Retreatment regimen (n)	Adequacy to resistance	SVR12
Genotype 1a (n = 9)					
WT (1)	WT (1)	WT (1)	SOF/SMV 24 wk (1)	Yes	Yes
M28T+Q30HR (2) [**HLR** to LDV, DCV, OMB, EBV; **ILR** to VEL-M28T-)]	WT (2)	WT (2)	**SOF/SMV/RBV 24 wk** (2)	Yes **Yes**	Yes **No**[1]
Q30DHKR (4) [**HLR** to DCV, LDV-30HKR, OMB-30KR-, EBV-30DR-, VEL-30 K-; **ILR** to EBV-30H-]	WT (4)	WT (4)	SOF/SMV 24 wk(1) SOF/SMV/RBV 24 wk (2) PrOD/RBV 24 wk(1)	Yes Yes Yes	Yes Yes Yes
Q30R+L31M (1) [**HLR** to LDV, DCV, OMB, EBV, VEL]	**V36M+R155K** (1) [**HLR** to GRZ; **ILR** to SMV, PTV]	WT (1)	PrOD/RBV 24 wk(1)	No	Yes
L31V (1) [**HLR** to DCV, LDV, OMB, EBV, VEL]	WT (1)	WT (1)	**SOF/LDV/RBV 24 wk** (1)	**No**	**No**[2]
Genotype 1b (n = 5)					
L31M (1)	WT (1)	WT (1)	PrOD/RBV 24 wk (1)	Yes	Yes
L31IMV+Y93H (2) [**HLR** to LDV, DCV, OMB, EBV; **ILR** to VEL	WT (2)	**C316N** (2) [DSV RAS in vitro]	**SOF/LDV 12 wk**(1) SOF/PrOD/RBV 24 wk(1)	**No** Yes[#]	**No**[3] Yes
L31M+Y93H (1) [**HLR** to LDV, DCV, OMB, EBV, VEL]	**Q80R+D168E** (1) [**HLR** to SMV; **ILR** to GRZ]	WT (1)	PrOD 12 wk(1)	No	Yes
A92K (1) [**HLR** to LDV, VEL]	WT (1)	WT (1)	SOF/LDV/RBV 12 wk (1)	No	Yes
Genotype 3 (n = 21)					
WT (2)		WT (2)	SOF/VEL 12 wk(1) SOF/VEL/RBV 24 wk (1)	Yes Yes	Yes Yes
A30K (2) [**HLR** to VEL; **ILR** to DCV]		WT (2)	SOF/LDV 24 wk(1) SOF/VEL 12 wk(1)	Yes No	Yes Yes
L31F (1) [**HLR** to DCV; **ILR** to VEL]		WT (1)	SOF/LDV/RBV 24 wk (1)	Yes	Yes
Y93H (16) [**HLR** to DCV, VEL]		WT (16)	**SOF/RBV 24 wk**(2)	**Yes** Yes	**No**[1] Yes
			SOF/PegINT/RBV 12 wk(2)	Yes No	Yes Yes
			SOF/DCV/RBV 24 wk (4)	**No** No (2)	**No** Yes
			SOF/VEL/RBV 12 wk (3)	No No **No**	(2) Yes Yes
			SOF/VEL 24 wk(1)	Yes[#]	**No**[4]
			SOF/VEL/RBV 24 wk (2)	Yes[#]	Yes Yes
			SOF/GRZ/EBV/RBV 12 wk(1) SOF/GRZ/EBV/RBV 16 wk(1)		
Genotype 4 (n = 1)					
Y93H (1) [**HLR** to DCV, LDV; **ILR** to EBV, VEL[+]] ([+]: only GT4a)	WT (1)	WT (1)	PrO/RBV 24 wk(1)	Yes	Yes

RASs determined *in vitro*.
WT, no RASs. [1]These patients were cirrhotic, HIV-coinfected and IFN-exposed; [2]cirrhotic; [3]IFN-exposed; [4]this patient was cirrhotic and IFN-exposed. For first generation DAAs (LDV, DCV, OMB, SMV, PTV, DSV): HLR, RASs with fold-change >100×; ILR, RASs with fold-change 20–100×. For second generation DAAs (EBV, VEL, GRZ): HLR, RASs with fold-change >10×; ILR, RASs with fold-change 2.6–9×. [#]3/4-drug regimen: resistance only to 1 of the components of the regimen and/or no further options at the time of retreatment. Patients that failed to achieve SVR12 are highlighted in bold.
DAA, direct-acting antiviral; DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; GT, genotype; HLR, high-level resistance; IFN, interferon; ILR, intermediate-level resistance; LDV, ledipasvir; OMB, ombitasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; PTV, paritaprevir; RAS, resistance-associated substitution; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir; WT, wild-type.

reported as susceptible by our initial report based on the information available in the Lontok *et al.* consensus[20] regarding drug-RASs in patients infected with HCV. However, the L31V substitution is reported as having HLR to ledipasvir (>100 fold-change) in the further update in 2018 conducted by Sorbo *et al.*[13] One patient was infected with genotype 1b and was erroneously retreated with sofosbuvir/ledipasvir for 12 weeks, as L31IMV+Y93H, conferring HLR to ledipasvir, were detected in

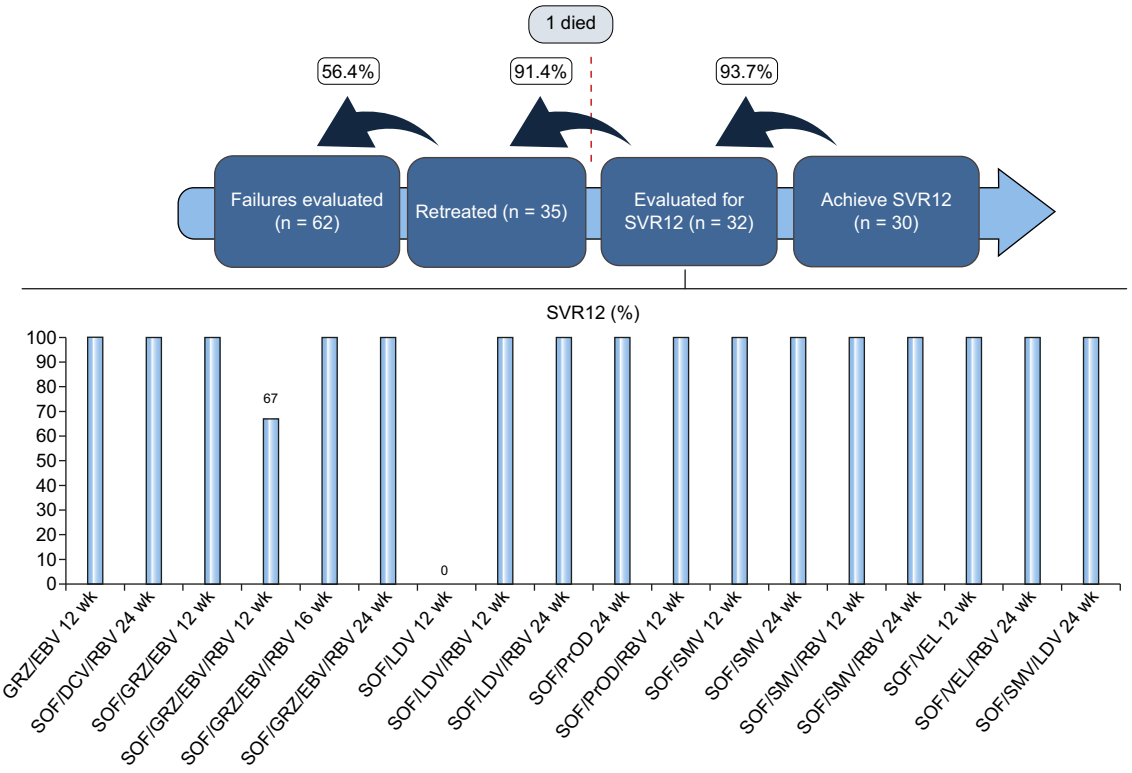

Fig. 3. Failures to paritaprevir-ritonavir/ombitasvir±dasabuvir±ribavirin: Efficacy of resistance-guided retreatment. DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; GT, genotype; LDV, ledipasvir; OMB, ombitasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; PTV, paritaprevir; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir.

NS5A at the time of first failure. The remaining patients not reaching SVR12 after resistance-guided retreatment were infected with genotype 3 and all harboured the Y93H substitution in NS5A. One patient, cirrhotic, HIV-infected and IFN-exposed was retreated with a suboptimal sofosbuvir/ribavirin for 24 weeks. The other 2 patients were treated with sofosbuvir/velpatasvir/ribavirin for either 12 or 24 weeks (cirrhotic and IFN-exposed). Y93H is a highly challenging RAS to re-treat because it confers HLR to all the approved NS5A inhibitors active against genotype 3, including velpatasvir (fold-change = 720). Pibrentasvir is the only NS5A inhibitor that is free of the Y93H resistance effect on genotype 3.[13]

Table 4 provides a detailed description of the RASs detected at failure, their *in vitro* impact on the activity of DAAs, the regimen used for retreatment, its adequacy in relation to the resistance-guided report and the efficacy of retreatment.

Failure to ombitasvir containing regimens

We were able to evaluate 62 patients (19.1%) failing a paritaprevir-ritonavir+ombitasvir±dasabuvir±ribavirin combination. The vast majority of these patients were infected with genotype 1a (59.7%) or 1b (25.8%). Only 12.9% of the patients were infected with genotype 4. A total of 1.6% patients were erroneously treated with these combinations because they were erroneously genotyped at origin. Genotypes 3 and 1a developed RASs at failure in a large proportion of patients (100.0% and 86.5%, respectively). The development of RASs in more than one gene was common across all genotypes. Table 2 summarizes these findings.

Fig. 3 shows the efficacy of resistance-guided retreatment of PrOD/PrO±ribavirin failures in our cohort. Thirty-five patients (56.5%) were retreated with conventional regimens (26.7% cirrhotic); 1 patient died on treatment and 32 patients have been evaluated for SVR12. Thirty of them have cleared HCV infection. On a modified intention-to-treat approach, the efficacy of resistance-guided retreatment of PrOD/PrO±ribavirin failures with conventional regimens was 93.7%.

Both patients that had previously failed a PrO regimen and did not achieve SVR after resistance-guided retreatment were infected with genotype 4. One patient was retreated with a suboptimal ledipasvir-based regimen in the presence of Y93H. The second patient, with a Y93C variant was treated with a potent triple drug (sofosbuvir, grazoprevir, elbasvir) and ribavirin regimen but only for 12 weeks; although Y93C has a high impact on elbasvir activity in genotype 1a, and the failure with Y93C is associated with GT4, its impact on genotype 3 has not been described yet.

Table 5 provides a detailed description of the RASs detected at failure, their *in vitro* impact on the activity of DAAs, the regimen used for retreatment, its adequacy in relation to the resistance-guided report and the efficacy of retreatment.

Discussion

Treatment of chronic hepatitis C with DAAs achieves high cure rates. Virological failure occurs in less than 5% of DAA-treated patients. In absolute numbers, a second-line therapy is needed to achieve viral eradication in a significant number of patients. Several clinical guidelines[4,5] recommend the use of a 3-drug

Table 5. RASs detected at failure of PrOD/PrO±RBV, *in vitro* impact on the activity of DAAs, the regimen used for retreatment, its adequacy and the efficacy of retreatment (SVR12).

RAS NS5A (n)	RAS NS3 (n)	RAS NS5B (n)	Retreatment regimen (n)	Adequacy to resistance	SVR12
Genotype 1a (n = 19)					
WT (6)	WT (6)	WT (5)	SOF/LDV/RBV 12 wk(2)	Yes	Yes
			SOF/LDV/RBV 24 wk(2)	Yes	Yes
			SOF/GRZ/EBV/RBV 12 wk(1)	Yes	Yes
		S556G (1) [DSV RAS *in vitro*]	SOF/SMV/RBV 24 wk(1)	Yes	Yes
M28T (3) [**HLR** to DCV, LDV, OMB, EBV **ILR** to VEL]	WT (2)	WT (2)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
			SOF/PrOD/RBV 12 wk(1)	Yes[#]	Yes
	R155K (1) [**ILR** to SMV, PTV, GRZ]	**S556G** (1) [DSV RAS *in vitro*]	SOF/GRZ/EBV/RBV 24 wk(1)	Yes[#]	Yes
M28TV+Q30R (2) [**HLR** to DCV, LDV, OMB, EBV **ILR** to VEL[+]] ([+] only M28T)	**V36M+R155K** (1) [**HLR** to GRZ **ILR** to SMV, PTV]	WT (2)	SOF/GRZ/EBV/RBV 16 wk(1)	Yes[#]	Yes
	WT (1)		SOF/SMV/RBV 24 wk(1)	Yes	Yes
Q30HR (4) **HLR** to DCV, LDV, OMB[+], EBV[+] ([+] only Q30R) **ILR** to EBV (Q30H)	WT (4)	WT (4)	SOF/SMV/RBV 24 wk(1)	Yes	Yes
			SOF/PrOD/RBV 12 wk(1)	Yes	Yes
			SOF/VEL 12 wk(1)	Yes	Yes
			SOF/VEL/RBV 24 wk(1)	Yes	Yes
Q30K (1) [**HLR** to DCV, LDV, OMB, VEL]	WT (1)	**C316Y** (1) [**HLR** to DSV]	SOF/LDV/RBV 12 wk(1)	No	Yes
Q30R (2) [**HLR** to DCV, LDV, OMB, EBV]	**S122G** (1) **D168V**(1) [**HLR** to SMV, PTV, GRZ]	**C316Y** (2) [**HLR** to DSV]	SOF/PrOD 24 wk(1)	Yes[#]	Yes
			SOF/GRZ/EBV/RBV 16 wk(1)	Yes[#]	Yes
Q30R+H58D (1) **HLR** to DCV, LDV, OMB, EBV **ILR** to VEL (only H58D)	WT (1)	WT (1)	SOF/SMV 24 wk(1)	Yes	Yes
Genotype 1b (n = 6)					
WT (1)	WT (1)	**C316N+S556G** [DSV RAS *in vitro*]	SOF/SMV 24 wk(1)	Yes	Yes
WT (1)	**S122T** (1)	WT (1)	SOF/GRZ/EBV/RBV 16 wk(1)	Yes	Yes
Y93H (4) **[HLR** to DCV, LDV, OMB, EBV **ILR** to VEL]	WT (3)	WT (1)	SOF/SMV/LDV 24 wk(1)	Yes[#]	Yes
		C316N (3) [DSV RAS *in vitro*]	SOF/SMV 12 wk(1)	Yes	Yes
			GRZ/EBV 12 wk(1)	No	Yes
	D168V (1) [**HLR** to SMV, PTV, GRZ]		SOF/GRZ/EBV 12 wk(1)	Yes[#]	Yes
Genotype 3* (n = 1)					
Y93H (1) [**HLR** to DCV, VEL]	~~	WT (1)	SOF/DCV/RBV 24 wk(1)	No	Yes
Genotype 4 (n = 6)					
WT (1)	WT (1)	WT (1)	SOF/GRZ/EBV/RBV 12 wk(1)	Yes	Yes
L28VS (2)	WT (1)	WT (2)	SOF/LDV/RBV 24 wk(1)	Yes	Yes
HLR to OMB for GT4d **ILR** to OMB for GT4a	**D168A** (1) [**HLR** to GRZ]		SOF/SMV/RBV 12 wk(1)	Yes	Yes
Y93CHS (3) [**HLR** to DCV, LDV **ILR** to DCV, EBV, VEL]	WT (3)	WT (3)	SOF/SMV 24 wk(1)	Yes	Yes
			SOF/LDV 12 wk(1)	**No**	**No[1]**
			SOF/GRZ/EBV/RBV 12 wk(1)	Yes[#]	**No**

RASs determined *in vitro*.

WT, no RASs; *This patient was genotyped at origin as GT3 and was initially treated erroneously with PrOD; [1]This patient was HIV-coinfected. HLR: high-level resistance; ILR: intermediate-level resistance. For first generation DAAs (LDV, DCV, OMB, SMV, PTV, DSV): HLR, RASs with fold-change >100×; ILR, RASs with fold-change 20–100×. For second generation DAAs (EBV, VEL, GRZ): HLR, RASs with fold-change >10×; ILR, RASs with fold-change 2.6–9×. [#]3/4-drug regimen: resistance only to one of the components of the regimen and/or no further options at the time of retreatment. Patients that failed to achieve SVR12 are highlighted in bold.

DAA, direct-acting antiviral; DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; GT, genotype; HLR, high-level resistance; IFN, interferon; ILR, intermediate-level resistance; LDV, ledipasvir; OMB, ombitasvir; PrO, paritaprevir-ritonavir/ombitasvir; PrOD, paritaprevir-ritonavir/ombitasvir/dasabuvir; PTV, paritaprevir; RAS, resistance-associated substitution; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR12, sustained virological response at 12 weeks after treatment completion; VEL, velpatasvir; WT, wild-type.

class combination of sofosbuvir, velpatasvir and voxilaprevir for retreatment. Preliminary data suggest that the retreatment regimen may be selected according to the RASs against the drugs included in the failing regimen.[22] In our study we show that the resistance-guided retreatment in conjunction with an interpreted report achieve efficacy rates close to 90% in patients who failed after a NS5A inhibitor-based regimen, who are the most difficult to retreat. We provide recommendations concerning the selection of the retreatment regimen based on the resistance findings. We believe that these recommendations may be of interest in those settings where sofosbuvir/velpatasvir/voxila previr may not be available.

We analyzed the failures to NS5A inhibitors-based regimens found in the GEHEP-004 cohort. This is the largest cohort study conducted in Spain and one of the largest international cohort studies regarding DAA failures. Although GEHEP-004 does not include centers throughout the whole country, the distribution by genotypes we have analyzed is similar to the distribution reported by the most recent Spanish molecular HCV epidemiologic studies. In fact, in the GEHEP-005 study,[23] the largest and most recent study conducted in Spain, the distribution of HCV genotypes for the years 2000–2015 was 66.9% for genotype 1 (24.9% 1a and 37.9% 1b), 17.3% for genotype 3 and 11.4% for genotype 4, whereas in our study the distribution was 58.0% for genotype 1, 21.6% for genotype 3 and 14.7% for genotype 4. This distribution is consistent with the most recent data reported in Europe.[24]

Several studies have analyzed the development of RASs in patients who fail after their first DAA regimen.[14–18] In line with our study, the development of RASs in patients failing NS5A inhibitors is usual. However, inclusion of a high proportion of HIV-coinfected population in our study may explain the higher RAS development rates, because the frequency of adverse events due to drug-drug interactions, which may lead to a lower adherence, is greater in the HIV-coinfected population than in monoinfected patients. Along with other recent studies,[15–17] our study is one of the first showing resistance data, retreatment and efficacy to first-line NS5A based regimens. The vast majority of patients in our cohort were retreated with ribavirin and for a longer period (24 weeks) because clinicians were following the recommendations of the previous versions of the EASL treatment guidelines available at that time.

We performed an in-depth analysis of how patients were retreated to attempt to produce guidance on how to use resistance data to provide retreatment indications. Our focus was those patients not achieving SVR after their second DAA treatment. We have also considered cirrhosis, HIV-coinfection and previous IFN exposure. All of which are known prognostic factors for low response to conventional DAA regimens. In our cohort, patients failing a ledipasvir, daclatasvir or PrOD regimen without RASs in NS5A, NS3 or NS5B that were retreated with sofosbuvir+NS5Ainhibitor+ribavirin for 12/24 weeks achieved very high rates of SVR12. Therefore, the sofosbuvir+NS5Ainhibitor+ribavirin regimen might be recommended for the retreatment of patients failing without any RASs. When available, velpatasvir should be the NS5A component of the new retreatment regimen; if not available, the previously used NS5A inhibitor may be recycled adding ribavirin and extending the duration for 24 weeks. For patients who failed with RASs only in NS5A, the majority of the non-genotype 3 patients were retreated and cured with a PrOD±sofosbuvir regimen, adding ribavirin. Simeprevir-based regimens, with ribavirin and for 24 weeks, were also highly effective, though suboptimal SVR rates were found in patients with one or more factors of low response, especially if they were cirrhotic. When possible, simeprevir-based regimens should be avoided, especially for patients with cirrhosis. Most of the patients infected with genotype 3 were retreated and cured with a sofosbuvir+NS5Ainhibitor+ribavirin 24-week regimen or a sofosbuvir plus 2/3 drug regimen, with additional ribavirin. These patients should be retreated with a sofosbuvir+NS5Ainhibitor+ribavirin 24-week regimen if Y93H is present. As these combinations may be less optimal if the patient is cirrhotic, a sofosbuvir+2/3-drugs regimen and ribavirin, if possible, is also recommended. Finally, when RASs in both NS5A and NS3 were detected at failure, patients were cured with a sofosbuvir based 3-drug regimen, with additional ribavirin.

Our study has several limitations. First, the study has been carried out within the GEHEP-004 Spanish cohort. Therefore, our data may not be representative at the European level. Secondly, our study may not have enough power to allow our conclusions to be extrapolated; although we include a large number of patients and we have probably one of the largest cohorts of DAA failures, the wide variety of genotypes, drugs, treatment duration options, and the use or not of ribavirin, lead to only a limited number of patients in each subgroup. Collaboration between researchers studying different resistance cohorts may be needed to give definitive recommendations on resistance guidance. Third, in our study we used the Lontok *et al.* consensus,[20] which, as shown in the results section, may have missed some important resistance findings; we fully agree with the EASL recommendation that retreatment based on resistance findings should be performed in the context of a multidisciplinary team including virologists with a deep knowledge of RASs' impact, a continuously updated list of RASs (like the Sorbo *et al.* 2018 update[13]) and the participation of experienced HCV-treating clinicians. Finally, and as the main limitation, the arrival of new molecules, especially the combination of sofosbuvir/velpatasvir/voxilaprevir, which has been approved for the retreatment of patients who fail DAAs may outdate our results.

In conclusion, we have shown that resistance findings in conjunction with an interpreted report allow patients to achieve SVR rates close to 90%. We believe that our data may be of special relevance for those countries where new drug combinations are still not available, and may enable treatment of patients at a lower cost, avoiding drug-drug interactions and preserving the 3-drug combination regimen. We hypothesize that SVR rates may even be improved if resistance data are discussed between experienced virologists and treating clinicians.

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Conflict of interest

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

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Supplementary data

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