

# Viral dynamics among HCV infected patients with different genotypes treated with genotypic specific or pan-genotypic direct-acting antiviral agent combinations

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**Background:** New hepatitis C virus (HCV) therapies have improved efficacy, allowed pangenotypic applications, increased barriers to drug resistance and shortened therapy duration.

**Methods:** Patients infected with different HCV genotypes were divided into two groups: group 1 included 169 patients receiving genotypic specific regimens (GSR), while group 2 included 186 patients receiving pan-genotypic regimens (PGR). Patient's HCV RNA was quantified and sequenced.

**Results:** Comparable sustained viral response (SVR) rates were observed in both GSR and PGR treated patients. Nevertheless, even if not significant, a greater proportion of non-detectable levels (NDL) of HCV RNA was observed in patients treated with PGR as compared with GSR. Overall, among patients in the GSR and PGR groups with residual viremia, 124/169 (73.4%) and 125/186 (67.2%) at four weeks, and 66/169 (39.1%) and 58/186 (31.2%) at eight weeks, achieved SVR. No difference was observed in the clinical outcome comparing patients in the GSR and PGR groups according to genotype. While, comparing patients between the two groups, the proportion of patients with NDL HCV RNA at four and eight weeks was higher in patients infected with genotype 1b treated with PGR ( $p=0.0015$ ). A significantly higher number of patients infected with 1b had RASs at baseline ( $p=0.0001$ ). In addition, the proportion of patients with treatment failure was higher in patients with RASs at baseline compared with those without ( $p=0.012$ ). Overall, 2.5% patients failed to achieve SVR after DAA treatment.

**Conclusion:** A sharp HCV RNA decrease was observed in patients treated with both GSR and PGR. However, even if comparable, a slightly greater number of patients treated with PGR achieved NDL HCV RNA as compared with GSR. A significant difference was observed in patients with baseline RASs, both in relation to treatment failure and genotype. In conclusion, the use of new DAA combinations helps patients achieve a more rapid virologic response.

**Keywords:** hepatitis C virus, drug resistance, DAA inhibitors, viral dynamics, treatment failure

## Introduction

Chronic infection with hepatitis C virus (HCV) affects more than 70 million individuals worldwide, and genotype distribution varies according to different geographic areas.<sup>1</sup> These subjects are at risk of liver cirrhosis, hepatocellular carcinoma and liver-related death.<sup>1,2</sup>

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After the introduction of direct-acting antivirals (DAAs), which specifically block key viral enzymes, chronic HCV infections have been successfully treated in cirrhotic, non-cirrhotic patients and those coinfecting with HIV-1 and/or HBV.<sup>3–5</sup>

Recently, improved DAA combinations have been approved and have a safer profile with minimal adverse events. Highly effective and safe interferon (IFN)-free DAA treatments are available, with a very high genetic barrier to resistance, antiviral potency and pangenotypic coverage.<sup>6–11</sup> These drugs are associated with a sustained virologic response (SVR) in an even higher percentage of patients (98–100%) in comparison with previously approved DAAs.<sup>6,7,12,13</sup> In addition, the duration of treatment has been shortened to eight weeks, which simplifies treatment decisions, adherence, tolerability and potentially allows for the treatment of more patients.<sup>14–16</sup> Although the efficacy of these new drugs is extremely high, DAA failure is an unfortunate event that can occur in different clinical situations, and it is frequently associated with the onset of HCV resistance-associated substitutions (RASs).<sup>17–20</sup> HCV RASs found after failure generally develop during or at the end of treatment, while in some patients they may even have been present at baseline.<sup>21–23</sup>

The aims of this study were to evaluate the dynamics of rapid virologic response (RVR) with respect to SVR in patients treated with different DAA combinations during the period from January 2017 to May 2018 and to illustrate the frequency of DAA-resistant variants at baseline in all patients and in patients failing treatment.

## Methods

### Patients

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Review Board of the Fondazione IRCCS Policlinico San Matteo (protocol no. 20080009620). Written informed consent was obtained from all study participants. Three hundred and fifty five HCV infected patients with HCV genotypes 1, 2, 3 and 4, referred to the Fondazione IRCCS Policlinico San Matteo, Pavia between January 2017 and September 2018 were enrolled following the inclusion criteria set by the Italian Medicines Agency (AIFA). According to the Italian guidelines patients with hepatocellular carcinoma patients were excluded because treatment were not refundable. Patients were divided into two groups:

group 1 included 169 patients receiving genotypic specific regimens (GSR): paritaprevir/ombitasvir/dasabuvir/ritonavir/ribavirin (par/omb/das/rtv/rbv), sofosbuvir/daclatasvir (sof/dac), sofosbuvir/ledipasvir (sof/led) or elbasvir/grazoprevir (elb/graz), and group 2 included 186 patients receiving pan-genotypic regimens (PGR): sofosbuvir/velpatasvir (sof/vel), or pibrentasvir/glecaprevir (pib/gle). Therapeutic decisions were made following the recommendations of the European Association for the Study of the Liver (EASL) clinical practice guidelines<sup>17</sup> and AIFA criteria for HCV treatment (<http://www.agenziafarmaco.gov.it>). Treatment was prolonged to 24 weeks when indicated. All patients were compliant to the therapy. Patients with any adverse event leading to discontinuation were excluded. Only serious adverse events were reported.

Serum samples from each patient were prospectively collected at baseline and at months 1, 2 and 3 to evaluate RVR during treatment depending on the different DAA combinations: Patients were monitored for at least three months after treatment completion to evaluate SVR.

### HCV-RNA quantification and genotyping

HCV viral load decreases in patients who achieved SVR and treatment-failing patients were compared at baseline and each month during and after stopping treatment using the Abbott HCV-RNA assay (Abbott Park, Illinois, U.S.A.), with a lower limit of detection of 12 IU/ml. To quantify HCV RNA levels <12 IU/ml, threshold cycles (CT) were used to obtain values with reference to a linear quantification curve. Non-detectable (NDL) HCV RNA levels were defined when target RNA samples were negative. HCV genotypes were defined using the Abbott RealTime HCV Genotype II assay (Abbott Park, Illinois, U.S.A.). In addition, when genotype results were ambiguous, the NS3/NS5B region was sequenced to further subtype HCV strains. Data were analysed using the Blast program (<http://blast.ncbi.nlm.nih.gov>).

Viral RNA was extracted from serum samples using the automatic Easy Mag extractor (Biomerieux, Lyon, France). Evaluation of RASs in the HCV NS5A, NS3 and/or NS5B genes was performed in 316/355 (89%) patients at baseline according to the drug class included in the DAA regimen.<sup>24</sup> Direct Sanger-sequencing of the NS3-protease (aa 1-181), NS5A domain I (aa 1–213), and NS5B (aa 1-591) genes was performed using an automatic sequencer (ABI PRISM 3130xl genetic analyzer DNA Sequencer, Applied Biosystems, Foster City, CA, USA) and the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA,

USA). RASs were defined according to the geno2pheno algorithm [http://hcv.geno2pheno.org/index.php] and other clinical and in vitro data for RAS interpretation.<sup>18,19,25,26</sup>

## Statistical analysis

Continuous variables (ie viral load) were compared using the Mann-Whitney U test for independent non-parametric data. Categorical variables were compared by the chi-square or Fisher's exact test, as appropriate. The Log-rank test was used to compare Kaplan-Meier curves. All of the analyses were two tailed and performed using GraphPad Prism version 5 (GraphPad Software Inc., CA, USA); *p*-values of  $\leq 0.05$  were considered statistically significant. The positive predictive value (PPV) and negative predictive value (NPV) of NDL HCV RNA (less than the lower limit of quantification (<LLOQ) or quantifiable

( $\geq$ LLOQ)) at four and eight weeks of treatment were calculated for all patients in the GSR and PGR groups.<sup>27</sup>

## Results

### Efficacy outcomes by different treatment and genotype

Patient demographic and virological characteristics as well as risk factors are reported in Table 1. All patients were DAA treatment naïve, except for two patients receiving the elb/graz and sof/vel regimen respectively, who had received a DAA protease inhibitor in 2016. The proportion of patients with cirrhosis in the GSR group was slightly higher than that treated with PGR (*p*=0.09) (Table 1). In detail, the fraction of patients with cirrhosis was higher in the sof/led group (33.3%), while it was lower in the sof/vel group (7.5%).

**Table 1** Patient characteristics

	Treatment		p-value*
	Genotypic specific Regimens (GSR) N° 169	Pan-genotypic Regimens (PGR) N° 186	
Gender			0.88
Male	85 (50.3%)	96 (51.6%)	
Female	84 (49.7%)	90 (48.4%)	
Nationality			0.74
Italian	164 (97.1%)	182 (97.8%)	
Other	5 (2.9%)	4 (2.2%)	
Genotype			
1a	20 (11.8%)	35 (18.8%)	
1b	85 (50.3%)	44 (23.6%)	
2	28 (16.5%)	76 (40.9%)	
3	12 (7.1%)	25 (13.5%)	
4	24 (14.3%)	6 (3.2%)	
HIV-1/HBVcoinfection	28 (16.6%)	40 (21.5%)	0.28
BL PLT, per cubic ml (range)	188,000 (26,000–940,000)	196,000 (64,000–870,000)	0.17
BL ALT, U/liter	51.4±49.7	64.3±93.1	0.22
BL AST, U/liter	41.8±33.8	53.8±85.1	0.54
BL Bilirubin, µmol/liter	14.0±7.8	41.0±330.0	0.67
Serious adverse event (grade 3–4, WHO)	11 (6.5%)	8 (4.3%)	0.48
Baseline median HCV viral load (IU/ml log <sub>10</sub> )	5.71 (range 3.1–7.1)	5.74 (range 3.0–6.9)	0.39
Naïve	139 (82.2%)	146 (78.5%)	0.42
IFN/RBV experienced	30 (17.8%)	40 (21.5%)	0.42
DAA experienced	1 (0.6%)	1 (0.5%)	1.00
Cirrhosis (>12.5 Kpa)	29 (17.1%)	20 (10.75%)	0.09
HCV RNA>6,000,000 IU/ml	10 (5.9%)	17 (9.1%)	0.32
Baseline drug RASs	9/140 (6.4%)	17/176 (9.7%)	0.41
Previous IFN-treatment	30 (17.7%)	40 (21.5%)	0.42

**Note:** \*Fisher's exact or Chi-square test.

**Abbreviations:** BL, baseline; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

A comparable sustained viral response (SVR) was observed in both GSR and PGR treated patients (Figure 1) (97.0% vs 97.8%;  $p=0.88$ ). However, in the elb/graz group we only observed SVR in 91.7% of the patients.

No differences in HCV RNA decay (delta log) between the two groups were observed at four weeks of therapy ( $p=0.35$ , data not shown). Nevertheless, even not significant, a slightly greater fraction of NDH HCV RNA was observed in patients treated with PGR as compared with GSR at all time points (27.2% vs 32.3% at four weeks; 62.7% vs 71.1% at eight weeks; and 88.8% vs 91.3% at 12 weeks;  $p=0.12$ ; Figure 2A). Among patients treated with GSR, the sof/led combinations showed a greater proportion of patients with NDH HCV RNA at four, eight and 12 weeks (respectively, 42.9%, 81.0% and 95.2%) as compared with the combinations: elb/graz (38.9%, 72.2% and 94.4%), sof/dac (31.0%, 64.3% and 90.5%) and par/omb/das (14.3%, 51.4% and 82.9%) ( $p=0.08$ ; Figure 2B). Among patients treated with PGR, a comparable percentage of NDH HCV RNA at four, eight and 12 weeks was observed in patients treated with the pib/gle (respectively, 34.0%, 78.6% and 94.7%) and sof/vel (31.6%, 68.1% and 90.9%) combinations, ( $p=0.23$ ; Figure 2C). HCV RNA levels at 24 weeks of treatment were always undetectable in SVR patients.

Overall, no significant difference in the proportion of patients with NDH HCV RNA was observed in the GSR and PGR therapy groups according to genotype (Figure 3A and B,  $p=0.24$  and  $p=0.76$ ). However, in the GSR group a higher fraction of patients with NDH HCV RNA at four weeks was observed in patients infected with genotypes 1a

and 2 (40.0% and 39.3%) and in patients infected with genotype 3 at eight weeks (83.3%; Figure 3A). The lowest proportion of patients with NDH HCV RNA was observed in patients infected with genotype 4 at four and eight weeks (12.5% and 50.0%). Among the PGR patients, a higher proportion with NDH HCV RNA at four weeks was observed in patients infected with genotypes 1b and 4 (34.1% and 33.3%), and at eight weeks in patients infected with genotype 1b (79.5%) (Figure 3B). While, the lowest proportion of patients with NDH HCV RNA at four weeks was observed in patients infected with genotype 3 (28.0%) and at eight weeks in patients infected with genotype 1a (54.3%; Figure 3B). A significant difference was observed only for genotype 1b ( $p=0.0015$ ); when comparing GSR and PGR groups according to genotype (Figure 3C–G). The proportion of patients with NDH HCV RNA at four and eight weeks was 24.9% and 61.5% in the GSR group and 34.1% and 79.5% in the PGR group.

### NDH HCV RNA predictive values for SVR at 4 and 8 weeks

In patients treated with GSR, 36.7% (62/169) had HCV RNA <LLOQ and 34.4% (45/169) had NDH HCV RNA at week 4. Among 62 patients with HCV RNA <LLOQ, 60 patients achieved SVR (PPV, 96.8%) and of the 45 patients with NDH HCV RNA, 44 patients achieved SVR (PPV, 97.8%). A total of 62 treated with GSR had RNA  $\geq$ LLOQ at week four, of whom two relapsed (NPV, 3.2%). Overall, 124/169 (73.4%) patients had HCV RNA  $\geq$ LLOQ ( $n=62$ )

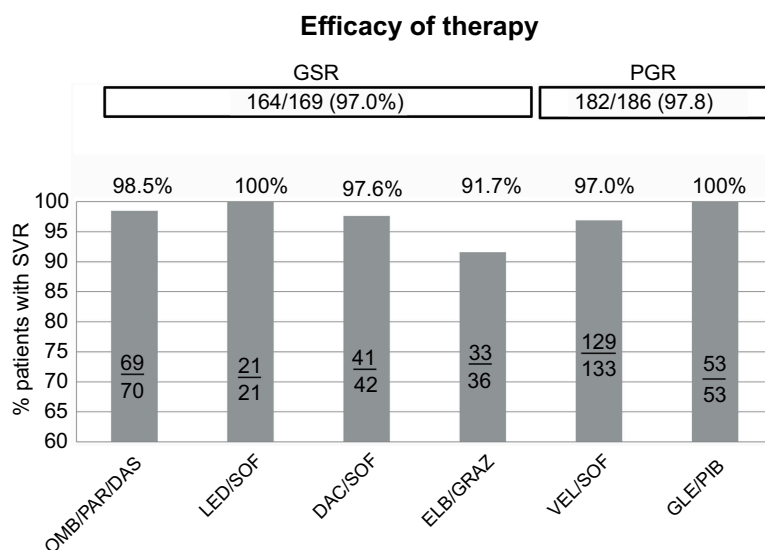
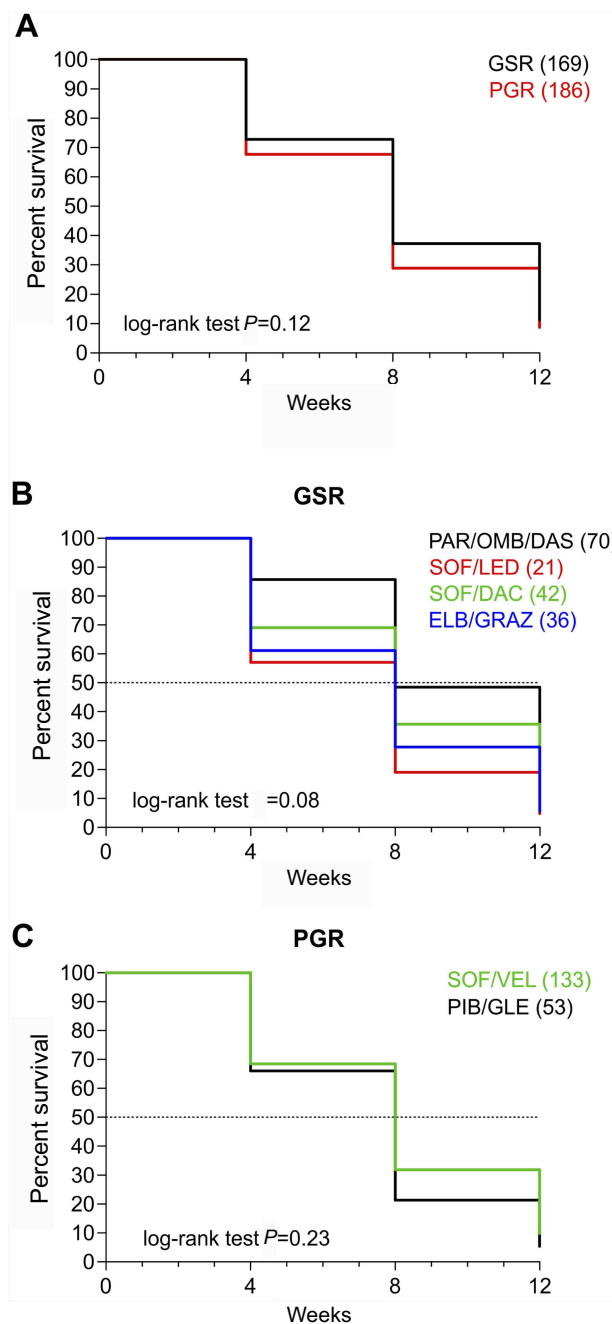


Figure 1 End of therapy SVR rates according to different treatments.



**Figure 2** Kaplan–Meier curves of cumulative undetectable HCV RNA levels in patients according to GSR vs PGR (A) or in patients grouped as having GSR (B) or PGR (C). *p*-values were obtained using the log-rank test.

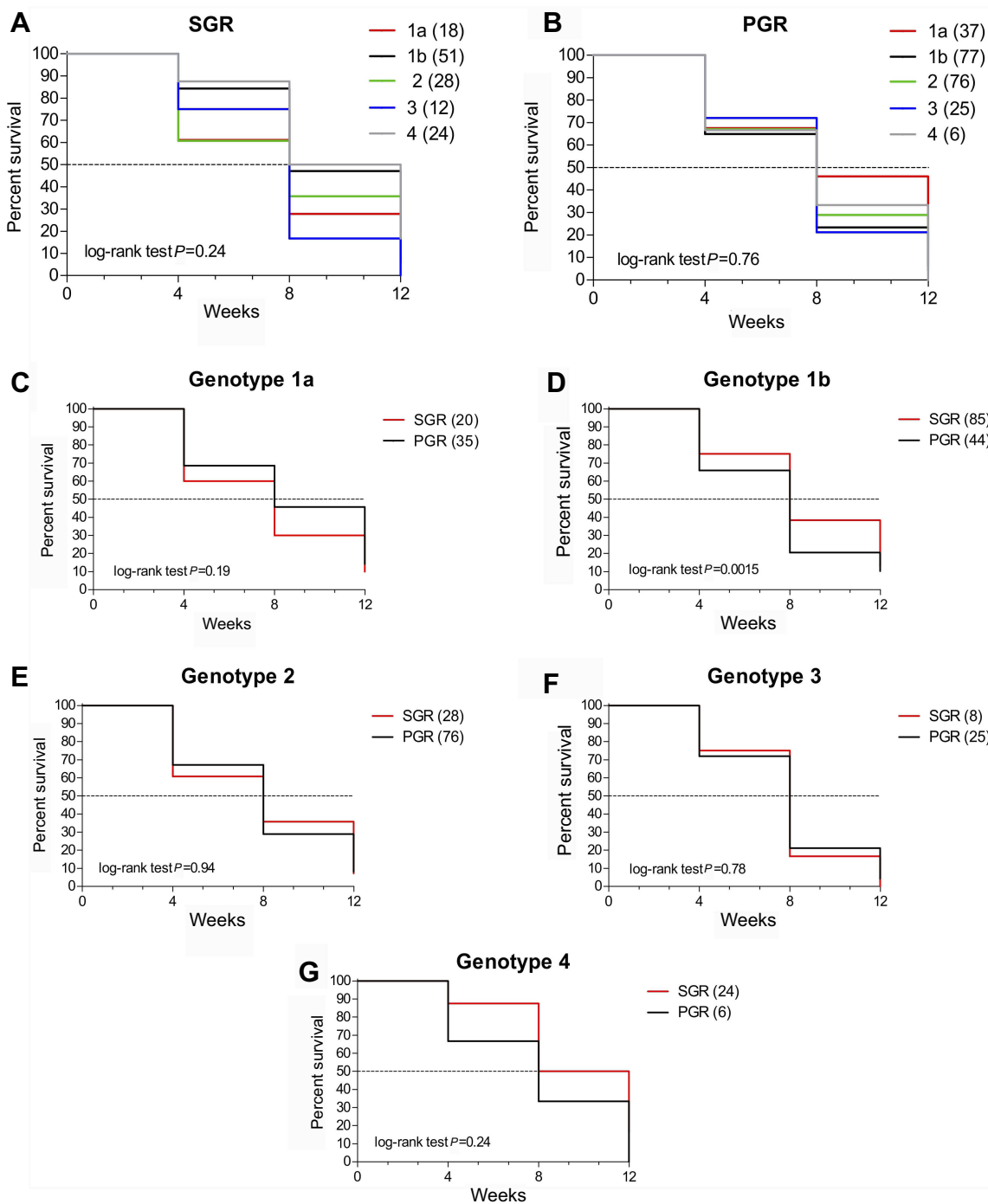
or HCV RNA <LLOQ (n=62), of whom only four relapsed (NPV, 3.2%). At week 8, among patients treated with GSR, 29.0% (49/169) had HCV RNA <LLOQ and 60.9% (103/169) had NDH HCV RNA. Among 49 patients with HCV RNA <LLOQ, 47 patients achieved SVR (PPV, 95.9%) and of the 103 patients with NDH HCV RNA, 100 patients achieved SVR (PPV, 97.1%). A total of 17 patients treated with GSR had RNA  $\geq$ LLOQ at week 8,

none of whom relapsed (NPV, 0%). Overall, 66/169 (39.1%) patients had HCV RNA  $\geq$ LLOQ (n=17) or HCV RNA <LLOQ (n=49), only two of whom relapsed (NPV, 3.0%).

In patients treated with PGR, 33.9% (63/186) had HCV RNA <LLOQ and 34.4% (64/186) had NDH HCV RNA at week 4. Among 63 patients with HCV RNA <LLOQ, 60 patients achieved SVR (PPV, 95.2%) and of the 64 patients with NDH HCV RNA, all achieved SVR (PPV, 100%). A total of 62 patients treated with PGR had RNA  $\geq$ LLOQ at week 4, of whom one relapsed (NPV, 1.6%). Overall, 125/186 (67.2%) patients had HCV RNA  $\geq$ LLOQ (n=62) or HCV RNA <LLOQ (n=63), of whom only four relapsed (NPV, 3.2%). At week 8, among patients treated with PGR, 22.0% (41/186) had HCV RNA <LLOQ and 68.8% (128/186) had NDH HCV RNA. Among 41 patients with HCV RNA <LLOQ, all achieved SVR (PPV, 100%) and of the 128 patients with NDH HCV RNA, 124 patients achieved SVR (PPV, 96.9%). A total of 17 patients treated with PGR had RNA  $\geq$ LLOQ at week 8, none of whom relapsed (NPV, 0%). Overall, 58/186 (31.2%) patients had HCV RNA  $\geq$ LLOQ (n=17) or HCV RNA <LLOQ (n=41), none of whom relapsed (NPV, 0%). The high PPV and low NPV values observed after both 4 and 8 weeks of treatment with GSR are similar to those observed in patients treated with PGR.

## Resistance analysis

An analysis of RASs was performed at baseline in 316/355 (89%) patients. In particular, 88/316 (27.8%) patients were infected with genotype 1b and 228/316 (72.2%) patients with other genotypes. A significantly higher number of patients infected with 1b (18/88, 20.5%) had RASs at baseline compared to those with other genotypes (8/228, 3.5%;  $p=0.0001$ ). Overall, 140/169 (82.8%) patients were analyzed in the GSR group and 176/186 (94.6%) in the PGR group. HCV RASs were observed in 9/140 (6.4%) in the GSR group and 17/176 (9.7%) in the PGR group. Of these, RASs were present in 4/26 (15.4%) patients with HCV genotype 1a, 18/26 (69.2%) patients with genotype 1b, 1/26 (3.8%) patient with genotype 2c and 3/26 (11.6%) patients with genotype 3a. Of these, RASs associated with the DAA regimen, in at least one of the three target genes, were observed in 21/26 (80.7%) patients, 8/21 (38.1%) patients in the GSR group and 13/21 (61.9%) patients in the PGR group (Table 2, in bold). In particular, one or more RASs associated with the DAA regimen were observed: 56F in the NS3 gene of 1/26 (3.8%) patients,



**Figure 3** Kaplan–Meier curves of cumulative HCV RNA undetectable levels according to genotype and patients grouped as having GSR (A), PGR (B). Kaplan–Meier curves of cumulative HCV RNA undetectable levels according to genotype (C–G). *p*-values were obtained using the log-rank test.

28M,30K/R,31M/T and 93H in the NS5A gene of 16/26 (61.5%) patients and 159F, 448H,451H/Y, 556G in the NS5B gene of 5/26 (19.2%) patients (Table 2).

Overall, 9/355 (2.5%) patients failed to achieve SVR after DAA treatment. The proportion of patients with treatment failure was higher in patients with RASs at baseline (14.3%) compared with those without (6/334,

1.8%) ( $p=0.012$ ). Of these, 5/169 (3.0%) patients treated with GSR and 4/186 (2.2%) patients treated with PGR, relapsed during the first month following treatment completion (Table 3). Among the 21 patients with baseline RASs to the ongoing DAA regimen, 3/21 (14.3%) patients failed treatment. Of these, a comparable number of patients, 2/8 (25%) in GSR and 1/13 (7.7%) PGR, failed

**Table 2** DAA baseline RASs in HCV genes

Group of treatment	# patient	Genotype	Therapy	Baseline RASs			Outcome
				NS3	NS5A	NS5B	
GSR	1	1a	Omb,Par,Das,RTV, RBV	None	31M	None	Responder
	2	1b	<b>Omb</b> ,Par,Das,RTV, RBV	None	<b>93H</b>	None	Responder
	3	1b	<b>Omb</b> ,Par,Das,RTV, RBV	None	<b>28M,93H</b>	None	<b>Relapser</b>
	4	1b	<b>Led</b> , Sof	None	<b>93H</b>	None	Responder
	5	1b	<b>Elb</b> +Graz	None	<b>93H</b>	None	Responder
	6	1b	Elb+ <b>Graz</b>	<b>56F</b>	None	None	Responder
	7	1b	<b>Elb</b> +Graz	None	<b>93H</b>	None	<b>Relapser</b>
	8	1b	<b>Elb</b> +Graz	None	<b>93H</b>	None	Responder
	9	1b	<b>Elb</b> +Graz	None	<b>93H</b>	None	Responder
PGR	10	1a	Sof + <b>Vel</b>	None	<b>31M</b>	None	Responder
	11	1a	Sof + <b>Vel</b>	None	<b>30R</b>	None	Responder
	12	1a	<b>Pib</b> +Gle	None	<b>28T+30R</b>	None	Responder
	13	1b	Sof +Vel	None	None	556G	Responder
	14	1b	Sof +Vel	None	None	448H, 451Y	Responder
	15	1b	Sof +Vel	None	None	451H, 556G	Responder
	16	1b	<b>Sof +Vel</b>	None	<b>93H</b>	<b>159F</b>	Responder
	17	1b	<b>Sof +Vel</b>	None	None	<b>159F</b>	Responder
	18	1b	Sof + <b>Vel</b>	None	<b>28M+93H</b>	None	Responder
	19	1b	<b>Sof +Vel</b>	None	None	<b>159F</b>	Responder
	20	1b	<b>Sof +Vel</b>	None	None	<b>159F</b>	Responder
	21	1b	<b>Sof +Vel</b>	None	None	<b>159F</b>	Responder
	22	1b	Pib+Gle	None	93H	None	Responder
	23	2c	Sof + <b>Vel</b>	None	<b>31M</b>	None	<b>Relapser</b>
	24	3a	Sof + <b>Vel</b>	None	<b>93H</b>	None	Responder
	25	3a	Sof + <b>Vel</b>	None	<b>93H</b>	None	Responder
	26	3a	Sof + <b>Vel</b>	None	<b>30K</b>	None	Responder

**Notes:** RASs to ongoing therapy and drug regimen-including combination with reduced sensitivity are in Bold; the RASs not correlated with the ongoing therapy are in plain style. **Abbreviations:** Omb, ombitasvir; Par, paritopravir; Das, dasabuvir; RTV, ritonavir; RBV, ribavirin; Led, ledipasvir; Sof, sofosbuvir; Elb, elbasvir; Graz, grazoprevir; Vel, velpatasvir; Pib, pibrentasvir; Gle, glecaprevir.

treatment ( $p=0.53$ ). While no RASs were observed at baseline in the remaining six patients with treatment failure (Table 3). RASs to ongoing therapy in the NS3 gene (56F/H, 168V) and/or in the NS5A gene (28M, 31M, 93H) were observed in 6/9 (66.7%) patients following treatment, four patients from the GSR group and two from the PGR group.

## Discussion

HCV RNA levels have been considered a predictive on-therapy marker of treatment outcome in patients receiving peg-IFN and ribavirin.<sup>28</sup> Currently, a number of studies with IFN-free DAA combination therapies reporting a rapid viral decline after a few weeks of treatment have shown SVR rates >90%.<sup>6,7,12,13,15</sup> However, it is still unclear whether early RVR during therapy with undetectable HCV RNA, measured with a highly sensitive assay, may be useful to predict

virological failure. In monotherapy DAA studies, residual detectable viremia at four weeks of protease inhibitor-based therapies was associated with higher relapse rates.<sup>29–31</sup> Recently, highly effective IFN-free DAA treatments have led to RVR in 4–8 weeks of treatment in a high portion of patients.<sup>27,32,33</sup> However, using more sensitive assays at early time points, it was shown that residual viremia was detectable as late as 12 weeks of treatment in a portion of patients,<sup>27,32,34</sup> who subsequently achieved SVR.

Our data showed that residual viremia, measured in patients treated with GSR and PGR, was detectable in different percentages of patients at 4 to 12 weeks of treatment. Nevertheless, the majority of patients with residual viremia at four and eight weeks achieved SVR and comparably high PPVs and low NPVs between the two groups were observed. In addition, despite a slight difference in the proportion of patients with cirrhosis at baseline

**Table 3** DAA RASs in patients with virologic failure

Patient	Genotype	Therapy (weeks)	RASs						Outcome	Risk factor	HCV RNA at 4 weeks
			NS3		NS5A		NS5B				
			Baseline	At Failure	Baseline	At Failure	Baseline	At Failure			
1	1b	<b>Omb,Par, Das,RTV</b> (12)	None	None	<b>28M,93H</b>	<b>28M,93H</b>	None	None	<b>Relapser</b>	IFN*	<12 IU/ml
2	2	Dac,Sof (12)	None	None	None	None	None	None	<b>Relapser</b>	Cirrhosis	NEG
3	1b	<b>E1b,Graz</b> (12)	174F	174F	None	<b>93H</b>	nd	None	<b>Relapser</b>	IFN	26 IU/ml
4	1b	E1b, <b>Graz</b> (12)	None	<b>56F</b>	None	31I	None	None	<b>Relapser</b>	None	<12 IU/ml
5	1b	<b>E1b,Graz</b> (12)	None	<b>56H,168V</b>	<b>93H</b>	<b>31V, 93H</b>	None	None	<b>Relapser</b>	Cirrhosis, IFN	20 IU/ml
6	1a	Sof,Vel (12)	None	None	None	174F	nd	None	<b>Relapser</b>	IFN	<12 IU/ml
7	3	Sof,Vel (12)	None	None	none	<b>93H</b>	None	None	<b>Relapser</b>	IFN	<12 IU/ml
8	2c	Sof,Vel (12)	None	None	<b>31M</b>	<b>31M</b>	None	None	<b>Relapser</b>	IFN	<12 IU/ml
9	2c	Sof,Vel (12)	None	None	None	None	None	None	<b>Relapser</b>	Cirrhosis	17 IU/ml
			None	None	None	None	None	None	<b>Relapser</b>	None	<12 IU/ml

**Notes:** RASs to ongoing therapy and drug regimen-including combination with reduced sensitivity are in Bold; \*Previous Interferon treatment. **Abbreviations:** Omb, ombitasvir; Par, paritaprevir; Das, dasabuvir; RTV, ritonavir; Dac, daclatasvir; Sof, sofosbuvir; E1b, elbasvir; Graz, grazoprevir; Vel, velpatasvir; nd, not done; NEG, not detected.

between the two groups, no differences were highlighted in the clinical outcome. Similarly, comparable HCV RNA decay and SVR ( $\geq 97\%$ ) were obtained. Thus, HCV RNA measurements during treatment didn't add significant advantages in predicting the outcome in this study. In addition, residual viremia at four and eight weeks of treatment was not predictive of relapse with GSR and PGR as also previously reported.<sup>27,32</sup> However, differences in RVR at four, eight and 12 weeks of treatment were observed. In fact, even if not significant, a slightly greater fraction of NDH HCV RNA, was observed in patients treated with PGR as compared with GSR. Even though, among the GSR, a comparable proportion of NDH HCV RNA at four, eight and 12 weeks was observed also in the sof/led treated. Therefore, overall PGR seems to be slightly more efficient in eliminating viral load at four, eight and 12 weeks.

Stratifying patients by genotype, it was shown that differences in the levels of HCV decay and proportion of residual viremia, at four, eight and 12 weeks of treatment, were not overall statistically significant in the GSR or PGR groups. Similarly, in these patient subgroups, SVR was achieved regardless of genotype. However, at four weeks, patients infected with genotypes 1a and 2 in the GSR group and with genotype 1b and 4 in the PGR group had a higher fraction of NDH HCV RNA. While, comparing genotypes between the GSR and PGR groups, a proportion of patients with NDH HCV RNA at four and eight weeks was significantly higher in patients infected with genotype 1b treated with PGR.

Among the nine patients with virologic treatment failure, one patient had NDH HCV RNA at four weeks of treatment, while at the same time point eight patients had detectable HCV RNA. In these eight patients, NDH HCV RNA was achieved after 8–12 weeks of treatment. Treatment failure was most likely caused by the onset of RASs (6 patients) or by incomplete HCV RNA suppression (3 patients). Virologic failure in patients treated with GSR and PGR was comparable (3.0% vs 2.2%). Nevertheless, regarding baseline RASs as a risk factor, a significant difference was observed in clinical outcome. In fact, the frequency of treatment failure was higher in patients with RASs at baseline compared to those without. Special attention must be paid to patients infected with genotype 1b who appear to have a more significant chance of having RASs at baseline. This suggests that baseline sequencing, especially in genotype 1b, may help define the most appropriate regimen. In addition, despite the low

number of patients in whom RASs were present at baseline in the GSR group a slightly higher proportion of patients failed treatment as compared with those treated with PGR (25.0% vs 7.7%).

In conclusion, considering all of the patients, a significant difference was observed in patients with baseline RASs, both in relation to treatment failure and genotype. Nevertheless, no significant differences were found in clinical outcome comparing the GSR and PGR groups, in relation to the therapy, genotype or baseline RASs. However, the introduction of new and more efficient DAA combinations with good pharmacodynamics has led to better response rates and more rapid decreases in viral load. On the other hand, in difficult to treat patients, or those treated with short-term therapies,<sup>14,15,33</sup> should be monitored carefully since short treatment duration may lead to an incomplete suppression of HCV replication or the onset of RASs. In addition, due to high cross-resistance among NS5A-inhibitors and the essential role of this class, NS5A experienced-patients are the most “difficult to retreat”. This evolution in treatment pharmacodynamics may still improve by using triple combination therapies that affect the three genes of the target virus resulting in more efficient virus elimination and shorter treatment duration.<sup>17,20</sup> Hence, the elimination of HCV RNA, which typically initially decreases rapidly, then more slowly with residual viremia,<sup>35</sup> may be accelerated. Importantly, this acceleration may also reduce the possibility of transmission in the highest at risk populations.

Strengths of this study are: i) number of patients analyzed with all HCV genotypes were considered; iii) only a limited number of reports considered pan-genotypic vs genotypic specific treatment. Limitations are: i) the number of failures (as expected) is low, then only few patients failing therapy (no. 9) could be included in the post treatment analysis; ii) the RASs analysis was performed using the Sanger technology with a resolution level lower than deep sequencing methodology. Then, some mutants could have been missed. iii) Hepatocellular carcinoma patients could not be included due to the Italian guidelines.

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## Author contributions

Stefania Paolucci: concept or design of the work, data analysis and interpretation, drafted the article. Federica Novazzi, Alice Fratini, Laura Bellotti: data collection. Renato Maserati: critical revision of the article. Roberto Gulminetti, Stefano Novati, Giorgio Barbarini, Paolo Sacchi: patient enrollment. Antonio Piralla: data analysis and interpretation. Fausto Baldanti: critical revision of the article and final approval of the version to be published. All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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