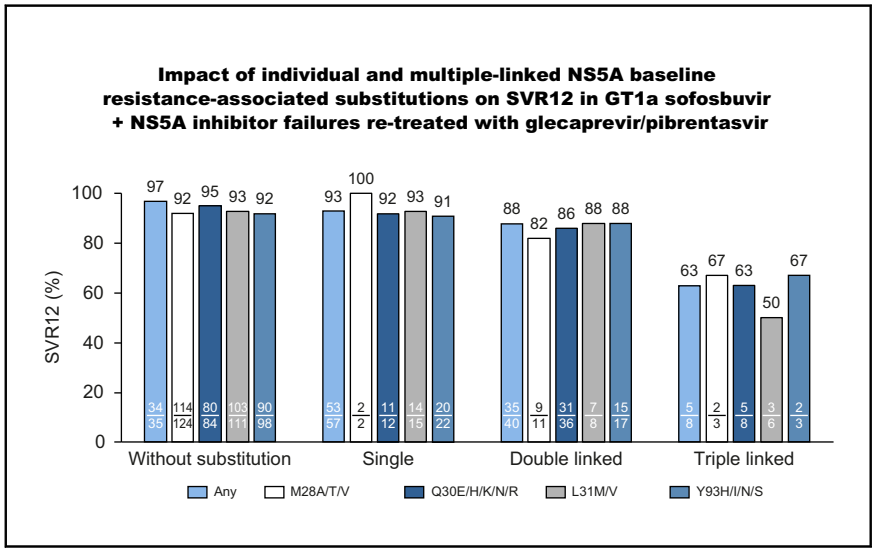
Research Article
Viral Hepatitis

JOURNAL
OF HEPATOLOGY

Linkage of resistance-associated substitutions in GT1 sofosbuvir + NS5A inhibitor failures treated with glecaprevir/pibrentasvir

Graphical abstract



Highlights

- Primer-ID NGS tracked multiply linked NS5A RASs in GT1 failures re-treated with G/P.
- Decreasing SVR12 rates with double- or triple-linked NS5A RASs.
- No single GT1a NS5A RAS or NS3 RAS was associated with a reduced SVR12.
- Among 13 failures, 4 had treatment-emergent NS3 RASs; 10 had additional NS5A RASs.
- 85% of GT1a with double- or triple-linked NS5A RASs achieved SVR12 with 16 weeks G/P.

Authors

Gary P. Wang, Gretja L. Schnell, Jens J. Kort, …, Joy A. Peter, Michael W. Fried, David R. Nelson

Correspondence

gary.wang@medicine.ufl.edu
(G.P. Wang).

Lay summary

Direct-acting antivirals have revolutionized the treatment of chronic hepatitis C infection, but treatment failure occurs in some patients. Retreatment of patients who previously failed a regimen consisting of sofosbuvir and an NS5A inhibitor with a regimen of glecaprevir and pibrentasvir (G/P) is >90% effective. Herein, we analyzed samples from these patients and showed that retreatment efficacy with G/P is lower in patients with double- or triple-linked NS5A resistance mutations than in patients with single or no NS5A resistance mutations.

https://doi.org/10.1016/j.jhep.2021.04.057
 J. Hepatol. 2021, 75, 820–828

Research Article
Viral Hepatitis

JOURNAL OF HEPATOLOGY

Linkage of resistance-associated substitutions in GT1 sofosbuvir + NS5A inhibitor failures treated with glecaprevir/pibrentasvir

Gary P. Wang[1,2,*], Gretja L. Schnell[3], Jens J. Kort[4], Gurjit S. Sidhu[1], Layla Schuster[1], Rakesh L. Tripathi[3], Lois Larsen[3], Larry C. Michael[5], Ken Bergquist[5], Ashley Magee[5], Chandni B. Patel[1], Joan A. Whitlock[1], Ryan Tamashiro[1], Joy A. Peter[6], Michael W. Fried[7], David R. Nelson[8]

[1]*Division of Infectious Diseases and Global Medicine, Department of Medicine, University of Florida, Gainesville, FL, USA;* [2]*Infectious Disease Section, Medical Service, North Florida/South Georgia Veterans Health System, Gainesville, FL, USA;* [3]*AbbVie Inc, North Chicago, IL, USA;* [4]*AbbVie Inc, Mettawa, IL, USA;* [5]*HCV-Target Data Coordinating Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA;* [6]*Hepatology Research, University of Florida, Gainesville, FL, USA;* [7]*Division of Gastroenterology and Hepatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA;* [8]*Department of Medicine, University of Florida, Gainesville, FL, USA*

Background & Aims: Retreatment with glecaprevir/pibrentasvir (G/P) resulted in a rate of sustained virologic response 12 weeks after treatment completion (SVR12) of >90% in HCV genotype 1 (GT1) patients who previously failed a regimen of sofosbuvir plus an NS5A inhibitor (NS5Ai). This study investigated the prevalence and impact of baseline NS3 and NS5A resistance-associated substitutions (RASs) on the efficacy of G/P in prior GT1 sofosbuvir+NS5Ai failures and the persistence of treatment-emergent RASs.

Methods: Longitudinal samples from 177 patients enrolled in a phase IIIb, randomized pragmatic clinical trial were analyzed. Patients without cirrhosis were randomized to 12 or 16 weeks of G/P, and patients with compensated cirrhosis were randomized to G/P and ribavirin for 12 weeks or G/P for 16 weeks. Linkage of RAS was identified using Primer-ID next-generation sequencing at a 15% cut-off.

Results: Of 177 patients, 169 (95.5%) were PI-naïve. All 33 GT1b-infected patients achieved SVR12. In GT1a-infected patients, baseline NS5A RASs were prevalent (74.5%, 105/141) but NS3 RASs were uncommon. Baseline NS3 RASs had no impact on G/P efficacy and patients with baseline NS5A RASs showed a numerically but not statistically significantly lower SVR12 rate compared to those without NS5A RASs (89% *vs.* 97%). SVR12 was achieved in 34 of 35 (97%) patients without NS5A baseline substitution, and 53 of 57 (93%), 35 of 40 (88%), 5 of 8 (63%) with single, double-linked, and triple-linked NS5A substitutions, respectively. Among 13 patients with virologic failure, 4 acquired treatment-emergent NS3 RASs and 10 acquired NS5A RASs.

Conclusion: Baseline NS5A RASs were highly prevalent. The presence of an increasing number of linked NS5A RASs in GT1a showed a trend in decreasing SVR12 rates, although no specific NS5A RASs or their linkage pattern were associated with lower SVR12 rates.

Lay summary: Direct-acting antivirals have revolutionized the treatment of chronic hepatitis C infection, but treatment failure occurs in some patients. Retreatment of patients who previously failed a regimen consisting of sofosbuvir and an NS5A inhibitor with a regimen of glecaprevir and pibrentasvir (G/P) is >90% effective. Herein, we analyzed samples from these patients and showed that retreatment efficacy with G/P is lower in patients with double- or triple-linked NS5A resistance mutations than in patients with single or no NS5A resistance mutations.

Clinical trial number: NCT03092375.

Introduction

About 1% of the world's population is infected with HCV, corresponding to approximately 71 million people with HCV viremia worldwide.[1] Chronic HCV infection is the most common cause of liver disease and is the leading cause of liver transplantation due to cirrhosis and hepatocellular carcinoma (HCC).[2] In the United States, HCV affects 4-5 million individuals[3] and is responsible for 15,000 deaths annually.[4] The morbidity and mortality associated with HCV infection is expected to increase over the next few decades.[5,6]

Direct-acting antivirals (DAAs) have revolutionized HCV treatment. Multiple DAA regimens are approved to treat HCV and achieve viral cure or sustained virologic response 12 weeks after treatment completion (SVR12) rates in treatment naïve-individuals of 95% or higher.[7–9] Three HCV non-structural proteins (NS3, NS5A, and NS5B) are major targets for pharmacotherapy and almost all DAA regimens utilized today include NS5A inhibitors (NS5Ai). Despite high cure rates, some patients fail treatment with DAA regimens and develop resistance-associated substitutions (RASs) that decrease retreatment efficacy.[10] While RASs in NS3 typically disappear within the first year after treatment failure, RASs selected by NS5Ai tend to persist for several years after failure.[11,12] Given the global burden of HCV and the increased use of DAA regimens, there is a growing need

Keywords: Glecaprevir/Pibrentasvir; Hepatitis C therapy; Multiple-linked substitutions; Next-generation sequencing; NS5A resistance; Persistence of treatment-emergent substitutions; Resistance-associated substitutions; Treatment-emergent RASs; Treatment failure.
Received 21 December 2020; received in revised form 15 April 2021; accepted 27 April 2021; available online 21 May 2021
* Corresponding author. Address: 1600 SW Archer Road, D2-14E, Gainesville, FL 32610, USA. Tel.: 352-294-5481, fax: 352-273-6526
E-mail address: gary.wang@medicine.ufl.edu (G.P. Wang).
https://doi.org/10.1016/j.jhep.2021.04.057

to expand the retreatment options for patients who have failed an NS5Ai-containing regimen.

Glecaprevir is an NS3/4A protease inhibitor (PI) and pibrentasvir is an NS5Ai with potent pangenotypic activity against common amino acid substitutions that confer resistance to approved NS3/4A and NS5A inhibitors.[13,14] In NS3, a subset of single substitutions that included A156T/V in genotype (GT) 1, 2 and 4, A156G in GT3 and D168H in GT6a conferred >100-fold resistance to glecaprevir.[14] In NS5A, the majority of individual substitutions at positions 24, 28, 30, 31, 58, 92, or 93 in GT1-6 did not confer resistance to pibrentasvir, while some combinations of ≥2 NS5A substitutions resulted in reduced pibrentasvir susceptibility.[13,15] In a pooled resistance analysis of NS3/4A PI- and NS5Ai-naive patients who received glecaprevir/pibrentasvir (G/P) for 8, 12, or 16 weeks in 8 phase II and III clinical studies, baseline RASs had no impact on treatment outcome in GT1, 2, 4, 5, and 6, while numerically lower SVR12 was observed in GT3 patients with baseline NS5A-A30K who received 8 weeks therapy.[10] A recent study examined G/P for 8 weeks in treatment-naïve patients with chronic HCV GT1-6 and compensated cirrhosis; this study reported high SVR12 of 99.7% with no impact of baseline RASs in any population.[16]

Given the high barrier to resistance, G/P was evaluated for HCV retreatment for patients who failed an NS5Ai-containing regimen. In MAGELLAN-1 (part 2), G/P demonstrated SVR12 of 94% (17/18) and 88% (14/16) for NS5Ai-experienced patients who received 16 or 12 weeks of treatment, respectively.[17] Baseline NS5A substitutions were common and substitutions in NS5A alone did not impact treatment outcome in patients treated for 16 weeks.[17] G/P is currently approved as a once-daily, regimen for 16 weeks in GT1 NS5Ai-experienced patients with or without compensated cirrhosis and without prior treatment with an NS3/4A PI.[15] However, the AASLD HCV treatment guidelines currently recommend this only as an alternative treatment option for NS5Ai-experienced patients.[18] A recent phase IIIb, open-label study evaluated the efficacy of G/P in 177 HCV GT1 patients who failed prior treatment with an NS5Ai plus sofosbuvir[19] to provide additional data in this population. Patients without cirrhosis were randomized to 12 or 16 weeks of G/P, and patients with compensated cirrhosis were randomized to G/P and weight-based ribavirin for 12 weeks or G/P for 16 weeks. Results following 16 weeks of G/P showed SVR12 rates of 97% and 94% in patients with and without cirrhosis, respectively. The study also included 8 patients who failed an NS3/4A PI-containing regimen prior to sofosbuvir+NS5Ai and all of whom achieved SVR12 after G/P retreatment.[19] The objective of the current study is to describe the prevalence and the impact of baseline substitutions in NS3 and NS5A on treatment outcome, as well as treatment-emergent RAS (TE-RAS) at the time of virologic failure, for these NS5Ai-experienced patients enrolled in the phase IIIb G/P retreatment study. We employed a Primer-ID barcode approach with next-generation sequencing of NS3 and NS5A genes to quantify single or multiple-linked RASs in individual viral genomes. Additionally, the evolution and persistence of TE-RASs and their linkage among G/P retreatment failures were evaluated.

Patients and methods

Patient population and study design

Plasma samples were obtained from all patients enrolled in a phase IIIb, open-label, randomized, pragmatic, study conducted in 30 centers in the United States (ClinicalTrials.gov number: NCT03092375). Patient population, inclusion and exclusion criteria, and efficacy and safety data have been reported previously.[19] The study protocol was approved by the Institutional Review Board of all participating centers and all patients provided written informed consent prior to enrollment. Eligible non-cirrhotic patients were randomized 2:1 to Arm A: G/P12 weeks and Arm B: G/P16 weeks, and compensated cirrhotic patients were randomized 1:1 to Arms C: G/P+RBV12 weeks and Arm D: G/P16 weeks. G/P was dosed once a day and RBV was administered 1,000 mg daily for weight <75 kg or 1,200 mg daily for weight ≥75 kg.

Sample collection and virologic failure

SVR12 was defined as HCV RNA below the lower limit of quantification (LLOQ) at 12 weeks post treatment (PTWk12). For 12 weeks G/P ± RBV, Arms A and C, samples were collected at screening, day 1, weeks 4, 8, 12, and PT weeks 4 and 12 (Fig. 1). For 16 weeks G/P, Arms B and D, samples were also collected at week 16. For patients who prematurely discontinued treatment, samples were collected at the time of discontinuation. HCV RNA was measured at a central laboratory (Cenetron Diagnostics, Austin, TX) using the AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0 (Roche Molecular Systems Inc., Branchburg, NJ) with both lower limit of detection (LLOD) and LLOQ being 15 IU/ml. Breakthrough was defined as confirmed HCV RNA ≥100 IU/ml after HCV RNA <LLOQ or confirmed increase in HCV RNA >1 log IU/ml from nadir at any time point during treatment. Laboratory assessments were repeated in patients with suspected breakthrough, and if confirmed, treatment was stopped, and patients were followed until PTWk12. Relapse was defined as confirmed HCV RNA ≥LLOQ between end of treatment and PTWk12 in patients with HCV RNA <LLOQ at the end of treatment.

HCV GT and subtype were determined using Versant® HCV genotype Inno LiPA assay, v2.0 or higher (LiPA, Siemens Healthcare Diagnostics, Tarrytown, NY). When GT results from the central laboratory were indeterminate, historical GT results at the local site were used for study enrollment. Two patients in Arm A were missing GT information; therefore, GT and subtype for both patients were determined by phylogenetic analysis (Fig. S1).

Resistance analyses

Illumina next-generation sequencing (NGS) was performed on baseline samples from all patients and post-baseline samples of patients of failures at Dr. Gary Wang's laboratory (University of Florida, Division of Infectious Diseases and Global Medicine, Gainesville, FL). Primer-ID was used to tag individual HCV RNA genomes,[20–22] utilizing reverse transcription (RT) primers for complementary DNA (cDNA) synthesis. Each RT primer molecule includes a random 14-nucleotide sequence constituting a unique Primer-ID Tag, flanked by a sequence at the 3' end that anneals to the RNA template and a sequence at the 5' end that serves as the annealing site for PCR primers. Viral RNA serves as the template for cDNA synthesis using amplicon (NS3 or NS5A)-specific RT primers that contained the Primer-ID tag. Each reaction contained 10^2-10^3-fold molar excess of primers to ensure that each RNA template was reverse transcribed to generate cDNA labeled with a unique Primer-ID tag. The resulting cDNA was purified, then amplified using forward and reverse PCR primers that included 4-8 variable length nucleotide barcode sequences

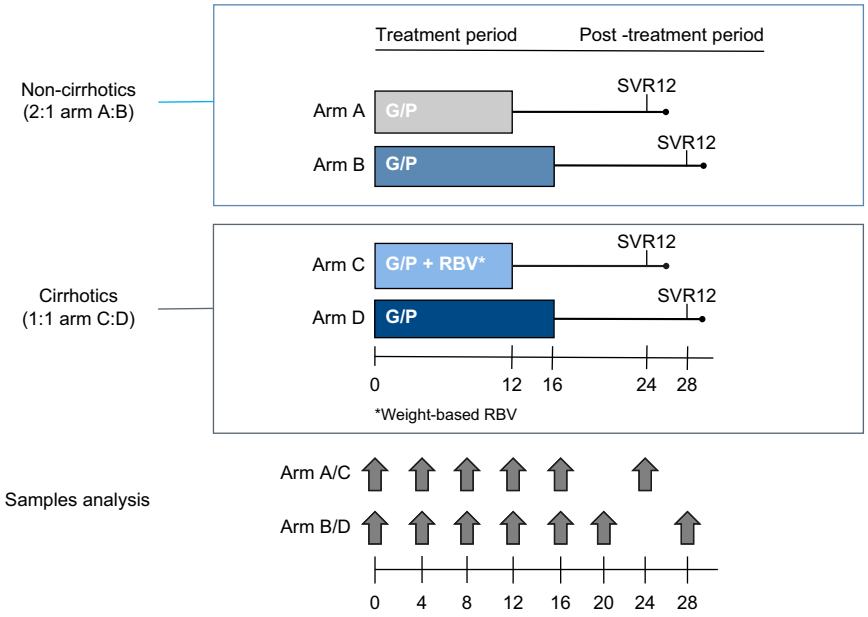


Fig. 1. Study design and time points of samples (arrows) for resistance analysis.

specific to each sample. The PCR products were purified and tailed with index sequences required for Illumina sequencing. Amplified DNA were combined in equimolar pool, gel purified and quantified by qPCR (KAPA Library Quantification kit for Illumina sequencing platforms, Kapa Biosystems), then sequenced on MiSeq using v3 600 cycle kit and a 20% PhiX spike-in. The regions sequenced encompassed NS3 amino acids 36-188 in GT1a and 36-201 in GT1b, and NS5A amino acids 13-132 in GT1a and 16-133 in GT1b. A 15% threshold was used to define baseline RASs relative to subtype-specific prototypic reference sequences (H77 for GT1a, accession number NC_004102; Con1 for GT1b, accession number AJ238799). TE-RAS were identified by comparing sequences in samples collected at failure to baseline.

Baseline RASs were assessed in NS3 at positions 155, 156, and 168, and in NS5A at positions 24, 28, 30, 31, 58, 92, and 93 for all patients. These specific NS3 and NS5A positions are known to confer resistance to at least 1 inhibitor in the protease or NS5A class. Among the analyzed NS5A positions in GT1a, Y93H/N is the only NS5A position with an increase of >2.5 fold in EC50 for pibrentasvir *in vitro*.[13] Polymorphism at the Q80 position in NS3 did not confer *in vitro* change in the glecaprevir EC50,[14] and did not impact SVR12 in phase II/III resistance analyses that included DAA treatment-naïve, and GT1-and GT4 DAA treatment-experienced patients.[10,17] Nevertheless, the impact of Q80 polymorphism and additional NS3 polymorphisms on SVR12 was studied in a supplementary analysis (Table S1). Two patients in Arm A were missing baseline sequencing data for both NS3 and NS5A. Among failure samples with HCV RNA ≥1,000 IU/ml, TE-RASs at a 15% threshold were assessed in NS3 positions 155, 156, and 168, and in NS5A positions 24, 28, 30, 31, 32, 58, 92, and 93. Phylogenetic analysis was performed to determine HCV re-infection or HCV subtype. Sequences were aligned to NS3 or NS5A reference sequences for genotypes 1a and 1b (H77 and Con1, respectively) using MUSCLE v.3.7.[23] Analyses were performed in IQ-TREE v.1.4.1[24] with 1,000 ultrafast bootstrap replicates and automated model fitting. A general time reversible model (GTR+G4) was selected for each phylogeny. NS3 and NS5A sequences from longitudinal samples were compared to baseline, as well as the prototypic reference sequence. Persistence of post-baseline NS3 and NS5A substitutions were compared through PTWk12.

Results

Prevalence of baseline RASs in NS3 and NS5A

Among 177 patients, 142 (80.2%) patients had GT1a, 34 (19.2%) GT1b, and 1 (0.6%) GT1 (subtype not determined) infection. The prevalence of NS3 and NS5A baseline RASs by study Arm is summarized in Table 1. Baseline RASs in NS3 positions 155, 156, and 168 were rare, occurring in 1.5% (2/137; both patients had NS3-R155K) of GT1a- and no (0/34) GT1b-infected patients, which is consistent with the NS5Ai-experienced population. Overall, baseline NS5A substitutions at positions 24, 28, 30, 31, 58, and 93 were common (substitutions at position 92 were not detected), occurring in 74.5% (105/141) of GT1a- and 96.8% (30/31) of GT1b-infected patients. Baseline RASs in NS5A in GT1a-infected patients included K24N/Q (2.1%, 3/141), M28A/T/V (11.3%, 16/141), Q30E/H/K/N/R (39.7%, 56/141), L31M/V (20.6%, 29/141), H58C/D/P (10.6%, 15/141), and Y93H/I/N/S (29.8%, 42/141). NS5A substitutions L28M (1/31), R30H/Q/L/S (8/31), L31I/M (14/31), P58A/S (4/31), and Y93H (87.1%, 27/31) were detected in GT1b-infected patients. Baseline RASs at position 32 in NS5A were not detected in any GT1a- or GT1b-infected patients. Two GT1a- (1.5%, 2/137) and no GT1b-infected patients (0/31) had baseline RASs detected in both NS3 and NS5A. One hundred and sixty-six (93.8%) patients had failed treatment with ledipasvir/sofosbuvir (132 GT1a and 34 GT1b), 10 (5.6%) patients had failed velpatasvir/sofosbuvir, and 1 patient had failed daclatasvir/sofosbuvir. For the 166 patients who failed ledipasvir/sofosbuvir, the prevalence of NS3 and/or NS5A

Table 1. Prevalence of baseline NS3 and/or NS5A substitutions.

		% (n/N)[a]				
HCV subtype	**Target**	**Arm A**	**Arm B**	**Arm C**	**Arm D**	**Overall**
GT1a						
	None	30.4 (17/56)	28.2 (11/39)	18.8 (3/16)	19.2 (5/26)	26.3 (36/137)
	NS3 only[b]	0 (0/56)	0 (0/39)	0 (0/16)	0 (0/26)	0 (0/137)
	NS5A only[b]	67.9 (38/56)	71.8 (28/39)	81.3 (13/16)	76.9 (20/26)	72.3 (99/137)
	NS3 + NS5A[c]	1.8 (1/56)	0 (0/39)	0 (0/16)	3.8 (1/26)	1.5 (2/137)
	Any NS3[d]	1.8 (1/56)	0 (0/39)	0 (0/16)	3.8 (1/26)	1.5 (2/137)
	Any NS5A[d]	71.2 (42/59)	71.8 (28/39)	82.4 (14/17)	80.8 (21/26)	74.5 (105/141)
GT1b						
	None	5.9 (1/17)	0 (0/9)	0 (0/3)	0 (0/2)	3.2 (1/31)
	NS3 only[b]	0 (0/17)	0 (0/9)	0 (0/3)	0 (0/2)	0 (0/31)
	NS5A only[b]	94.1 (16/17)	100 (9/9)	100 (3/3)	100 (2/2)	96.8 (30/31)
	NS3 + NS5A[c]	0 (0/17)	0 (0/9)	0 (0/3)	0 (0/2)	0 (0/31)
	Any NS3[d]	0 (0/17)	0 (0/10)	0 (0/4)	0 (0/3)	0 (0/34)
	Any NS5A[d]	94.1 (16/17)	100 (9/9)	100 (3/3)	100 (2/2)	96.8 (30/31)

Arm A: G/P for 12 weeks in non-cirrhotics; Arm B: G/P for 16 weeks in non-cirrhotics; Arm C: G/P plus RBV for 12 weeks in cirrhotic; Arm D: G/P for 16 weeks in cirrhotics.
[a]Baseline substitutions by NGS at 15% threshold are included for NS3 positions 155, 156, and 168, and NS5A positions 24, 28, 30, 31, 58, 92, and 93. n = number of patients with substitution, N = total number of patients sequenced.
[b]'Only' indicates total number of patients with baseline substitution within the indicated target and no substitution in the other target. Patients for whom both NS3 and NS5A sequences were available are included in the analysis.
[c]'NS3 + NS5A' indicates the total number of patients with baseline substitutions in both NS3 and NS5A, and only includes the patients for whom both NS3 and NS5A sequences were available.
[d]'Any' indicates the total number of patients with any baseline substitution within the indicated target at the amino acid positions analyzed. All patients with available sequences were included in the analysis. Baseline NS3 data was not available in 6 patients and baseline NS5A data was not available in 5 patients.

baseline RASs was similar to the overall population. Nine out of 10 patients who failed velpatasvir/sofosbuvir (all GT1a) had sequencing data available, and 8 (88.9%) had baseline RASs in NS5A (Fig. 2).

Impact of NS3 and NS5A baseline RASs on response to G/P

Except for 8 patients with prior sequential NS3 PI and NS5Ai experience, 169 patients were PI-naïve (NS5Ai only experienced). NS3 baseline RASs were rare and those at positions 155, 156, and 168 were limited to R155K, which does not confer resistance to glecaprevir.[14] In the 2 patients with GT1a and R155K, 1 achieved SVR12 and 1 relapsed. The presence of NS3 polymorphisms at Q80K/R, T54S, V55I, and Y56F which does not cause glecaprevir resistance *in vitro,* had no impact of SVR12 rates (Table S1). Due to the small number of patients with NS3 baseline RASs, the impact of pre-existing NS3 substitutions on response to G/P could not be ascertained.

NS5A baseline RASs were detected in 96.8% (30/31) of GT1b-infected patients and had no impact on treatment outcome, as no GT1b-infected patients experienced failure. Overall, SVR12 for GT1a in the presence or absence of NS5A baseline RASs at positions 24, 28, 30, 31, 58, and 93 was 88.6% (93/105; 95% CI 81%–93%) or 97.1% (34/35; 95% CI 85%–99%), respectively (Fig. 2A). The SVR12 rate for the 166 patients with ledipasvir/sofosbuvir failure, with and without NS5A baseline RASs (Fig. 2B), was similar to the overall population. Among velpatasvir/sofosbuvir failures (all GT1a) with NS5A baseline RASs, 7/8 achieved SVR12 (Fig. 2C). In patients who had both NS3 and NS5A sequences available, SVR12 in those with NS5A but no NS3 baseline RASs was not significantly different from SVR12 in patients without NS3 or NS5A substitutions (Table 2).

Impact of individual and multiple NS5A baseline RASs on response to G/P

Due to numerically lower SVR12 rate of 88.9% (88 of 99) in patients with GT1a with NS5A baseline RASs only, the impact of individual and multiple NS5A baseline RASs on outcome was evaluated utilizing a 15% NGS cut-off. Among 141 GT1a-infected patients, 1 had re-infection and was excluded from subsequent analyses. The impact of any single position NS5A polymorphism on SVR12 is reported by treatment group in Table S1. A comparison of SVR12 with and without baseline RASs by position revealed 4 positions, M28, Q30, L31, and Y93, that had any numerical reduction in SVR12 rates (Fig. 3A). Thus, we focused on these 4 positions for multiple linkage analyses.

The Primer-ID approach with Illumina sequencing allowed us to quantify single or multiple-linked RASs in individual viral genomes. Among the 140 GT1a-infected patients, 35 (25%) had no NS5A baseline RASs, 57 (41%) had single, 40 (29%) had double-linked, and 8 (5.7%) had triple-linked substitutions (Fig. 3B). Considering that 48/105 (46%) patients with NS5A baseline RASs had multiple-linked substitutions, an assessment of linked substitutions was necessary. Compared to a SVR12 rate of 93% (95% CI 83%–97%) in patients with single and 97% (95% CI 85%–99%) in those with no NS5A baseline RASs, numerically lower SVR12 rates of 88% (95% CI 74%–95%) were observed in patients with any double-linked baseline RASs, and 63% (95% CI 31%–86%) in patients with any triple-linked baseline RASs at positions 24, 28, 30, 31, 58, and 93. When examining specific RAS positions that had numerical reductions in SVR12 rates (M28, Q30, L31, and Y93), similar reductions in SVR12 rates were seen with double-linked (82%–88%) and triple-linked (50%–67%) RASs (Fig. 3B), compared to the SVR12 rates examining all RASs at positions 24, 28, 30, 31, 58, and 93. These results did not differ when utilizing a lower, 2% NGS cut-off (data not shown).

Baseline and TE-RAS in patients with virologic failure

Among 177 patients, 15 failed to achieve SVR12, including 13 with GT1a (4 breakthroughs and 9 relapses), 1 with GT1b who died from HCC prior to assessment of SVR12, and 1 with GT1a who was re-infected based on phylogenetic analysis (Fig. S2).

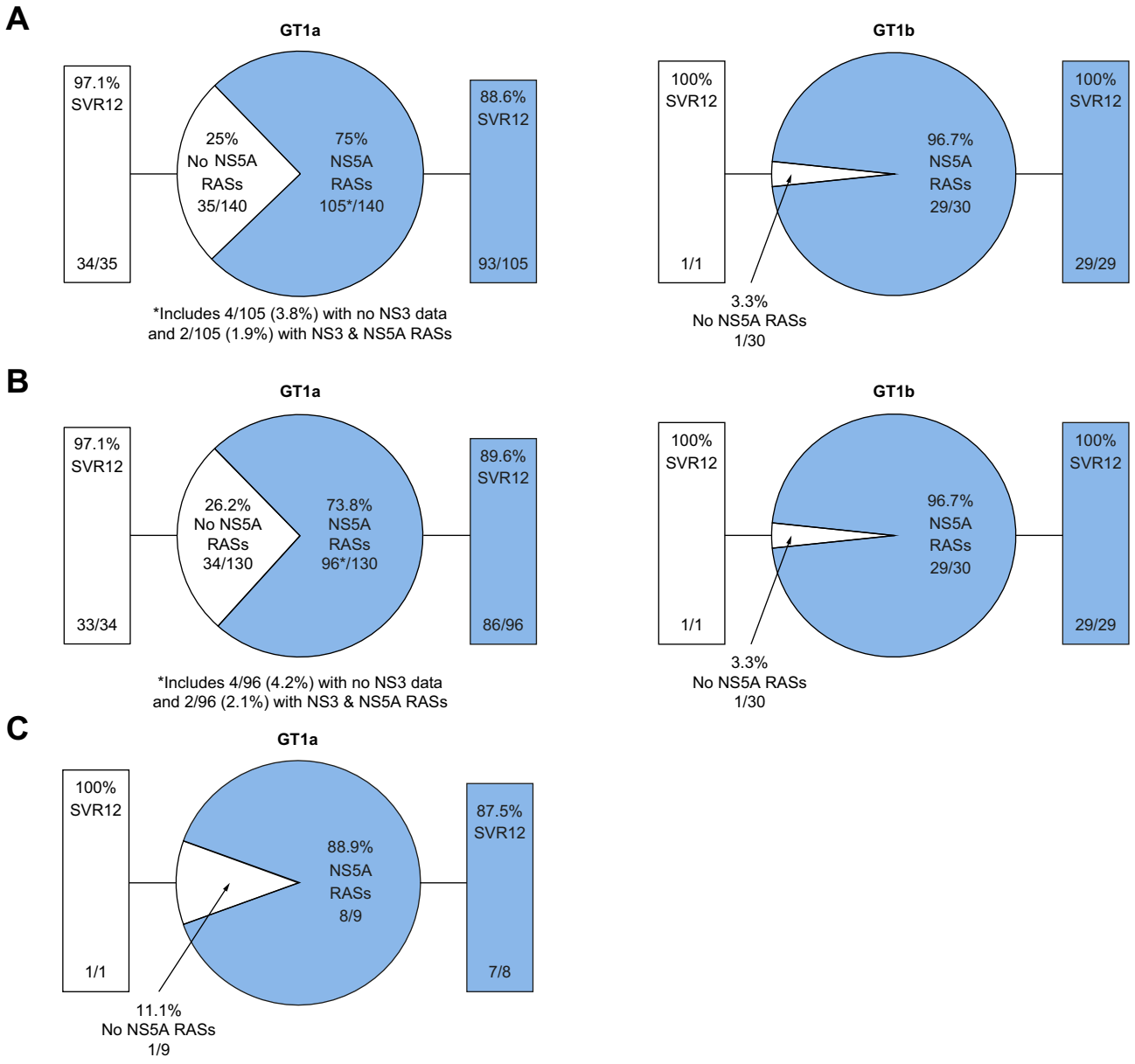


Fig. 2. Prevalence and impact of baseline NS5A substitutions on SVR12. (A) All patients, (B) ledipasvir/sofosbuvir failures, and (C) velpatasvir/sofosbuvir failures and includes all subjects for whom NS5A sequences were available. One GT1a subject with HCV re-infection and one GT1b subject who died from HCC prior to SVR12 were excluded. The number and % of patients include patients with both NS3 and NS5A RASs as well as patients who had no available NS3 data, and are shown as subgroups within the NS5A RASs. SVR12 for patients with and without NS5A RASs are shown adjacent to the pie charts.

Substitutions at baseline, virologic failure, and at PTWk12 are shown in Table 3 for the 13 failures and the patient with re-infection (No.14).

Among 13 failures, 1 had a NS3 baseline RAS (R155K) which does not confer resistance to glecaprevir.[14] NS3 TE-RASs A156V (n = 2) or R155W + A156G (n = 2) were observed in 4 patients. In contrast, 12 of 13 failures had ≥1 NS5A baseline RASs M28V/T, Q30E/H/N/R, L31M/V, H58D, or Y93H/N. Among them, 8 had multiple-linked baseline RASs. At failure, additional NS5A TE-RASs were observed at positions 28, 30, 31, 32, and 58 in 10/13 patients, all of which resulted in linked NS5A substitutions. One patient (No.9) had no baseline or TE-RASs in NS5A at failure and was evaluated for re-infection by phylogenetic analysis. However, it was determined to be a relapse based on genetic similarity between the baseline and PT sequences.

Persistence of TE-RAS

Persistence of TE-RAS in NS3 and NS5A (15% threshold) was examined through PTWk12 for 6 patients with TE-RAS and at least 1 follow-up time point after failure. Among 2 patients with TE-RASs R155W + A156G in NS3, R155W was no longer detected (Table 3), while A156G was detected at 30% prevalence in 1/2 patients at PTWk12 (No.1). Treatment-emergent A156V in NS3 was detected in 2 patients at failure, with A156V no longer

Table 2. Overall SVR12 rate in patients with and without baseline RASs in NS3 and/or NS5A.

Target	% SVR12 (n/N)[a]	
	GT1a	GT1b
NS3 only[b]	–	–
NS5A only[b]	88.9 (88/99) [NS]	100 (29/29) [NS]
NS3 + NS5A[c]	50 (1/2)	–
None	97.1 (34/35)	100 (1/1)

NS: Comparison of SVR12 between 'NS5A only' and 'none' by Fisher's exact test was not statistically significant.
[a]Baseline substitutions by NGS at 15% threshold at the following positions were included: 155, 156, and 168 in NS3, and 24, 28, 30, 31, 58, 92, and 93 in NS5A. n = number of patients achieving SVR12 with or without baseline substitutions, N = total number of patients with or without baseline substitutions.
[b]'Only' indicates total number of patients with baseline substitutions within the indicated target, and no substitution in the other target. Analysis only includes patients for whom both NS3 and NS5A sequences were available.
[c]'NS3 + NS5A' indicates the total number of patients with baseline substitutions in both NS3 and in NS5A. Analysis only includes patients for whom both NS3 and NS5A sequences were available.

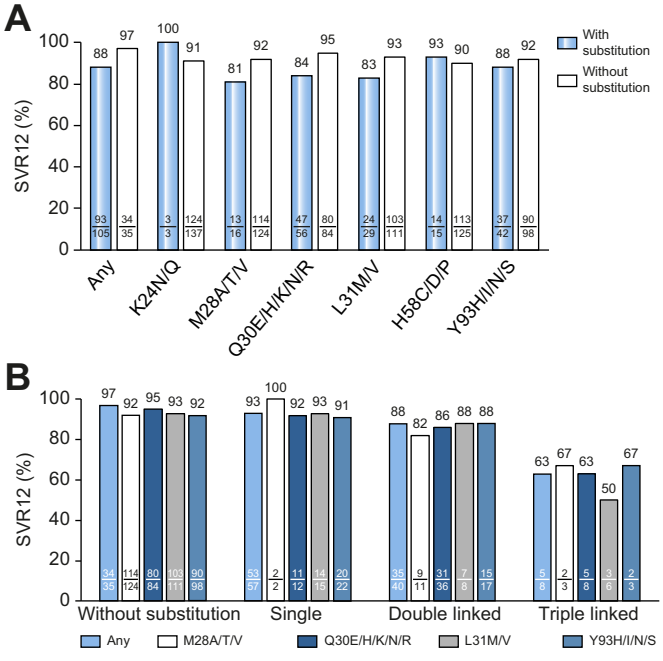


Fig. 3. Impact of individual and multiple NS5A baseline RASs on SVR12 in GT1a. Baseline substitution at position 92 was not observed and is thus not shown. Subject with re-infection was excluded from the analysis. (A) Overall analysis by NS5A position. (B) NS5A multiple analysis (Any, M28, Q30, L31 and Y93 positions). "Any" denotes any baseline RAS at positions 24, 28, 30, 31, 58 or 93. M28, Q30, L31, or Y93 indicate single-, double-, or triple-linked baseline RASs that include those positions.

detectable at PTWk12 in 1 patient (No.4), while the second patient did not have another PT sample for analysis.

In contrast, NS5A baseline- and TE-RASs were detectable through PTWk12 in all 6 patients with available persistence data (Table 3 and Fig. S3). One (No.3) with triple-linked NS5A baseline RASs did not acquire any TE-RASs, and the baseline RASs persisted through PTWk12 (Table 3 and Fig. S3A). Among 5 patients with NS5A TE-RASs, 3 (Nos. 1, 4, and 13) acquired treatment-emergent M28T or H58D resulting in double- or triple-linked substitutions persisting through PTWk12. Patient 4 also had M28T and Q30R detectable at baseline, which increased to 100% prevalence at failure (Table 3 and Fig. S3A). Two (Nos. 10 and 11) had baseline RASs M28V or M28T that changed to M28A or M28S at failure, respectively; these TE-RASs, as well as other NS5A-linked baseline RASs (Q30R and L31V) remained detectable at PTWk12 (Table 3 and Fig. S3C).

Discussion

In a recent published report,[19] G/P was found to be highly efficacious and safe in GT1 NS5Ai+sofosbuvir-experienced patients, with and without compensated cirrhosis, treated in a real-world setting. Results of this study added evidence for the retreatment of GT1 NS5Ai+sofosbuvir ±RBV failures with G/P in a diverse US population, which included patients with prior treated HCC (n = 17), liver transplantation (n = 15), and HIV/HCV coinfection (n = 9). However, detailed analyses on the prevalence and impact of baseline RASs on the efficacy of G/P and the evolution of TE-RASs was not presented. This analysis fills this knowledge gap.

Among the 177 patients in this study, 80.2% of patients were GT1a, and 19.2% GT1b. Baseline NS3 substitutions were uncommon, which was consistent with this population of NS5Ai-experienced patients. In contrast, NS5A baseline RASs were found in 105 of 141 (74.5%) GT1a- and 30 of 31 (96.8%) GT1b-infected patients. Two GT1a- and no GT1b-infected patients had baseline RASs detected in both NS3 and NS5A. These data are comparable to results from Sarrazin *et al.*,[25] where 79% of NS5Ai-experienced patients had NS5A baseline RASs. Among the 141 GT1a-infected patients, baseline RASs at NS5A position 30 were the most prevalent (40%; 56/141), followed by 93 (30%, 42/141) and 31 (21%, 29/141). Patients with ledipasvir/sofosbuvir failure comprised the majority (93.8%, 166 of 177) of patients and the prevalence of specific NS5A substitutions among this group was similar to the overall population. Among the 9 GT1a velpatasvir/sofosbuvir failures with available sequencing data, 5 had single, 2 had dual-linked, and 1 had triple-linked substitutions. This was comparable to the overall GT1a population for which multiple NS5A substitutions were detected in 34% (48/140). NS5A substitutions at 30 or 31 were most common among velpatasvir/sofosbuvir failures (4/9 patients each), and 1/9 patients had Y93H (which was linked to Q30H). The low prevalence of Y93H among velpatasvir/sofosbuvir failures was unexpected, as Y93H was the most commonly selected substitution (observed in 3/5) following velpatasvir/sofosbuvir failure in a pooled analysis of phase III clinical trials.[26]

Since all GT1b-infected patients (n = 33) achieved SVR12, the analysis of the impact of NS3 and/or NS5A baseline RASs on SVR12 was focused on GT1a. NS3 baseline RASs at 155, 156, and 168 were infrequent among GT1a-infected patients, and Q80K was a highly prevalent polymorphism. None of these NS3 RASs/polymorphism had an impact on SVR12. Since 46% (48/105) of GT1a-infected patients with NS5A baseline RASs had multiple-linked NS5A polymorphisms, we conducted additional linkage analysis comparing single, double- and triple-linked substitutions involving any NS5A RASs at positions 24, 28, 30, 31, 58, and 93, as well as RASs at the 4 specific positions M28, Q30, and L31, and Y93 that were associated with any reduction in SVR12. In our analysis of all G/P treatment arms combined, patients with triple-linked NS5A baseline RASs had numerically lower SVR12 rates than patients with double-linked substitutions, which in turn were lower than SVR12 for single or no NS5A baseline RASs (Fig. 3B). However, we did not identify a NS5A position that was significantly associated with not achieving SVR12. While it may be tempting to suggest that baseline RAS

Table 3. Baseline and treatment-emergent substitutions in patients who experienced virologic failure.

Patient no.	Arm[a]	GT	Prior treatment regimen	Outcome	Amino acid substitution (% prevalence within patient's viral population)[b]					
					NS3			NS5A		
					BL	Time of VF	PTW12[c]	BL	Time of VF	PTW12[c]
1	A	1A	SOF/LDV	Breakthrough	None	R155W + A156G (100)	A156G (30)	Q30N + Y93H (100)	M28T + Q30N + Y93H (100)	M28T + Q30N + Y93H (98)
2	B	1A	SOF/LDV	Breakthrough	None	A156V (100)	—	Q30R + L31M + H58D (100)	Q30R + L31M + H58D (100)[d]	—
3	C	1A	SOF/LDV	Breakthrough	None	R155W + A156G (100)	None	Q30H + L31M + Y93H (100)	Q30H + L31M + Y93H (100)	Q30H + L31M + Y93H (98)
4	C	1A	SOF/LDV	Breakthrough	None	A156V (100)	None	M28T + Q30R (28)	M28T + Q30R + H58D (100)	M28T + Q30R + H58D (100)
5	A	1A	SOF/LDV	Relapse	None	None	—	Q30R + L31M (100)	Q30R + L31M + H58D (97)	—
6	A	1A	SOF/LDV	Relapse	None	—	None	Y93N (100)	—	L31M + Y93N (100)
7	A	1A	SOF/VEL	Relapse	None	None	—	Q30H + Y93H (93)	Q30N + Y93H (40); Q30H + L31V + Y93H (60)	—
8	A	1A	SOF/LDV	Relapse	R155K (100)	—	R155K (100)	Q30E (99)	—	Q30E + H58D (100)
9	A	1A	SOF/LDV	Relapse	None	—	None	None	—	none
10	B	1A	SOF/DCV	Relapse	None	None	None	M28V + Q30R + L31V (100)	M28A + Q30R + L31V (100)	M28A + Q30R + L31V (100)
11	B	1A	SOF/LDV	Relapse	None	None	None	M28T + Q30R (100)	M28S + Q30R (98)	M28S + Q30R (97)
12	C	1A	SOF/LDV	Relapse	None	—	None	L31M (100)	—	L31M + P32-DEL (98)
13	D	1A	SOF/LDV	Relapse	None	None	None	Y93N (100)	H58D + Y93N (100)	H58D + Y93N (100)
14	A	1A	SOF/LDV	Re-infection	None	None	—	None	None	—

BL, baseline; BT, breakthrough; LDV, ledipasvir; PTW, post-treatment week; SOF, sofosbuvir; SVR12, sustained virologic response 12 weeks after treatment completion; VEL, velpatasvir; VF, virologic failure.
[a]Arm A: G/P for 12 weeks in non-cirrhotics; Arm B: G/P for 16 weeks in non-cirrhotics; Arm C: G/P plus RBV for 12 weeks in cirrhotics; Arm D: G/P for 16 weeks in cirrhotic.
[b]Substitutions at 15% threshold at positions 155, 156, and 168 in NS3, and 24, 28, 30, 31, 32, 58, 92, and 93 in NS5A relative to subtype-specific reference sequences are listed. At time of VF and PTW12, treatment-emergent substitutions and pre-existing baseline substitutions are listed. Linked substitutions detected at 15% threshold are listed.
[c]PTW12 scheduled visit corresponds to post-treatment nominal day (study drug end day) 84 and time window (study drug end day range) 57 to 126. VFs that occurred during the SVR12 time window only have post-treatment sequence data reported in the SVR12 column.
[d]Patient failed treatment at week 8, but sequencing data for NS5A were only available from the week 12 timepoint due to technical difficulties.

linkage analysis may be of value prior to retreatment with G/P in GT1a, we caution that these data were based on a small number of patients with double- and triple-linked substitutions who were treated with varying durations of G/P with or without ribavirin. Indeed, a not-recommended 12-week retreatment duration with G/P for these GT1a sofosbuvir±NS5Ai±RBV failures was associated with a lower SVR12 rate (87%; 67/77) when compared to 16 weeks of G/P (94%; 61/65).[19] In addition, linkage testing of RASs is currently not commercially available. Thus, additional studies in a larger cohort of patients with multiple-linked baseline RASs are needed to confirm these observations in order to guide clinical practice. We also note that despite multiple-linked RASs in nearly half of patients with NS5A baseline RASs, 35/40 patients with double-linked and 5/8 with triple-linked substitutions achieved SVR12. Importantly, SVR12 was achieved in all prior sofosbuvir+NS5Ai failures with GT1b infection treated with G/P.

Thirteen GT1a-infected patients experienced failure, including 4 breakthroughs and 9 relapses. One patient was found to be re-infected with GT1a. All 8 patients with prior sequential failure to a PI/no NS5Ai regimen followed by a NS5Ai/no PI regimen achieved SVR12. No failures had a NS3 baseline RAS that decreased susceptibility to glecaprevir, and 8 had no baseline- or TE-RAS in NS3. NS3 TE-RASs A156V (n = 2) or R155W + A156G (n = 2) were observed in 4 patients at the time of failure. Among them, R155W was no longer detected at PTWk12 in the 2 patients, A156G was detected in 1 patient but at lower prevalence (30%). The rapid disappearance of R155W + A156G and A156V is consistent with the rapid loss of NS3 RASs selected by other PIs observed in previous studies,[12,27,28] and suggests reduced viral fitness of treatment-emergent NS3 variants.

As expected in this population of NS5Ai-experienced patients, 12/13 failures had NS5A baseline RASs. Among the 13 failures, double- or triple-linked RASs were identified in 8 patients at baseline and in 10 at treatment failure. Of the 10 patients with TE-RASs, 4 acquired linked H58D, 2 acquired L31M or L31V, 1 acquired M28T, and 1 acquired the rare P32 deletion, all of which resulted in double- or triple-linked substitutions. For the remaining 2 patients (Nos. 10 and 11), the TE-RASs involved an amino acid change in their double- or triple-linked baseline RASs. All 10 TE-RASs were dominant viral populations (detected at >97% in 9 patients and 60% in 1 patient). Two patients with triple-linked baseline RASs (Nos. 2 and 3) did not acquire additional TE-RASs. One patient (No. 9) had no NS3 or NS5A baseline-

or TE-RASs, and phylogenetic analysis was consistent with relapse. Among the 6 patients with available persistence data, double- or triple-linked NS5A substitutions persisted through PTWk12. These results are consistent with the long-term persistence of NS5A RASs reported in previous studies.[11,12,28]

This study had several limitations. The design included 4 different treatment groups with 2 different durations and the inclusion of ribavirin in 1 treatment group among patients with compensated cirrhosis. This led to limited power for assessment of the impact of individual or linked RASs on G/P efficacy when administered for 12 or 16 weeks, with or without ribavirin. Given these limitations, we focused this analysis on the combined data from all treatment arms and examined the impact of NS3 and NS5A positions known to be commonly associated with resistance to the PI- and NS5A-inhibitor classes. We further determined the SVR12 rates according to the presence of each of an extended set of polymorphisms in NS3 and NS5A on SVR12 rates, without new findings (Table S1). Although it may be of interest to explore other potential predictors of SVR12, such as fibrosis stage, HCC, and IL28B status, this was beyond the objective of this study. Among patients with and without compensated cirrhosis, SVR12 rates with 16 weeks G/P were similar, 97%, 28/29 and 94%, 46/49, respectively.[19] Due to the timeline of the study, the vast majority of our patients (93.8%) were prior ledipasvir/sofosbuvir failures, and a limited number (n = 10) of sofosbuvir/velpatasvir failures were included. As such, future studies are warranted to determine whether our results can be generalized to other sofosbuvir+NS5Ai regimen failures, including sofosbuvir/velpatasvir.

In summary, our study provided a detailed analysis of NS3 and NS5A baseline- and TE-RASs in GT1a-infected patients retreated with G/P who had failed treatment with sofosbuvir+NS5Ai. Although results are presented with a 15% NGS threshold, using a 2% threshold demonstrated similar results (not shown) and did not affect our conclusions. For the first time, we provide detailed information on the linkage of NS5A RASs in the setting of DAA-retreatment in the real world. We found that NS5A baseline RASs, including multiple-linked RASs, were highly prevalent in this sofosbuvir+NS5Ai-experienced population with GT1a infection. SVR12 in patients with baseline double- and triple-linked NS5A substitutions were numerically lower than in patients with single or no NS5A substitutions. However, given the small number of patients in different subgroups with specific individual and/or double- or triple-linked RASs, we could not draw conclusions on the impact of specific individual RASs and their linkage on the efficacy of G/P. Nonetheless, despite double- or triple-linked NS5A baseline RASs in 48 GT1a-infected patients, 40 (83%) achieved SVR12, which is respectable given that 28/48 (58%) received a suboptimal G/P treatment duration of 12 weeks, and the SVR12 rate with the recommended 16-week duration was 85% (17/20). Among GT1a failures, nearly all acquired additional treatment-emergent NS3 and/or NS5A RASs. In contrast to NS5A RASs which persisted through PTWk12, NS3 TE-RASs disappeared by PTWk12 in the majority of patients.

Abbreviations

cDNA, complementary DNA; DAA, direct-acting antiviral; G/P, glecaprevir/pibrentasvir; GT, genotype; HCC, hepatocellular carcinoma; LLOD, lower limit of detection; LLOQ, lower limit of quantification; NS, non-structural; NS5Ai, NS5a inhibitor; PI, protease inhibitor; PT, post-treatment; PTWk12, post-treatment week 12; RAS, resistance-associated substitution; RBV, ribavirin; RT, reverse transcription; SVR, sustained virologic response; TE-RAS, treatment-emergent resistance-associated substitution.

Financial support

This study is a collaboration between AbbVie and the University of Florida. AbbVie provided funding and study drug.

Conflict of interest

Gary Wang: Has a patent issued. Gretja Schnell: Personal fees from AbbVie, during the conduct of the study; Personal Fees and other from AbbVie, outside the submitted work. Jens Kort: Personal fees from AbbVie; Personal fees and other from AbbVie, outside the submitted work; has a patent issued. Gurjit Sidhu: Nothing to disclose. Layla Schuster: Nothing to disclose. Rakesh Tripathi: Personal fees and other from AbbVie, outside the submitted work. Lois Larsen: Personal fees from AbbVie, during the conduct of the study; Personal Fees and other from AbbVie, outside the submitted work. Larry Michael: Nothing to disclose. Ken Bergquist: Nothing to disclose. Ashley Magee: Nothing to disclose. Chandni Patel: Nothing to disclose. Joan Whitlock: Nothing to disclose. Joy Peter: Domestic Partner is an AbbVie Stockholder. Michael Fried: Grants from AbbVie, Bristol-Myers Squibb, Merck, and Gilead; Personal Fees from AbbVie, Bristol-Myers Squibb, Merck, Roche; Personal fees and other from TARGET PharmaSolutions, outside the submitted work. David Nelson: Grants from AbbVie, Gilead and Merck during the conduct of the study; Stockholder of TARGET PharmaSolutions, outside the submitted work.

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

Authors' contributions

Study concept and design: GPW, JJK, JAP, MWF, DRN.
Acquisition of Data: GPW, LS, CBP, JAW, JAP.
Analysis and Interpretation of data: GPW, GLS, JJK, LL, GSS, LCM, KB, AM, JAP.
Drafting of manuscript: GPW, GLS, JJK.
Critical revision of manuscript: GPW, GLS, JJK, RT, LL, LCM, KB, AM, JAP, MWF, DRN.

Data availability statement

Study data is available upon request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.04.057.

References

Author names in bold designate shared co-first authorship

[1] Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol 2017;2:161–176.
[2] Rosen HR. Clinical practice. Chronic hepatitis C infection. N Engl J Med 2011;364:2429–2438.
[3] Edlin BR, Eckhardt BJ, Shu MA, Holmberg SD, Swan T. Toward a more accurate estimate of the prevalence of hepatitis C in the United States. Hepatology 2015;62:1353–1363.
[4] Ly KN, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. Ann Intern Med 2012;156:271–278.

[5] Millman AJ, Nelson NP, Vellozzi C. Hepatitis C: Review of the epidemiology, clinical care, and continued challenges in the direct acting antiviral era. Curr Epidemiol Rep 2017;4:174–185.
[6] Thomas DL. Global control of hepatitis C: where challenge meets opportunity. Nat Med 2013;19:850–858.
[7] Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014;370:1889–1898.
[8] Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014;370:211–221.
[9] Feld JJ, Jacobson IM, Hezode C, Asselah T, Ruane PJ, Gruener N, et al. Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infection. N Engl J Med 2015;373:2599–2607.
[10] Krishnan P, Pilot-Matias T, Schnell G, Tripathi R, Ng TI, Reisch T, et al. Pooled resistance analysis in patients with hepatitis C virus genotype 1 to 6 infection treated with glecaprevir-pibrentasvir in phase 2 and 3 clinical trials. Antimicrob Agents Chemother 2018;62. https://doi.org/10.1128/AAC.01249-18. Print 2018 Oct.
[11] Wyles D, Mangia A, Cheng W, Shafran S, Schwabe C, Ouyang W, et al. Long-term persistence of HCV NS5A resistance-associated substitutions after treatment with the HCV NS5A inhibitor, ledipasvir, without sofosbuvir. Antivir Ther 2018;23:229–238.
[12] **Jeong Y**, **Jin B**, **Lee HW**, Park HJ, Park JY, Kim DY, et al. Evolution and persistence of resistance-associated substitutions of hepatitis C virus after direct-acting antiviral treatment failures. J Viral Hepat 2018;25:1251–1259.
[13] Ng TI, Krishnan P, Pilot-Matias T, Kati W, Schnell G, Beyer J, et al. In Vitro antiviral activity and resistance profile of the next-generation hepatitis C virus NS5A inhibitor pibrentasvir. Antimicrob Agents Chemother 2017;61. https://doi.org/10.1128/AAC.02558-16. Print 2017 May.
[14] Ng TI, Tripathi R, Reisch T, Lu L, Middleton T, Hopkins TA, et al. In Vitro antiviral activity and resistance profile of the next-generation hepatitis C virus NS3/4A protease inhibitor glecaprevir. Antimicrob Agents Chemother 2017;62. https://doi.org/10.1128/AAC.01620-17. Print 2018 Jan.
[15] Abbvie Inc. Mavyret (glecaprevir and pibrentasvir). Package insert. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/209394s008lbl.pdf 2019.
[16] Brown RS, Buti M, Rodrigues L, Chulanov V, Chuang WL, Aguilar H, et al. Glecaprevir/pibrentasvir for 8weeks in treatment-naive patients with chronic HCV genotypes 1-6 and compensated cirrhosis: the EXPEDITION-8 trial. J Hepatol 2020;72:441–449.
[17] Poordad F, Pol S, Asatryan A, Buti M, Shaw D, Hezode C, et al. Glecaprevir/Pibrentasvir in patients with hepatitis C virus genotype 1 or 4 and past direct-acting antiviral treatment failure. Hepatology 2018;67:1253–1260.
[18] AASLD-IDSA. HCV Guidance: Recommendations for testing, managing, and treating hepatitis C. https://www.hcvguidelines.org. accessed May 11, 2020.
[19] Lok AS, Sulkowski MS, Kort JJ, Willner I, Reddy KR, Shiffman ML, et al. Efficacy of glecaprevir and pibrentasvir in patients with genotype 1 hepatitis C virus infection with treatment failure after NS5A inhibitor plus sofosbuvir therapy. Gastroenterology 2019;157. 1506-1517.e1.
[20] Wang GP, Terrault N, Reeves JD, Liu L, Li E, Zhao L, et al. Prevalence and impact of baseline resistance-associated substitutions on the efficacy of ledipasvir/sofosbuvir or simeprevir/sofosbuvir against GT1 HCV infection. Sci Rep 2018;8:3199–3213.
[21] Brown AN, Liu L, Rodriquez JL, Zhao L, Schuster L, Li E, et al. Sofosbuvir (SOF) suppresses ledipasvir (LDV)-resistant mutants during SOF/LDV combination therapy against genotype 1b hepatitis C virus (HCV). Sci Rep 2017;7:14421–14431.
[22] Liu L, Nardo D, Li E, Wang GP. CD4+ T-cell recovery with suppressive ART-induced rapid sequence evolution in hepatitis C virus envelope but not NS3. AIDS 2016;30:691–700.
[23] Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32:1792–1797.
[24] Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 2015;32:268–274.
[25] Sarrazin C, Cooper CL, Manns MP, Reddy KR, Kowdley KV, Roberts SK, et al. No impact of resistance-associated substitutions on the efficacy of sofosbuvir, velpatasvir, and voxilaprevir for 12weeks in HCV DAA-experienced patients. J Hepatol 2018;69:1221–1230.
[26] Hezode C, Reau N, Svarovskaia ES, Doehle BP, Shanmugam R, Dvory-Sobol H, et al. Resistance analysis in patients with genotype 1-6 HCV infection treated with sofosbuvir/velpatasvir in the phase III studies. J Hepatol 2018;68:895–903.
[27] Sullivan JC, De Meyer S, Bartels DJ, Dierynck I, Zhang EZ, Spanks J, et al. Evolution of treatment-emergent resistant variants in telaprevir phase 3 clinical trials. Clin Infect Dis 2013;57:221–229.
[28] Lahser F, Galloway A, Hwang P, Palcza J, Brunhofer J, Wahl J, et al. Interim analysis of a 3-year follow-up study of NS5A and NS3 resistance-associated substitutions after treatment with grazoprevir-containing regimens in participants with chronic HCV infection. Antivir Ther 2018;23:593–603.