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Retreatment With Sofosbuvir and Simeprevir of Patients With Hepatitis C Virus Genotype 1 or 4 Who Previously Failed a Daclatasvir-Containing Regimen

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Failure to achieve sustained virological response (SVR) with hepatitis C virus (HCV) direct-acting antiviral-based regimens is commonly associated with emergence of resistance-associated variants (RAVs). To avoid cross-resistance, recent guidelines recommend that patients who have failed on nonstructural protein 5A (NS5A) inhibitors should be retreated with sofosbuvir (SOF; NS5B inhibitor) combined with simeprevir (SIM; protease inhibitor [PI]); however, supporting evidence is lacking. This "real-world" study comprised patients who had failed to achieve SVR on previous NS5A-based therapy with daclatasvir (DCV) plus pegylated interferon (Peg-IFN) and ribavirin (RBV), with (n = 3) or without (n = 13) asunaprevir (ASV; PI). All 16 patients were retreated for 12 weeks with SOF plus SIM, without RBV. Antiviral efficacy was evaluated using the primary endpoint of SVR12 (SVR 12 weeks post-treatment); on-treatment response was also assessed. Patients (N = 16; 13 male; mean age: 54 years [range, 43-73]) were chronically infected with HCV genotype (GT) 1 (1a, n = 11; 1b, n = 3) or 4 (n = 2); they had advanced fibrosis or compensated cirrhosis (FibroScan, 9.6-70 kPa; cirrhosis, n = 9); median baseline HCV-RNA level was 1.38 × 10^6 IU/mL. No patient discontinued treatment because of adverse events or virological failure. All patients achieved HCV RNA below lower limit of quantification (<12 IU/mL) by end of treatment (EOT) and 10 of 16 had a rapid response (week 4). SVR12 was achieved by 14 of 16 patients; the remaining 2 relapsed by 4 weeks post-EOT (both were GT 1a infected with cirrhosis; 1 had previously failed DCV-ASV plus Peg-IFN and RBV). Presence of SIM RAVs/polymorphisms (R155K and Q80K) at study baseline did not predict retreatment failure. *Conclusion:* Our findings support the concept of retreating NS5A inhibitor failures with SOF combined with SIM. However, the most difficult-to-cure patients may need more than 12 weeks of treatment and/or the addition of RBV. (HEPATOLOGY 2016;63:1809-1816)

Treatment of chronic hepatitis C virus (HCV) infection has advanced significantly over the last 5 years, with the introduction of drug combination regimens based on direct-acting antiviral (DAA) agents. Sustained virological response (SVR) rates of the order of 60%-100% have been achieved when one DAA is combined with pegylated interferon (Peg-IFN) alpha and ribavirin (RBV), with SVR rates varying according to HCV genotype, disease severity, the DAA used, and the preexistence at treatment baseline of resistance-associated variants (RAVs).[1]

Abbreviations: AEs, adverse events; ASV, asunaprevir; CI, confidence interval; DAA, direct-acting antiviral; DCV, daclatasvir; EASL, European Association for the Study of the Liver; EOT, end of treatment; GT, genotype; HCV, hepatitis C virus; IFN, interferon; kPa, kilopascals; MELD, Model for End-Stage Liver Disease; NS5A, nonstructural protein 5A; OBV, ombitasvir; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; PI, protease inhibitor; RAVs, resistance-associated variants; RBV, ribavirin; SAEs, serious adverse events; SD, standard deviation; SVR, sustained virological response; SVR12, SVR 12 weeks post-treatment; VFs, virological failures; VRs, virological responses.

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The increased number of HCV DAAs available is now associated with the possibility of interferon (IFN)-free, and possibly RBV-free, regimens, with the potential for greatly improved tolerability. DAAs currently approved in Europe include: daclatasvir (DCV; nonstructural protein 5 A [NS5A] inhibitor); sofosbuvir (SOF; nucleotide analog inhibitor of HCV-RNA-dependent RNA polymerase); simeprevir (SIM; NS3/4A protease inhibitor [PI]); single-pill combination of SOF and ledipasvir (NS5A inhibitor); and single-pill combination of paritaprevir (ritonavir-boosted NS3/4A protease inhibitor) and ombitasvir (OBV; NS5A inhibitor), used with or without dasabuvir (nonnucleoside polymerase inhibitor), according to the HCV genotype.

Despite improved SVR rates with DAA-based combination regimens, treatment fails to eradicate HCV infection in a substantial proportion of patients (5%-15%, dependent on the treatment regimen and treated population). Treatment failure is generally associated with the selection of HCV RAVs, that is, viral variants that have reduced susceptibility to the DAA(s) administered.[1-4] NS5A inhibitors have a low barrier to resistance, and the variants they select confer cross-resistance across all members of the drug class.[3,5] Thus, NS5A resistance currently appears as the principal challenge of IFN-free, DAA-based therapy.

Because most current DAA-based treatment regimens include an NS5A inhibitor, RAVs tend to persist for several years after treatment failure.[1,6-9] In contrast, RAVs selected by NS3/4A protease inhibitors persist for a much shorter time and are progressively replaced by wild-type virus within a few months post-therapy.[6,10] Additionally, RAVs selected by SOF (a drug with a high barrier to resistance) have poor viral fitness; thus, they rarely emerge in the presence of the drug and tend to rapidly disappear if selected.[1,11] Therefore, high barrier to resistance of SOF coupled with the lack of cross-resistance between NS5A inhibitors and PIs provide a rationale for retreating NS5A-containing regimen failures with a combination of SOF and a PI.

Recent international guidelines recommended that patients infected with genotypes (GTs) 1 or 4 who have failed treatment on a DCV plus Peg-IFN and RBV regimen should be retreated with a combination of SOF and SIM, generally with RBV.[1] However, although this recommendation seems intuitively reasonable, there is no published evidence to support this strategy. Therefore, we conducted a "real-life" pilot study of SOF and SIM combination without RBV for retreatment of patients with chronic HCV GT 1 or 4 infection, who had previously failed to achieve SVR with a DCV-containing regimen.

Patients and Methods

PATIENTS AND TREATMENT

Patients with chronic HCV who were previous participants in phase II or III trials assessing DCV in combination with Peg-IFN and RBV (n = 13), or DCV in combination with asunaprevir (ASV), Peg-IFN, and ribavirin (n = 3), and failed to achieve an SVR were included. All patients were treated in the context of the French early access program, with DAA access limited to patients with severe fibrosis or cirrhosis (FibroScan $\geq$9.6 kPa). The latter patients had compensated cirrhosis (Child-Pugh A) at the time of treatment, without a past episode of decompensation. Child-Pugh score B or C patients were excluded. All patients were enrolled in the ANRS CO22 HEPATHER cohort[12] and provided written informed consent. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in *a priori* approval by ethics committee Ile de France III.

Patients were treated for 12 weeks with a combination of SOF and SIM without RBV. SOF was taken as

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TABLE 1. Primers Specific for the HCV NS3 protease, NS5A, and NS5B Polymerase Genes, According to HCV GT (1a, 1b, or 4)

HCV GT	Primer	5′-3′ Sequence	Gene
1	NS3G1FI-M13*	GTA AAA CGA CGG CCA GCT BCT SGG RCC RGC CGA	NS3
1	NS3G1RI-M13*	CAG GAA ACA GCT ATG ACG CCA CYT GGW AKS TCT GSG G	NS3
4	NS3G4FI*	ATC TTG CTC GGG CCG GCC GA	NS3
4	NS3G4RI*	GCG ACC TGR TAG GTC TGR GGC A	NS3
1b	NS3-1b-1s†	GGC GTG TGG GGA CAT CAT C	NS3
1b-4	NS3-1b-4a†	CAT ATA CGC TCC AAA GCC CA	NS3
1a	NS3-1a-1s‡	CCG GGA GAT ACT GCT CGG AC	NS3
1a	NS3-1a-1a‡	GCT CTG GGG CAC TGC TG	NS3
1a	NS3-1a-2s‡	CCG ATGGAATGG TCT CCA AGG	NS3
1a	NS3-1a-2a‡	GAG AGG AGT TGT CCG TGA ACA C	NS3
1	NS5A-G1F	TGG ATG AAC CGG YTR ATW GC	NS5A
1	NS5A-G1R	ACG TAR TGR AAR TCC CCC ACC	NS5A
4	NS5A-G4F	GAR GGR GCY GTS CAR TGG AWG AAY C	NS5A
4	NS5A-G4R	KRC GAA CYT CCA MGT ABT CCT C	NS5A
1-4	Sn755§	TAT GAY ACC CGC TGY TTT GAC TC	NS5B
1-4	5B-SI766‖	CTG YTT TGA CTC CAC NGT RAC	NS5B
1-4	GEN1A.R1¶	CCG GGC AYG AGA CAC GCT GTG ATA AAT G	NS5B
1-4	GEN1B.R1¶	TGC GGC ACG AGA CAV GCT GTG ATA TG	NS5B

Underlined nucleotides correspond to M13 universal primers.
*Previously published primers.[23]
†Previously published primers.[24]
‡Previously published primers.[25]
§Previously published primers.[26]
‖Previously published primers.[27]
¶Previously published primers.[28]

one 400-mg capsule once-daily and SIM as one 150-mg capsule once-daily with food, as recommended.

ASSESSMENTS

HCV-RNA levels were measured by means of the Abbott RealTime Assay (Abbott Molecular, Des Plaines, IL), with a lower limit of quantification equal to the lower limit of detection of 12 IU/mL (i.e., 1.1 log IU/mL). Antiviral efficacy was assessed by determining on-treatment responses at weeks 4, 8, and 12 (end of treatment; EOT), and 4 and 12 weeks after treatment cessation. Virological responses (VRs) were defined as: (1) rapid response: HCV RNA undetectable (<12 IU/mL) at week 4; (2) early response: HCV RNA detectable at week 4, but undetectable at week 8; and (3) late response: HCV RNA detectable at weeks 4 and 8, but undetectable at week 12. The primary efficacy endpoint was an SVR (HCV RNA <12 IU/mL) 12 weeks after EOT (SVR12), which corresponds to a definitive cure of infection.

Genotypic resistance was assessed in all patients at baseline of retreatment, and in those patients who failed to eliminate HCV at the time of their virological breakthrough or post-treatment relapse, and on subsequent samples. Sequence analysis was based on population sequencing of three viral regions, including the NS3 protease (target of SIM and ASV), the NS5A protein (target of DCV), and the NS5B polymerase (target of sofosbuvir) coding regions. Briefly, HCV RNA was extracted with the QIASymphony DSP Virus/Pathogen kit on a QIASymphony device (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. Complementary DNA synthesis was performed with the OneStep RT-PCR kit (QIAGEN GmbH) with sets of primers adapted to the viral regions targeted (Table 1).[13-18] Nested polymerase chain reaction (PCR) was then performed, if needed, with primers specific for GTs 1a, 1b, or 4 (Table 1). PCR products were purified with Amicon Ultra-0.5 mL Centrifugal Filters (EMD Millipore, Darmstadt, Germany) and sequenced with the BigDye Terminator Cycle Sequencing Kit v3.1 on an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, CA).

Safety and tolerability were monitored and managed as per routine clinical practice, with regular physical examination, review of any adverse events (AEs), and blood samples taken for clinical laboratory testing. Serious adverse events (SAEs), treatment discontinuations, and laboratory abnormalities were recorded.

STATISTICAL ANALYSIS

Results are presented as mean ± 1 standard deviation (SD) or median with interquartile range for continuous data and number (percentage) for categorical data. The main criterion for efficacy was estimated with its 95% confidence interval (CI).

Results

BASELINE CHARACTERISTICS AND DISPOSITION

A total of 16 patients previously exposed to DCV were retreated with SOF in combination with SIM. Most patients were male, with a mean age of 54.9 years (Table 2). Patients were most commonly infected with HCV GT 1a (11 of 16) and 14 of 16 had baseline HCV RNA >800,000 IU/mL. FibroScan analysis revealed that 9 patients had cirrhosis and 7 had severe fibrosis (Table 1). Median FibroScan score was 13.6 kilopascals (kPa), and mean Model for End-Stage Liver Disease (MELD) score was 7.3 for patients with cirrhosis. No patient had decompensated cirrhosis and/or Chid-Pugh B or C score.

The previous treatment regimens were DCV plus Peg-IFN and RBV (13 patients) and DCV combined with ASV with Peg-IFN and RBV (3 patients). A mean of 31.5 ± 11.8 months had elapsed between the end of the previous DCV-based regimen and initiation of the new treatment with SOF and SIM. All 16 patients completed the 12-week retreatment course, and 12 weeks of post-EOT follow-up were available for all patients.

TABLE 2. Demographics and Baseline Characteristics

Parameter	Patients (N = 16)
Male, n (%)	13 (81)
Mean age, years (SD)	54.9 (7.8)
HCV GT, n (%)	
1a	11 (69)
1b	3 (19)
4	2 (12)
Median HCV RNA (range)	
$\times 10^6$ IU/mL	1.38 (0.86-2.47)
Log_{10} IU/mL	6.14 (5.93-6.39)
HCV RNA $>0.8 \times 10^6$ IU/mL, n (%)	13 (81)
Median FibroScan value, kPa (range)	13.6 (9.6-70)
Compensated cirrhosis (FibroScan >12.5 kPa), n (%)	9 (56)
Child-Pugh score in patients with cirrhosis, n	
A5	8
A6	1
MELD score in patients with cirrhosis, mean (SD)	7.3 (1.2)
Severe fibrosis (FibroScan >9.5-≤12.5 kPa), n (%)	7 (44)
Past treatment in combination with DCV	
Peg-IFN and RBV, n (%)	13 (81)
ASV plus Peg-IFN and RBV, n (%)	3 (19)
Patients with ≥1 NS3 RAVs at baseline, n (%)	8 (50)
Patients with ≥1 NS5A RAVs or deletion at baseline, n (%)	12 (75)
Patients with ≥1 NS5B RAVs at baseline, n (%)	3 (18.7)
Mean creatinine, μmol/L (SD)	78.1 (12.9)
Mean albumin, g/L (SD)	40.6 (1.5)
Mean hemoglobin, g/dL (SD)	15.3 (1.5)
Mean platelet count $\times 10^3/\mu L$ (SD)	205 (68)
Prothrombin INR, mean (SD)	1.0 (0.1)
Mean total bilirubin, μmol/L (SD)	11.7 (4.8)

Abbreviation: INR, international normalized ratio.

EFFICACY

All patients achieved VR (HCV RNA <12 IU/mL) by EOT (Table 3). Ten of the sixteen patients were rapid responders, achieving undetectable HCV RNA at week 4. An early response (undetectable HCV RNA at week 8) was observed in 1 patient, whereas the 5 remaining patients were late responders (HCV RNA detectable at weeks 4 and 8, but undetectable at week 12).

The primary efficacy endpoint, SVR12 (HCV RNA <12 IU/mL 12 weeks post-EOT) was achieved by 14 of 16 patients (87.5%; 95% CI: 61.7-98.4). The 2 patients who relapsed post-EOT were found to have HCV RNA >12 IU/mL at week 4 post-EOT (Table 3). SVR12 was achieved by all rapid and early responders and by 3 of the 5 late responders.

The 2 patients who relapsed were late on-treatment responders. Both patients were infected with HCV GT 1a, had relatively high HCV-RNA levels at baseline, had cirrhosis with a high FibroScan value, and had at least one RAV detected in both the NS3 and NS5A regions of the viral genome at the start of retreatment (Fig. 1; Tables 4 and 5). One of the two patients had been previously exposed to ASV.

INFLUENCE OF BASELINE RAVS ON VIROLOGICAL OUTCOME

NS5A RAVs at baseline were observed in 13 of 16 patients. All of the amino acid substitutions have been previously reported to be associated with NS5A inhibitor-containing regimen failures *in vivo*,[19] except two: Q30L and a deletion at position 30. However, the Q30L substitution was shown to confer *in*

TABLE 3. VR and Timing of Response in Patients Retreated with SIM Combined with SOF

	SIM+SOF for 12 Weeks (N = 16)
Patients with HCV RNA <12 IU/mL during treatment phase, n (%)	
Week 4	10 (63)
Week 8	11 (69)
Week 12 (EOT)	16 (100)
Patients with HCV RNA <12 IU/mL post-EOT, n (%)	
SVR4	14 (88)
SVR12 (primary endpoint)	14 (88)
Patients with VF (HCV RNA >12 IU/mL), n (%)	
During treatment phase	0
Relapse post-EOT	2 (12)

vitro resistance to OBV,[20] whereas the deletion at position 30 was shown to confer resistance to DCV *in vitro*.[21] Among the 13 patients with NS5A RAVs at baseline, 11 (84.6%) achieved SVR12. The 2 patients who failed to achieve SVR had Q30K and L31M substitutions as the dominant viral populations at retreatment baseline, respectively (Table 5).

NS3 protease RAVs at baseline were observed in 8 patients, including 2 who had been exposed to ASV. The most frequent amino acid substitutions were at positions 155 (R155K in 3 patients) and 80 (Q80K in 2 patients; Table 4); both substitutions are known to confer resistance to SIM *in vitro*[10] (Table 5). Among the 2 patients who failed to achieve SVR12, 1 had detectable Q80K and the other detectable R155K at baseline. The remaining 3 patients with 155 or 80 substitutions achieved SVR12 on SOF-SIM. Other NS3 protease substitutions were present at baseline in patients who ultimately achieved SVR12; they included V55A/I and S122T, not known to confer reduced susceptibility to SIM. S122T has recently been shown to be a natural polymorphism with no substantial effect on SIM half maximal effective concentration (fold-change $\leq$2).[10] Overall, 6 of 8 patients with NS3 RAVs detectable by population sequencing at baseline (75%) achieved SVR12.

NS5B polymerase RAVs were present in 3 patients, including C316N in 1 infected with GT 1b and A421V in 2 infected with GT 1a. The C316N substitution has been reported to be associated with reduced susceptibility to SOF.[22] The A421V substitution has been shown to be associated with reduced susceptibility to beclabuvir, a non-nucleoside inhibitor of the RNA polymerase.[19] These 3 patients with NS5B polymerase RAVs achieved SVR12.

SELECTION OF RAVS IN PATIENTS WHO FAILED TO ACHIEVE SVR

Two patients infected with GT 1a failed to achieve SVR on SOF-SIM combination therapy. The first patient harbored a dominant R155K population in the protease region and a dominant Q30K population in the NS5A region at baseline, which both remained unchanged at the time of relapse (Table 5). The second patient was infected with a dominant Q80K population in the protease region and a dominant L31M population in the NS5A region at baseline. At relapse, the L31M substitution was still dominant in the NS5A region, whereas both Q80K and D168E substitutions in the NS3 protease region had been selected by SIM.

SAFETY AND TOLERABILITY

No patient discontinued treatment and no SAEs were reported. There were no reports of grade 3 or 4 adverse events (AEs), nor grade 3 or 4 laboratory abnormalities.

Discussion

The combination of SIM and SOF was highly effective and well tolerated in this population of HCV GT 1- or 4-infected patients who had previously failed an IFN-containing regimen including the NS5A inhibitor, DCV. All patients achieved VR by EOT, despite some harboring Q80K and R155K mutations, which have been associated with SIM treatment failure in previous studies.[6] However, 2 patients subsequently relapsed and failed to achieve SVR12. These patients are considered difficult to cure; 1 patient had advanced cirrhosis (FibroScan = 33 kPa), and the other patient had cirrhosis and had been previously

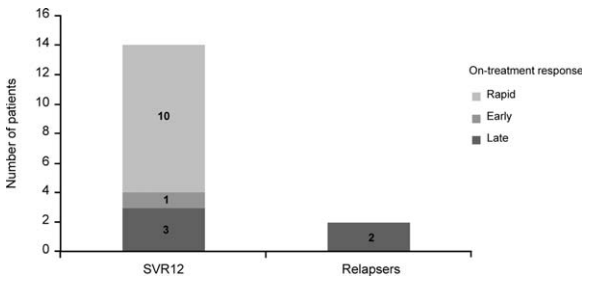

FIG. 1. VR by presence of baseline resistance mutations.

TABLE 4. Virological Outcome According to Baseline Factors

Parameter	Patients Achieving SVR12 (N = 14)	Patients Not Achieving SVR12 (N = 2)
Male, n	12/14	1/2
Mean age, years	56	48
HCV GT, n		
1a	9/14	2/2
1b	3/14	0
4	2/14	0
Median HCV RNA $\times 10^6$ IU/mL (range)	1.32 (1.94-5.98)	2.86 (2.08-3.63)
Median FibroScan value, kPa (range)	12.4 (9.6-70)	23.8 (14.9-32.8)
Cirrhosis*, n	7/14	2/2
Child-Pugh score in patients with cirrhosis, n		
A5	7	1
A6	0	1
MELD score in patients with cirrhosis, mean	6.9	9.0
Past treatment with DCV		
Peg-IFN and RBV, n	12/14	1/2
ASV plus Peg-IFN and RBV, n	2/14	1/2
Presence of $\geq$1 NS5A RAVs†, n	10/14	2/2
Q30E/R/K	5	1
L30S	1	0
L31M	5	1
Y93C/H	4	0
Deletion at amino acid position 30	1	0
Presence of $\geq$1 NS5B RAVs	3/14	0/2
C316N/A421V	1/2	0
Presence of $\geq$1 NS3 polymorphisms/RAVs†, n	6/14	2/2
Q80K	1	1
V55A/I	2	0
S122T	1	0
R155K	2	1

*Defined as FibroScan >12.5 kPa.
†Some patients harbored more than one amino acid substitution.

exposed to the PI, ASV, in combination to DCV. One of these patients had dominant R155K and Q30K substitutions in the protease region and NS5A region at baseline, respectively, and the other harbored dominant Q80K and L31M polymorphisms in the NS3 and NS5A regions, respectively, at baseline. Thus, both harbored RAVs known to confer reduced susceptibility to SIM at baseline of retreatment.

Our real-life study included a patient population characterized by advanced liver disease and high baseline HCV-RNA levels. The population also included 2 patients with HCV GT 4 infection, who both achieved SVR12. These findings should be noted given that the efficacy of SIM has not been extensively studied in patients other than those infected with HCV GT 1, and this is the first study to assess the efficacy of SIM and SOF retreatment in patients who have failed past DAA-based regimens. Because we included patients from our routine clinical practice, the study population was more diverse than those in clinical trials, which usually have restrictive inclusion criteria. Additionally, as per other clinical trials with SIM and SOF, the patients in our study had good adherence to, and compliance with, medication and dosing requirements, although this was not formally assessed. However, wider application of the findings should be done cautiously because of the small sample size and because only patients failing past daclatasvir-based treatment were included.

The American Association for the Study of Liver Disease and the European Association for the Study of the Liver (EASL) both recommend the administration of SIM and SOF after DCV treatment failure.[1,23] However, there are currently no data on retreatment of virological failures (VFs) subsequent to DCV-containing regimens in the literature. Our findings showing that an SVR rate of 87.5% are in keeping with results of studies assessing the combination of SOF and SIM as a retreatment strategy for other types of treatment failures.[24,25] The phase II COSMOS study with SOF and SIM reported SVR12 in 92% of GT 1-infected patients who had previously failed a Peg-IFN and RBV regimen.[24] In another real-life study, with a heterogeneous population including treatment-experienced patients, an SVR rate of 82% was reported in GT 1 patients receiving SOF and SIM, with or without RBV.[25]

The number of patients in this study is too small to establish a direct relationship between the individual NS34A RAVs present at baseline and retreatment outcome. However, it is interesting that 3 of the 5 patients

TABLE 5. Virological Outcome and Occurrence of RAVs for the 2 Patients Who Failed Retreatment

	Baseline	12 Weeks Post-EOT
Patient 1 (female, age 47 years with advanced cirrhosis*)		
HCV-RNA x10^6 IU/mL	2.08	1.90†
NS3 RAVs	R155K	R155K
NS5A RAVs	Q30K	Q30K
NS5B RAVs	—	—
Patient 2 (male, age 48 years with past treatment failure on a regimen including a PI)		
HCV-RNA $\times 10^6$ IU/mL	3.63	7.07‡
NS3 RAVs	Q80K	Q80K D168E
NS5A RAVs	L31M	L31M
NS5B RAVs	—	—

*FibroScan: 33 kPa; serum albumin: 32 g/L; platelets count: 76 $\times 10^3/\mu$L.
†HCV-RNA at week 4 post-EOT was 2.9x10^6 IU/mL.
‡HCV-RNA at week 4 post-EOT was 5.7x10^6 IU/mL.

harboring the R155K or Q80K substitutions at baseline achieved SVR12, despite that these substitutions confer reduced susceptibility to SIM. This suggests that combination with SOF restores SIM susceptibility by dramatically reducing levels of resistant viruses within the quasi-species population, thereby improving the balance with exposure. A relatively high rate of SVR has also been reported with SOF and SIM in patients with baseline Q80K in the COSMOS study.[3] Unsurprisingly, no SOF RAVs emerged in the 2 patients who failed to achieve SVR12. SOF is known to have a high barrier to resistance, and SOF-resistant viruses are exceptionally selected because their replicative fitness is poor.[22] Overall, our results suggest that baseline resistance testing would not have been useful in identifying which patients would succeed or fail on this retreatment regimen.

Despite the overall high retreatment success rate, 2 patients relapsed. The first patient was a female, infected with HCV GT 1a with advanced cirrhosis who had previously failed on a DCV plus Peg-IFN and RBV regimen. At baseline, she harbored a Q30R substitution in the NS5A region, which likely contributed to past DCV-based treatment failure and persisted after retreatment with SOF and SIM. At baseline, she also harbored the known SIM RAV, R155K,[10] although she had never been previously exposed to PIs. This substitution persisted and was present as the dominant species at the time of relapse. The second patient was a male, also infected with HCV GT 1a who had previously failed on a regimen including DCV, ASV, Peg-IFN, and RBV. As with other patients from this cohort, he harbored the known DCV RAV, L31M, at baseline. L31M was presumably selected by the previous DCV-containing regimen and subsequently persisted, because NS5A RAVs have been shown generally to persist after treatment failure.[1,6,7] At baseline, this patient also harbored a Q80K substitution in the NS3 protease region. Q80K is a polymorphism found in 19%-48% of treatment-naïve patients infected with HCV GT 1a.[3,26] Q80K reduces SIM activity *in vitro* by 9-fold when present alone and by 2,000-fold when combined with R155K. However, the effect of Q80K on drug susceptibility depends on drug exposure; thus, Q80K alone is unlikely to confer *in vivo* resistance when SIM is used at the dose of 150 mg/day.[10,27] This is consistent with our observation of another patient with Q80K at retreatment baseline who achieved SVR12. In the second patient who relapsed, past exposure to ASV could have selected a fitter Q80K variant, more likely to be selected by SIM retreatment.

Both patients who failed to achieve SVR12 at 12 weeks of SOF and SIM retreatment could have benefited from a longer duration of therapy and/or addition of RBV. Indeed, both options, which are not mutually exclusive, have been shown to improve SVR rates in patients with cirrhosis and in those with baseline RAVs receiving DAA-based therapies in various studies.[28,29] In this respect, it is noteworthy that the EASL 2015 guidelines recommend that any patient with cirrhosis should receive either a 12-week course of therapy with RBV or a 24-week course without RBV.[1] Furthermore, retreatment should always include RBV and should be prolonged to 24 weeks in patients with advanced fibrosis or cirrhosis, pending more data in patients who failed a DAA-based regimen.[1] In this respect, it is interesting to note that 7 of the 9 patients with cirrhosis and all patients with severe fibrosis achieved SVR12 despite only 12 weeks of therapy and the lack of RBV.

In conclusion, these real-life findings suggest high efficacy, good tolerance, and feasibility of a combination regimen of SOF and SIM in patients infected with chronic HCV GT 1 or 4 infection who have failed a previous DCV-based regimen. The study shows that patients who achieved rapid or early responses were more likely to achieve SVR than those achieving late responses. Of the 16 patients with DAA-based treatment failures evaluated in this study, 14 achieved SVR12 despite some having R155K and Q80K polymorphisms known to be associated with failure of SIM treatment. These results support the concept of retreating NS5A inhibitor failures with SOF combined with SIM and provide a signal as to which patient profiles could require longer duration of therapy and or addition of RBV. Such patients may include those with cirrhosis and/or pre-existing RAVs.

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