Received: 28 December 2017 | First decision: 17 January 2018 | Accepted: 11 February 2018

DOI: 10.1111/apt.14592

WILEY AP&T Alimentary Pharmacology & Therapeutics

High efficacy of sofosbuvir/velpatasvir and impact of baseline resistance-associated substitutions in hepatitis C genotype 3 infection

J. von Felden[1,2] | J. Vermehren[3] | P. Ingiliz[4] | S. Mauss[5] | T. Lutz[3] | K. G. Simon[6] | H. W. Busch[7] | A. Baumgarten[4] | K. Schewe[1] | D. Hueppe[8] | C. Boesecke[9] | J. K. Rockstroh[9] | M. Daeumer[10] | N. Luebke[5] | J. Timm[5] | J. Schulze zur Wiesch[1] | C. Sarrazin[3,11] | S. Christensen[7]

[1]Hamburg, Germany
[2]New York City, NY, USA
[3]Frankfurt, Germany
[4]Berlin, Germany
[5]Duesseldorf, Germany
[6]Leverkusen, Germany
[7]Muenster, Germany
[8]Herne, Germany
[9]Bonn, Germany
[10]Kaiserslautern, Germany
[11]Wiesbaden, Germany

Correspondence
Dr. J von Felden, I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
Email: j.von-felden@uke.de

Funding information
JvF and JSzW are supported by Deutsche Forschungsgemeinschaft (German Research Foundation grants FE1746/1-1, SFB841 A6). JSzW and CS are supported by the German Center for Infection Research (DZIF).

Summary

Background: Twelve weeks of the pangenotypic direct-acting antiviral (DAA) combination sofosbuvir/velpatasvir (SOF/VEL) was highly efficient in patients with hepatitis C virus (HCV) genotype 3 (GT3) infection in the ASTRAL-3 approval study. However, presence of resistance-associated substitutions (RASs) in the HCV non-structural protein 5A (NS5A) was associated with lower treatment response.

Aim: To assess the efficacy and safety of SOF/VEL ± ribavirin (RBV) and the impact of NS5A RASs and RBV use on treatment outcome in HCV GT3 infection in a real-world setting.

Methods: In this multicentre cohort study, GT3 patients from ten treatment centres across Germany were included. Sustained virological response was assessed 12 weeks after end-of-treatment (SVR12) in modified intention-to-treat (mITT) and per-protocol analysis (PP). NS5A RASs were tested by population-based sequencing.

Results: A total of 293 GT3 patients were included. The median age was 48 years, 70% were male, 25.3% were cirrhotic, 9.2% were HCV/HIV co-infected and 21.8% were treatment-experienced, including 4.1% with DAA experience. Baseline NS5A RASs (Y93H, A30K, L31M) were detected in 11.2%. RBV was added in 5% of non-cirrhotic and 58.9% of cirrhotic patients, respectively. SVR12 rates for SOF/VEL±RBV were 95.9% (mITT) and 99.5% (PP), respectively. Only 1 virological relapse occurred in a cirrhotic patient previously treated with SOF/RBV. No treatment-related major adverse events occurred.

Conclusion: Twelve weeks of SOL/VEL±RBV was safe and highly efficient in HCV GT3 across a diverse patient population. Baseline NS5A RASs were rarely observed and presence did not seem to impact SVR, regardless of the use of RBV.

J. von Felden and J. Vermehren share first authorship.
C. Sarrazin and S. Christensen share senior authorship.
The Handling Editor for this article was Professor Grace Wong, and it was accepted for publication after full peer-review.
Authors' complete affiliations are listed in Appendix section.

1 | INTRODUCTION

Chronic hepatitis C virus (HCV) infection affects approximately 71 million patients worldwide, who are at risk of developing progressive liver disease, including cirrhosis and hepatocellular carcinoma.[1] HCV genotype 3 (GT3) is the second-most prevalent genotype, accounting for 25% of all infections, and has a particularly high prevalence among European drug users and in Southern Asia.[2,3] Moreover, recent evidence suggests that HCV GT3 infection is associated with higher rates of liver steatosis, more rapid fibrosis progression, and development of hepatocellular carcinoma compared to infection with other HCV genotypes.[4-6]

Historically, HCV GT3 infection was considered an easy-to-treat genotype with sustained virological response (SVR) rates of approximately 70% following 24-48 weeks of interferon-based therapy in noncirrhotic patients.[7,8] More recently, development of interferon-free direct-acting antiviral (DAA) drug combinations has marked a significant breakthrough in antiviral therapy for chronic HCV infection with high SVR rates.[9] However, most first-generation HCV protease inhibitors and nonstructural protein 5A (NS5A) inhibitors were less effective in GT3 infection, particularly in the presence of other negative predictive factors, including cirrhosis, prior interferon treatment failure and/or resistance-associated substitutions (RASs).[3,10] Thus, HCV GT3 infection was still regarded as one of the more "difficult-to-treat" genotypes in the era of interferon-free treatments.

Velpatasvir (VEL), a second-generation, pangenotypic HCV NS5A inhibitor in combination with the HCV NS5B polymerase inhibitor sofosbuvir (SOF) was recently introduced as an interferon-free, oral, once daily regimen for the treatment of HCV genotypes 1-6.[11,12] SOF/VEL demonstrated potent antiviral activity and a high barrier to resistance in vitro, and high SVR rates of 95%-99% were achieved across all HCV genotypes following 12 weeks of SOF/VEL therapy.[11,12] However, SVR rates were lower in HCV GT3 infection compared to other genotypes, particularly in treatment-experienced cirrhotic patients.[11,13] These observations prompted the European Medicines Agency to recommend that ribavirin (RBV) may be added in patients with HCV GT3 infection and cirrhosis although no data are available to support this recommendation.[14]

While rarely observed in the approval trials, presence of the highly resistant NS5A substitution Y93H at baseline was associated with lower SVR, and Y93H was also observed as the predominant variant in all GT3 failures at time of treatment failure.[13]

So far, no large cohort studies outside of clinical trials are available for GT3 patients, and thus, it is unclear whether equally high SVR rates reported in clinical trials are achievable under real-world conditions.

In our multicentre cohort study, we aimed to assess the efficacy and safety of 12-week therapy with SOF/VEL ± RBV in patients with HCV GT3 infection, including patients with HCV and human immunodeficiency virus-1 (HIV) co-infection, prior HCV-treatment experience and/or cirrhosis in a real-world setting. In addition, impact of baseline NS5A RASs on treatment outcome was analysed.

2 | PATIENTS AND METHODS

For the present study, all consecutive patients with chronic HCV GT3 infection treated with SOF/VEL with or without RBV for 12 weeks within the German Hepatitis C Cohort (GECCO) and at the outpatient clinic of the University Hospital Frankfurt were included. GECCO is an ongoing prospective, multicentre study including patients from 9 hepatitis C treatment centres in Germany (Berlin, Bonn, Duesseldorf, Frankfurt, Hamburg, Herne, Leverkusen, and Muenster) who initiated treatment with various DAA-based regimens since February 2014.[15-18]

Individual patient treatment regimens were chosen at the discretion of the treating physician with regard to the patient's prior treatment experience, presence or absence of cirrhosis, liver function tests, presence of NS5A RASs at baseline (if available), comorbidities and concurrent medications. Of note, DAA-experienced patients were also included in this study.

Clinical, laboratory and virological parameters were assessed at baseline, treatment week 4, 8, and 12 (if available), as well as 4, 12 and 24 (if available) weeks after the end of therapy.

Cirrhosis was diagnosed through biopsy or clinical assessment, including liver stiffness >12.5 kPa according to transient elastography (FibroScan, Echosens, Paris, France) and/or aspartate transaminase/platelet-ratio index (APRI > 2).

2.1 | Resistance testing

Resistance testing was performed in all available serum samples, which were collected prior to treatment initiation. HCV ribonucleic acid (RNA) extraction, amplification of the NS5A target region as well as population-based sequencing were performed as previously described.[19] The viral load sensitivity threshold of the resistance assay was approximately 1000 IU/mL HCV RNA or greater. Detected resistance-associated substitutions (RASs) were classified as low (<10-fold), medium (10-100-fold) or high (>100-fold) according to their change in VEL susceptibility (EC_{50}) in comparison to a wild type reference strain in in vitro replicon assays.[10,20] HCV GT3 subtypes were re-evaluated as part of the study based on nucleotide sequences.

2.2 | Study endpoints and statistical analyses

Primary endpoint of the study was sustained virological response defined as undetectable HCV RNA 12 weeks (SVR12) after end of therapy. Additional endpoints of our study were undetectable HCV RNA 4 weeks after end of therapy (SVR4) and overall safety of SOF/VEL therapy. Virological failure, ie, relapse, was defined as undetectable HCV RNA at end of therapy which became detectable again thereafter without proven re-infection.

The safety analysis included all patients who started SOF/VEL treatment and in whom at least a baseline visit was documented (*overall cohort*). Safety analysis assessed severe adverse events, ie, death or pre-terminal cessation of the medication due to adverse events. SVR12 rates were calculated according to the modified

intention-to-treat (mITT, *mITT population*) and per-protocol analysis (PP, *PP population*). The *mITT population* included all patients who should have reached a follow-up visit of at least 12 weeks after end of therapy at the time of final data analysis (October 31st, 2017), including patients who did not attend their scheduled follow-up visit and were lost to follow-up, had cessation of treatment for any reason or death during treatment or until follow-up week 12. The *PP population* included all patients from the *mITT population*, except nonvirological failures. Patients who have not reached their scheduled 12-week follow-up were not eligible for mITT or PP analysis.

We performed further subgroup analyses on outcome of patients with cirrhosis, HCV/HIV co-infection, pegylated interferon (PEG-IFN) and DAA-experience as well as for patients with confirmed NS5A RASs at baseline. For statistical analysis, we used Pearson's Chi Square test, two-way ANOVA, or Fisher's exact test, if applicable. A *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 24 (IBM Corporation, New York, NY, USA).

3 | RESULTS

3.1 | Baseline characteristics

A total of 293 patients with HCV GT3 infection started SOF/VEL therapy with or without RBV between July 2016 and August 2017 and were included in our study (*overall cohort*). Median age of the *overall cohort* was 48 years (range 18-77 years) with 205 (70%) male patients. A total of 74 patients had cirrhosis (25.3%), predominantly Child-Pugh-Turcotte (CPT) score A (n = 65, 87.8%). The median liver stiffness assessed with transient elastography was 6.9 kPa (range 3.0-75.0 kPa) for the *overall cohort*, and 6.1 kPa (range 3.0-12.4 kPa) and 18.0 kPa (range 11.1-75 kPa) for noncirrhotic and cirrhotic patients ($P < 0.001$), respectively. Detailed baseline characteristics of the *overall cohort*, including liver function tests are displayed in Table 1.

Sixty-four patients (21.8%) had previously received antiviral HCV therapy. Among these, 49 patients had received (PEG)-IFN with or without RBV, 3 patients had previously taken part in a clinical HCV trial with the cyclophilin inhibitor alisporivir plus RBV, and 12 patients had received SOF-based DAA regimens. Twenty-eight patients (9.6%) were HCV/HIV co-infected.

A total of 222 and 214 out of 293 patients were eligible for mITT and PP analysis, respectively (see Figure 1).

3.2 | Efficacy outcome and safety analysis

Overall, 95.9% (213/222) of the *mITT population* and 99.5% of the *PP population* achieved SVR12 (see Figure 2). In the entire cohort, there was only 1 patient with virological relapse, which occurred 4 weeks after end of therapy. This was a 53-year-old male patient with compensated cirrhosis (CPT score A) who had previously been treated with sofosbuvir and RBV for 24 weeks and in whom a virological relapse had occurred thereafter. Following negative screening for NS5A RASs at baseline, this patient was now retreated with SOF/VEL without RBV for 12 weeks at the discretion of the treating physician.

TABLE 1 Characteristics of patients at baseline

Characteristic	Sofosbuvir/velpatasvir ± RBV Overall cohort, n = 293
Age, years	48 (18-77)
Male	205 (70.0%)
HCV RNA, IU/mL	1.28×10^6 ($0.06 \times 10^3 - 51.8 \times 10^6$)
Liver stiffness, kPa	6.9 (3.0-75.0)
Cirrhosis	74 (25.3%)
CPT score A	65 (87.8%)
CPT score B	9 (12.2%)
SOF/VEL without RBV	239 (81.6%)
SOF/VEL with RBV	54 (18.4%)
Previous HCV therapy	64 (21.8%)
DAA-based	12 (18.8%)
PEG-IFN ± RBV	49 (76.6%)
Other regimen	3 (4.7%)
HCV/HIV co-infection	28 (9.2%)
HIV RNA, copies/mL	<LLOQ (<LLOQ – 1.54×10^6)
$CD4^+$ T-cells, cells/μL	624 (137-2487)
On stable ART[a]	26 (92.9%)
Haemoglobin, g/dL	14.4 (10.0-19.2)
Platelets, cells/nL	197 (39-471)
ALT, ×-fold ULN	1.7× (0.3-8.3)
Bilirubin, mg/dL	0.5 (0.1-3.4)
Albumin, g/dL	41.7 (19-51.7)
INR	1.03 (0.85-2.70)

ALT, alanine transaminase; ART, antiretroviral therapy; CD, cluster of differentiation; DAA, direct-acting antiviral; HCV, hepatitis C virus; HIV, human immunodeficiency virus-1; INR, international normalised ratio; LLOQ, lower limit of quantification; RNA, ribonucleic acid; RBV, ribavirin; ULN, upper limit of normal.
Continuous variables are displayed as median and range, and categorical variables as n and percentage.
[a]ART regimen was recently switched in 1 patient.

Subgroup analysis revealed SVR12 rates (mITT and PP, respectively) of 93.2% (55/59) and 98.2% (55/56) in cirrhotic patients, 97% (162/167) and 100% (162/162) in treatment-naive patients, and 97.7% (43/44) and 100% (43/43) in non-DAA treatment-experienced patients, respectively. All HCV/HIV co-infected patients achieved SVR12 (100% in mITT and 100%, in PP, 19/19 each). In patients with NS5A RASs at baseline, 95.5% (21/22) and 100% (21/21) achieved SVR12 in mITT and PP, respectively. Patients who had previously received DAA-based therapy, ie, SOF-containing regimens, showed statistically lower SVR12 rates of 72.7% (8/11) and 88.9% (8/9) in mITT and PP, respectively.

There were 2 deaths in the *mITT population*, which occurred during treatment and were judged as not treatment-related by the treating physician (cardiac arrest at week 4 and fatal gastrointestinal bleeding at week 8 of antiviral treatment, both patients had compensated cirrhosis), and 6 patients were lost to follow-up (2.7%). There was no pre-terminal cessation of the medication due to adverse events observed in the *safety population* (n = 293).

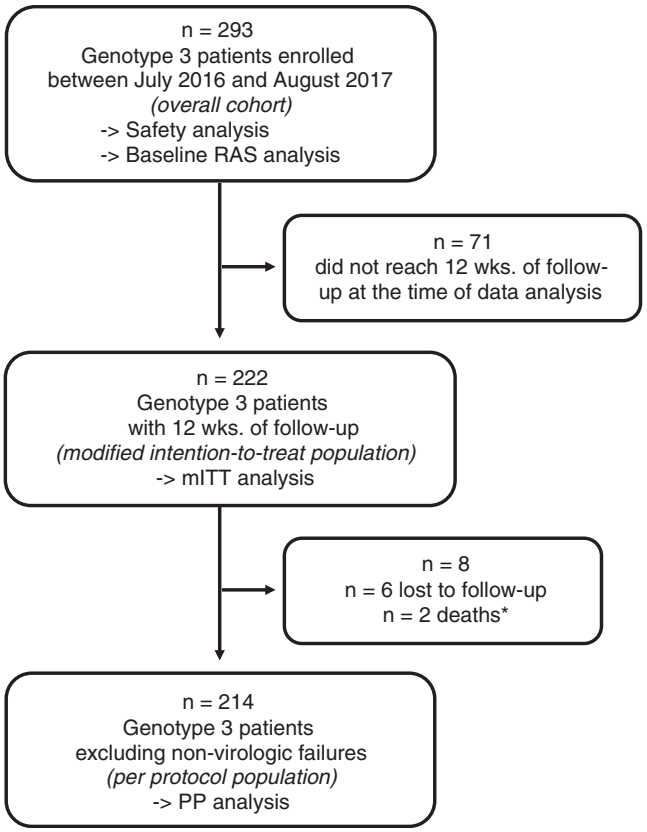

FIGURE 1 Study flow design. Distribution of patients into overall cohort, modified intention-to-treat population (mITT) and per-protocol population (PP) with indication of statistical analysis for each population. *Judged as not treatment-related

Subgroup analysis of patients who had available follow-up data at 4 weeks after end of treatment showed SVR rates of 96.2% (230/239) and 99.6% (230/231) in *mITT and PP populations*, respectively (see Figure S1 for details).

3.3 | Use of ribavirin and impact on treatment outcome

To investigate the impact of RBV on treatment outcome, we assessed the use of RBV in cirrhotic and noncirrhotic patients and compared SVR12 rates between these groups. In the *overall cohort*, 54 patients (18.9%) were treated with SOF/VEL plus RBV. Of note, the decision whether RBV was added or not was at the discretion of the treating physician. RBV was used more frequently in cirrhotic patients compared to noncirrhotic patients (43/73 vs 11/219, $P < 0.001$). In order to test whether the decision to add RBV to SOF/VEL in patients with compensated cirrhosis (CPT score A) was influenced by severity of liver disease or patient history, we compared baseline characteristics between these 2 patient groups. Platelet count at baseline was significantly lower in compensated cirrhotic patients who received RBV compared to those who did not (102 vs 156 cells/nL, $P = 0.010$), whereas other markers for severity of liver disease (eg, liver stiffness, bilirubin, albumin, INR), previous HCV therapy, baseline NS5A RASs or HCV/HIV co-infection were not significantly different between the 2 groups. As expected, RBV led to a decrease in haemoglobin levels, but without clinical significance (see Table S2). Among compensated and decompensated cirrhotic patients who were available for per-protocol analysis (n = 56), all patients treated with RBV achieved SVR12 (35/35, 100% [PP]), whereas the only relapse was in the subgroup of cirrhotic patients who were treated without RBV (n = 21, SVR12: 20/21, 95.2% [PP]).

In noncirrhotic patients, RBV was more frequently used in DAA-pre-treated patients compared to all other noncirrhotic patients (3/5 vs 8/214, $P = 0.001$) and in patients with NS5A RASs at baseline compared to patients without (6/22 vs 3/153, $P < 0.001$). All noncirrhotic patients who were available for per-protocol analysis (n = 158) achieved SVR12, independent of RBV (8/8 with RBV and 150/150 without RBV, 100% [PP], respectively).

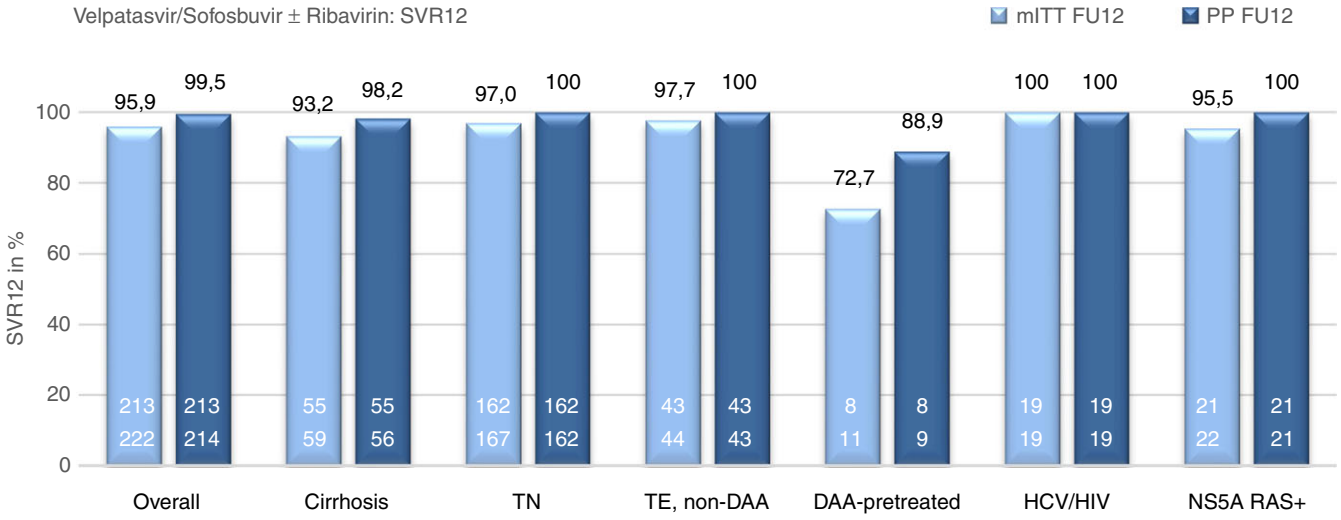

FIGURE 2 SVR12 rates. Modified intention-to-treat (mITT) and per-protocol (PP) analysis of SVR12 rates of SOF/VEL ± RBV of the entire cohort (overall), patients with cirrhosis (cirrhosis), treatment-naive patients (TN), treatment-experienced patients without DAA-based regimen (TE, non-DAA), patients previously treated with DAA-based regimen (DAA-pre-treated), patients with HCV/HIV co-infection (HCV/HIV), and patients with detection of NS5A baseline RAS (NS5A RAS+). Of note, only 1 virological failure occurred in a cirrhotic, DAA-pre-treated patient

3.4 | Prevalence of baseline RASs and impact on treatment outcome

In a total of 251/293 patients (85.7%), baseline serum samples were tested for NS5A RASs. RASs could not be evaluated in 14 patients due to technical failure of the sequence analysis assay, resulting in a total of 237/293 patients (80.9%) with successful RAS testing.

The overall prevalence of clinically relevant NS5A RASs was 11.8% (28/237). High-level RASs (Y93H) were found in 4.6% (11/237) and medium-level RASs (A30K, L31M) were found in 7.2% (17/237), 2 of whom had both RASs detectable. There were no VEL-specific low-level RASs detected in this study population.

All patients with baseline NS5A RASs achieved SVR12 in the PP analysis (21/21, see Table 2). Among noncirrhotic patients with baseline Y93H, 4 patients received RBV and another 4 patients did not, all 8 of whom achieved SVR12. Among cirrhotic patients, there was only 1 patient with baseline Y93H. This patient received SOF/VEL plus RBV for 12 weeks and achieved SVR12. All ten patients with baseline A30K RAS achieved SVR12.

TABLE 2 Impact of baseline NS5A RAS on treatment outcome

	SVR12 (PP)			
	Without RBV		With RBV	
	n/n (TE)	%	n/n (TE)	%
Noncirrhotic patients				
High level NS5A RAS (Y93H)	4/4 (1)	100	4/4 (0)	100
Medium-level NS5A RAS (A30K, L31M)[a]	8/8 (2)	100	0	–
Cirrhotic patients				
High level NS5A RAS (Y93H)	0	–	1/1 (1)	100
Medium-level NS5A RAS (A30K, L31M)[a]	0	–	4/4 (2)	100

NS5A, nonstructural protein 5A; RAS, resistance-associated substitution; RBV, ribavirin; SVR, sustained virological response.
Detailed efficacy of sofosbuvir/velpatasvir in patients with baseline NS5A RASs according to utilisation of RBV in noncirrhotic and cirrhotic patients (n = 21, per-protocol analysis [PP]). Number of treatment-experienced (TE) patients in each subgroup is displayed in parenthesis.
[a]One patient had both NS5A RAS detectable.

3.5 | Efficacy and safety in patients with HCV/HIV co-infection

Overall, 28 patients (9.6%) were co-infected with HCV/HIV (detailed characteristics in Table S1). Cirrhosis was present in 5 patients with HIV/HCV co-infection (17.9%), and 8 patients (28.6%) had received previous antiviral HCV therapy, including 1 patient with previous SOF/LDV therapy. Intravenous drug abuse was the most frequent route of infection in this subgroup (57.1%). Twenty-six patients (92.9%) were on stable antiretroviral therapy (ART), in 2 patients ART had been switched or started a few weeks prior to the start of SOF/VEL therapy, respectively. Not surprisingly, nucleoside/nucleotide reverse transcriptase inhibitor were part of the majority of ART regimens (24/28, 85.7%). The NRTI tenofovir disoproxil fumarate (TDF) was included in 8 regimens, but switched to tenofovir alafenamide in 1 case at start of SOF/VEL. Twenty-two, eleven, and four regimens included integrase inhibitors, protease inhibitors and non-nucleoside/nucleotide reverse transcriptase inhibitors, respectively.

A total of 19 patients were available for mITT and PP analysis, all of whom achieved SVR12 (100%, see Figure 2) whereas 3 additional patients achieved SVR4 and 6 patients were still on treatment at time of data analysis. No safety issues were reported in the subgroup of patients with HIV/HCV co-infection.

3.6 | Efficacy and safety in patients with previous DAA-based therapy

Due to our observational study design, our cohort also contained patients who had previously been treated with DAA-based regimens at the discretion of their treating physicians. In detail, our cohort included a total of 12 DAA-pre-treated patients, all of whom had received SOF-based regimens in the past (detailed characteristics are shown in Table 3). This subgroup predominantly consisted of male patients (91.7%). Cirrhosis and baseline NS5A RASs were present in this subgroup in 58.3% and 18.2%, respectively. Nine patients were available for PP. RBV was used in 5 out of 9 patients. Eight out of nine patients achieved SVR12 in the PP analysis (88.9% [PP], see Figure 2). As already mentioned, the relapse occurred in a patient who was treated with SOF/VEL without RBV. Apart from the 2 patients who died during treatment due to nontreatment-related events (see above), no severe adverse events occurred in this subgroup.

3.7 | Efficacy and safety in patients with decompensated cirrhosis

Our cohort included 9 patients with decompensated cirrhosis (CPT score B, 12.3% of all cirrhotic patients), and all of them achieved SVR12 (100% [PP]). Eight out of nine patients were treated with SOF/VEL plus RBV, of whom 2 patients had medium-level NS5A RASs (A30K) at baseline. Of note, there were no liver-related events or other safety concerns in this subgroup.

4 | DISCUSSION

In this study, we assessed for the first time the real-world efficacy of SOF/VEL and the impact of baseline NS5A RASs on treatment outcome in HCV GT3 infection.

Twelve weeks of SOF/VEL with and without RBV resulted in 95.9% (mITT analysis) and 99.5% (PP analysis) SVR12. Previous real-world data of patients with HCV GT3 infection, who were treated with SOF-based regimens not containing VEL, had shown inferior efficacy compared to the respective approval trials, particularly in patients with cirrhosis and/or other negative predictive baseline factors.[18,21,22] In contrast, our data mirror the results of the respective SOF/VEL ASTRAL-3 study with an overall SVR rate of 95%.[11]

TABLE 3 Characteristics of patients with prior DAA-based antiviral therapy

			Previous HCV-treatment		Current HCV-treatment				
Age, Gender	Cirrhosis	HIV co-infection	DAA	RBV	DAA	RBV	Duration (weeks)	Baseline NS5A RAS	Outcome
38, m	None	None	SOF/DCV	No	SOF/VEL	None	12	None[a]	SVR12
43, m	None	Yes CDC C3	SOF/LDV	No	SOF/VEL	None	12	None	SVR24
56, m	None	None	SOF/DCV	No	SOF/VEL	Yes	24	A30K	On treatment
74, m	None	None	SOF/LDV	No	SOF/VEL	Yes	12	None	SVR12
35, m	None	None	SOF	Yes	SOF/VEL	Yes	12	None	SVR24
53, m	Yes, CPT A	None	SOF	Yes	SOF/VEL	None	12	None	Relapse FU12
63, m	Yes, CPT A	None	SOF	Yes	SOF/VEL	None	12	None	SVR24
57, f	Yes, CPT A	None	SOF	Yes	SOF/VEL	Yes	12	None	Death week 4
55, m	Yes, CPT A	None	SOF/LDV	Yes	SOF/VEL	Yes	24	None	Death week 8
53, m	Yes, CPT A	None	SOF	Yes	SOF/VEL	Yes	12	None	SVR12
59, m[b]	Yes, CPT B	None	SOF/LDV	Yes	SOF/VEL	Yes	12	A30K	SVR12
64, m	Yes, CPT A	None	SOF	Yes	SOF/VEL	Yes	12	Not tested	SVR12

CDC, U.S. Centers for Disease Control and Prevention classification; CPT, Child Pugh Turcotte classification; DAA, direct-acting antiviral; DCV, daclatasvir; f, female; HCV, hepatitis C virus; HIV, human immunodeficiency virus-1; LDV, ledipasvir; m, male; NS5A, nonstructural protein 5A; RAS, resistance-associated substitution; SOF, sofosbuvir; SVR, sustained virological response, VEL, velpatasvir.

Displayed are patients with prior DAA-based therapy ordered by presence of cirrhosis, utilisation of ribavirin (RBV) and outcome of current HCV therapy.

[a]After SOF/DAC, Y93H was detectable, but was repeatedly tested negative over a time of >6 months before SOF/VEL was started.

[b]This patient possibly suffered from re-infection after initial HCV therapy.

In our entire study population, there was only 1 patient who experienced virological relapse. This patient suffered compensated cirrhosis and had previously been treated with SOF plus RBV for 24 weeks. He was now retreated with SOF/VEL without RBV for 12 weeks, which, in retrospect, may have been suboptimal as recent data suggest that 12 weeks of SOF/VEL alone may be insufficient to cure patients with previous SOF experience.[23] Moreover, recent guidelines recommended that patients with DAA failure should be retreated with SOF plus NS5A inhibitor, or SOF plus NS5A- and NS3 protease inhibitor plus RBV for 12-24 weeks, if classified as "difficult-to-cure", ie, patients with advanced fibrosis or cirrhosis.[24] However, these recommendations were based on pharmacological and virological considerations rather than evidence from clinical trials. More recently, a triple DAA combination therapy containing SOF/VEL and the NS3 protease inhibitor voxilaprevir was shown to be superior to SOF/VEL alone in patients who failed a DAA-based regimen without NS5A inhibitor, and superior to matching placebo in patients who failed an NS5A inhibitor containing regimen in 2 phase 3 clinical trials with SVR12 rates of 96%-98%.[23] This regimen was recently approved for the treatment of DAA-experienced patients by the European Medicines Agency and U.S. Food and Drug Administration.

The use of RBV in combination with SOF/VEL in genotype 3 has so far only been studied in decompensated cirrhosis and in a 24-week retreatment study after previous SOF/VEL failure. Interestingly, both studies showed SVR rates below 90%.[25,26]

In our study, 8 out of 9 SOF-pre-treated patients achieved SVR12, despite receiving only 12 weeks of therapy. Although the patient numbers are too small to draw any definitive conclusions, we believe that retreatment with SOF/VEL plus RBV for 12 weeks in SOF-pre-treated patients may be feasible in some cases, particularly when there are contraindications to voxilaprevir or glecaprevir/pibrentasvir (eg, decompensated cirrhosis) or availability is limited, but larger datasets are needed in order to give firm recommendations.

Presence of baseline RASs (Y93H and A30K) in the NS5A gene has been associated with decreased in vitro activity of the NS5A inhibitors daclatasvir and VEL, and lower SVR rates were reported in clinical trials.[27,28] In the ASTRAL-3 study, patients who were treated with SOF/VEL achieved 84% SVR in the presence of Y93H compared to 97% in patients without Y93H.[11] Moreover, Y93H could be detected in all 10 GT3 patients from ASTRAL-3 who experienced virological relapse.[13] Based on these findings, recent clinical practice guidelines recommend RAS testing before the initiation of SOF/VEL therapy in treatment-experienced patients with or without cirrhosis, and addition of RBV is recommended in noncirrhotic and cirrhotic patients in the presence of baseline Y93H.[24,29]

In our study, neither the high-level NS5A RAS Y93H, nor the medium-level NS5A RASs A30K and L31M were associated with lower SVR12, regardless of the use of RBV. This may be due to the fact that the RAS prevalence was rather low in our study. However, our findings are in line with recent findings that showed a low NS5A RAS prevalence in Germany, ranging from 2% to 7% only.[30] Thus, our findings need to be handled with caution and no final recommendation can be made in respect to RAS testing prior to starting SOF/VEL therapy.

In our study, the use of RBV did not seem to benefit patients with or without compensated cirrhosis. Interestingly, cirrhotic patients who received RBV tended not to have significantly more

severe liver disease compared to those without RBV. The only relapse in our study occurred in a SOF-experienced patient who had cirrhosis but did not receive RBV.

Our cohort contained 9 patients with decompensated cirrhosis (CPT score B), 8 of whom received SOF/VEL plus RBV according to current guideline recommendations,[24,29] and all achieved SVR12. In the ASTRAL-4 study, GT3 patients who did not receive RBV had significantly lower SVR compared to patients with RBV (13/26 [50%] vs 11/13 [85%]).[26] Despite the small number of patients, our data are encouraging and confirm the high efficacy of SOF/VEL + RBV in decompensated cirrhosis.

All 28 patients with HCV/HIV co-infection achieved SVR12. This is in line with recent data from our own group and others showing equally high SVR rates in HCV mono-infected and HCV/HIV co-infected patients, although the number of co-infected patients is clearly underrepresented in the present study.[16,18] The potential for drug-drug interactions between DAAs and ART has raised concerns in the past.[31] It was particularly noted that TDF plasma exposure may increase in combination with NS5A inhibitors.[32] However, we did not observe any significant decrease in estimated glomerular filtration rate in the 7 patients who were treated with TDF-containing ART regimens during therapy with SOF/VEL (data not shown).

Our study is subject to common limitations of real-world studies. Particularly, results of comparative analyses of 2 subgroups, eg, treatment with and without RBV, need to be interpreted with caution because randomised controls are missing in a real-world observational setting. The decision to add RBV to treatment was done at the treating physician's discretion, which poses a risk for selection bias.

In conclusion, we confirm an overall very high efficacy and safety of 12 weeks of SOF/VEL in patients with HCV GT3 infection in a real-world setting. While the prevalence of clinically relevant NS5A RASs was low, our data indicate that their impact may be of less importance than previously expected. Thus, addition of RBV may only be required in certain subgroups, including patients with previous DAA-experience and/or decompensated cirrhosis.

ACKNOWLEDGEMENTS

We thank Florian Berger for assistance in statistical analysis, and Axel Adam, Thomas Buhk, Felix Piecha, Michael Sabranski, Guenther Schmutz and Hans-Juergen Stellbrink for their help in data acquisition and/or patient management.

Declaration of personal interests: Dr. Vermehren reports personal fees from Abbott, AbbVie, Bristol Myers-Squibb, Gilead, Medtronic, Merck/MSD and Roche. Dr. Ingiliz reports personal fees from Gilead, ViiV, Abbvie, BMS, MSD and Janssen. Dr. Mauss reports personal fees from AbbVie, Gilead, Janssen, and Merck/MSD. Dr. Baumgarten reports personal fees from Abbvie, BMS, Gilead, Janssen-Cilag, MSD and ViiV. Dr. Simon reports personal fees from AbbVie, BMS, Gilead, Janssen, Merck/MSD, Merz and Norgine. Dr. Schewe reports personal fees from Abbvie, BMS, Gilead, Hexal, Janssen-Cilag, MSD and ViiV. Dr. Luebke reports personal fees from ViiV. Dr. Schulze zur Wiesch reports personal fees from AbbVie, Gilead and Merck/MSD. Dr. Sarrazin reports personal fees from AbbVie, Abbott, BMS, Gilead, Janssen, Merck, and grants from Abbott, Gilead, Janssen and Siemens. Dr. Christensen reports personal fees from AbbVie, Bristol Myers-Squibb, Gilead, Indivior, Janssen, Merck/MSD and ViiV. The remaining authors have nothing to disclose regarding this manuscript.

AUTHORSHIP

Guarantor of the article: Johann von Felden.

Author contributions: JvF, JV, PI, SM, JSzW, SC: concept and design. JvF, JV, PI, SM, TL, KGS, HWB, AB, KS, DH, CB, JKR, MD, NL, JT, JSzW, CS, SC: data acquisition. JvF, JV: Data analysis and drafting the manuscript. PI, SM, JSzW, CS, SC: Critical review of the manuscript for intellectual input.

All authors have read and approved the final version of the manuscript.

ORCID

J. von Felden http://orcid.org/0000-0003-2839-5174
J. Vermehren http://orcid.org/0000-0002-3287-0815

REFERENCES

1. *Global Hepatitis Report 2017*. Geneva: World Health Organization; 2017.
2. Polaris Observatory HCVC. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol*. 2017;2:161-176.
3. Goossens N, Negro F. Is genotype 3 of the hepatitis C virus the new villain? *Hepatology*. 2014;59:2403-2412.
4. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA*. 2012;308:2584-2593.
5. Probst A, Dang T, Bochud M, Egger M, Negro F, Bochud PY. Role of hepatitis C virus genotype 3 in liver fibrosis progression–a systematic review and meta-analysis. *J Viral Hepat*. 2011;18:745-759.
6. Leandro G, Mangia A, Hui J, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology*. 2006;130:1636-1642.
7. Andriulli A, Mangia A, Iacobellis A, Ippolito A, Leandro G, Zeuzem S. Meta-analysis: the outcome of anti-viral therapy in HCV genotype 2 and genotype 3 infected patients with chronic hepatitis. *Aliment Pharmacol Ther*. 2008;28:397-404.
8. Schulze Zur Wiesch J, Pudelski N, Hoepner L, et al. "Real-Life" comparison of pegylated-interferon 2a versus 2b combination therapy of chronic hepatitis C virus. *Hepatology*. 2011;53:1405-1406; author reply 7.
9. Berden FA, Aaldering BR, Groenewoud H, IntHout J, Kievit W, Drenth JP. Identification of the best direct-acting antiviral regimen for patients with hepatitis C virus genotype 3 infection: a systematic review and network meta-analysis. *Clin Gastroenterol Hepatol*. 2017;15:349-359.
10. Sarrazin C. The importance of resistance to direct antiviral drugs in HCV infection in clinical practice. *J Hepatol*. 2016;64:486-504.
11. Foster GR, Afdhal N, Roberts SK, et al. Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. *N Engl J Med*. 2015;373:2608-2617.

12. Feld JJ, Jacobson IM, Hezode C, et al. Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infection. *N Engl J Med*. 2015;373:2599-2607.
13. Hezode C, Reau N, Svarovskaia ES, et al. Resistance analysis in patients with genotype 1-6 HCV infection treated with sofosbuvir/velpatasvir in the phase 3 studies. *J Hepatol*. 2017; https://10.1016/j.jhep.2017.11.032. [Epub ahead of print].
14. European Medicines Agency: Epclusa - EMEA/H/C/004210 - II/0012 - EPAR - Product Information, 2016. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004210/WC500211151.pdf. Nov 2017
15. Boesecke C, Ingiliz P, Berger F, et al. Liver cirrhosis as a risk factor for direct-acting antiviral therapy failure in real-life hepatitis C virus/human immunodeficiency virus coinfection. *Open Forum Infect Dis*. 2017;4:ofx158.
16. Ingiliz P, Christensen S, Kimhofer T, et al. Sofosbuvir and ledipasvir for 8 weeks for the treatment of chronic hepatitis C virus (HCV) infection in HCV-monoinfected and HIV-HCV-coinfected individuals: results from the German hepatitis C cohort (GECCO-01). *Clin Infect Dis*. 2016;63:1320-1324.
17. Mauss S, Berger F, Wehmeyer MH, et al. Effect of antiviral therapy for HCV on lipid levels. *Antivir Ther*. 2017;21:81-88.
18. Wehmeyer MH, Ingiliz P, Christensen S, et al. Real-world effectiveness of sofosbuvir-based treatment regimens for chronic hepatitis C genotype 3 infection: results from the multicenter German hepatitis C cohort (GECCO-03). *J Med Virol*. 2018;90:304-312.
19. Dietz J, Susser S, Berkowski C, Perner D, Zeuzem S, Sarrazin C. Consideration of Viral resistance for optimization of direct antiviral therapy of hepatitis C virus genotype 1-infected patients. *PLoS ONE*. 2015;10:e0134395.
20. Lawitz EJ, Dvory-Sobol H, Doehle BP, et al. Clinical resistance to velpatasvir (GS-5816), a novel pan-genotypic inhibitor of the hepatitis C virus NS5A protein. *Antimicrob Agents Chemother*. 2016;60:5368-5378.
21. Feld JJ, Maan R, Zeuzem S, et al. Effectiveness and safety of sofosbuvir-based regimens for chronic HCV genotype 3 infection: results of the HCV-TARGET study. *Clin Infect Dis*. 2016;63:776-783.
22. Cornberg M, Petersen J, Schober A, et al. Real-world use, effectiveness and safety of anti-viral treatment in chronic hepatitis C genotype 3 infection. *Aliment Pharmacol Ther*. 2017;45:688-700.
23. Bourliere M, Gordon SC, Flamm SL, et al. Sofosbuvir, velpatasvir, and voxilaprevir for previously treated HCV infection. *N Engl J Med*. 2017;376:2134-2146.
24. European Association for the Study of the Liver. Electronic address eee. EASL Recommendations on Treatment of Hepatitis C 2016. *J Hepatol*. 2017;66:153-194.
25. Gane EJ, Shiffman ML, Etzkorn K, et al. Sofosbuvir-velpatasvir with ribavirin for 24 weeks in hepatitis C virus patients previously treated with a direct-acting antiviral regimen. *Hepatology*. 2017;66:1083-1089.
26. Curry MP, O'Leary JG, Bzowej N, et al. Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis. *N Engl J Med*. 2015;373:2618-2628.
27. Hernandez D, Zhou N, Ueland J, Monikowski A, McPhee F. Natural prevalence of NS5A polymorphisms in subjects infected with hepatitis C virus genotype 3 and their effects on the antiviral activity of NS5A inhibitors. *J Clin Virol*. 2013;57:13-18.
28. Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*. 2015;61:1127-1135.
29. AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology*. 2015;62:932-954.
30. Dietz J, Susser S, Vermehren J, et al. Patterns of resistance-associated substitutions in patients with chronic hcv infection following treatment with direct-acting antivirals. *Gastroenterology*. 2017; https://10.1053/j.gastro.2017.11.007. [Epub ahead of print].
31. Soriano V, Labarga P, Fernandez-Montero JV, et al. Drug interactions in HIV-infected patients treated for hepatitis C. *Expert Opin Drug Metab Toxicol*. 2017;13:807-816.
32. Mogalian E, Daryani V, Osinusi AO, et al. Pharmacokinetics of sofosbuvir/velpatasvir and tenofovir in subjects with HCV/HIV coinfection using boosted or unboosted antiretroviral regimens. *Hepatology*. 2016;64:419a-420a.

SUPPORTING INFORMATION

Additional Supporting Information will be found online in the supporting information tab for this article.

How to cite this article: von Felden J, Vermehren J, Ingiliz P, et al. High efficacy of sofosbuvir/velpatasvir and impact of baseline resistance-associated substitutions in hepatitis C genotype 3 infection. *Aliment Pharmacol Ther*. 2018;00:1–8. https://doi.org/10.1111/apt.14592

APPENDIX

AUTHORS' COMPLETE AFFILIATIONS

Johann von Felden: I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany and Divisions of Liver Diseases and Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA. Johannes Vermehren: Department of Internal Medicine 1, University Hospital Frankfurt, Frankfurt, Germany. Patrick Ingiliz and Axel Baumgarten: Center for Infectiology Berlin (CIB), Berlin, Germany. Stefan Mauss: Center for HIV and Hepatogastroenterology, Duesseldorf, Germany. Thomas Lutz: Infektiologikum, Frankfurt, Germany. Karl G. Simon: Practice for Gastroenterology Leverkusen, Leverkusen, Germany. Heiner W. Busch and Stefan Christensen: CIM Infectious diseases, Muenster Germany. Knud Schewe: Infektionsmedizinisches Centrum Hamburg (ICH), Hamburg, Germany. Dietrich Hueppe: Practice for Gastroenterology Herne, Herne, Germany. Christoph Boesecke and Juergen K. Rockstroh: I. Department of Medicine, University Hospital Bonn, Bonn, Germany. Martin Daeumer: Institute of Immunology and Genetics, Kaiserslautern, Germany. Nadine Luebke and Joerg Timm: Institute of Virology, Heinrich-Heine-University, University Hospital Duesseldorf, Duesseldorf, Germany. Julian Schulze zur Wiesch: I. Department of Medicine, University Medical Center Hamburg-Eppendorf and German Center for Infection Research (DZIF), Hamburg, Germany. Christoph Sarrazin: Department of Internal Medicine 1, University Hospital Frankfurt and St. Josef's Hospital, Wiesbaden, Germany