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Durability of SVR in chronic hepatitis C patients treated with peginterferon-α2a/ribavirin in combination with a direct-acting anti-viral

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SUMMARY

Background
The introduction of direct-acting anti-virals has increased sustained virological response (SVR) rates in chronic hepatitis C genotype 1 infection. At present, data on long-term durability of viral eradication after successful triple therapy are lacking.

Aim
To evaluate the long-term durability of viral eradication in patients treated with triple therapy, including direct-acting anti-virals.

Methods
Patients who participated in randomised, controlled trials or an extended access programme of treatment with peginterferon-α2a/ribavirin in combination with a direct-acting anti-viral (telaprevir, danoprevir, faldaprevir, simeprevir, mericitabine, balapiravir) were followed after achieving SVR. The median follow-up after the patients was 21 (range: 7–64) months.

Results
One hundred and three patients with chronic hepatitis C genotype 1 infection [f/m: 34/69; GT-1b: 67 GT-1a: 34, GT-4: 2; mean age: 47.6 years (45.5–49.7; 95% CI)] achieving a SVR triple therapy were followed. Two cases of late relapses (2/103, 1.9%; 95% CI: 0.24–6.8) were observed. One patient was cirrhotic, both carried the genotype 1b and completed the prescribed treatment. The relapses occurred 8 and 12 months after cessation of anti-viral treatment. Cloning sequencing revealed identical sequence in both patients. Resistance analysis revealed no presence of viral resistance.

Conclusion
Like the SVR after peginterferon-α2/ribavirin combination treatment, HCV eradication after triple therapy remains durable after long-term follow-up.

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INTRODUCTION

Eradication of HCV is the ultimate goal of anti-viral therapy. The commonly used parameter for viral eradication is a sustained virological response defined by undetectable HCV-RNA 6 months after end of successful therapy. Sustained virological response is commonly considered as parameter for cure of chronic hepatitis C. Long-term follow-up studies in patients treated by peginterferon/ribavirin have shown that HCV eradication is durable with follow-up periods of more than 20 years.[1–5] Achieving a SVR prevents progression to end stage liver disease and occurrence of hepatocellular carcinoma.[6–8] Moreover, successful treatment of HCV is associated with reduced hepatic morbidity, liver related deaths and improvement of health-related quality of life.[7–11] Recent reports even documented a reversal of cirrhosis many years after achieving sustained virological response (SVR).[12–14]

Currently we are witnessing the development of potent anti-viral drugs for treatment of chronic hepatitis C. The first two licensed protease inhibitors almost doubled the rate of SVR both in treatment-naïve[15, 16] and treatment-experienced patients.[17, 18] It is unknown whether the encouraging long-term data of dual combination therapy can be extrapolated to triple therapy with direct-acting anti-virals. So far, no data on the long-term outcome of patients with SVR following triple therapy have been reported. Aim of our study was to analyse virological follow-up of a large cohort of SVR patients participating in phase 2 or 3 trials or an extended access programme treated with peginterferon/ribavirin in combination with different direct-acting anti-virals.

PATIENTS AND METHODS

Patients

Patients participated in prospective randomised trials of treatment with pegylated interferon-alfa, ribavirin (PEG-IFN/RBV) with a direct-acting anti-viral[15, 18–20] or in a named patient early access programme for telaprevir and were routinely invited for follow-up examinations.[3] Patients were selected according to the inclusion and exclusion criteria of the respective trial. All patients were Caucasians. Patients receiving placebo or no ribavirin were excluded from this analysis. A total of 103 out of 113 patients (91.2%) with SVR after DAA containing therapy attended follow-up visits at the out-patient clinics of the 4 participating tertiary referral centres. SVR was achieved in 43 patients receiving telaprevir, 30 on faldaprevir,[21, 22] 20 on simeprevir[23], 3 on danoprevir, 5 on ritonavir boosted danoprevir, 5 on mericitabine[24], 3 on ritonavir boosted danoprevir and mericitabine and 2 on balapiravir.[25] For details see Table 1.

Routine laboratory parameters like alanine aminotransferase (ALT) and alpha-fetoprotein (AFP) were determined at every follow-up visit. EDTA blood was used for genetic testing. The SNPs for IL28B rs 12979860 were analysed by the StepOnePlus Real time PCR System (Applied Biosystems, Foster City, CA, USA) as described previously.[26] Baseline data are summarised in Table 1.

Virological tests. HCV was quantified during the studies in central laboratories using different PCR assays described in subscript Table 1. During follow-up, viral load of HCV was quantified by real-time PCR (COBAS TaqMan HCV Test Version 2; Roche Diagnostics, Pleasanton, CA, USA) in all patients. Genotyping of HCV was determined by Versant genotype assay (LiPA 2.0; Bayer HealthCare LLC, Subsidiary of Bayer Corporation, Tarrytown, NY, USA). Sequencing of HCV-RNA was performed for one patient by Böhringer-Ingelheim Canada (by Marquis Martin) using the ABI 3730 Genetic Analyzer (Applied Biosystems) detection system. The second sequencing of HCV-RNA was performed by the department of virology (HH) using the ABI 3130XL Genetic Analyzer (Applied Biosystems).

Clinical follow-up. All patients were asked to return to follow-up visits after 6 months and then at least once a year. Out of 113 patients, 103 patients returned to follow-up visits. At the clinical visits, routine blood tests, including alfa-1-fetoprotein and quantitative HCV-RNA determination (by TaqMan HCV Test), were obtained. Patients with pre-treatment cirrhosis were asked to perform a liver ultrasound examination prior to the visit. If necessary [in case of hepatic decompensation or suspicion for hepatocellular carcinoma (HCC)], further examinations were performed as needed. The institutional review board approved this retrospective non-interventional analysis.

Statistical analysis

Database management and statistical analysis were performed using commercially available software systems (Microsoft Office Excel 2010; Microsoft Corporation, USA and SPSS 2006 for Windows version 16). Quantitative variables were expressed as mean with the 95% confidence interval or as median (range). Data were analysed by using Student's *t*-test for Gaussian variables, Mann–Whitney *U*-test for non-Gaussian variables as well

Table 1 | Patients with SVR in triple therapy studies and long-term follow-up

Drug	Study	No. recruited*	All with SVR 24; *n* (%)	No. with SVR and FU†
Telaprevir	Prove 2,[19]¶ C208,[20]‡ ADVANCE,[15]‡ REALIZE,[18]‡ EAP	53	43 (81.1)	40
Simeprevir	ASPIRE (NCT00980330),‡ PILLAR (NCT00882908),‡	26	20 (77.0)	19
Faldaprevir	SILEN 1,[25]‡ SILEN 3 (NCT00984620)‡	39	30 (76.9)	26
Danoprevir	ATLAS (NCT00869661)§	3	3	3
Ritonavir boosted Danoprevir	DAUPHINE (NCT01220947)‡	10	5	5
Mericitabine	PROPEL,[21]§	8	5	5
Ritonavir boosted Danoprevir + Mericitabine	MATTERHORN (NCT01331850)‡	9	5	3
Balapiravir [22]§		11	2	2

EAP, extended access programme.

* Only patients receiving PEGIFN/RBV plus a direct-acting anti-viral.

† Patients completed treatment and long-term follow-up. Virological test used in the central laboratories of the studies.

‡ Roche COBAS Taqman HCV/HPS assay: limit of quantification <25 IU/mL; limit of detection 10 IU/mL.

§ Roche COBAS Ampliprep/COBAS, limit of detection 15 IU/mL.

¶ Roche COBAS Taqman version 1.0, limit of quantification 30 IU/mL; lower limit of detection 10 IU/mL.

as Chi-squared test. To determine, whether variables were normally distributed or not, the Kolmogorow–Smirnow test was applied. All *P*-values were two-tailed and assumed to be statistically significant if $P \leq 0.05$.

RESULTS

Characteristics of patients

A total of one hundred and three patients (f/m = 34/69, age: 47.6 ± 8.7; years ± s.d.) with a sustained virological response (SVR) were evaluated. Patient's characteristics are shown in Table 2. A total of 23 patients (17 Non-responder/6 Relapser) were treatment experienced, while 80 were treatment naive.

One hundred and one patients were chronically infected with HCV-genotype 1 (HCV-1a: 34; HCV-1b: 67), two patients with HCV-genotype 4 (both received mericitabine). Ninety-three patients with HCV-genotype 1 received a protease inhibitor, seven patients a NS5B inhibitor (balapiravir or mericitabine) and three patients a combination of protease and NS5B inhibitor together with pegylated interferon-alfa and ribavirin at the four Austrian study sites (for details see Table 1).

Two relapses (2/103, 1.9%) occurred during follow-up after achieving a sustained virological response: One late relapse was seen in a 51-year-old female noncirrhotic patient (Fibroscan: 5.8 ± 0.5 kPa) infected with genotype 1b. She was a carrier of the IL-28B rs12979860 T/C genotype. She was treatment naïve and received triple

Table 2 | Patient characteristics

	n (%)
n (f/m)	103 (34/69)
Age (mean ± s.d.)	47.6 ± 8.7
BMI (kg/m^2)	24.7 + 3.6
HCV-Genotype	
HCV-1a	34 (33.0)
HCV-1b	67 (65.1)
HCV-4	2 (1.9)
Cirrhosis (liver biopsy available in 98*)	18 (18.4)
Follow-up [mo, median(range)]‡	21 (7–64)
IL28 rs12979860 available in 79†	
CC	23 (29.1%)
C/T	44 (55.7%)
T/T	10 (12.7%)
Treatment	
Protease inhibitor	93 (90.3)
NS5B Polymerase inhibitor	7 (6.8)
Both	3 (2.9)

* In five patients fibrosis grade was assessed by Fibroscan, none of them had F4.

† IL28 rs12979860 was not available in all patients.

‡ Follow-up [months, median (range)] after achieving SVR (=24 weeks after end of treatment).

therapy (3 days lead in with peginterferon/ribavirin followed by peginterferon/ribavirin/faldaprevir 120 mg daily) for 12 weeks and peginterferon/ribavirin for a total of 24 weeks. HCV-RNA became undetectable at week 4 and remained undetectable throughout the whole therapy. She was HCV-RNA negative after 24 weeks of follow-up (SVR24) and had aminotransferases in normal range. Two months later, she came to the out-patient unit because of abdominal pain. Laboratory test showed detectable HCV-RNA (=8 months after end of treatment; viral load: 1.33 E5 IU/mL). The positive HCH RNA was confirmed several times.

Cloning sequencing in this patient showed the same sequence in the sample taken after relapse as in the sample obtained at screening. Resistance analysis revealed no presence of viral resistance. She received a retreatment with pegIFN/RBV and telaprevir for a total of 24 weeks and was HCV-RNA undetectable at end of treatment. She is currently on treatment-free follow-up.

The second late relapse occurred in a 59-year-old treatment-naïve, cirrhotic, male patient, who was chronically infected by HCV-genotype 1b and carrier of IL-28B genotype rs12979860 T/C. He received triple therapy (peginterferon/ribavirin/faldaprevir 120 mg daily for 24 weeks and peginterferon/ribavirin for a total of 48 weeks). HCV-RNA became undetectable at week 4 and remained undetectable throughout the whole therapy. After 24 weeks of follow-up, HCV-RNA was not detected (SVR24) and aminotransferases were normal. At a routine control examination 12 months after end of treatment, increased ALT was detected. HCV quantification revealed a viral load of 288 IU/mL. The genotype of the pre-treatment and the postrelapse sample was HCV-1b and cloning sequencing revealed identical sequence in both samples. Resistance analysis revealed no presence of viral resistance. No signs of hepatic decompensation or HCC occurrence were observed.

In both patients, HCV-RNA increased to pre-treatment levels within the next months. Of the remaining 101 patients, 90 (89.1%) had aminotransferases within the normal range at the last follow-up. AFP levels were available in 60 patients and were within the normal range in 54/60 (90%) patients. In the patients with slight increase AFP levels abdominal ultrasound revealed no signs of hepatic mass. No patient took immunosuppressive medication in the posttreatment follow-up period.

DISCUSSION

This study confirms that SVR equals permanent HCV eradication by whatever interferon-based anti-viral treatment it was achieved. Our data indicate that the favourable long-term outcome reported after peginterferon/ribavirin combination therapy seems to hold true for patients treated with a triple therapy with peginterferon/ribavirin in combination with a direct anti-viral agent. To the best of our knowledge, this is the first study reporting long-term virological outcomes in patients with hepatitis C after successful anti-viral triple therapy.

There are theoretical concerns regarding the durability of HCV eradication after successful direct-acting anti-viral-based triple therapy. During direct-acting anti-viral treatment resistance-associated variants with reduced replication fitness compared with the wild type virus may emerge. If these resistance-associated variants cannot be eliminated by the required peginterferon/ribavirin backbone, strains with reduced replication fitness may persist in low concentration and may account for late relapses.

In the ADVANCE study, 1 of 357 patients evaluated had a confirmed late relapse after early discontinuation of treatment (randomised to the arm 8 weeks of telaprevir followed by dual therapy) at week 12.[15] In the PROVE 2 study, 2 late relapses were observed 36 and 48 weeks after the end of treatment.[19] In a phase 2 trial of an interferon-free regime (ritonavir boosted protease inhibitor ABT-450 combined with the nonnucleoside inhibitor of HCV NS5B polymerase ABT-072), one patient had a late relapse 36 weeks after end of treatment.[27] Thus data on longer follow-up are of major importance in the era of direct-acting anti-virals in HCV treatment.

In our study, two patients with a sustained virological response (SVR24) experienced a relapse 2 and 6 months later. Both patients completed a full course of anti-viral treatment without dose modifications or discontinuations of medications with peginterferon/ribavirin and faldaprevir. The serum samples of both patients (taken at baseline and after relapse) revealed no viral resistance and showed viral homology to samples collected at screening. Neither of the two patients showed a risk behaviour regarding HCV infection before reappearance of HCV. Thus, in both patients a late relapse rather than a newly acquired infection seems to be the reason for reappearance of hepatitis C virus. Obviously, an ongoing occult HCV infection cannot be excluded with certainty.[28]

Overall, our data show that it seems appropriate to extrapolate the encouraging long- term data of dual combination therapy to triple therapy with direct-acting anti-virals. The late relapse rate was 1.9% (95% CI: 0.24–6.8) as compared to 0.18% (0.004–1.01) in a much larger cohort of patients with SVR after dual therapy.[29] As all patients treated with direct-acting anti-virals in combination with

PEGIFN/RBV achieving similar rates of SVR were followed prospectively, a potential selection bias is unlikely. However, in parallel to reported late relapses after successful dual therapy), late relapses after triple therapy – although a rare event – seem to occur within the first months after SVR like in the cases in our cohort. The fact that both relapses were observed in patients receiving faldaprevir occurred possibly just by chance, as the mode of action and the efficacy of the protease inhibitors are similar. Nevertheless, it seems advisable to confirm a successful HCV eradication within the first year of follow-up after achieving a sustained virological response. From our data, no impact on the durability of SVR after interferon-free treatments can be inferred.

In conclusion, our study shows that HCV eradication by triple therapy remains durable and confirms an excellent long-term prognosis of HCV patients with SVR. To assess the long-term clinical benefit of triple therapy, studies with a longer follow-up and larger patient numbers are needed.

AUTHORSHIP

Guarantor of the article: Peter Ferenci.

Author contributions: Karoline Rutter: data collection, data analysis, writing of the manuscript. Albert Friedrich Stättermayer, Sandra Beinhardt, Thomas-Matthias Scherzer, Melisa Dulic, Michael Gschwantler, Andreas Maieron, Hermann Laferl, Petra Steindl-Munda: acquisition of data, critical revision of the manuscript for important intellectual content. Robert Strassl and Heidemarie Holzmann performed the virological assays. Peter Ferenci and Harald Hofer: study concept and design, analysis and interpretation of data, outlining and revising the manuscript. All authors approved the final version of the article.

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REFERENCES

1. Marcellin P, Boyer N, Gervais A, *et al.* Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997; **127**: 875–81.
2. Reichard O, Glaumann H, Fryden A, Norkrans G, Wejstal R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. *J Hepatol* 1999; **30**: 783–7.
3. Formann E, Steindl-Munda P, Hofer H, *et al.* Long-term follow-up of chronic hepatitis C patients with sustained virological response to various forms of interferon-based anti-viral therapy. *Aliment Pharmacol Ther* 2006; **23**: 507–11.
4. Swain MG, Lai MY, Shiffman ML, *et al.* A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. *Gastroenterology* 2010; **139**: 1593–601.
5. Veldt BJ, Heathcote EJ, Wedemeyer H, *et al.* Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677–84.
6. 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**: 2584–93.
7. Bruno S, Stroffolini T, Colombo M, *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45**: 579–87.
8. Singal AG, Volk ML, Jensen D, Di Bisceglie AM, Schoenfeld PS. A sustained viral response is associated with reduced liver-related morbidity and mortality in patients with hepatitis C virus. *Clin Gastroenterol Hepatol* 2010; **8**: 280–8, 288 e1.
9. Yoshida H, Arakawa Y, Sata M, *et al.* Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology* 2002; **123**: 483–91.
10. Innes HA, Hutchinson SJ, Allen S, *et al.* Excess liver-related morbidity of chronic hepatitis C patients, who achieve a sustained viral response, and are discharged from care. *Hepatology* 2011; **54**: 1547–58.
11. Bonkovsky HL, Woolley JM. Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy. The Consensus Interferon Study Group. *Hepatology* 1999; **29**: 264–70.
12. D'Ambrosio R, Aghemo A, Rumi MG, *et al.* A morphometric and immunohistochemical study to assess the benefit of a sustained virological

response in hepatitis C virus patients with cirrhosis. *Hepatology* 2012; **56**: 532–43.
13. Poynard T, McHutchison J, Manns M, *et al.* Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303–13.
14. Shiratori Y, Imazeki F, Moriyama M, *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517–24.
15. Jacobson IM, McHutchison JG, Dusheiko G, *et al.* Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405–16.
16. Poordad F, McCone J Jr, Bacon BR, *et al.* Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195–206.
17. Lee SS, Roberts SK, Berak H, *et al.* Safety of peginterferon alfa-2a plus ribavirin in a large multinational cohort of chronic hepatitis C patients. *Liver Int* 2012; **32**: 1270–7.
18. Zeuzem S, Andreone P, Pol S, *et al.* Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417–28.
19. Hezode C, Forestier N, Dusheiko G, *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839–50.
20. Marcellin P, Forns X, Goeser T, *et al.* Telaprevir is effective given every 8 or 12 hours with ribavirin and peginterferon alfa-2a or -2b to patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 459–68.
21. Sulkowski M AT, Lalezari J, Ferenci P, Fainboim H, Muñizõ FJ, Leggett B. Faldaprevir combined with peginterferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype-1 HCV: SILEN-C1 Trial. *Hepatology* 2013; doi: 10.1002/hep.26276.
22. Ferenci P, Asselah T, Foster GR, Zeuzem S, Sarrazin C. Faldaprevir plus pegylated interferon alfa-2a and ribavirin in chronic HCV genotype-1 treatment-naïve patients: final results from startverso1, a randomised, double-blind, placebo-controlled phase III trial. *J Hepatol* 2013; **58**(Suppl. 1): S569–70.
23. Manns M, Marcellin P, Poordad F, Affonso de Araujo S, Buti M, Horsmans Y. Simeprevir (TMC435) with peginterferon/ribavirin for treatment of chronic HCV genotype-1 infection in treatment-naïve patients: results from QUEST-2, a phase III trial. *J Hepatol* 2013; **58**(Suppl. 1): S568.
24. Wedemeyer H, Jensen D, Herring R Jr, *et al.* PROPEL: a randomized trial of mericitabine plus peginterferon alfa-2a/ribavirin therapy in treatment-naive HCV genotype 1/4 patients. *Hepatology* 2013; doi: 10.1002/hep.26274.
25. Nelson DR, Zeuzem S, Andreone P, *et al.* Balapiravir plus peginterferon alfa-2a (40KD)/ribavirin in a randomized trial of hepatitis C genotype 1 patients. *Ann Hepatol* 2012; **11**: 15–31.
26. Stättermayer AF, Stauber R, Hofer H, *et al.* Impact of IL28B genotype on the early and sustained virologic response in treatment-naive patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2011; **9**: 344–50 e2.
27. Lawitz E PF, Kowdley KV, Jensen D, Cohen DE, Siggelkow S. A 12-week interferon-free regimen of ABT-450/r, ABT-072, and ribavirin was well tolerated and achieved sustained virologic response in 91% treatment-naïve HCV IL28B-CC genotype-1-infected subjects. *J Hepatol* 2012; **56** (Suppl. 2): S7.
28. De Marco L, Manzini P, Trevisan M, *et al.* Prevalence and follow-up of occult HCV infection in an Italian population free of clinically detectable infectious liver disease. *PLoS ONE* 2012; **7**: e43541.
29. Rutter K, Stättermayer AF, Ferenci P, *et al.* Successful HCV eradication due to antiviral therapy is associated with improved long term outcome of patients with chronic hepatitis C. *J Hepatol* 2013; **58**(Suppl. 1): S369.