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Original article

Intravenous silibinin as 'rescue treatment' for on-treatment non-responders to pegylated interferon/ribavirin combination therapy

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Background: Intravenous silibinin (ivSIL) is a potent antiviral agent against HCV. *In vitro* silibinin (SIL) inhibits viral replication, possibly by inhibiting HCV RNA polymerase. In this proof-of-concept study, ivSIL was tested in on-treatment non-responders to full-dose of pegylated interferon-α2a/ribavirin (standard of care [SOC]).

Methods: A total of 27 treatment-naive patients with <2 log drop in viral load after 12 weeks or still detectable HCV RNA after 24 weeks of SOC treatment (mean age 54.4 ±6.8 years, male/female 19/8, HCV genotype 1 *n*=19, 3a *n*=4 and 4 *n*=4, liver fibrosis F4 *n*=14, F3 *n*=5 and F2 *n*=3, and interleukin 28B polymorphism C/C *n*=1, T/C *n*=22 and T/T *n*=4) received additionally 20 mg/kg/day SIL (Legalon-SIL®; Rottapharm-Madaus, Monza, Italy) intravenously for 14 or 21 days. Thereafter, pegylated interferon/ribavirin was continued. HCV RNA was quantified by TaqMan® (Roche Diagnostics, Pleasanton, CA, USA).

Results: At the end of ivSIL treatment, 23/27 (85.1%) patients had undetectable HCV RNA. In one of the four remaining patients HCV RNA became undetectable 8 weeks after ivSIL on SOC. Five patients relapsed after ivSIL, three of them responded to repeated administration of ivSIL, but relapsed again. The best predictor of response was a low pre-ivSIL HCV RNA level. A total of 19 patients reached one treatment end point (end of SOC treatment HCV RNA undetectable *n*=11 and non-response *n*=8); 8 patients were still on SOC (all HCV-RNA-negative). All 11 patients with end-of-treatment response completed 24 weeks of follow-up; 7 patients remained HCV-RNA-negative and 4 relapsed. Except for a slight increase in bilirubin (mean ±SD 0.98 ±0.35 to 2.12 ±0.99 mg/dl), treatment was well-tolerated.

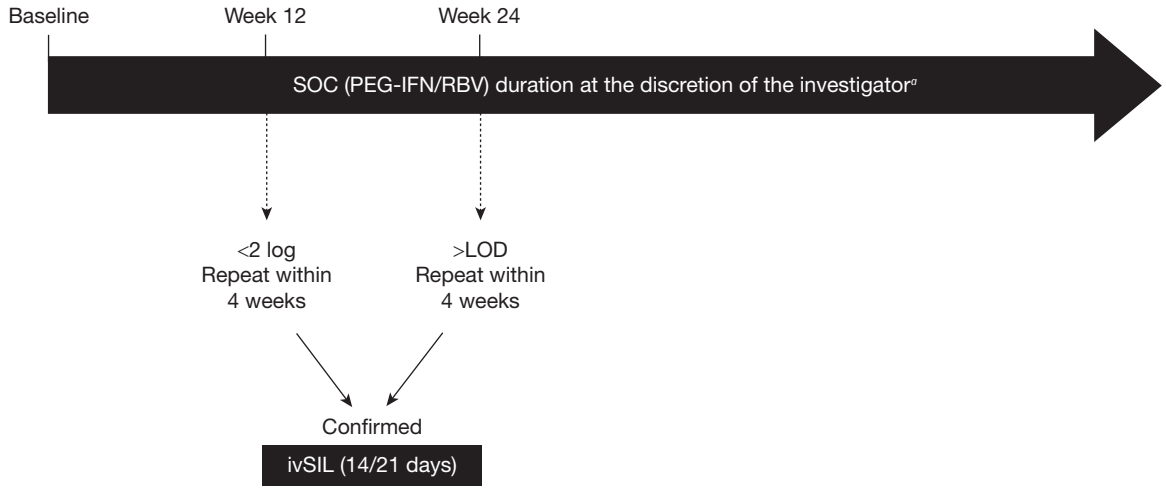
Conclusions: ivSIL is an effective 'rescue treatment' for on-treatment non-responders to full-dose of SOC.

Introduction

The current standard of care (SOC) for chronic hepatitis C is the combination of pegylated interferon-α and weight-based ribavirin. About one-half of the patients infected with genotypes 1 or 4 achieve a sustained virological response (SVR) [1,2]. For non-responders, treatment options are limited at present [3–5]. Persistence of HCV may lead to progression of liver fibrosis, cirrhosis and hepatocellular carcinoma [6]. Thus, there is an unmet medical need for new treatments strategies in this patient group. Currently, several direct acting antivirals (DAAs) are under clinical development [7–9].

Approximately 3 years ago, we described the potent antiviral activity of intravenous silibinin (ivSIL) against HCV [10]. The observation was confirmed also by others [11]. Silibinin (SIL) is derived from silymarin, an extract from the seeds of the milk thistle (*Silybum marianum Gaertneri*) [12]. ivSIL (Legalon SIL®, Rottapharm-Madaus, Monza, Italy) is a 1:1 mixture of SIL A and SIL B, and is available as an intravenous therapeutic agent for treatment of mushroom poisoning. The antiviral effects of SIL were further reconfirmed by *in vitro* studies [13,14]. SIL inhibits the HCV NS5B polymerase activity directly [15] or by interfering with the binding of RNA to this enzyme [16]. Furthermore, the mechanisms of the antiviral action of SIL appear also to include blocking of virus entry, transmission and secretion [17]. In addition, SIL has strong antioxidative [18] and antifibrotic [19,20] properties. The suppression of virus was independent to the inhibition of oxidative stress [21]. Oral silymarin may improve the long-term

Figure 1. Study protocol



[a]Minimum 36 weeks if HCV RNA does not become negative. ivSIL, intravenous silibinin; PEG-IFN, pegylated interferon; LOD, limit of detection; SOC, standard of care; RBV, ribavirin.

outcome of patients with cirrhosis [22], but the blood concentrations after oral administration of silymarin are too low to exert its antiviral effects [23]. Therefore the drug has to be given intravenously.

Based on studies on viral kinetics, stopping rules and treatment individualization were developed for pegylated interferon/ribavirin therapy [24]. Treatment should be discontinued in patients with a viral decline of <2 log after 12 weeks of SOC therapy or with detectable HCV RNA after 24 weeks of therapy. These patients are unlikely to benefit from extended SOC therapy. Before discontinuing treatment in such patients we investigated whether ivSIL could induce a viral response, allow continuation of therapy ('rescue therapy') and enable the patient to achieve an SVR.

Methods

Patients

All patients were treatment-naive. Standard inclusion and exclusion criteria for pegylated interferon and ribavirin therapy were applied. All patients received 180 μg/week pegylated interferon-α2a (Pegasys®; Roche, Basel CH, Switzerland) and 1–1.2 g/day ribavirin (Copegus®; Roche) for ≥14 weeks. Non-response to SOC was defined by the lack of a >2 log drop of viral load after 12 weeks of therapy and/or by not achieving undetectable HCV RNA at week 24 of full-dose of pegylated interferon-α2a and weight-based ribavirin combination therapy.

Study protocol

A total of 27 on-treatment non-responders were included and received 20 mg/kg SIL (Legalon SIL®; Rottapharm-Madaus) for either 14 days (n=12) or 21 days (n=15) as shown in Figure 1. SIL was infused every day for ≥1–4 h. Blood was collected predose at day 1, 8, 15, 22 and at week 5, and thereafter every 4 weeks. Duration of SOC treatment after SIL infusion therapy depended on treatment response. Patients with a treatment response (HCV RNA undetectable) continued SOC therapy for a minimum of 24 weeks. In case of breakthrough after infusion therapy the patient had the option to stop or to receive a retreatment with SIL. After the end of treatment, patients were followed for 24 weeks without treatment. Details of the study were explained to the patients and all patients signed an informed consent. The protocol (NCT00684268) was approved by the Ethics Committee of the Medical University of Vienna (Vienna, Austria).

Laboratory methods

Serum HCV RNA level was determined by the TaqMan PCR assay (Cobas Ampliprep/Cobas TaqMan HCV Test; Roche Diagnostics, Pleasanton, CA, USA; limit of detection [LOD] 10 IU/ml; limit of quantification [LOQ] 15 IU/ml). The predictive marker rs12979860 in the region of the IL-28B gene was investigated as described before [25] by the StepOnePlus Real time PCR System (Applied Biosystems, Foster City, CA, USA) using published primers. Transient elastography

(Fibroscan®) was performed in five patients as described by Castéra *et al*. [26].

Statistics

This was a proof-of-concept study. The primary study end point was the HCV RNA response at end of SIL administration; secondary end points were tolerability, safety and SVR. Data analysis was performed with commercially available software (SPSS version 14.0; SPSS Inc., Chicago, IL, USA). Comparison between means was done with Student's *t*-test.

Results

Demographics

The demographics of the 27 on-treatment non-responders to pegylated interferon-α2a/ribavirin combination therapy investigated are shown in Table 1.

The mean ±SD duration of SOC before start of SIL was 30.4 ±7.8 weeks. The mean ±SD log drop on SOC was 1.31 ±0.5 after 4 weeks, 2.97 ±1.1 after 12 weeks and 2.58 ±1.4 after 24 weeks of standard therapy. Five patients were null responders (<2 log decline in HCV RNA level in the first 12 weeks of treatment) and 22 patients had a partial response (≥2 log decline of HCR RNA in 12 weeks but not negative at week 24 after treatment initiation) on standard therapy. The median viral load at the beginning of SIL was 19 IU/ml (range 15–8.0×10^5 IU/ml). A total of 13 patients started ivSIL therapy with detectable but unquantifiable viral load (<15 IU/ml).

Viral kinetics on silibinin

After 7 days of ivSIL, 16 (59.3%) patients had undetectable HCV RNA. After 14 days of ivSIL these 16 patients and 7 additional patients showed undetectable HCV RNA. After 21 days of treatment, 23 patients (85.1%) were HCV-RNA-negative (14 of 15 patients of the group with 21 days and 9 of 12 patients of the group with 14 days of SIL treatment. After the end of SIL infusions HCV RNA became detectable again in five patients (three patients with 14 days ivSIL treatment, two patients with 21 days ivSIL treatment); all of them had a high viral load at the beginning of ivSIL treatment (mean ±SD 3.2 ±3.3 MIU/ml). Three of these patients received a second and third course of ivSIL and all of them became HCV-RNA-negative again. One patient became HCV-RNA-negative 8 weeks after ivSIL on SOC.

End-of-treatment response

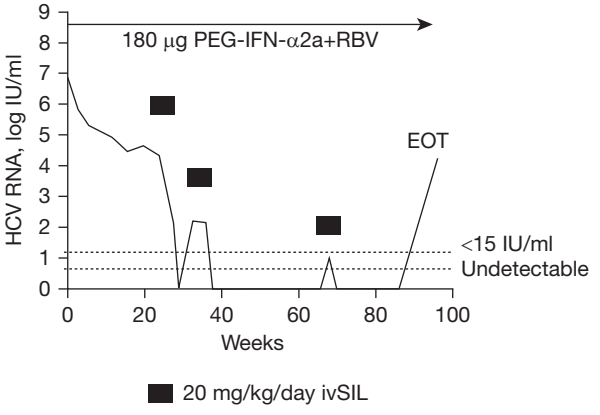
In 19 patients, end of SOC treatment data are available. At the end of SOC treatment, 11 out of 19 patients (57.9%) were HCV-RNA-negative and 8 patients were HCV-RNA-positive. Three patients received a retreatment with SIL (one patient three times), due to virological breakthrough. Figure 2 shows the course of one patient who achieved a complete suppression of viral replication after every course of SIL therapy but had a repeated virological breakthrough.

Table 1. Demographics

Characteristic	Value
Patients, *n*	27
Male/female patients, *n*	19/8
Mean age, years (±SD)	54.4 (6.8)
HCV genotype	
1, *n*	19
3, *n*	4
4, *n*	4
Metavir fibrosis score[a]	
F4, *n* (%)	14 (51.9)
F3, *n* (%)	5 (18.5)
F2, *n* (%)	3 (11.1)
IL-28B SNP rs1297986	
T/C, *n*	22
T/T, *n*	4
C/C, *n*	1
Mean baseline viral load, IU/ml (±SD)[b]	3.9×10^6 (2.7×10^6)
Median viral load at ivSIL start, IU/ml (range)	19 (15–8.0×10^5)
Mean baseline ALT level, U/l (±SD)[b]	77.4 (24.5)
Mean ALT at ivSIL start, U/l (±SD)	50.7 (28.6)
Mean body mass index, kg/m² (±SD)	24.2 (2.9)

[a]Transient elastography instead of liver biopsy was done in five patients (9.0 ±2.8, 7.7 ±0.9, 12.9 ±4.8, 3.8 ±0.6 and 11.6 ±0.8 kPa). [b]Baseline represents the start of standard of care therapy. ALT, alanine aminotransferase; IL, interleukin; ivSIL, intravenous silibinin.

Figure 2. Viral kinetics in patient number 9



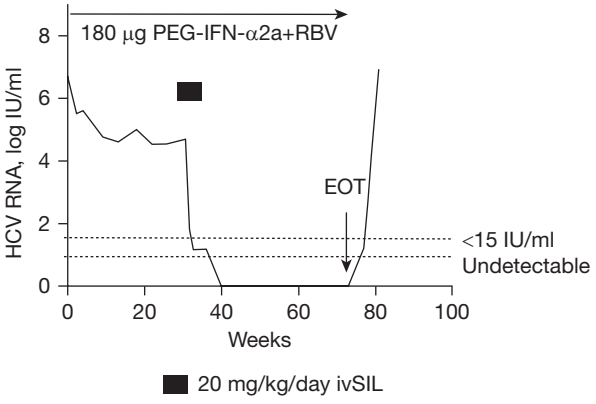
Results of a 56-year-old male patient with advanced cirrhosis (genotype 1b). After non-response to standard of care, he received 3×20 mg/kg/day intravenous silibinin (ivSIL) for 21 days. Standard of care was stopped 24 weeks after the last course of ivSIL. EOT, end of treatment; PEG-IFN, pegylated interferon; RBV, ribavirin.

Table 2. Patients with end of follow-up

Patient	Age, years	Sex	GT	IL-28	Fibrosis[a]	ivSIL, days	HCV RNA, IU/ml							Outcome
							Baseline	Week 12[b]	Day 0[c]	Day 7	Day 14	Day 21	EOT	
2	62	M	1	T/C	4	14	8.39×10^6	615	15	Neg	Neg	Neg	Neg	SVR
3	54	M	4	T/C	12.9 ±4.8[d]	14	3.43×10^6	455	2.40×10^4	15	Neg	Neg	Neg	SVR
4	50	F	1	T/T	4	14	7.66×10^6	4.94×10^6	15	15	Neg	Neg	Neg	SVR
8	51	F	1	T/C	3	14	2.65×10^6	1.09×10^3	15	Neg	Neg	Neg	Neg	SVR
11	42	M	1	T/C	7.7 ±0.9[d]	14	6.73×10^5	15	15	Neg	Neg	Neg	Neg	SVR
17	62	M	1	T/C	3.8 ±0.6[d]	21	7.10×10^6	1.02×10^3	15	Neg	Neg	Neg	Neg	SVR
24	40	M	1	T/C	2	14	6.38×10^6	2.01×10^3	15	Neg	Neg	Neg	Neg	SVR
1	53	M	3	T/T	4	14	7.52×10^3	2.30×10^3	19	Neg	Neg	Neg	Neg	REL
7	47	F	1	T/C	4	14	4.76×10^6	1.02×10^3	4.96×10^4	52	30	15	Neg	REL
15	48	M	1	T/C	4	21	4.31×10^6	3.72×10^3	15	Neg	Neg	Neg	Neg	REL
22	51	M	4	T/C	4	21	2.32×10^5	15	30	Neg	Neg	Neg	Neg	REL
6	71	F	1	C/C	4	14	2.87×10^5	1.99×10^3	5.84×10^4	357	34	15	1.31×10^5	BT
9	56	M	1	T/T	3	14	7.20×10^6	1.30×105	2.20×10^4	120	Neg	Neg	1.70×10^4	BT
10	48	M	3	T/T	4	14	1.20×10^6	2.04×10^3	1.25×10^3	10	Neg	Neg	1.87×10^5	BT
12	45	M	1	T/C	2	21	7.19×10^6	5.99×10^4	2.41×10^5	81	Neg	Neg	9.22×10^3	BT
13	70	F	1	T/C	4	21	8.70×10^6	3.96×10^3	9.98×10^3	111	Neg	Neg	915	BT
14	60	M	3	T/C	4	21	2.26×10^6	1.05×10^6	6.64×10^5	1.95×10^3	38	15	4.40×10^5	BT
18	65	F	1	T/C	3	21	1.90×10^6	690	38	Neg	Neg	Neg	5.09×10^4	BT
5	53	M	1	T/C	4	14	1.93×10^6	7.11×10^5	8.00×10^5	4.34×10^3	1.81×10^3	3.67×10^3	6.48×10^4	BT

Total *n*=19. HCV RNA 15 IU/ml means detectable but unquantifiable HCV RNA. [a]Stage of fibrosis is according to the Metavir Scoring System. [b]Week 12 is standard of care (SOC). [c]Day 0 marks the start of intravenous silibinin (ivSIL) treatment. [d]Transient elastography, kPa. BT, breakthrough; EOT, end of treatment; F, female; GT, genotype; IL, interleukin; M, male; Neg, negative; REL, relapse; SVR, sustained virological response.

Figure 3. Viral kinetics in patient number 7



Results of a 47-year-old female patient with cirrhosis (genotype 1b), inborn immunoglobulin deficiency syndrome and lichen planus with large oral ulcers (treated with 20–80 mg methylprednisolon/day and local cyclosporine while on standard of care [SOC]). The patient did not respond to SOC and became HCV-RNA-undetectable after 20 mg/kg/day intravenous silibinin (ivSIL) for 15 days. SOC treatment was stopped after 48 weeks and she relapsed 4 weeks thereafter. EOT, end of treatment; PEG-IFN, pegylated interferon; RBV, ribavirin.

Sustained virological response

All 11 patients with end-of-treatment response completed 24 weeks of treatment-free follow-up. Overall, 7 (63.6%) patients remained HCV-RNA-negative (6 of 7 patients received 14 days of ivSIL) and 4 relapsed (2 patients with 21 days of ivSIL and 2 patients with 14 days of treatment). Six of the seven patients with SVR had detectable, but unquantifiable HCV RNA before ivSIL treatment (<15 IU/ml; Table 2). One patient who relapsed (Figure 3) was infected by intravenous immunoglobulin substitution for an inborn immunoglobulin deficiency in the 1980s. She had cirrhosis and also suffered from severe lichen planus with large ulcers in her mouth (treated with high dose steroids and local cyclosporine). A second patient relapsed after termination of treatment earlier than recommended (17 weeks after ivSIL). Eight patients had not yet completed SOC treatment; all patients were HCV-RNA-negative at this time (Table 3).

Predictors of response to silibinin

A total of 13 patients (48.1%) had a detectable but unquantifiable HCV RNA (<15 IU/ml) at the beginning of ivSIL. After SIL (14 days treatment in five patients and 21 days treatment in nine patients) all patients had undetectable HCV RNA. Overall, 14 patients had quantifiable HCV RNA levels before ivSIL (median 22,000, range 19–800,000 IU/ml). In 9 (64.3%) of them, HCV RNA became undetectable after ivSIL, and 4 did not achieve HCV RNA negativity after ivSIL treatment (Figure 4). The majority of patients had the unfavourable interleukin (IL)-28B T-allele (T/C=22 and T/T=4). The IL-28B polymorphism did not predict response on SIL in this small cohort.

Table 3. Patients on treatment

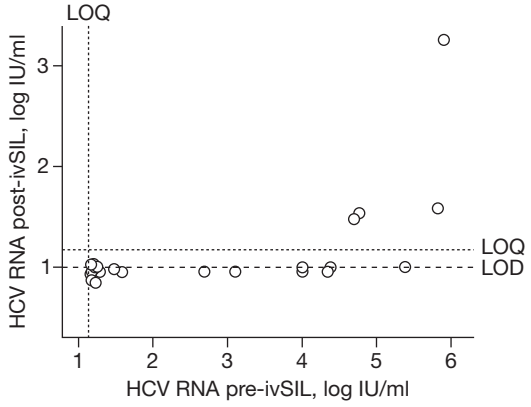
Patient	Age, years	Sex	GT	IL-28	Fibrosis[a]	ivSIL, days	HCV RNA, IU/ml						
							Baseline	Week 12[b]	Day 0[c]	Day 7	Day 14	Day 21	Last HCV RNA
16	55	M	3	T/C	4	21	7.61×10^5	46	15	Neg	Neg	Neg	Neg
19	64	M	1	T/C	3	21	1.15×10^6	1.32×10^3	15	Neg	Neg	Neg	Neg
20	56	F	1	T/C	3	21	6.01×10^6	9.15×10^5	15	Neg	Neg	Neg	Neg
21	70	F	1	T/C	4	21	1.11×10^6	1.53×10^3	15	Neg	Neg	Neg	Neg
23	53	M	1	T/C	11.5 ±8.5[d]	21	2.15×10^6	2.72×10^3	1.02×10^4	45	Neg	Neg	Neg
25	54	M	4	T/C	4	21	2.94×10^5	37	15	Neg	Neg	Neg	Neg
26	44	M	4	T/C	9.0 ±2.8[d]	21	3.65×10^6	954	15	Neg	Neg	Neg	Neg
27	49	M	1	T/C	2	21	6.47×10^6	906	488	Neg	Neg	Neg	Neg

Total *n*=8. [a]Stage of fibrosis according to the Metavir Scoring system. [b]Week 12 is standard of care (SOC). [c]Day 0 marks the start of intravenous silibinin (ivSIL) treatment. [d]Transient elastography, kPa. F, female; GT, genotype; IL, interleukin; M, male; Neg, negative; SOC, standard of care.

Safety and side effects

Safety and tolerability was assessed by physical examinations and laboratory tests. ivSIL was well-tolerated. A total of 12 (44.5%) patients felt a heat sensation during first infusion therapy. This was sometimes associated with mild sweating. On the first infusion day, 15 (55.6%) patients further reported about gastrointestinal symptoms like nausea, abdominal pain and diarrhoea. The symptoms were self-limiting and decreased in following treatment days. Side effects could be avoided by slow application of SIL. All other side effects (flu-like symptoms [*n*=4], depression [*n*=7] and exanthema [*n*=7]) had already occurred before ivSIL treatment and were therefore considered not associated. A slight increase of bilirubin was seen in all patients. Mean ±SD pre-ivSIL bilirubin was 0.98 ±0.35 mg/dl. After ivSIL treatment there was a significant increase of bilirubin to mean ±SD 2.12 ±0.98 mg/dl ($P\leq0.001$; in three patients bilirubin increased to 5.0–5.5 mg/dl). Within 2 weeks after ivSIL, bilirubin levels decreased to 1.31 ±0.6 mg/dl.

Figure 4. HCV RNA viral load before and after intravenous silibinin



ivSIL, intravenous silibinin; LOD, limit of detection; LOQ, limit of quantification.

Discussion

The present study shows that by ivSIL administration HCV RNA became undetectable in 85.1% of patients with insufficient viral response on SOC treatment. These findings reconfirm the antiviral activity of ivSIL in a difficult-to-treat patient group, which included mostly patients with advanced liver disease and predominantly IL-28B T-allele carriers. Furthermore, the antiviral effect was repeatable in relapsing patients. So far no viral resistance has been detected [15,27]. At this planned interim analysis, the overall SVR rate was 36.8% (7 out of 19 patients), but 8 SIL responders are still on treatment with SOC and all of them are HCV-RNA-negative. Thus, SIL is a potentially valuable treatment option for on-treatment non-responders to SOC.

Currently, other curative treatment options for these patients are limited. Retreatment with SOC only leads to SVR rates of 8–12% [4,28,29]. On triple therapy with pegylated interferon, ribavirin, and telaprevir or boceprevir [30,31] only 38% of non-responders achieved an SVR. At the moment DAAs are only available in a few patients participating in clinical studies. Furthermore, safety in patients with advanced liver disease is still unknown. Most of our patients suffered from advanced liver disease (Metavir score F3/F4, *n*=19 [73%]) with a high risk to develop end-stage liver failure and/or hepatocellular carcinoma. Thus, there is an urgent medical need to develop new treatment strategies in this particular patient group.

SIL may be also a rescue treatment for patients not responding to combined SOC/DAA therapy [32]. This was shown in four patients not responding to 4 weeks of triple therapy. Three of them achieved a complete suppression of viral replication by intravenous SIL.

The use of ivSIL has not been explored by a systematic study so far. Our protocol is a 'learning by doing'

approach. Since our first publication on the antiviral effect of SIL [10], several improvements were added. The prolongation of the infusions to 21 days in retreatment of pedigreed non-responders (instead of 14 days) increased the rate of on-treatment response from 32% to 50% [33]. The duration of infusion therapy was shortened to 1 h (initially the infusion was given over 4 h) without any change in tolerability. The present data show that maintained HCV RNA negativity can only be achieved if HCV RNA becomes undetectable by SIL infusions. Most of the successfully treated patients achieved a very low viral load already on SOC. If HCV RNA remained detectable after ivSIL treatment, no patient achieved SVR. In such patients, longer administration may be necessary. Even in patients with undetectable HCV RNA, it is unknown how long SIL and SOC has to be continued to prevent a viral breakthrough. Even 21 days of ivSIL treatment appears to be too short in some patients. This is not surprising since DAAs are given for much longer time periods (12–48 weeks) [8]. The intravenous route of application is certainly a limitation to use the drug for longer periods. Furthermore, slow responders may benefit from extension of SOC treatment to 72 weeks to achieve a SVR [34].

In summary, ivSIL may 'rescue' non-responders to ongoing SOC therapy. Based on our observations, there is no need to stop unsuccessful SOC treatment according to established stopping rules, especially for patients who reach a profound viral suppression but fail to accomplish undetectable HCV RNA on SOC treatment, because ivSIL can still induce a viral response in 85% of patients and allow continuation of therapy ('rescue therapy'). ivSIL may also become a treatment option for patients who fail on triple combination therapy that includes a DAA. The optimal way to reach SVR has to be investigated.

Acknowledgements

KR was involved in data collection, analysis of data and writing of the manuscript. T-MS and HH were involved in the acquisition of data and critical revision of the manuscript for important intellectual content. PS-M was involved in the acquisition of data and critical revision of the manuscript for important intellectual content. SB and AFS were involved in the acquisition of data. TP-K and HK performed the virological assays. PF was involved in the study concept and design, was the principal investigator, performed the analysis and interpretation of data, and outlining and revising of the manuscript.

Disclosure statement

PF is a member of the global advisory board and of the speaker's bureau of Roche (Basel CH) and Rottapharm-Madaus (Monza, Italy). He receives an unrestricted research grant from Roche Austria. He is one of the patent holders for ivSIL for treatment of hepatitis C. PS-M and HH serve as speakers for Roche Austria. All other authors declare no competing interests.

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