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No beneficial effect of all-trans retinoic acid in previous non-responder patients with chronic hepatitis C: The ATRACTION study, a phase II randomised trial

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ABSTRACT

Background: Preclinical data suggested all-trans retinoic acid (tretinoin) as a potential antiviral agent against chronic hepatitis C infection.
Aims: To assess efficacy, safety, and tolerability of tretinoin in combination with peg-interferon and ribavirin in genotype-1 infected patients with prior non-response.
Method: We performed an open-label multicentre clinical trial. Patients were randomised to either receive additional tretinoin (45 mg/m^2/day) for 12 weeks (arm A), or peg-interferon and ribavirin alone (arm B). Primary endpoint was the slope of the third phase of viral decline (Mδ) as determined in an established kinetic model known to correlate with treatment outcome. Secondary endpoints were additional kinetic parameters, viral response rates, safety, and tolerability.
Results: 27 patients in arm A and 30 patients in arm B were treated per protocol until week 12. Viral kinetic parameters did not differ. Rates of early virological response (>2 log$_{10}$ drop at week 12) were similar (10/27 versus 11/30 patients). In arm A, patients experienced a higher rate and intensity of adverse events, most commonly skin and mucosal dryness, and headache.
Conclusion: Addition of tretinoin was safe and acceptably well tolerated. However, it did not influence viral kinetics and thus cannot be further considered as a treatment option.

1. Introduction

Hepatitis C virus (HCV) still causes a significant burden of morbidity and mortality in Europe [1]. In the next years, a further increase of patients with HCV-associated cirrhosis is expected [2]. Thus, treatment is mandatory before complications of long-term infection become manifest. The introduction of pegylated interferon (peg-IFN) in combination with ribavirin has been a major step forward, however, patients who did not respond to this therapy had only marginal chances to achieve sustained virological response (SVR) with a repeated therapy [3–5]. Also after protease inhibitors became available, responsiveness to peg-IFN and ribavirin remains an important predictive factor for success of treatment [6,7]. Thus, new substances are urgently needed.

All-trans retinoic acid (ATRA), pharmacologically available as tretinoin, is a naturally occurring derivative of vitamin A, playing an important role in several biological processes including cell differentiation [8]. Due to this property, ATRA is the agent of choice

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to induce remission in acute promyelocytic leukaemia (PML) [9]. In vitro findings suggested that ATRA is effective against hepatitis C: In the replicon system, glutathione peroxidase (GI-GPx), one of the main cellular enzymes to encounter oxidative stress, was down-regulated 20-fold in the presence of HCV replication. The addition of ATRA induced GI-GPx expression and significantly reduced HCV replication [10], presumably by binding on retinoic acid receptors in the GI-GPx-promotor region [11]. Furthermore, ATRA may exert immunological effects by inducing the expression of RIG-1, a protein which shares functional similarity with toll-like receptor-3 [12,13]. In addition, ATRA can be speculated to increase the expression of interferon receptors on hepatocytes in a similar fashion as cis-retinoic acid [14].

Based on these in vitro findings, a pilot trial in 20 patients with previous non-response has been performed [15]. Monotherapy with ATRA for 12 weeks induced a viral decay by >1 $\log_{10}$ in 5 out of 10 patients, and the combination with peg-IFN after 12 weeks of treatment led to a transient viral clearance in 3 out of 10 patients in this difficult-to-treat patient group.

The safety profile of ATRA needs specific attention: up to 25% of patients treated for PML experience the so-called retinoic acid syndrome (RAS), characterised by hyperleukocytosis, fever, inflammatory lung infiltrates, serositis, hypotension, and oedema, with a fatal outcome in some cases [16,17]. Although a pathogenetic link with the PML-associated chromosomal translocation *t*(15;17) involving a retinoic acid receptor exists [18], it remains unknown whether RAS may occur also in patients without PML. In addition, retinoic acid derivatives are potentially hepatotoxic: more than 10% of treated patients developed elevated transaminases [17,19,20]. Other potential side effects of retinoic acid derivatives are less serious, but potentially affect the patients' quality of life, mainly skin and mucosal dryness, pruritus, and neuropsychiatric symptoms like headache, fatigue, or depression [19].

After start of therapy with peg-IFN and ribavirin, viral kinetics are characterised by an early rapid decline, attributed to the blockade of viral production. A second, flattened phase is attributed to the loss of infected cells, followed by a third phase of steeper decline, which, interestingly, in several studies was positively correlated with viral success rates [21–23]. A detailed and well-validated mathematical model is available to determine viral kinetic parameters from a limited number of assessments [22]. Using viral kinetic parameters as a primary endpoint in a clinical trial provides the substantial advantage to generate valid results by only exposing a limited number of patients to experimental treatment strategies.

This randomised clinical trial was conducted to reveal whether the addition of ATRA to peg-IFN and ribavirin influences viral kinetics during the first 12 weeks of treatment in HCV patients infected with genotype 1 and previous non-response to peg-IFN and ribavirin. The slope of viral decline in the third phase Mδ was used as the primary endpoint. Secondary endpoints were the slope of the first and the second phase, viral success rates, safety, and tolerability.

2. Patients and methods

This was a prospective, multicentre, randomised open-label phase II clinical trial. The protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study was conducted in compliance with guidelines for Good Clinical Practice. The protocol a priori was approved by the ethics committee of the Ärztekammer Rhineland-Palatinate, Mainz, number 837.117.07 (5652), and by the ethics committees at each of the participating institutions. The full trial protocol can be accessed by contacting the corresponding author. The study is registered at EudraCT, Nr. 2006-005500-14.

Patients were recruited from the outpatient clinics of the following German university hospitals: Aachen, Charité Berlin, Essen, Frankfurt, Kiel, Mainz, Ulm, Heidelberg, and Tübingen.

Inclusion criteria were as follows: Male or female patients with chronic hepatitis C of genotype 1, age 18–65 years, with previous documented non-response to peg-IFN and ribavirin, defined as the failure to achieve at least a 2 $\log_{10}$ drop in viral load at week 12 or detectable viral load at week 24. During previous therapy, patients had to have received standard dosages of peg-IFN and ribavirin (i.e. peg-IFN alfa-2a ≥135 μg or peg-IFN alfa-2b ≥1.0 μg/kg body weight/week) plus ribavirin ≥800 mg/day as a starting dose) for at least 80% of the time. Availability of liver histology not older than 24 months was required, revealing at least minimal inflammatory activity and/or mild fibrosis.

Patients with the following characteristics were not eligible for randomisation: Current or history of decompensated liver disease (i.e. Child-Pugh B or C), suspicion of hepatocellular carcinoma (defined as cirrhosis in combination with α-fetoprotein >100 ng/ml), and patients with preexisting eye disease or active skin disorder.

Patients on continuous medication with the following drugs were excluded: Vitamin A, in order to avoid hypervitaminosis, and tetracyclines, which, similar to tretinoin, potentially increase intracranial pressure.

Further exclusion criteria comprised the contraindications for peg-IFN and ribavirin as labelled [24,25], known allergy to retinoic acid derivatives, or soy bean oil which is an ingredient of tretinoin.

Informed consent was obtained from every patient prior to any study procedure.

The starting dose of peg-IFN alfa-2a (Pegasys®) was 180 μg/week plus ribavirin (Copegus®) (both Roche Pharma AG, Grenzach-Wyhlen, Germany) adapted to body weight (<75 kg: 1000 mg, ≥75 kg: 1200 mg/day, divided in two doses) as licensed.

Patients were assigned to treatment groups by central FAX randomisation at Interdisciplinary Centre for Clinical Trials (IZKS) in Mainz. Randomisation was stratified according to viral load (<400,000 versus ≥400,000 IU/ml) and loss of viral load during previous therapy (<0.5 $\log_{10}$ versus ≥0.5 $\log_{10}$). The randomisation ratio was 1:1. Within strata, randomisation was performed using 20 blocks of length 4. Randomisation lists were generated by means of a validated SAS program developed at IZKS Mainz and used since 2006.

Patients assigned to arm A were planned to additionally receive therapy with tretinoin (Vesanoid®, 10 mg capsules, Roche Pharma AG, Grenzach-Wyhlen, Germany) for 12 weeks. The planned dose of tretinoin was 45 mg/m^2 body surface area, to be taken with food. Patients in arm B were planned to receive treatment with peg-IFN and ribavirin only.

To determine viral kinetic parameters during the first 12 weeks, assessment of HCV RNA was scheduled at baseline and on days 1, 2, 3, 7, 14, 21, 28, 42, 56, and 84. HCV RNA was quantified centrally at Mainz University Hospital from deep-frozen plasma samples (−20 °C) using Roche COBAS Ampliprep®/COBAS TaqMan®test (Roche Diagnostics) with a lower limit of quantification of 15 IU/ml.

The study scheme is depicted in Fig. 1. In arm A, patients who showed a drop of HCV RNA > 2 $\log_{10}$ as compared to baseline (early virological response, EVR), received peg-IFN and ribavirin for additional 36 weeks. If no EVR was achieved, or viral load was still detectable at week 24, therapy was stopped. Patients in arm B who did not achieve an EVR received additional tretinoin for 12 weeks (arm B2). For these patients, the same stopping rules were applied with a 12 weeks delay, i.e. viral load had to decrease by >2 $\log_{10}$ at week 24 and had to be non-detectable at week 36. Thus, maximum duration of therapy in arm B was 60 weeks. In all patients, HCV RNA assessment was scheduled at week 12 and 24 after end of treatment (follow-up). Individual viral kinetic parameters were

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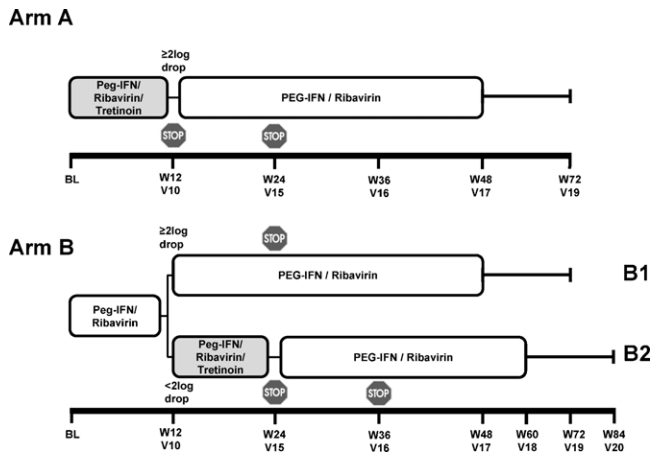

Fig. 1. Study protocol (Peg-IFN: pegylated interferon, BL: baseline, W: week, v: visit).

calculated using the equations established by Herrmann et al. [22] (Fig. 2).

The slope of viral decay in the third phase, expressed as the inflation factor Mδ, was used as the primary endpoint. As secondary endpoints, the "efficiency factor" ε, representing the slope of the first decline, the slope of the second phase named δ, and the clearance of free virus named c, were determined. Numeric solution of the differential equations and least square fitting were calculated using R 2.10.0 software. Virological outcome was analysed using the common definition of SVR (HCV RNA not detectable 24 weeks after end of treatment).

For calculation of sample size, a difference of $\geq$0.4 in mean $\log_{10}$ Mδ (SD $\pm$ 0.6) on a two-sided significance level of 0.05 was assumed based on previous data [16], corresponding to an effect size of 0.67. To detect this difference with a power of 80%, a sample of 37 patients in each arm was required. To compensate drop-outs, a sample size of 40 patients in each group was planned. Results in viral kinetic parameters were initially planned to be compared using *t*-tests and changed into Mann–Whitney-*U*-Tests for unpaired nonparametric samples since the paper describing the mathematical model to determine the viral kinetic parameters [22] used this test as well. Chi-Square-test was used to compare the frequency of a third phase of viral decline. In case of skewed distribution of the results, median and quartiles are to be displayed.

Safety and tolerability were analysed using data on frequency and intensity of adverse events as assessed during patient visits and laboratory abnormalities rated as "clinically significant" by the investigators. Adverse events (AEs) were coded with MedDRA 14.0 (Medical dictionary for regulatory activities, http://www.ich.org/products/meddra.html). Intensity of adverse events had to be categorised as "mild", "moderate" or "severe". The presumed relation to trial medication was assessed in a "yes" or "no" decision. Compliance was assessed counting returned medication.

Serious adverse events (SAEs) according to ICH-GCP were reported to the sponsor and to the drug safety of Roche Pharma AG. For any SAE, causal relation was assessed. Reference safety documents were the summaries of product characteristics (SPCs) of Pegasys®, Copegus®, and Vesanoid®. Safety data were reviewed by an independent Data and Safety Monitoring Board (DSMB) every 3 months. Annual safety reports were submitted to the Ethics Committee and competent authority during the clinical conduct of the trial. Clinical monitoring was planned on a regular basis.

According to the investigational plan, the intent to treat population should comprise all randomised patients. The safety population was defined as all patients who had received at least one dosage of study medication, and the per-protocol population comprises all patients on treatment with available viral load samples until week 12 with at least 80% of compliance as documented.

$$V'(t) = (1-\epsilon)\, p\, I(t) - c\, V(t)$$

$$I'(t) = (1-\eta)\, \beta T(t)\, V(t) - \delta I(t)$$

$$T'(t) = \gamma(T(0) + I(0) - T(t) - I(t))$$

After start of treatment, the infected cell loss changes to Mδ and, therefore, the second equation becomes

$$I'(t) = (1-\eta)\, \beta T(t)\, V(t) - M\, \delta I(t)$$

Fig. 2. Equations of the applied mathematical model on viral kinetics [22] (I: productively infected hepatocytes, T: uninfected hepatocytes, c: clearance of free virus, δ: rate of infected cell loss, p: virus production, β: de novo infection, ε: antiviral effects during initial phase (efficiency factor), η: antiviral effects on de novo infection during initial phase, M: inflation factor $\geq$1, starting at some delayed time point $t_1 > t_0$).

3. Results

3.1. Study population

The first patient was screened on September 26, 2007, and the last patient left the study on February, 23, 2011. Recruitment lasted until March 2010 and was regularly ended as the planned number of patients was included. 90 patients were screened, and 82 patients were randomised.

Patients in all consecutive study phases are shown in Fig. 3. At one trial side, severe malpractice and missing documentation occurred, and it was decided to exclude all 13 patients recruited there. With the data available on the primary endpoint for this modified intent to treat population of 69 patients, we did not replace these patients by additional recruitment. Baseline characteristics are shown in Table 1. Of those, one patient withdrew consent before receiving any study medication, leaving 68 patients (34 patients in each arm) who started treatment, constituting the safety population. All patients started peg-IFN and ribavirin as planned, except for one patient receiving 400 mg of ribavirin only. Patients in arm A and B2 received oral tretinoin in a range of 60–110 mg per day. Dose reduction of any medication became necessary during the study in 10/34 patients (29.4%) in arm A and 3/34 patients (8.8%) in arm B.

Data for compliance with intake of medication are available only for 16 patients in arm A and 20 patients in arm B which was mainly due to insufficient return of medication to clinical visits. Available

Table 1
Baseline characteristics of patients (modified intent to treat population, $n = 69$).

	Arm A $n = 34$	Arm B $n = 35$
Female gender	10 (29%)	15 (43%)
Mean age (years) $\pm$ SD	50.5 $\pm$ 8.0	50.3 $\pm$ 9.2
Ethnicity: caucasian	34 (100%)	35 (100%)
Mean body weight (kg) $\pm$ SD	85.8 $\pm$ 13.3	79.3 $\pm$ 15.4
Mean body height (cm) $\pm$ SD	177 $\pm$ 9	171 $\pm$ 9
Baseline viral load < 400.000 IU/ml	6 (18%)	5 (14%)
Viral load decline during previous therapy $\geq$0.5 $\log_{10}$	29 (85%)	27 (77%)
Alanine aminotransferase (U/l)	108 $\pm$ 83	110 $\pm$ 85
Bilirubin (mol/l)	15.8 $\pm$ 7.0	13.3 $\pm$ 6.9
Liver histology: bridging fibrosis/cirrhosis	11 (32%)	6 (17%)

SD: standard deviation, kg: kilogram, cm: centimetres, IU: international units, ml: millilitre, U: units, l: litre.

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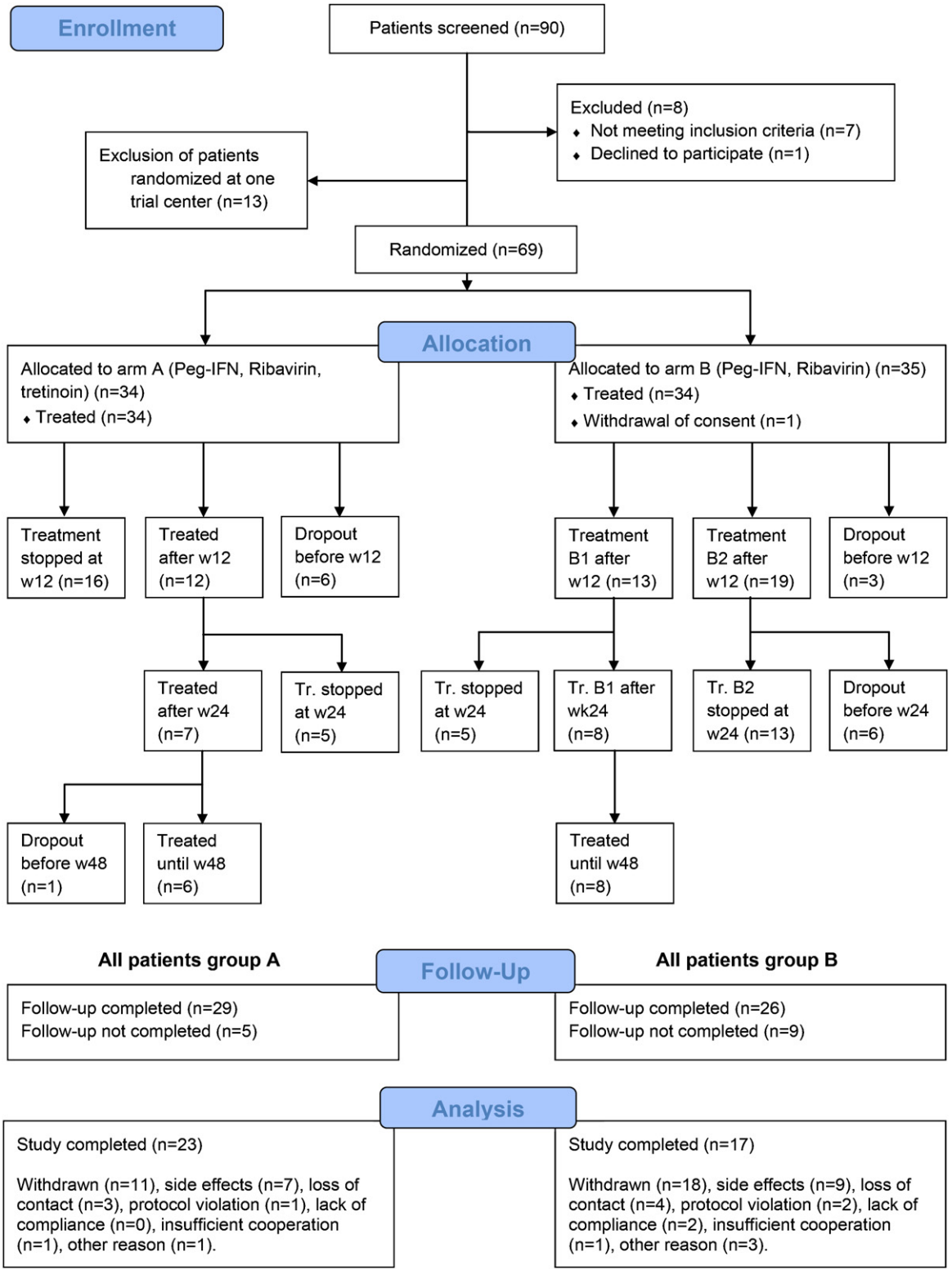

Fig. 3. Number of patients in different study phases (peg-IFN: pegylated interferon, w: week).

data demonstrated a mean compliance of 101.3% in arm A and 98.0% in arm B.

Until week 12, 27 patients in arm A and 30 in arm B were treated per protocol and were available for analysis of the primary endpoint. The percentage of patients with a baseline viral load <400,000 IU/ml was comparable (arm A: 15%, arm B: 17%), and baseline viral load did not differ (arm A: median 6.4 $\log_{10}$, Q1 (25% quartile) 6.0, Q3 (75% quartile) 6.7; arm B: 6.4 $\log_{10}$, Q1 6.1, Q3 6.7 $\log_{10}$, $p = 0.860$). In arm A, a maximal decrease of HCV RNA $\geq$0.5 $\log_{10}$ during previous treatment had been observed in 23 patients (85%) versus 24 patients in arm B (80%). The following protocol deviations were tolerated and did not lead to exclusion from the per-protocol population: $\geq$1 visit differing $\geq$7 days from the scheduled date (19 patients in arm A and 22 patients in Arm B),

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Table 2
Primary endpoint (Mδ) and other viral kinetic parameters as analysed in the per-protocol population until week 12.

		Mδ	ε	δ	c
A (n = 27)	Q1	0.0223	0.1237	0.0014	0.73
	Median	0.0729	0.6054	0.0186	2.25
	Q3	0.1109	0.7561	0.0692	10.00
B (n = 30)	Q1	0.0225	0.0317	0.0050	0.35
	Median	0.0425	0.6504	0.0279	1.88
	Q3	0.1057	0.8728	0.0651	16.42
p-Value (Mann–Whitney-U)		0.554	0.576	0.774	0.643

Q1: 25% quartile, Q3: 75% quartile, Mδ: Modifyed rate of infected cell loss, ε: antiviral effects during initial phase, δ: rate of infected cell loss, c: clearance of free virus.

intake of forbidden co-medication (1 patient in arm B), no effective contraception (1 patient in arm B), age <18 or >65 (3 patients in arm A and 2 patients in arm B), preexisting retinopathy (2 patients in arm B), Diabetes mellitus with HBA1c >7% (4 patients in arm B), and no anti-HCV test at screening (1 patient in arm B).

3.2. Efficacy

Mδ did not differ significantly between both arms, neither did any of the assessed viral kinetic parameters (Table 2). EVR was observed in 10/27 patients (37.0%) in arm A and in 11/30 patients in arm B (36.7%, $p = 0.977$). At the end of treatment, in each arm 6 patients were negative for HCV RNA. In addition, the frequency of a triphasic decline was comparable (63% in arm A versus 66.7% in arm B, $p = 0.77$). Two patients, both in arm B1, achieved SVR.

As expected, patients achieving EVR had higher Mδ (median 0.0951, Q1 = 0.0778, Q3 = 0.1377 versus 0.0345, Q1 = 0.0156, Q3 = 0.0589, $p < 0.0005$) and a higher ε (median 0.85, Q1 = 0.41, Q3 = 0.96 versus 0.51, Q1 = −0.13, Q3 = 0.71, $p = 0.005$).

3.3. Adverse events

Seven SAEs were reported affecting five patients. One patient in arm A was hospitalised due to severe headache after first intake of tretinoin (considered as related by the investigator); this patient discontinued treatment but experienced two further SAEs during follow-up (constipation and ureteric calculus, considered as not related). In arm B, 2 patients were hospitalised due to pneumonia (1 considered as related, 1 as not related), one patient was admitted with intestinal campylobacter infection (considered as not related), and 1 patient with dyspnoea at rest (considered as related). No pregnancy occurred. No patient developed symptoms similar to RAS. No suspected unexpected serious adverse reaction (SUSAR) was observed, and no patient died during the study.

All except two patients in arm B experienced at least one episode of an adverse event (AE). The intensity of most AEs was mild to moderate. Only singular AEs were of severe intensity (headache ($n = 4$), stomatitis ($n = 2$) in arm A, and pruritus ($n = 1$) in arm B).

Table 3 lists all AEs until week 12 that affected more than 10% of patients in at least one of the treatment arms. In the patients receiving tretinoin, a higher frequency of neurological AEs (headache, insomnia, dizziness), AEs concerning ear, nose and throat (mucosal dryness, oropharyngeal pain, dry lips, epistaxis), gastrointestinal (nausea and vomiting), and skin disorders (pruritus, dry skin, cheilitis) was observed.

One patient in arm A accidentally swallowed 48 capsules of tretinoin and 33 tablets of ribavirin within 48 h. He was monitored, but developed only dry mouth, dry skin, and mild to moderate exanthema.

The addition of tretinoin did not influence any routine laboratory parameter including blood cell counts (data not shown). Of special interest, mean levels of alanine aminotransferase (ALT) at the end of week 12 were comparable, accounting for 70.5 ± 76.8 U/L in arm A and 68.0 ± 59.7 U/L in arm B.

Table 3
Adverse events until week 12.

General symptoms	Arm A (n = 34)		Arm B (n = 34)	
	n	%	n	%
Headache	28	82	13	38
Feeling cold	18	53	19	56
Fatigue	18	53	19	56
Musculosceletal disorders	14	47	15	44
Chills	14	41	5	15
Influenca like illness	10	29	7	21
Cough	8	24	7	21
Insomnia	7	21	2	6
Dizziness	7	21	2	6
Pyrexia	6	18	4	12
Irritability	4	12	1	3
Dyspnoa	4	12	3	9
Night sweats	4	12	2	6
Disturbance in attention	2	6	4	12
Ear, nose and throat				
Ear and labyrinth disorders	14	41	3	9
Epistaxis	10	29	4	12
Oropharyngeal pain	5	15	0	0
Nasopharyngitis	4	12	1	3
Gastrointestinal disorders				
Nausea	13	38	6	18
Vomiting	9	26	3	9
Upper abdominal pain	4	12	2	6
Diarrhoea	3	9	4	12
Hair, skin, and mucous membranes				
Pruritus	16	47	5	15
Dry skin	15	44	7	21
Dry lips	13	38	0	0
Cheilitis	10	29	3	9
Mucosal dryness	8	24	2	6
Alopecia	8	24	3	9
Dry mouth	7	21	7	21
Hyperhidrosis	6	18	4	12
Rash	5	15	3	9
Aphtous stomatitis	4	12	2	6
Gingival bleeding	4	12	0	0
Erythema	4	12	1	3

4. Discussion

The aim of this study was to assess whether ATRA exerts an antiviral effect when combined with peg-IFN and ribavirin in patients with previous non-response. The concept was based on promising in vitro data and results from a pilot clinical trial. However, in this randomised study, we could not observe an influence of ATRA on viral decline in this combination therapy.

Until today, a previous non-response to peg-IFN and ribavirin reduced the likelihood of achieving SVR [6,7]. In the era of dual therapy, re-treatment has been effective only in a minority of patients [3–5] which was not surprising as the main determinants of SVR, host genetics like polymorphisms in the IL-28B promotor region [26] and viral properties [27], remained unchanged. Thus, this population was in the most urgent need for new treatment options which was a major motivation for this and many other clinical trials. However, for the current trial, the low responsiveness to peg-IFN and ribavirin may have been disadvantageous for the discovery of presumed synergistic effects of ATRA with peg-IFN and ribavirin.

HCV replication is associated with an increased level of oxidative stress [28,29]. Since viral proteins contribute to the increased production of reactive oxygen species [30,31], it can be speculated that this situation is beneficial for HCV replication. This would be in accordance with results from the replicon system showing that

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ATRA reduces oxidative stress which in turn leads to a decrease in viral replication [10]. However, although ATRA has been demonstrated to possess antioxidant effects in animal models [32], its antioxidative effect in humans may be less pronounced. Until today, it remains a challenging question how to effectively modify oxidative stress levels in chronic liver disease [33].

Recent data underline the central role of vitamin A in the innate and the adaptive immune system [13,34–36], and it was debated whether ATRA increases antiviral immune responses [12]. However, in our study, viral kinetics were similar in both treatment arms, which allows to conclude that no functionally significant improvement in HCV-related immunity had emerged during treatment with ATRA.

Some patients did not fulfil all in- and exclusion criteria. However, since deviations laid nearby the limit values (age, HBA1c), they were not excluded from the analysis. Fortunately, neither the intake of forbidden comedication, insufficient contraception, nor the presence of retinopathy was followed by associated adverse events. In general, it seems very unlikely that primary or secondary endpoints were significantly influenced.

During the study, no major safety concerns were raised, and, most importantly, no patient developed retinoic acid syndrome (RAS). However, this does not exclude the possibility of its occurrence in hepatitis C patients at low frequencies. Recently, RAS was observed in two patients with dermatological disorders receiving retinoic acid, emphasising that it is not exclusively attributed to PML [37,38].

Tolerability was acceptable, but severity and frequency of adverse events were higher in patients treated with ATRA, comprising mainly of well-known retinoic acid associated symptoms. In both arms, the drop-out rate was increased during the periods with additional ATRA treatment. Thus, triple therapy required a close clinical monitoring and, if needed, supportive medication, but was acceptably well tolerated by the majority of patients. In our study, ATRA did not exert a significant hepatotoxic effect which was of special concern in patients with preexisting liver disease.

Finally, this randomised controlled clinical trial provides no evidence that co-administration of ATRA with peg-IFN and ribavirin in patients with previous non-response influences viral kinetics. It is not expected that results would have changed if the per-protocol population had comprised a higher number of patients. However, ATRA was safe and acceptably well tolerated. As a consequence, ATRA should not be further considered as a treatment option for patients with chronic hepatitis C.

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