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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