Peginterferon alfa-2a plus tenofovir disoproxil fumarate for hepatitis D (HIDIT-II): a randomised, placebo controlled, phase 2 trial

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Summary

Background Hepatitis D is the most severe form of chronic viral hepatitis. Treatment guidelines recommend 1 year of peginterferon alfa, which is effective in 25–30% of patients only. Whether prolonged therapy with peginterferon alfa-2a for 96 weeks and combination therapy with tenofovir disoproxil fumarate (TDF) would increase hepatitis D virus (HDV) RNA suppression is unknown. We aimed to explore whether prolonged treatment of HDV with 96 weeks of peginterferon would increase HDV RNA response rates and reduces post-treatment relapses.

Methods We did two parallel, investigator-initiated, multicentre, double-blind randomised, controlled trials at 14 study sites in Germany, Greece, Romania, and Turkey. Patients with chronic HDV infection and compensated liver disease who were aged 18 years or older were eligible for inclusion. All patients were HBsAg positive for at least 7 months, anti-HDV positive for at least 3 months, and HDV-RNA positive at the local laboratory at the screening visit. Patients were ineligible if alanine aminotransferase levels were higher than ten times above the upper limit of normal and if platelet counts were lower than 90 000 per µL, or if they had received interferon therapy or treatment with a nucleoside and nucleotide analogue within the preceding 6 months. Patients were randomly assigned by blinded stratified block randomisation (1:1) to receive 180 µg of peginterferon alfa-2a weekly plus either TDF (300 mg once daily) or placebo for 96 weeks. The primary endpoint was the percentage of patients with undetectable HDV RNA at the end of treatment assessed by intention to treat. The trials are registered as NCT00932971 and NCT01088659.

Findings Between June 24, 2009, and Feb 28, 2011, we randomly assigned 59 HDV RNA-positive patients to receive peginterferon alfa-2a plus TDF and 61 to receive peginterferon alfa-2a plus placebo, including 48 (40%) patients with cirrhosis to the two treatment groups (23 in the peginterferon alfa-2a plus TDF group and 25 in the peginterferon alfa-2a plus placebo group). The primary endpoint was achieved in 28 (48%) of 59 patients in the peginterferon alfa-2a plus TDF group and in 20 (33%) of 61 patients in the peginterferon alfa-2a plus placebo group (odds ratio 1·84, 95% CI 0·86–3·91, p=0·12). We recorded 944 adverse events (459 in the peginterferon alfa-2a plus TDF group and 485 in the peginterferon alfa-2a plus placebo group). The most common adverse events were haematological, behavioural (eg, fatigue), musculoskeletal, influenza-like syndromes, and psychiatric complaints.

Interpretation Addition of TDF resulted in no significant improvement in HDV RNA response rates at the end of treatment. These findings highlight that alternative treatment options are needed for hepatitis D.

Funding The HepNet Study-House (a project of the German Liver Foundation founded by the German Liver Foundation, the German Ministry for Education and Research, and the German Center for Infectious Disease Research), Hoffmann-La Roche, and Gilead Sciences.

Lancet Infect Dis 2019; 19: 275–86

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Introduction

Hepatitis D (also known as hepatitis delta) infection is caused by co-infection of patients with the hepatitis B virus (HBV) and the hepatitis D virus (HDV). HDV is encapsidated by HBsAg, and HDV infection can therefore occur only in individuals infected with HBV. An estimated 10–20 million patients worldwide are infected with HDV.[1] Eight different HDV genotypes have been described, with HDV genotype 1 being the most common in central Asia, Europe, and North America.[2] HBV–HDV co-infection is the most severe form of viral hepatitis, frequently leading to progressive liver disease with rapid development of liver cirrhosis and an increased risk for hepatocellular carcinoma.[2] Studies have shown the particular severity of both HDV genotype 1[3–6] and HDV genotype 3 infection.[7] Notably, patients with HBV and HDV co-infection have a higher risk of liver-related clinical events than do mono-infected patients with chronic hepatitis B.[6,8]

Treatment of hepatitis D is challenging, with no approved regimen. Because HDV uses host polymerases

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See Online for appendix

Research in context

Evidence before this study

Hepatitis D virus (HDV) infection has been treated with interferon alfa-based therapies for almost 30 years, even though no drug is approved for the treatment of HDV infection. We searched PubMed with the terms "HDV OR hepatitis delta" AND "interferon" for papers published in English between Jan 1, 1985, and March 10, 2018. Several systematic reviews and published studies showed that hepatitis D can be treated successfully with 48–72 weeks of interferon alfa-based therapies in 25–30% of cases. The investigator-initiated, randomised, placebo-controlled Hep-Net International Delta Hepatitis Intervention Trial (HIDIT)-I achieved a response at post-treatment week 24 in 28% of patients receiving a course of 48 weeks of therapy with peginterferon alfa. However, further long-term follow-up of responding patients revealed late HDV RNA relapses in more than 50% of those who responded at post-treatment week 24. The combination of peginterferon alfa-2a with adefovir dipivoxil increased HBsAg decline in patients with hepatitis D. No previous study has investigated combination therapy with peginterferon alfa-2a and tenofovir disoproxil fumarate in patients with HDV infection.

Added value of this study

The HIDIT-II trial showed that extending peginterferon alfa-2a therapy to 96 weeks is possible in most patients with an acceptable safety profile leading to on-treatment HDV RNA suppression rates of about 40%. Prolonged administration of peginterferon alfa-2a for 96 weeks was associated with an improvement in histological fibrosis scores in most patients. However, post-treatment HDV RNA relapses occurred in more than a third of responding patients despite prolonged antiviral therapy. Combination therapy with tenofovir disoproxil fumarate does not improve HBsAg declines in patients with hepatitis D.

Implications of all the available evidence

Pegylated interferon therapy for 48 weeks remains the recommended standard of care for patients with HDV infection. Neither prolonged administration of peginterferon alfa-2a for 96 weeks nor combination therapy with tenofovir disoproxil fumarate can be recommended. Prolonged treatments could be considered in the subgroup of patients who show a pronounced HBsAg decline, with the aim to induce HBsAg loss. Alternative treatments for HDV infections should be developed because few patients benefited from pegylated interferon therapy. However, our data give important information and will guide future trials exploring novel HDV therapies with and without peginterferon.

for replication,[2] various nucleoside and nucleotide analogues used to treat HBV infection have been tested as treatment for HDV but all have been shown to be ineffective.[9] By contrast, high doses of interferon alfa reduced levels of alanine aminotransferase and HDV RNA,[9] which has been associated with improved clinical long-term outcomes.[10] Peginterferon given for 48–72 weeks induced off-treatment HDV RNA responses (defined as undetectable HDV RNA 24 weeks after the end of treatment) in 17–43% of patients.[9] The investigator-initiated, randomised, placebo-controlled Hep-Net International Delta Hepatitis Intervention Trial (HIDIT)-I[11] achieved a response at 24 weeks post-treatment in 28% of patients receiving 48 weeks of therapy with peginterferon alfa. However, long-term follow-up of responding patients revealed late HDV RNA relapses in more than 50% of those who responded at 24 weeks post-treatment,[12] highlighting that a sustained virological response is difficult to achieve with a 1 year course of peginterferon therapy.

Beyond HDV RNA suppression, a main goal of hepatitis D treatment is serological cure from HBV infection because a decrease in levels of HBsAg is also associated with HDV reduction. One strategy to further decrease HBsAg might be the combination of peginterferon alfa with HBV polymerase inhibitors. In HIDIT-I,[11] combination with adefovir dipivoxil was associated with a slightly greater decline of HBsAg levels compared with therapy with peginterferon alfa alone. In HBV mono-infection, the combination of peginterferon alfa with the nucleotide analogue tenofovir disoproxil fumarate (TDF) reduced levels of HBsAg.[13] However, no study has investigated the combination of peginterferon alfa and TDF therapy in patients with hepatitis D.

We hypothesised that extending peginterferon alfa therapy would increase HDV RNA response rates and that combination with TDF would enhance HBsAg suppression. Our first objective was therefore to explore whether prolonged therapy of HDV with 96 weeks of peginterferon would increase on-treatment HDV RNA response rates and reduce post-treatment relapses. The second objective was to investigate whether the combination of peginterferon with TDF would increase HDV RNA and HBsAg responses in patients with hepatitis D.

Methods

Study design and participants

HIDIT-II was an individually randomised, placebo-controlled trial, which included 120 patients from six hospitals in Germany, one centre in Greece, two centres in Romania, and five centres in Turkey (appendix). HIDIT-II consists of two parallel trials with identical protocols, a combined analysis, and a joint central laboratory (at Hannover Medical School, Hannover, Germany) to establish the primary virological

efficacy parameter. In Germany, Greece, and Romania, HIDIT-II was an investigator-initiated trial with Hannover Medical School (MHH) as the formal regulatory legal sponsor. In Turkey, the trial was done under the sponsorship of Roche. Identical screening standards, case report forms, and monitoring procedures were applied in both studies. Central and independent combined efficacy and safety assessments were applied for all patients in both studies.

Patients with chronic HDV infection and compensated liver disease who were aged 18 years or older were eligible for inclusion. All patients were HBsAg positive for at least 7 months, anti-HDV positive for at least 3 months, and HDV RNA positive at the local laboratory at the screening visit. Patients were ineligible if alanine aminotransferase levels were higher than ten times above the upper limit of normal and if platelet counts were lower than 90 000 per µL. Interferon therapy or treatment with a nucleoside and nucleotide analogue within the preceding 6 months was an exclusion criterion. A detailed description of all eligibility criteria is listed in the appendix, which includes the full study protocol.

All patients provided written informed consent before enrolment.

Randomisation and masking

Patients were randomly assigned to receive either subcutaneous peginterferon alfa-2a (180 µg once weekly) plus TDF (300 mg daily) for 96 weeks or subcutaneous peginterferon alfa-2a (180 µg once weekly) plus placebo for 96 weeks. Investigators were masked to treatment. Patients were stratified according to previous treatment with interferon alfa (yes *vs* no), sex, and country before randomisation. We used blinded stratified block randomisation with a block size of two across all strata. Randomisation was done by designated staff of the HepNet Study-House using the Hep-Net online randomisation tool. Staff who did the randomisation were not involved in the assessments of endpoints. The placebo was identical to TDF in shape, size, and colour.

Procedures

Patients were followed up for a total of 245 weeks after the end of therapy until study week 365. Assessments for virological and biochemical endpoints were made at treatment weeks 12, 24, 48, 72, and 96, as well as 24 weeks after the end of treatment (study week 120).

HDV RNA was measured at the central virology laboratory using the Cobas TaqMan system with an in-house assay (Roche Diagnostics, Mannheim, Germany), with stored frozen serum samples as described previously.[14] HDV RNA levels are provided in copies per mL because the study was initiated in 2009 when no WHO HDV RNA standard was available. A conversion factor was later determined: one copy per mL in this assay equals to 62 IU per mL.

Histological examination (grading and staging according to the Ishak score[15]) was done in pre-treatment samples as well as end-of-treatment liver biopsies by two experienced pathologists (JS and HPD), who were masked for clinical and virological data and for the study group. For statistical and safety analysis, cirrhosis was defined as histological staging F5 or F6, or in the case of missing biopsy slides, previous histology results indicating liver cirrhosis according to the local pathology reading. Additionally, cirrhosis was assumed to be present if at least two of the following criteria were fulfilled: ratio of aspartate aminotransferase to alanine aminotransferase greater than 1, bilirubin concentrations of greater than 1·5 times the upper limit of normal, albumin concentrations less than 35 g/L, platelet counts of less than 100 000 per µL, or presence of oesophageal varices.

Safety was assessed by the study investigators on the basis of adverse events reported by study participants. Additionally, haematological and biochemical parameters including full blood counts, liver aminotransferases, bilirubin concentrations, γ-glutamyl transferase concentration, prothrombin time, and creatinine concentrations were measured as indicated in the study protocol. Grading of adverse events was based on predefined criteria listed in the study protocol. Flares in

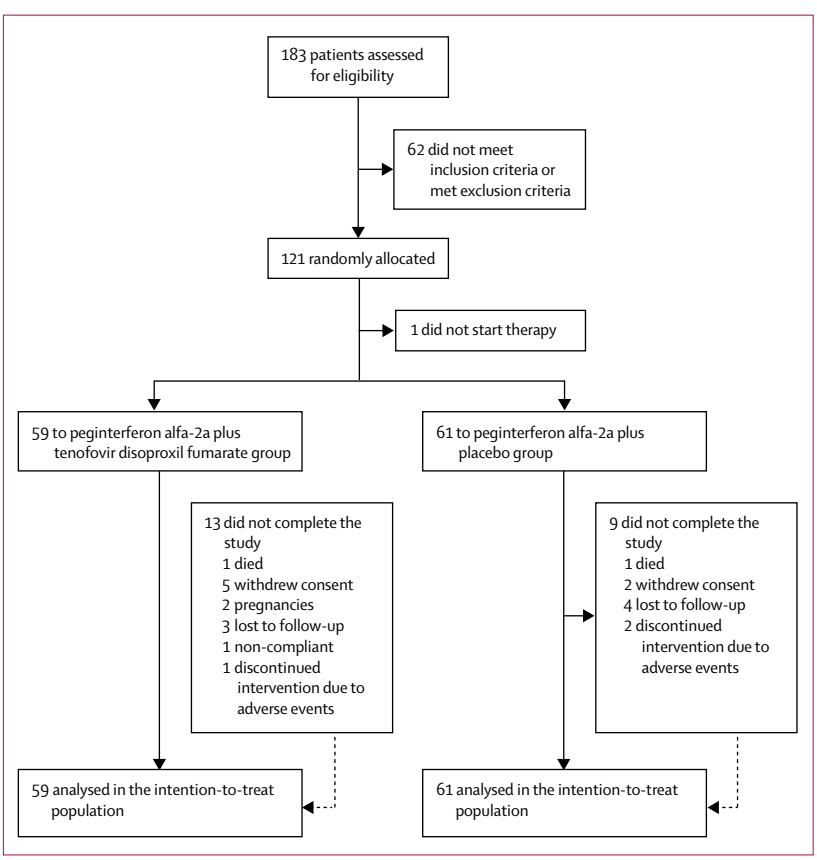

Figure 1: **Trial profile**

	Peginterferon alfa-2a plus TDF (n=59)	Peginterferon alfa-2a plus placebo (n=61)
Age (years)		
Mean (SD)	38·1 (12·3)	42·1 (10·3)
Range	20–64	20–60
Sex		
Male	38 (64%)	41 (67%)
Female	21 (36%)	20 (33%)
HBV DNA		
Negative	2 (3%)	7 (12%)
Mean log_{10} IU/mL (SD)	2·6 (1·3)	3·1 (1·6)
<100 UI/mL	31 (53%)	23 (38%)
>2000 UI/mL	10 (17%)	14 (23%)
HDV RNA		
Negative	1 (1·7%)	0 (0·0%)
<300 copies per mL	2 (3%)	2 (3%)
Mean log_{10} IU/mL (SD)	5·2 (1·0)	4·9 (1·1)
$>10^5$ copies per mL	31 (53%)	34 (56%)
HBsAg		
Mean log_{10} IU/mL (SD)	3·8 (0·5)	3·8 (0·6)
<1000 IU/mL	5 (9%)	6 (10%)
HBeAg		
Positive	12 (20%)	8 (13%)
Missing	7 (12%)	6 (10%)
Anti-HCV		
Positive	5 (9%)	4 (7%)
Missing	1 (2%)	0 (0%)
Alanine aminotransferase		
Mean IU/L (SD)	110·4 (92)	122·0 (74)
>5 times ULN	7 (12%)	4 (7%)
Cirrhosis	23 (39%)	25 (41%)
HDV genotype 1	56 (95%)	60 (99%)
IL28 genotype		
Missing	31 (53%)	34 (56%)
CC	7 (12%)	10 (16%)
CT	16 (27%)	15 (25%)
TT	5 (9%)	2 (3%)
Previous interferon treatment	29 (49%)	31 (51%)

Data are n (%) unless otherwise indicated. TDF=tenofovir disoproxil fumarate. HBV=hepatitis B virus. HDV=hepatitis D virus. HCV=hepatitis C virus. ULN=upper limit of normal.

Table 1: **Baseline characteristics**

alanine aminotransferase levels were defined as increases above ten times the upper limit of normal or increases of more than 2·5 times above baseline or nadir values. An independent international data safety monitoring board reviewed safety data.

Outcomes

The primary efficacy endpoint was the achievement of undetectable levels of HDV RNA at the end of treatment at study week 96 by intention-to-treat analysis. Secondary efficacy endpoints were HDV RNA negativity at treatment week 48 and post-treatment week 24 (study week 120), HBsAg loss, HBsAg declines of more than 0·5 log_{10} from baseline to treatment weeks 48 and 96 anxost-treatment week 24, and alanine aminotransferase normalisation at weeks 48 and 96 and post-treatment week 24. Analysis of HBV DNA levels below 2000 IU per mL and below 100 IU per mL were not specified in the protocol but defined before database lock.

Statistical analysis

Detailed information on the statistical analysis is available in the appendix, including the study protocol. The study includes centres from the EU and Turkey and was done for legislative reasons according to two separate protocols, foreseeing the combined analysis of both regional trials. The sample size of this pilot trial was feasibility driven. The primary efficacy assessment was based on the number of patients testing negative for HDV RNA at the end of treatment. Before the transmission of data to the biostatistics institute details of the analysis have been clarified (ie, the number of patients reaching the primary endpoint; virological, biochemical baseline, and end of treatment data; and adverse event reconciliation, etc) without any knowledge of the data.

Logistic regression was planned as the primary analysis. Analyses were stratified for sex, country, and previous interferon therapy. In line with the intention-to-treat principle in a double-blind clinical trial, all randomly assigned patients who received study medication at least once were included into the analysis. Patients with a missing primary endpoint were categorised as not having a response. In the secondary analysis, the same logistic regression model was applied to the analysis of the HDV RNA negative-patients at week 120. Additionally, further exploratory analyses with HDV RNA negativity at week 96 or week 120 as a dependent variable were done with univariate logistic regression models. Odds ratios and 95% CIs were calculated from these logistic regression models. In all secondary analyses, "p<0·05" and "significant" were used interchangeably. Statistical analyses were done in SAS (version 9.3).

Both HIDIT-II studies (MHH-sponsored HIDIT-II and Roche-Turkey sponsored HIDIT-II) are registered at ClinicalTrials.gov (numbers NCT00932971 and NCT01088659). The study protocol was approved by the respective national competent authorities and was approved by the ethics committees at each participating institution according to applicable national and European law, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use good clinical practice, and the Declaration of Helsinki (EudraCT number 2008-005560-13).

	Baseline	Week 12	Week 24	Week 48	Week 72	Week 96	Week 120
HDV RNA negative							
Peginterferon alfa-2a plus TDF (n=59)	1 (2%)	14 (24%)	21 (36%)	25 (42%)	23 (39%)	28 (48%)	18 (31%)
Peginterferon alfa-2a plus placebo (n=61)	1 (2%)	9 (15%)	18 (30%)	21 (34%)	19 (31%)	20 (33%)	14 (23%)
OR (95% CI), p value	..	1·71 (0·67–4·41), 0·26	1·32 (0·60–2·89), 0·49	1·60 (0·73–3·48), 0·24	1·66 (0·74–3·71), 0·22	1·84 (0.86–3·91), 0·1154	1·46 (0·64–3·31), 0·37
HBsAg decline ≥0·5% from baseline							
Peginterferon alfa-2a plus TDF (n=59)	..	4 (6·8%)	10 (16·9%)	14 (23·7%)	11 (18·6%)	17 (28·8%)	12 (20·3%)
Peginterferon alfa-2a plus placebo (n=61)	..	4 (6·6)	18 (29·5)	15 (24·6)	9 (14·8)	12 (19·7)	14 (23·0%)
OR (95% CI), p value	..	0·90 (0·21–3·90), 0·89	0·40 (0·15–1·03), 0·057	1·08 (0·41–2·86), 0·88	1·60 (0·54–4·67), 0·40	1·74 (0·67–4·51), 0·25	0·85 (0·32–2·26), 0·75
Normal ALT values							
Peginterferon alfa-2a plus TDF (n=59)	8 (14%)	12 (20%)	12 (20%)	18 (31%)	21 (36%)	26 (44%)	27 (46%)
Peginterferon alfa-2a plus placebo (n=61)	3 (5%)	10 (16%)	15 (25%)	16 (26%)	23 (38%)	23 (38%)	16 (26%)
OR (95% CI), p value	3·18 (0·79–12·82), 0·10	1·30 (0·51–3·34), 0·58	0·76 (0·30–1·94), 0·56	1·44 (0·61–3·37), 0·40	1·12 (0·49–2·57), 0·79	1·58 (0·72–3·48), 0·26	3·42 (1·38–8·47), 0·008

HDV=hepatitis D virus. TDF=tenofovir disoproxil fumarate. OR=odds ratio. ALT=alanine aminotransferase.

Table 2: **Virological and biochemical treatment response**

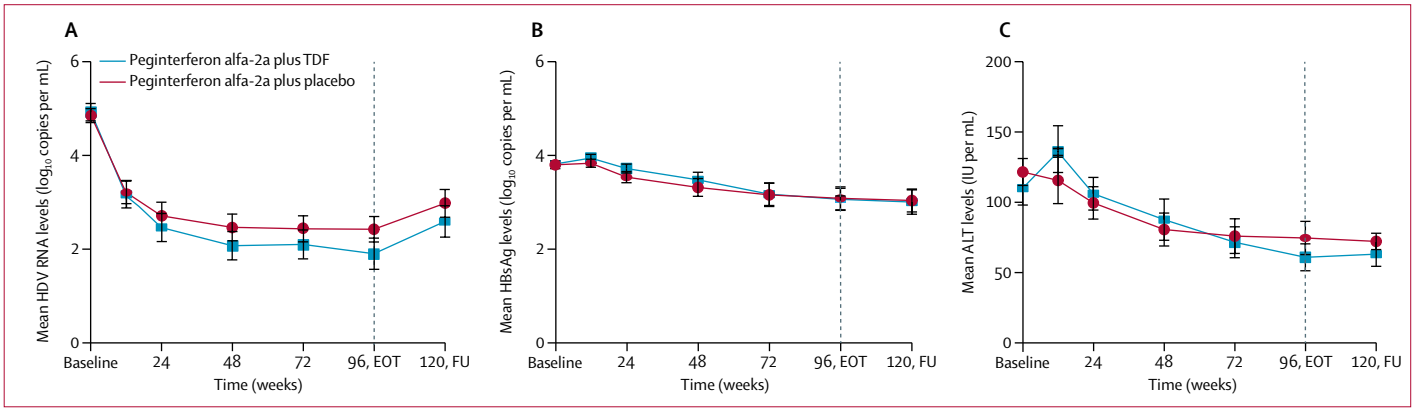

Figure 2: **Virological and biochemical response to treatment, according to treatment group**
(A) Mean HDV RNA levels over time. HDV RNA levels were significantly lower at treatment weeks 48 and 96 than at baseline (p<0·0001 for both groups) but were similar at treatment weeks 48 and 96. (B) Mean HBsAg levels over time. HBsAg levels were significantly lower at treatment weeks 48 (p=0·0001) and 96 (p=0·0002) than at baseline for both groups but were similar at treatment weeks 48 and 96. (C) Mean ALT levels over time. ALT levels were significantly lower at treatment weeks 48 (p=0·0091) and 96 (p<0·0001) than at baseline for both groups and continued to decrease between weeks 48 and 96. ALT=alanine aminotransferase. EOT=end of treatment. FU=follow-up. HDV=hepatitis D virus. TDF=tenofovir disoproxil fumarate.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Lead authors were employees of Hannover Medical School, one of the legal trial sponsors. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Between June 24, 2009, and Feb 28, 2011, we screened 183 patients at 16 centres (in two centres none of the screened patients were eligible for the study); 62 patients were ineligible at screening (figure 1). One patient was randomly assigned but did not start therapy. Baseline characteristics for the remaining 120 patients are shown in table 1. 60 (50%) patients had previously been treated with interferon alfa-based therapies. Liver cirrhosis was present in 48 (41%) patients, mean alanine aminotransferase levels were 116·3 IU/mL (SD 82·74), and 11 (9%) patients had high biochemical disease activity with alanine aminotransferase levels five times above the upper limit of normal. Re-testing of HDV RNA at the central virology laboratory confirmed HDV RNA positivity in 119 patients (one patient tested negative), with mean viral loads of 5·04 log_{10} copies per mL (SD 1·05, range 2·6–7·0 log_{10} copies per mL). HDV genotyping revealed HDV genotype 1 in all but two patients, who had HDV genotype 5. HBeAg was positive in 20 (17%) patients and most had low HBV viral loads with mean HBV DNA

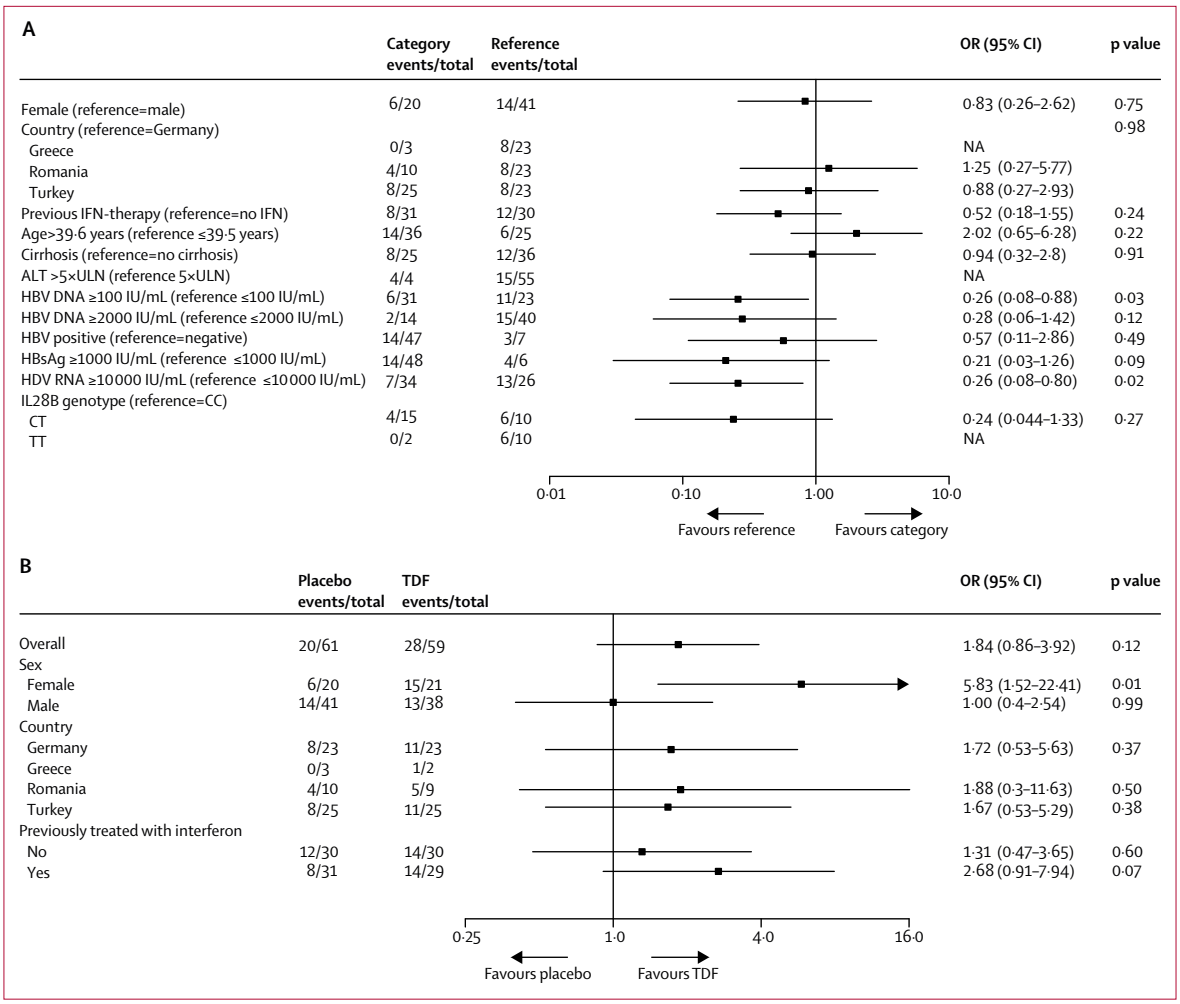

Figure 3: **Factors associated with undetectable HDV RNA results at week 96 and effects of treatment group**
(A) Exploratory analysis of individual factors and associations with undetectable HDV RNA at study week 96. (B) Subgroup analysis for sex, country, and previous interferon treatment (stratification factors) and effects of treatment group. ALT=alanine aminotransferase. HBV=hepatitis B virus. HDV=hepatitis D virus. IFN=interferon. OR=odds ratio. TDF=tenofovir disoproxil fumarate. ULN=upper limit of normal.

levels of 2·8 $\log_{10}$ IU/mL (SD 1·48). 24 (20%) patients had HBV viral loads above 2000 IU/mL. Mean HBsAg levels were 3·81 $\log_{10}$ IU/mL (SD 0·57); 11 (9·2%) patients presented with HBsAg levels below 1000 IU/mL.

97 patients completed 96 weeks of the therapy as well as 24 weeks of follow-up (study week 120). Reasons for not completing 120 study weeks were withdrawal of consent (n=7), non-compliance (n=1), loss-to-follow-up (n=8), death (n=2), adverse events (n=3), or pregnancies (n=2).

The primary endpoint (undetectable HDV RNA at the end of treatment at study week 96) was achieved in 28 (48%) of 59 patients receiving TDF and in 20 (33%) of 61 patients receiving placebo (table 2; odds ratio [OR] 1·84, 95% CI 0·86–3·91, p=0·12). The proportion of all patients testing HDV RNA negative increased during the first 48 weeks of therapy (table 2). Only two patients became HDV RNA negative during the second 48 weeks of treatment (48 [40%] patients were HDV RNA negative at treatment week 96). These findings are in line with changes in mean HDV RNA levels ($\log_{10}$ copies per mL), which decreased from baseline in both treatment groups until week 48 but plateaued thereafter (figure 2A).

Factors associated with response to peginterferon therapy were analysed in patients receiving peginterferon and placebo (figure 3A). Likelihood of response was higher in patients with HDV RNA viral loads above 100 000 copies per mL (OR 0·26, 95% CI 0·08–0·80, p=0·0194) and with HBV DNA levels greater than 100 IU/mL (OR 0·26, 95% CI 0·08–0·88, p=0·0299).

There was no significant difference between the groups for HDV RNA response at week 96 (peginterferon alfa-2a plus TDF group *vs* peginterferon alfa-2a plus placebo group; OR 1·84, 95% CI 0·86–3·91, p=0·12; table 2, figure 2A). Comparing HDV RNA response rates in the two treatment groups did not show significant treatment effects in the subgroup of patients who had previously

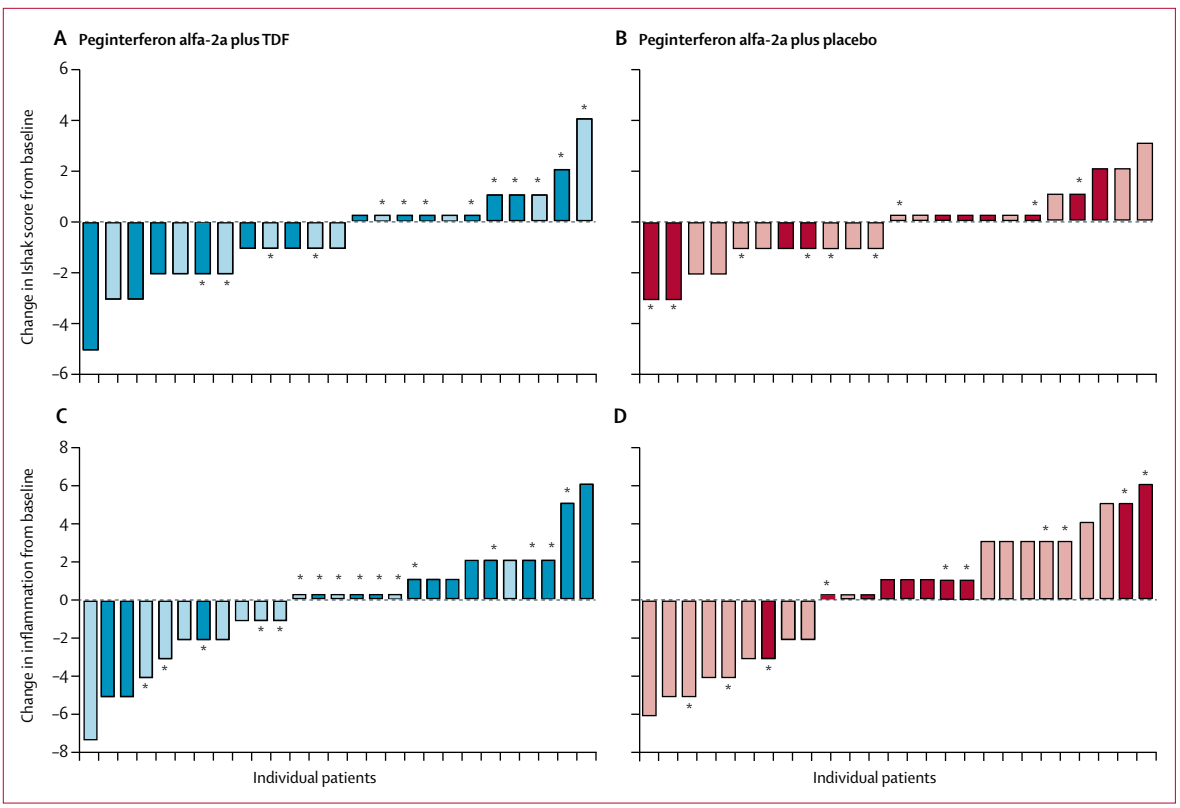

Figure 4: **Histological response to treatment as measured by Ishak fibrosis and activity scores**
(A and B) Individual changes in Ishak fibrosis scores for each patient (n=46) in whom secondary central pathology was possible between pretreatment liver biopsies and end-of-treatment biopsies. (C and D) Changes in histological activity scores (n=53) between pretreatment liver biopsies and end-of-treatment biopsies. Dark blue and dark red columns show patients who are HDV RNA-negative at end of treatment (week 96). HDV=hepatitis D virus. ALT=alanine aminotransferase. TDF=tenofovir disoproxil fumarate. *Patients with normal ALT at week 96.

been treated with interferon and in patients recruited in different countries in the post-hoc analysis (figure 3B). However, women in the TDF group were more likely to achieve an HDV RNA response at treatment week 96 than were women in the placebo group, although this was not the case for male patients (figure 3B).

19 (40%) of 48 patients with an end-of-treatment response and follow-up HDV RNA values available relapsed at study week 120 (11/28 [40%] patients in the peginterferon alfa-2a plus TDF group and eight/20 [40%] patients in the peginterferon alfa-2a plus placebo group). By contrast, three patients spontaneously lost HDV RNA during post-treatment follow-up after being HDV RNA positive at the end of therapy (one patient treated in the TDF group and two patients in the placebo group). Overall, the response rates at post-treatment week 24 were 31% in the TDF group and 23% in the placebo group (OR 1·46, 95% CI 0·64–3·31, p=0·37; table 2). Factors associated with an HDV RNA response at post-treatment week 24 are shown in the appendix. HDV was undetectable at week 96 in 18 (35%) of 52 patients who required peginterferon dose reduction during the trial and in 30 (45%) of 67 patients who maintained the peginterferon alfa dose.

A decline in HBsAg levels of at least 0·5 $\log_{10}$ IU/mL compared with baseline values was achieved in 29 (24%) patients at the end of treatment, with no significant difference between groups (OR 1·74, 95% CI 0·67–4·51, p=0·25) and in 26 (31·3%) at post-treatment week 24 with no significant difference between groups. A decline of greater than 0·5 $\log_{10}$ IU/mL was found in 29 (24%) of 120 patients (OR 0·85, 95% CI 0·32–2·26, p=0·75; table 2). There was no statistical difference in mean HBsAg levels between the two groups at any time during the study (figure 2B). Overall, there was a significant HBsAg decline of 0·37 $\log_{10}$ IU/mL from baseline to treatment week 48 (p=0·0101). During the second half of therapy HBsAg levels declined only slightly further, with mean HBsAg levels of 3·50 (SD 1·02) $\log_{10}$ IU/mL at week 48 and 3·48 (1·06) $\log_{10}$ IU/mL at week 96 (p=0·0200), which also did not change after therapy once peginterferon alfa-2a was stopped (figure 2B). Notably, there were no statistical differences in mean HBsAg levels nor relative declines from baseline between the two study groups at any timepoint during the study (table 2, figure 2B). Seven patients lost HBsAg during therapy (two patients in the TDF group and five in the placebo group). At

	Peginterferon alfa-2a plus placebo (n=61)	Peginterferon alfa-2a plus TDF (n=59)
Total adverse events	485	459
Patients with at least one adverse event	59	52
Type of adverse event		
Blood and lymphatic system disorders	19 (31·1%)	21 (35·6%)
Anaemia	6 (9·8%)	8 (13·6%)
Leucopenia	7 (11·5%)	3 (5·1%)
Neutropenia	6 (9·8%)	4 (6·8%)
Thrombocytopenia	12 (19·7%)	12 (20·3%)
Other	1 (1·6%)	2 (3·4%)
Endocrine disorders	4 (6·6%)	3 (5·1%)
Eye disorders	9 (14·8%)	6 (10·2%)
Gastrointestinal disorders	31 (50·8)	26 (44·1%)
Abdominal pain	11 (18·0%)	13 (22·0%)
Diarrhoea	6 (9·8%)	4 (6·8%)
Dry mouth	4 (6·6%)	2 (3·4%)
Dyspepsia	5 (8·2%)	6 (10·2%)
Nausea	12 (19·7%)	10 (16·9%)
Other	16 (26·2%)	24 (40·7%)
General disorders and administration site conditions	43 (70·5%)	41 (69·5%)
Asthenia	12 (19·7%)	7 (11·9%)
Fatigue	12 (19·7%)	14 (23·7%)
Influenza-like illness	26 (42·6%)	25 (42·4%)
Pyrexia	12 (19·7%)	14 (23·7%)
Other	9 (14·8%)	11 (18·6%)
Hepatobiliary disorders	6 (9·8%)	3 (5·1%)
Immune system disorders	1 (1·6%)	5 (8·5%)
Infections and infestations	21 (34·4%)	23 (39·0%)
Investigations	11 (18·0%)	14 (23·7%)
γ-glutamyltransferase increased	2 (3·3%)	2 (3·4%)
Aminotransferases increased	5 (8·2%)	10 (16·9%)
Weight decreased	3 (4·9%)	3 (5·1%)
Other	5 (8·2%)	4 (6·8%)
(Continued from previous column)		
Metabolism and nutrition disorders	19 (31·1%)	14 (23·7%)
Decreased appetite	12 (19·7%)	13 (22·0%)
Other	6 (9·8%)	1 (1·7%)
Musculoskeletal and connective tissue disorders	25 (41·0%)	23 (39·0%)
Nervous system disorders	19 (31·1%)	22 (37·3%)
Dizziness	6 (9·8%)	5 (8·5%)
Headache	13 (21·3%)	15 (25·4%)
Other	2 (3·3%)	12 (20·3%)
Psychiatric disorders	15 (24·6%)	14 (23·7%)
Reproductive system and breast disorders	6 (9·8%)	3 (5·1%)
Respiratory, thoracic, and mediastinal disorders	16 (26·2%)	10 (16·9%)
Cough	5 (8·2%)	2 (3·4%)
Epistaxis	6 (9·8%)	5 (8·5%)
Oropharyngeal pain	5 (8·2%)	2 (3·4%)
Other	4 (6·6%)	3 (5·1%)
Skin and subcutaneous tissue disorders	27 (44·3%)	15 (25·4%)
Alopecia	7 (11·5%)	6 (10·2%)
Pruritus	13 (21·3%)	11 (18·6%)
Other	26 (42·6%)	13 (22·0%)

TDF=tenofovir disoproxil fumarate.

Table 3: **Adverse events**

post-treatment week 24, two patients in the placebo group became HBsAg-positive again, while one patient in the TDF group lost HBsAg. Of the six patients who were HBsAg-negative at post-treatment week 24, four had also seroconverted to anti-HBs. Individual characteristics of patients who lost HBsAg are provided in the appendix.

The proportion of patients whose alanine aminotransferase levels returned to normal gradually increased during therapy. At the end of treatment 49 (41%) of 120 patients had normal alanine aminotransferase levels (26/59 [44·1%] in the TDF group and 23/61 [38%] in the placebo group, p=0·30; table 2). Proportions of patients who normalised their alanine aminotransferase levels at week 46 and 6 months post-treatment were similar in the two treatment groups (table 2). Similarly, mean alanine aminotransferase values decreased from baseline at study weeks 48, 96, and 120 in both study groups (figure 2C). Post-treatment increases of alanine aminotransferase of at least two-times above end-of-treatment values occurred in 17 (14·2%) of 120 patients (five/59 [8·5%] in the TDF group and 12/61 [19·7%] in the placebo group, p=0·12) with no differences between cirrhotic and non-cirrhotic patients (data not shown).

HBV DNA was suppressed to levels below 100 IU/mL more often in patients in the peginterferon alfa-2a plus TDF group than in patients in the peginterferon alfa-2a plus placebo group (51/59 [86%] patients *vs* 43/61 [71%] patients at treatment week 96; OR 2·67, 95% CI 1·06– 6·74, p<0·0032; appendix). None of the patients in the TDF group and seven (12%) in the placebo group had HBV viral loads above 2000 IU/mL at the end of treatment. At post-treatment week 24, HBV DNA levels remained suppressed (<100 IU/mL) in 29 (49%) patients in the TDF group and 38 patients (62%) in the placebo group. High viral loads (>2000 IU/mL) were detectable in

17 (14%) patients at post-treatment week 24 (11 in the TDF group and six in the placebo group). However, at this point, 11 patients were prescribed TDF medication at the discretion of the local investigator (TDF prescriptions were initiated between study weeks 108 and 120). Changes in mean HBV DNA viral loads are shown in the appendix.

Paired liver biopsies from before treatment and end of therapy that were sufficient for histological grading and staging were available for 65 (54%) patients. Fibrosis scores in patients, in whom secondary central pathology was possible, remained stable or even improved in most patients (36 [78%]) and only ten patients showed a worsening of fibrosis scores (figure 4A, 4B). Grouping liver biopsy readings as mild, moderate, or severe fibrosis showed a significant improvement of liver fibrosis in end-of-treatment biopsies ($p<0·0057$, data not shown). By contrast, histological disease activity was unchanged between pretreatment and end-of-treatment samples (figure 4C, 4D). There were no differences between treatment groups in histological outcomes (data not shown).

Of the 120 patients included in the study, 22 patients (19%; nine in the peginterferon alfa-2a plus placebo group and 13 in the peginterferon alfa-2a plus TDF group) prematurely discontinued the study before study week 120. Two (2%) patients died (one patient in each study group); one patient had a mitral valve rupture after 48 weeks of treatment and the other patient died from pneumonia and sepsis at treatment week 121. Both patients had compensated liver cirrhosis at baseline and fatal events were judged as possibly related to peginterferon alfa-2a by the investigator. The remaining 98 (80%) patients completed 120 study weeks. Overall, 111 patients reported at least one adverse event and a total of 944 adverse events were recorded (459 in patients in the TDF group and 485 in the placebo group; table 3). The adverse events were mainly those typically associated with interferon alfa treatment. The most common adverse events were haematological, behavioural (eg, such as fatigue), musculoskeletal, influenza-like syndromes, and psychiatric complaints. A total of 45 serious adverse events occurred in 31 (26%) patients (25 in the TDF group and six in the placebo group, the most common being elevated transaminases and thrombocytopenia); we considered 18 serious adverse events as possibly being related to the study drugs (eight in the placebo group and nine in the TDF group). No serious adverse event could be attributed to TDF (appendix).

Transient flares of alanine aminotransferase levels occurred in 28 patients (16 in the TDF group and 12 in the placebo group), mainly between weeks 4 and 24 of peginterferon alfa-2a therapy. Seven (24%) of 29 patients with cirrhosis had alanine aminotransferase flares. These flares were similar in magnitude to those in patients without cirrhosis (21/24 [24%]). All alanine aminotransferase flares resolved spontaneously and required transient dose adjustments of peginterferon alfa-2a in only nine patients. Only one patient who had liver cirrhosis developed elevated international normalised ratio (1·5–1·7) and bilirubin concentrations (4·2 mg/dL) after an alanine aminotransferase flare. Isolated bilirubin increases above 3·0 mg/dL were observed in two additional patients (3·3 mg/dL in one patient with cirrhosis and 4·4 mg/dL in one patient without cirrhosis at baseline).

Discussion

Treatment of hepatitis D is a major challenge. Peginterferon alfa is recommended by most national and international guidelines[16] because only interferon alfa has been shown to have some antiviral efficacy against HDV.[9,17] However, the optimal duration of peginterferon treatment as well as the possible usefulness of combination therapies with HBV polymerase inhibitors are not well defined. Our results show that: (1) extending peginterferon alfa-2a therapy to 96 weeks is possible in most patients, with an acceptable safety profile leading to rates of on-treatment HDV RNA suppression of about 40%, (2) prolonged administration of peginterferon alfa-2a is associated with stabilisation or even improvement in histological fibrosis scores in most patients (figure 4), (3) HDV RNA relapses after treatment occur in more than a third of responding patients, despite prolonged antiviral therapy, and (4) combination therapy with TDF does not increase HBsAg decline in patients with hepatitis D.

Peginterferon alfa therapies have been used to treat hepatitis B or C for more than 25 years and recommended treatment durations are usually 24–48 weeks. Longer treatment with interferon has been suggested for hepatitis D to achieve HBsAg loss in addition to HDV RNA undetectability.[9] However, extended peginterferon therapy has been possible in only selected patients in previous studies.[18] We show that up to 80% of patients with HDV could be treated for at least 96 weeks with an acceptable safety profile. This extended treatment was possible even though half of the patients had liver cirrhosis, in whom interferon alfa is usually less well tolerated. However, two patients died during peginterferon alfa-2a therapy, with events possibly being related to peginterferon alfa-2a therapy. In the HALT-C trial,[19] which explored 3·5 years of low peginterferon therapy in patients with hepatitis C, excess short-term mortality caused by peginterferon could not be excluded.

In the HIDIT-I trial, 24% of patients became HDV RNA negative during 48 weeks of therapy in the intention-to-treat analysis.[11] In HIDIT-II, 38% of patients were HDV RNA negative at treatment week 48. Extending peginterferon alfa-2a treatment to 96 weeks only slightly increased HDV RNA response rates and mean HDV RNA levels almost plateaued during the second year of therapy. These findings suggest that prolonged treatment

beyond 48 weeks is usually not needed if only suppression of HDV RNA is the main treatment goal.

An important finding of our trial was that 96 weeks of peginterferon alfa-2a treatment was associated with an improvement in histological fibrosis scores, which contrasts with our previous findings from 1 year of treatment in HIDIT-I.[11] Prolonged peginterferon alfa-2a therapy was also associated with a reduction in liver fibrosis in most patients with cirrhosis and hepatitis C.[20] We provide evidence to suggest that similar effects might be observed in HDV infection, even though no clear association with virological and biochemical responses could be established. These findings are in line with studies suggesting that interferon-based therapies of hepatitis D might translate into improved long-term clinical outcomes.[21,22] Still, studies are needed to establish correlations between virological and biochemical responses and histological outcomes.

Previous exposure to interferon alfa was not a negative response predictor, which somewhat contrasts with results for hepatitis C virus (HCV) infection, for which peginterferon re-treatment of interferon non-responder patients is ineffective. Thus, mechanisms of interferon treatment failure might be different between HCV and HDV. This difference is supported by the observation that—again, by contrast with hepatitis C—*IFNL3* genotyping did not identify treatment responders in this trial. Moreover, the presence of liver cirrhosis correlated with an increased likelihood for an HDV RNA response at post-treatment week 24. In hepatitis C, the presence of liver cirrhosis is strongly associated with reduced antiviral treatment efficacy. However, hepatitis B patients with advanced fibrosis or compensated cirrhosis more frequently showed a virological response to peginterferon alfa therapy,[23] which would be in line with our observations in hepatitis D. From a clinical perspective, retreatment of patients with hepatitis D should be considered if peginterferon treatment was tolerated and if progression of liver disease is likely. In this respect, calculation of the baseline event anticipation score might be helpful to identify which patients are at a higher risk of clinical complications of liver disease.[5]

There was no significant difference between the peginterferon alfa-2a with TDF group and the peginterferon alfa-2a alone group in end-of-treatment HDV RNA responses. Women seemed to benefit from TDF therapy, whereas men did not. As HDV does not encode for its own viral enzymes, TDF is unlikely to have any direct antiviral effect on HDV replication. This assumption is supported by our observation that monotherapy with adefovir, the other approved nucleotide analogue against HBV, was ineffective against HDV.[11] Similarly, an analysis of patients co-infected with HIV–HBV–HDV who received tenofovir-containing antiretroviral therapy for HIV showed that most patients had stable HDV RNA levels during long-term TDF therapy.[24] An alternative explanation for the non-significant increased HDV RNA response could be that TDF could have immunomodulatory properties[25] and could also induce an interferon lambda response, as shown in patients with HBV and HIV,[26] which in turn could have increased suppression of HDV RNA. Indeed, a study in humanised mice showed that HDV is sensitive to interferon lambda.[27] Why these effects should be evident in women but not in men—which could be due to chance—is unclear and requires further investigation.

We found no difference in HBsAg declines between the two study groups; however, peginterferon alfa plus adefovir was slightly more effective in reducing HBsAg levels than was peginterferon alfa alone in the HIDIT-I trial.[11] However, in HBV monoinfection, peginterferon alfa plus TDF was more effective regarding HBsAg loss than was peginterferon alfa monotherapy[13] (although in absolute numbers this difference was minor). Thus, combination therapy of peginterferon alfa plus HBV nucleotide analogous does not seem to greatly increase HBsAg seroclearance and can therefore not be recommended as standard of care. However, this trial might provide an opportunity to investigate factors associated with HBsAg loss in more detail, including flares in alanine aminotransferase during or after therapy. The role of alanine aminotransferase flares during peginterferon treatment of hepatitis D is being investigated in more detail in a subanalysis of the HIDIT-I and HIDIT-II studies.

Limitations of this trial need to be considered. We did not directly compare 48 weeks with 96 weeks peginterferon alfa-2a treatment. Also, not all patients agreed to undergo a second liver biopsy at the end of therapy and the findings on the effect of peginterferon alfa-2a therapy on histological grading and staging might therefore be biased, in that patients with alanine aminotransferase normalisation could have been more motivated to accept a second liver biopsy than were those without. Other intrahepatic markers of HBV activity such as HBV covalently closed circular DNA levels were not investigated. Finally, the study included almost exclusively patients with HDV genotype 1 infection and the findings cannot necessarily be transferred to individuals infected with other HDV genotypes. The efficacy of peginterferon might therefore be different in patients with hepatitis D in endemic areas such as the Amazonas region, sub-Saharan Africa, or eastern Asia and in patients with other routes of transmission because reliable information on the route of HDV acquisition was not available.

This study highlights limitations of peginterferon therapy in patients with hepatitis D. Thus, alternative treatment options for HDV infection are urgently needed. Novel treatment approaches, including prenylation inhibition,[28] blockade of particle secretion by nucleic acid polymers,[29] or HBV and HDV entry

inhibition,[30] are in clinical development. Still, the HIDIT-II trial is highly relevant for future treatment concepts because peginterferon alfa will probably be used as backbone therapy for upcoming future therapies.

In conclusion, this pilot trial showed that extended peginterferon alfa-2a treatment for more than 1 year is possible in most patients with hepatitis D but prolonged administration of peginterferon alfa-2a for 96 weeks does prevent post-treatment HDV RNA relapse. Combination therapy with TDF did not significantly affect end-of-treatment response rates but the clinical meaning is unclear. We suggest that until alternative treatment options become available, 48 weeks of peginterferon alfa-2a therapy remains the standard of care for hepatitis D for most patients with HDV. Individualised treatment durations could be considered in the subgroup of patients who show a pronounced HBsAg decline with the aim to induce HBsAg loss.

Contributions

The study was planned and initiated by HW, CY, and MPM. The study protocol was written, reviewed, and amended by HW, CY, MPM, IM, BH, UW, HvL, and SH. Central virological testing was coordinated by HW, IM, BH, and SH. Statistical analysis was done by KW, AK, BH, and HW. Central histological reading of liver biopsy slides was done by JS and HPD. Patients were recruited and treated by HW, CY, FAC, MGC, KY, USA, SG, SZ, AE, SL, GVP, OK, KP, MR, MKC, RI, MC, IM, BH, and MPM. The first version of the manuscript was written by HW with the support of SH, MC, and BH. Additional versions of the paper were first reviewed by AK, CY, MC, SH, and MPM and subsequently by all other authors.

Declaration of interests

HW reports honoraria for speaking or consulting from Abbott, Abbvie, BMS, Boehringer Ingelheim, Eiger, Gilead Sciences, Janssen, Merck Sharp & Dohme, Myr GmbH, Novartis, Novira, Roche Diagnostics, Roche, Siemens, and Transgene, and research support from Abbott, BMS, Gilead Sciences, Novartis, Roche Diagnostics, and Roche. CY reports honoraria from, Abbvie, Eiger, Gilead Sciences, Merck Sharp & Dohme, Bristol-Myers Squibb, and Janssen, and research grant from Roche, Bristol-Myers Squibb, and Eiger. FAC reports personal fees and non-financial support from Abbvie and Gilead Sciences. SG reports personal fees from Abbvie, and Gilead Sciences. SZ reports honoraria for speaking or consulting from Abbvie, Gilead Sciences, Janssen, Merck Sharp & Dohme, and Intercept. GVP reports honoraria for speaking or consulting from Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, Novartis, and Roche, and research support from BMS and Gilead Sciences. MR reports non-financial support from Abbvie and Gilead Sciences. MC reports honoraria for speaking or consulting from Abbvie, Bristol-Myers Squibb, Boehringer Ingelheim, Biogen, Gilead Sciences, Falk, Janssen, Merck Sharp & Dohme, Roche Diagnostics, Roche, and Siemens. MPM reports honoraria for speaking or consulting from Eiger, Gilead Sciences, Roche, Novartis. All other authors declare no competing interests.

Acknowledgments

The study was supported by the HepNet Study-House (a project of the German Liver Foundation founded by the German Liver Foundation, the German Ministry for Education and Research, and the German Center for Infectious Disease Research), Hoffmann-La Roche, and Gilead Sciences. We thank all HIDIT-II study team members, which included Birgit Bremer, Martina Darnedde, Regina Raupach, and Patrick Lehmann (Central Virology Lab, Hannover Medical School), Undine Baum, Kathrin Peter, Michael Wöbse, and Lewon Lutterkort (HepNet Study-House), the Hannover Clinical Trial Center HIDIT-II project team, Ulrich Alshuth (F Hoffmann La-Roche Germany), and all study nurses, trial coordinators, and sub-investigators involved in the trial. We express our very sincere appreciation and gratitude to all patients who gave informed consent to participate in the study.

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