Research Article

EASL JOURNAL OF HEPATOLOGY

Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: First results of a phase Ib/IIa study

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Background & Aims: The therapeutic option for patients with chronic hepatitis delta virus infection (CHD) is limited to interferon alpha with rare curative outcome. Myrcludex B is a first-in-class entry inhibitor inactivating the hepatitis B virus (HBV) and hepatitis D virus (HDV) receptor sodium taurocholate co-transporting polypeptide. We report the interim results of a pilot trial on chronically infected HDV patients treated with myrcludex B, or pegylated interferon alpha (PegIFNα-2a) or their combination.

Methods: Twenty-four patients with CHD infection were equally randomized (1:1:1) to receive myrcludex B, or PegIFNα-2a or their combination. Patients were evaluated for virological and biochemical response and tolerability of the study drugs at weeks 12 and 24.

Results: Myrcludex B was well tolerated and no serious adverse event occurred. Although hepatitis B surface antigen levels remained unchanged, HDV RNA significantly declined at week 24 in all cohorts. HDV RNA became negative in two patients each in the Myrcludex B and PegIFNα-2a cohorts, and in five patients of the Myrcludex B + PegIFNα-2a cohort. ALT decreased significantly in the Myrcludex B cohort (six of eight patients), and HBV DNA was significantly reduced at week 24 in the Myrcludex B + PegIFNα-2a cohort. Virus kinetic modeling suggested a strong synergistic effect of myrcludex B and PegIFNα-2a on both HDV and HBV.

Conclusions: Myrcludex B showed a strong effect on HDV RNA serum levels and induced ALT normalization under monotherapy. Synergistic antiviral effects on HDV RNA and HBV DNA in the Myr-IFN cohort indicated a benefit of the combination of entry inhibition with PegIFNα-2a to treat CHD patients.

Lay summary: Myrcludex B is a new drug to treat hepatitis B and D infection. After 24 weeks of treatment with myrcludex B and/or pegylated interferon α-2a, HDV R NA, a relevant marker for hepatitis D infection, decreased in all patients with chronic hepatitis B and D. Two of eight patients which received either myrcludex B or pegylated interferon α-2a, became negative for HDV RNA, and five of seven patients who received both drugs at the same time became negative. The drug was well tolerated.

Keywords: Chronic hepatitis B; Entry inhibitor; Hepatitis B virus receptor; Hepatitis B treatment; Hepatitis D; Hepatitis D treatment; Myrcludex B; Sodium taurocholate co-transporting polypeptide (NTCP; SLC10A1); Virus kinetic modeling.

Received 23 December 2015; received in revised form 19 March 2016; accepted 19 April 2016; available online 27 April 2016

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, bovine serum albumin; blq, below limit of quantification; cccDNA, covalently closed circular deoxyribonucleic acid; CHB, chronic hepatitis B; CHD, chronic hepatitis D; d, hepatocyte death rate; DNA, deoxyribonucleic acid; HBeAg, HBe antigen; HBsAg, HBs antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; IFN, interferon; lod, limit of detection; loq, limit of quantification; NOEL, no observed effect level; NLME, non-linear mixed effects; NTCP, sodium taurocholate co-transporting polypeptide; PCR, polymerase chain reaction; PegIFNα-2a, pegylated interferon α-2a; R0, basic reproductive ratio; RNA, ribonucleic acid; s, hepatocyte production rate; SC, subcutaneously; ULN, upper limit of normal.

Introduction

The treatment of chronic hepatitis delta (CHD) infection is an area of unmet medical need. Hepatitis D virus (HDV), the

causative agent of CHD, is a small, viroid-like single stranded, circular ribonucleic acid (RNA) virus, which acquires the envelope proteins from Hepatitis B virus (HBV) infected cells to assemble and disseminate [1,2]. An estimated 15 to 20 million patients worldwide are infected with HDV via parenteral routes of infection, sexual transmission or contact with infectious blood. The prevalence of HDV is increasing, and there is presently no cure for CHD [3–7]. Co-Infections of HBV and HDV occur either simultaneously resulting in severe and occasionally fulminant hepatitis, with a high rate of HBsAg seroconversion and elimination of both viruses following recovery. Alternatively, HDV occurs as superinfection in individuals who already suffer from CHB. The clinical outcome of superinfection is characterized by a high rate of HDV chronification and worsening of the disease with accelerated progression to liver cirrhosis and hepatocellular carcinoma [3,8].

Vaccination against HBV is the most effective way to protect against HDV and has helped to limit its prevalence in those countries where vaccination programs have been successfully implemented [7]. Current treatment options for CHD are sparse with no approved agents specifically addressing HDV replication. Currently approved drugs only indirectly interfere with virus replication by modulation of the immune system or by influencing the concurring HBV replication [2,3,8–11]. In eligible patients, the first line therapy is treatment with pegylated interferon alpha (PegIFNα) for 12 months or longer, if tolerated. Sustained virological response has been observed to various extents in treated patients; however, response rates rarely exceed 25%. Longer treatment, combination or alternative treatment with nucleoside analogues such as adefovir, lamivudine, or entecavir did not increase response rates; only tenofovir showed some effect on HDV serum RNA in HIV co-infected patients with improvement in liver fibrosis [6,10,12–18]. Moreover, late relapses were demonstrated in patients who initially achieved sustained response to IFN therapy and who were HDV RNA negative 24 weeks after the end of treatment. This indicated that the virus could not be eliminated by this drug [11].

Novel early clinical treatment approaches for CHD are currently being studied: lonafarnib, a prenylation inhibitor originally tested for its antineoplastic potency, affects the post-translational modification of the large HDV antigen and thereby interferes with the envelopment and release of the viral ribonucleoprotein [19,20]. Recent clinical data revealed that lonafarnib dose-dependently reduced HDV serum RNA levels [21]. Another approach is based on intravenous administration of highly negatively charged nucleic acid polymers that interfere with the attachment of HBV/HDV to heparan sulfate proteoglycans [22]. This interaction is required prior to specific receptor binding. An additional effect of these drugs might be attributed to a putative effect on virus assembly [23,24].

A promising approach used in our study aims at specific inhibition of the essential hepatic HBV and HDV virus receptor sodium taurocholate co-transporting polypeptide (NTCP) [25], by an optimized HBV envelope protein-derived lipopeptide, myrcludex B. This approach addresses a crucial and highly specific early step in the life cycle of HDV and HBV. According to its mode of action, myrcludex B blocks cccDNA formation and formation of HDV replicative intermediates in naïve or non-infected regenerated hepatocytes. Depending on the turnover dynamics of HDV and/or HBV infected cells (by either immune mediated cell killing, cytolytic effects of replicating HDV, or natural cell death) this would result in an overall decrease of infected cells. Thus, continuous administration of myrcludex B contributes to a decrease of the fraction of infected cells, which in the long-term may eventually lead to eradication of the infection [3,4,6,8,26,27]. This principle that continuous entry inhibition even in an immune deficient animal model can lead to clearance of infection in the absence of any direct antiviral acting agents, has recently been proven in an animal model for hepatitis C virus (HCV) infection [28].

Myrcludex B is a myristoylated peptide of 47 amino acids derived from the preS1-domain of the HBV large surface (L-) protein. It efficiently blocks entry of HBV and HDV with IC_{50}s of about 80 pM in primary human hepatocyte cultures, in cell lines, and *in vivo* in a humanized mouse model [26,29–34]. A first assessment on safety and pharmacokinetics of myrcludex B has been carried out in a dose escalating trial in healthy volunteers. The drug was excellently tolerated and pharmacokinetics followed a target mediated drug disposition [35]. In this paper we report the interim findings at week 24 of a pilot study in patients with CHB/CHD co-infection, who were subcutaneously (SC) treated with myrcludex B, PegIFNα-2a, or their combination.

Patients and methods

Study design and setting

This pilot study was a sub-study of a phase Ib/IIa randomized, open-label clinical trial of daily myrcludex B *vs.* entecavir administration in patients with CHB. The study was registered and approved by the competent authority, the Russian Ministry of Health (registration and authorization number 736/November 29th 2013) and was approved by the Ethics Council of the Ministry of Health of the Russian Federation" (Ethics Council approval #73, November 19th 2013) and the Ethics Committee of the Centrosoyuz Clinical Hospital (ethics committee approval #15, February 7, 2014). The study is registered at clinicaltrials.gov under NCT02637999.

A single center, randomized, 3-arm, parallel, open-label clinical trial was performed at Centrosoyuz Clinical Hospital in Moscow. The trial followed the principles of good clinical practice and was carried out in accordance with the ethical principles of the pertinent version of the Declaration of Helsinki. The objective of this study was to evaluate the effect of myrcludex B in patients with CHD infection either administered alone or in combination with PegIFNα-2a as compared to patients only treated with PegIFNα-2a. The primary endpoint was the HBsAg response at week 12 of therapy. HBsAg response was defined as serum HBsAg decline of at least 0.5 logs IU/ml at any time during the study. Secondary endpoints were the responses of HBsAg (24 weeks), HDV RNA, HBV DNA, and of alanine aminotransferase (ALT) to therapy at 24 and 48 weeks of therapy and at the end of the treatment-free 24 weeks follow up period. Safety analyses included the monitoring of adverse events, the assessment of immunogenicity of myrcludex B, possible interactions with PegIFNα-2a, and the assessment of bile acid levels. After obtaining written informed consent, each participant was screened and patients were equally randomized (1:1:1) into three cohorts. One cohort received 2 mg myrcludex B daily (SC) for 24 weeks followed by 180 μg PegIFNα-2a (SC) weekly for 48 weeks (Myr cohort). A second cohort received 2 mg myrcludex B daily (SC) in combination with 180 μg PegIFNα-2a weekly (SC) for 24 weeks followed by 24 weeks of PegIFNα-2a alone (Myr-IFN cohort). And finally there was a cohort which received 180 μg PegIFNα-2a weekly (SC) without co-medication of myrcludex B (INF cohort). Patients in the Myr and the Myr-IFN cohort received myrcludex B for the first 24 weeks. Each patient in the study received a total of 48 weeks of PegIFNα-2a. Hence, the overall duration of treatment was 72 weeks in the Myr cohort and 48 weeks in the Myr-IFN and IFN cohort (Fig. 1). The treatment duration for myrcludex B was chosen to allow the assessment of first pilot efficacy result without exposing patients for an extended period to a drug of unproven efficacy and safety. The patients visited the site for clinical and laboratory assessments 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 weeks after the baseline visit. A follow up was planned 6 months after the end of treatment. The estimation of efficacy parameters was planned to be performed after 24, 48 weeks of therapy and after the end of follow up. This paper presents the results of the 12 and 24 week interim assessments while the trial is still ongoing.

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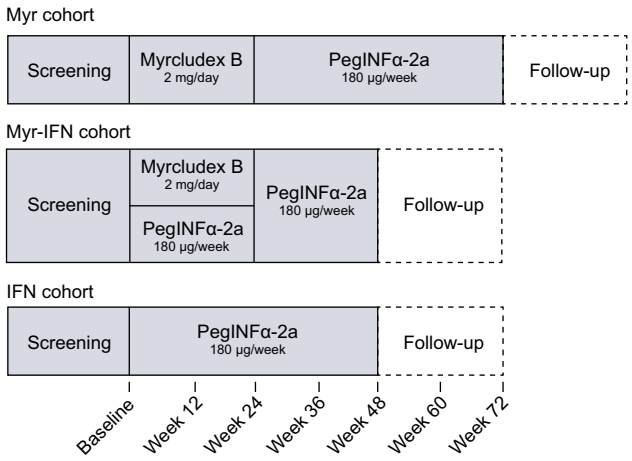

Fig. 1. Cohorts and respective treatment schedules of the trial. (myr, myrcludex B; PegIFNα-2a, pegylated interferon alpha 2a; Scr, screening).

Patient population

Patients between 18 to 65 years of age were eligible if they had CHB (presence of HBsAg for at least 6 months before screening), were HBeAg negative or anti-HBeAg positive, and co-infected with HDV (positive anti-HDV antibodies for at least 3 months and positive HDV RNA at screening). Detailed inclusion and exclusion criteria are described in the Supplementary material.

Study drugs

Myrcludex B was provided as dry powder (lyophilisate) in vials of 1 mg by Bachem (Bubendorf, Switzerland). Vials were reconstituted in 1 ml water for injection to reach a final concentration of 1 mg/ml. The 2 mg dose was administered once daily as two consecutive SC injections, each containing 1 mg peptide. PegIFNα-2a (Pegasys®, Roche, manufactured in Switzerland, labeled in Russia) was supplied by prescription from the treating physician, the recommended dose was 180 µg/week. Depending on individual tolerability of the drug and clinical situation PegIFNα-2a doses could be reduced according to the manufacturers recommendations (e.g. for low absolute neutrophil count, low platelet count, or elevated serum ALT). Patients received the first dose in an in-patient period of 48 h and were trained to self-administer both drugs subsequently.

Myrcludex B dose selection

The myrcludex B dose was selected considering the data from *in vitro* binding studies, *in vivo* pharmacology, toxicology and the pharmacokinetic studies performed in animal models. In addition pharmacokinetic and tolerability data from the recently performed first-in-human study were considered [35]. The study was designed to yield myrcludex B plasma concentrations of >0.8 ng/ml at steady-state, a concentration which was previously calculated to lead to a 50% hepatic receptor occupation in chimpanzees (unpublished data). A minimum dose of 1 mg SC daily was estimated to reach this goal and a dose of 2 mg daily was selected. This dose was approximately 90 times lower than the dose, which was defined as the 'no observed effect level' (NOEL) of 2.5 mg/kg in both rat and dog (i.e., 2 mg/70 kg resulting in ~0.028 mg/kg). Doses above 2 mg had already been proven to be well tolerated in the first-in-human trial in healthy volunteers [35,36].

Laboratory assessments

HBsAg and HBV DNA were measured at the central laboratory of the Heidelberg University Hospital using commercially available assays (Chemilum. Mikroparticle Immuno Assay, and Abbott RealTime HBV, both Abbott, Ludwigshafen, Germany). Limit of detection (lod) for HBV DNA was 34 copies/ml. For calculations values below lod were set to lod/2. ALT was measured at the study site laboratory by multifunctional biochemical analyzer (Olympus, Hamburg, Germany). The normal value for ALT was defined ⩽40 IU/L for male and female patients.

HDV RNA measurements

HDV RNA and HDV antigen antibodies at screening were determined by commercially available assays at the study center's local laboratory (Amplisense® HDV-FL, Central Research Institute of Epidemiology, Moscow, Russia; Vectohep-D IgM, Vector Best, Moscow, Russia). HDV RNA levels for efficacy analyses were measured by a quantitative PCR assay at the central laboratory of the Hannover Medical School, Germany [37]. Limit of detection (lod) for HDV RNA was 15 copies/ml. For calculations values below lod were set to lod/2.

Virus kinetic modeling

HBV and HDV kinetics were modeled using the HCV kinetic model introduced earlier [38,39]. R0 (basic reproductive ratio) and drug effect were assumed to be similar for HBV and HDV. The hepatocyte production rate (s) and the hepatocyte death rate (d) were fixed to $61.7 * 10^3$ (hepatocytes/ml/day) and 0.003 (1/day) as published earlier [40,41]. The drug effect was assumed to be constant over the treatment period. Modeling was performed using the non-linear mixed effects (NLME) modeling technique implemented in NONMEM 7.3 (ICON development solutions, San Antonio, USA).

Immunogenicity

Non-myristoylated myrcludex B with two additional C-terminal residues (YC) was synthesized by standard solid phase peptide synthesis and characterized by mass spectroscopy. 50 µl of a 2 µM solution in coating buffer (13 mM Na_2CO_3, 88 mM $NaHCO_3$, pH 9.2) of the peptide was coated in a 96-well ELISA plate (Greiner Bio-One, Frickenhausen, Germany) overnight, washed and blocked with 3% bovine serum albumin (BSA). The patients' sera were diluted 1:5000 in dilution buffer (Phosphate Buffer Solution, 0.05% Tween-20, 0.1% BSA) and incubated on the ELISA plate for 1 h at 37 °C. After extensive washing, peroxidase-coupled secondary antibody goat-anti-human IgA + IgG + IgM (H + L) (Jackson ImmunoResearch, West Grove, USA) was incubated for 1 h at 37 °C, washed, and the color reaction was performed by addition of TMB-ELISA substrate solution (eBioscience, San Diego, USA) for 10 min at room temperature. After stopping of the color reaction, absorbance was measured at 450 nm in a plate reader (TECAN, Crailsheim, Germany).

Bile acids

Bile acids were evaluated in an explorative way predose, at week 12, and at week 24 for the Myr and Myr-IFN cohorts, and pre-dose and at week 12 only for the IFN cohort. Food intake was not controlled. Bile acids were quantified as described earlier [42]. For statistical analysis the sum of the amount of individual bile acids belonging to the group of unconjugated, taurine- conjugated or glycine-conjugated species were considered. Results below the limit of quantification (blq) were set to blq/2.

Statistical analysis

Because this was a pilot study, there was no sample size calculation. The number of 8 participants per cohort was assumed to be sufficient to give first indications on efficacy, safety and tolerability of myrcludex B. All included patients were analyzed for all parameters assessed. The secondary endpoint HDV RNA, however, could only be assessed in those patients with measurable HDV RNA. Comparison between baseline, week 12, and week 24 was carried out by one-way ANOVA on log transformed data with correction for multiple comparisons. If only data for baseline and week 12 were available a two-tailed paired *t* test was used. A two-tailed *p* value <0.05 was considered significant. Statistics and graphs were generated using GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, California, USA) and SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Eight patients were included in each cohort. Eight patients each at week 12 and 24 could be evaluated in the Myr cohort and the IFN cohort for all parameters except HDV RNA, because in both cohorts one patient each had no measurable HDV RNA at

baseline in the central research laboratory. Seven patients only could be evaluated at week 12 and 24 in the Myr-IFN cohort because one patient terminated the study prior to week 12 due to a rash. Of the overall 24 patients at baseline, all were HBeAg negative, three had liver cirrhosis (12.5%) and nine (37.5%) had a history of prior PegIFNα-2a treatment. The demographics and characteristics at screening are summarized in Table 1. The medical history did not show any relevant ongoing diseases, 10 patients with a history of prior HCV infection were included, all of them were HCV RNA negative at screening.

The primary endpoint of the main study, HBsAg did not show any relevant changes after 12 or 24 weeks of treatment in all three treatment arms (Fig. 2). A slight, but significant transient HBsAg increase of $10^{0.23}$ IU/ml at week 12 ($p = 0.03$) in the Myr-IFN cohort was not present at week 24 anymore. However, HDV RNA decreased at week 24 $\geqslant$1 log in six of seven patients of the Myr cohort, and became undetectable in two patients during treatment with myrcludex B alone. Mean change from baseline was a reduction of $10^{1.67}$ copies/ml ($p = 0.002$). In the Myr-IFN cohort seven of seven patients had a decline of $\geqslant$1 log at week 24 and five patients became negative at that time. Mean change from baseline was a decline of $10^{2.59}$ copies/ml (p <0.001). In the IFN cohort, six of seven patients had a decline of $\geqslant$1 log at week 24 and two became negative for HDV RNA. The mean change from baseline was a decline of $10^{2.17}$ copies/ml ($p = 0.005$, Fig. 3). Baseline mean HDV RNA are shown in Table 1. Individual changes for HDV RNA are shown in Supplementary Table 1.

HBV DNA declined $\geqslant$1 log in two out of eight patients in the Myr cohort, with a mean decline of $10^{0.29}$ copies/ml, which was not significant ($p = 0.2$). There was a significant mean reduction of $10^{1.28}$ ($p = 0.04$) in the Myr-IFN cohort, where six of seven patients showed a decline $\geqslant$1 log. The decline in three out of eight patients in the IFN cohort also remained non-significant (mean reduction $10^{0.07}$, $p = 0.91$, Fig. 4).

Patients started with mean ALT levels of 90 (± 61), 79.9 (± 40), and 85.6 (± 25) IU/ml in the Myr, Myr-IFN and IFN cohort respectively. In six of eight patients in the Myr cohort ALT normalized (mean at week 24 = 40.9 (± 14.6) IU/ml, $p = 0.04$). In both cohorts with PegIFNα-2a treatment, ALT normalized only in one patient, which was a non-significant change for ALT levels (Fig. 5).

HBV and HDV kinetics were modeled simultaneously by the virus kinetic model (Fig. 6) [38]. Under IFN treatment the virion production rate was reduced by 74.9%. Under treatment with myrcludex B, the infection rate of healthy hepatocytes was reduced by 64.7%. All model parameters were estimated with good precision. Goodness-of-fit plots and individual plots showed that the virus kinetic was well described by the model (Table 2, Supplementary Fig. 1). A visual predictive check, stratified by virus and treatment arm, showed a good descriptive performance with neither bias nor under- or over-estimation of the model variability (Supplementary Fig. 2A–C). A simulation of a 1-year treatment with placebo, myrcludex B, PegIFNα-2a or their combination revealed significant synergistic effects of the combination treatment on the viral decline of HDV (−7.5 log, Supplementary Fig. 3).

Patients experienced a total of 96 adverse events within the first 24 weeks of their treatment, but none was serious. 61 events in 19 patients were mild, 27 events in 12 patients were moderate, and 8 events in 4 patients were severe according to the Common Terminology Criteria for Adverse Events v4.0. The severe cases occurred in the Myr cohort (2), Myr-IFN cohort (2), IFN cohort (4), were all adjudicated as unrelated to myrcludex B and comprised two ALT and aspartate aminotransferase (AST) increases, one ALT, AST, and gamma-glutamyltransferase increase each, and one thrombocytopenia. From all adverse events (Supplementary Table 2) four events were considered related to myrcludex B treatment, all of which were transient mild laboratory parameter deviations (thrombocytopenia, lymphopenia, eosinophilia, and neutropenia). There were no dose reductions or treatment interruptions for myrcludex B. Eighty-seven adverse events were related to the PegIFNα-2a treatment. One patient in Myr-INF cohort discontinued participation due to a rash, which was attributed to PegIFNα-2a.

In the Myr cohort three out of seven patients showed antibodies ($p = 0.20$), whereas six out of seven in the Myr-IFN cohort developed antibodies against Myr B (p <0.001). There was no apparent correlation between the appearance of antibodies and efficacy or safety profile of the drug. No patient in the IFN cohort developed antibodies against myrcludex B at week 12 (Supplementary Fig. 4).

Bile acids were evaluated in an explorative way. There was no increase in unconjugated bile acids in any of the cohorts (non-significant mean increase of $10^{0.24}$, $10^{0.46}$ in week 24 for the Myr and Myr-IFN cohort respectively, and $10^{0.04}$ in the IFN cohort at week 12). Taurine-conjugated bile acids had a significant increase of $10^{0.47}$ ($p = 0.049$) and $10^{0.74}$ ($p = 0.009$) in the Myr and Myr-IFN cohort respectively at week 24. Glycine-conjugated bile acids had a mean increase by $10^{0.52}$ ($p = 0.03$) and $10^{0.71}$ ($p = 0.002$) at week 24 in the Myr and Myr-IFN cohort, respectively (Supplementary Fig. 5). No increase of any conjugated bile acids occurred in the IFN cohort at week 12. There was no apparent correlation between the elevated bile acids and any efficacy or safety profile of the drug.

Table 1. Patient demographics and characteristics at baseline.

Cohort	Age mean (min-max) [years]	Height [cm] ± SD	Weight [kg] ± SD	Female/ male	HBsAg mean [U/ml] ± SD	HDV RNA mean [copies/ml] ± SD	HBV DNA mean [copies/ml] ± SD	ALT mean [U/ml] ± SD	Liver cirrhosis [%]	Prior interferon treatment [%]
Myr cohort (8/7*)	38.3 (29-55)	179.6 ± 13.0	81.6 ± 13.6	3/5	$10^{3.9} \pm 10^{0.56}$	$10^{4.14} \pm 10^{0.96}$	$10^{3.29} \pm 10^{2.33}$	90.5 ± 61.0	25	25
Myr-IFN cohort (7/7*)	33.0 (23-39)	171.9 ± 8.7	74.5 ± 16.1	1/6	$10^{4.18} \pm 10^{0.56}$	$10^{4.21} \pm 10^{1.08}$	$10^{2.78} \pm 10^{1.13}$	79.9 ± 40.0	0	43
IFN cohort (8/7*)	42.1 (34-61)	172.8 ± 8.0	81.0 ± 5.6	2/6	$10^{3.20} \pm 10^{1.57}$	$10^{4.20} \pm 10^{0.95}$	$10^{1.89} \pm 10^{0.77}$	85.6 ± 25.0	12.5	25

ALT, alanine aminotranferase; HBV DNA, hepatitis B deoxyribonucleic acid; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HDV RNA, hepatitis delta ribonucleic acid; SD, standard deviation.
*Evaluable patients for HDV RNA.

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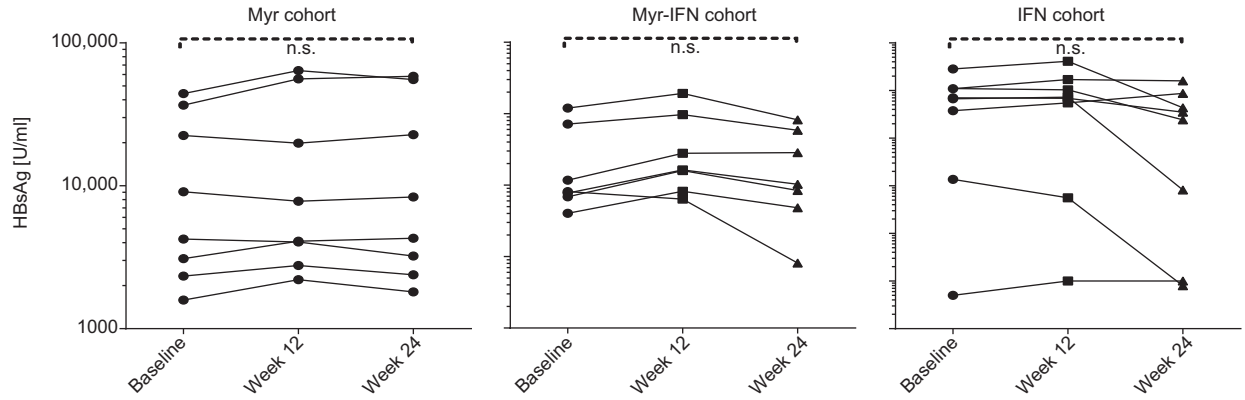

Fig. 2. Virological response to antiviral treatment with myrcludex B and/or pegylated interferon alpha. Expressed as HBsAg (hepatitis B surface antigen) at baseline and after 12 and 24 weeks of treatment in 8 patients (Myr cohort, IFN cohort) and 7 patients (Myr-IFN cohort).

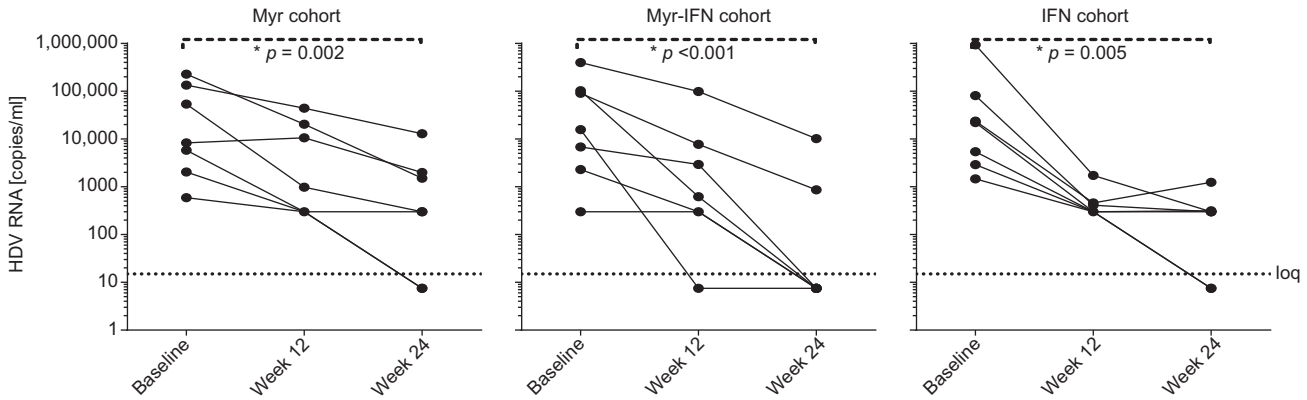

Fig. 3. Virological response to antiviral treatment with myrcludex B and/or pegylated interferon alpha. Expressed as HDV RNA (hepatitis delta ribonucleic acid) at baseline and after 12 and 24 weeks of treatment in 7 patients (Myr cohort, IFN cohort, Myr-IFN cohort).

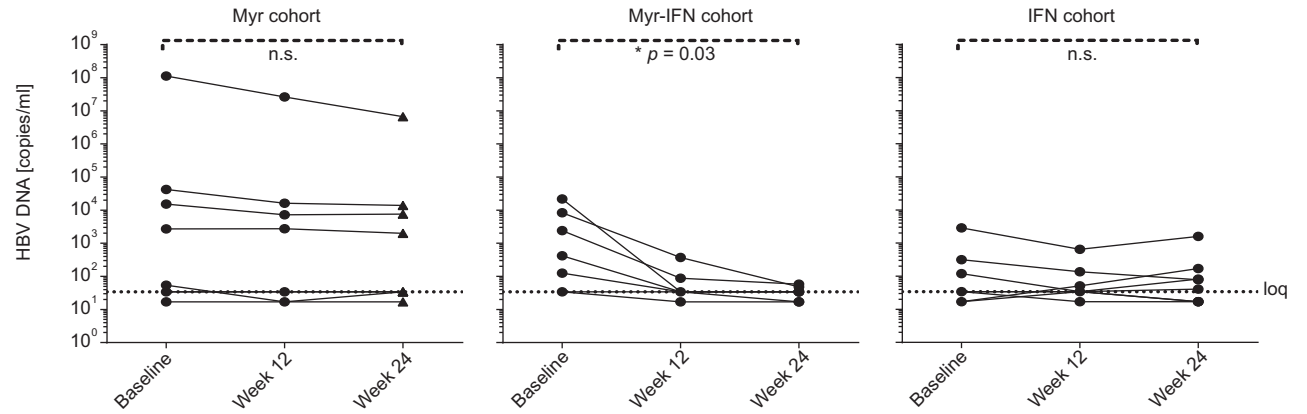

Fig. 4. Virological response to antiviral treatment with myrcludex B and/or pegylated interferon. Expressed as HBV DNA (hepatitis B deoxyribonucleic acid) at baseline and after 12 and 24 weeks of treatment in 8 patients (Myr cohort, IFN cohort) and 7 patients (Myr-IFN cohort).

Discussion

Our study demonstrated for the first time the clinical 'proof of concept' of entry inhibition for CHD patients. As monotherapy myrcludex B was very well tolerated. The combination with PegIFNα-2a did apparently not increase the frequency or severity of the mainly hematological adverse events, which were mostly related to the PegIFNα-2a treatment. All patients with measurable HDV RNA in the Myr cohort experienced a decline of HDV RNA under myrcludex B monotherapy, with two of seven patients

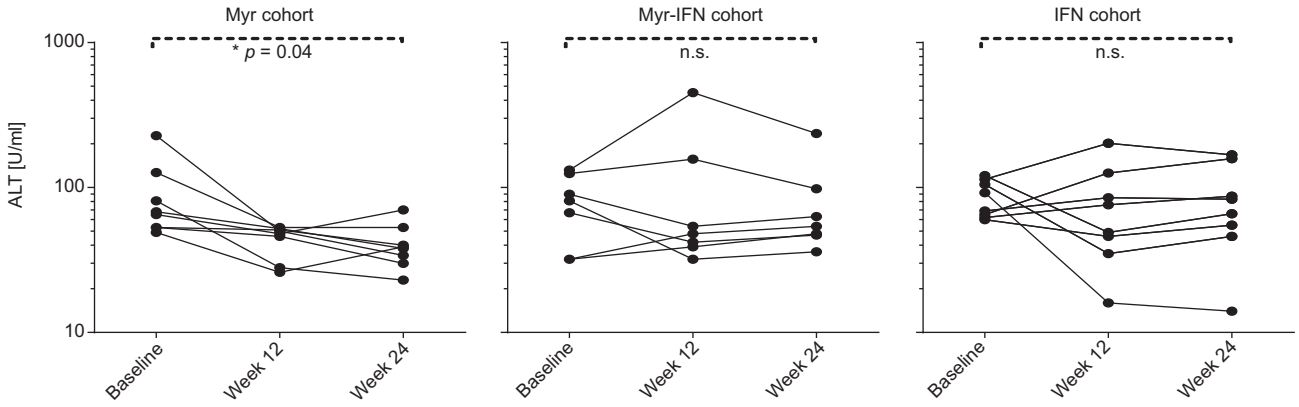

Fig. 5. Biochemical response to antiviral treatment with myrcludex B and/or pegylated interferon. Expressed as ALT (alanine aminotransferase) at baseline and after 12 and 24 weeks of treatment in 8 patients (Myr cohort, IFN cohort) and 7 patients (Myr-IFN cohort).

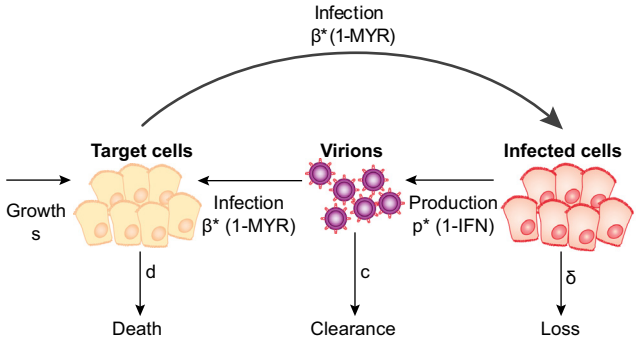

Fig. 6. Schematic view of the viral kinetic model. The model shows the natural turnover of target cells (growth, death), the possible exit ways of virus (loss of infected cells, clearance of virus) and the assumed mode of action of the used drugs myrcludex B (infection inhibition) and interferon α-2a (virion production).

reaching HDV RNA negativity at 24 weeks of treatment. It is to note that these were the two patients with the lowest HDV RNA levels. Remarkably, the combination of myrcludex B with PegIFNα-2a profoundly enhanced the antiviral effect and all subjects experienced a decline >1 log in HDV RNA, and five of seven patients became HDV RNA negative at week 24. As expected, PegIFNα-2a alone also decreased HDV in all patients and led to HDV RNA negativation in two of seven patients, which is in line with published data [11,43]. Notably, ALT levels, a clinically relevant and predictive marker for HDV-related disease progression, significantly declined and reached normal levels in six of eight patients of the Myr cohort. The absence of this tendency in both cohorts with PegIFNα-2a is consistent with previous findings probably indicating an ongoing stimulatory effect of PegIFNα-2a on immune mediated cell killing [10]. Elevated ALT levels reflect the ongoing immune reaction against infected cells, especially de novo infected cells. The normalization of ALT levels by myrcludex B therefore potentially marks the reduction of de novo infections and the resulting decreased hepatocyte turnover. Moreover, the incessant reduction of the number of infected cells by either apoptosis or immune mediated cell killing may also result in a decreased hepatic ALT release. Baseline levels of HBV DNA were generally lower in the majority of the study participants, which is not unexpected because HDV co-infection is thought to interfere with HBV replication [44].

Table 2. Parameter estimates of the HBV and HDV kinetic model including residual standard errors (RSE).

Parameter	Unit	HBV (RSE)	HDV (RSE)	Definition
R0		6.12 (48%)*		Basic reproductive ratio§
c	1/day	0.0309 (41%)	0.0915 (24%)	Virion clearance
δ	1/day	0.0171 (33%)	0.101 (38%)	Loss rate of infected cells
s	hepatocytes/ml/day	$61.7*10^3$		Hepatocyte growth rate
d	1/day	0.003*#		Hepatocyte death rate
MYR		0.647 (10%)*		Myrcludex B drug effect (infection inhibition)$
IFN		0.749 (8%)*		Pegylated interferon α2a drug effect (virion production inhibition)$
IIV R0	% CV	156 (33%)*		Interindividiual variability on R0

§Average number of newly infected cells that arise from any one infected cell in the beginning of the infection when almost all cells are uninfected and defined as: $R0 = \frac{p*\beta*s}{c*\delta*d}$.
*Assumed to be identical for HDV and HBV; #fixed to literature values; $see also Fig. 6 for details. For detailed description of the model parameters see [39].

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Guedj and colleagues have modeled early virus kinetics and have found a biphasic effect on HDV viremia during IFN treatment with an even higher overall efficacy of 96% on HDV production and release reduction [45]. Because our virus kinetic model is only based on data determined at week 12 and 24 we cannot compare directly with the early phase HDV kinetics [45]. Also their patients showed much higher HDV RNA- and ALT levels at baseline, which further limits the comparison with our data. However, our model highlighted another interesting observation: There was a more profound suppression of HDV RNA levels in the Myr-IFN combination cohort. In the simulation of a 1-year treatment, viral decline for HDV RNA in combination therapy is predicted to be more than four times as high as for each monotherapy (Supplementary Fig. 3). Also HBV declined slightly more under combination treatment (Supplementary Fig. 3). As one cannot expect that HDV has a direct influence on the HBV infection cycle it would also be an interesting option to investigate the effect of myrcludex B with PegIFNα-2a in HBV mono-infected patients.

Despite the clear antiviral effect as measured by HDV RNA, HBV DNA and ALT decline, HBsAg remained unaffected. Because myrcludex B as a NTCP-specific entry inhibitor does not interfere with late steps in the replication cycle like HDV or HBV production [30,31,46], the decline of viral loads under therapy probably reflects a corresponding reduction of the infected virus-producing cells. Evidence for this assumption came from *in vitro* and *in vivo* studies where myrcludex B induced a clear reduction of infected cells. [31,33]. However, it remains an open question, why HBsAg levels do not follow the same kinetics. One explanation could be that HBsAg secretion might be disconnected from HBV replication e.g., through production from integrated DNA independent of cccDNA. This might be even more likely in later phases of infection in HBeAg negative patients [44,47]. Furthermore, HBsAg is assumed to be an important factor modulating the mechanism of productive immune surveillance [48,49]. Accordingly, HBsAg levels in a chronically infected patient might be thoroughly regulated by transcriptional control of cccDNA. Clearance might therefore not proceed linearly with the loss of infected cells but could proceed by a more abrupt process when transcriptional compensation cannot be upheld anymore.

The appearance of antibodies from the peptidic drug myrcludex B was not unexpected following multiple dose administration of the peptide and could be demonstrated in some patients in the Myr cohort and as a significant regular trend in the Myr-IFN cohort (Supplementary Fig. 4). The high titers of antibodies in the latter might be related to a general immunostimulatory effect of PegIFNα-2a. The antibodies did not affect safety or efficacy of the drug, although a correct correlation analysis is impossible with the small sample size. Because myrcludex B contains essential and adjacent amino acids of the receptor binding site of the virus, antibodies against the peptide may exhibit virus-neutralizing potential, but may also neutralize the effect of the peptide itself. However, myrcludex B contains epitopes that have been described to induce highly potent neutralizing antibodies [50,51]. Further evaluations of the immunogenicity of the peptide for safety and efficacy, as well as the potential of antibodies to influence the therapeutic efficacy of the drug, are currently ongoing.

The myrcludex B dose of 2 mg does not completely saturate NTCP [35]. Patients under myrcludex B showed a very moderate increase in taurine-conjugated and glycine-conjugated bile acids, which were not associated with any clinical events. There are no normal ranges for bile acids, and the physiological consequences of increased bile acids remain unclear. A recent report of one patient with a non-functional NTCP showed clearly but asymptomatically raised bile acid levels [52]. Other studies, however, showed that bile acids might have possible adverse effects on the cardiovascular system [53,54]. However, *in vitro* HBV and HDV infection inhibition can be achieved at sub-saturating concentrations of NTCP. It remains to be investigated if clinical side effects possibly arising from long-term treatment with myrcludex B might be manageable by dose reduction, without loss of the therapeutic effect.

In conclusion entry inhibition of HBV and HDV in chronically co-infected patients has – for the first time – been shown to be associated with HDV RNA and HBV DNA declines and improvement of biochemical disease activity (ALT) after a 24-week treatment course. The antiviral effect of myrcludex B is more pronounced in combination with PegIFNα-2a indicating that a combination of both drugs is promising for future clinical trials. Myrcludex B was safe and well tolerated as monotherapy and in combination with PegIFNα-2a in patients with HDV co-infection. A comprehensive phase II program will further explore optimal dose-range and frequency, as well as the most effective duration of therapy for the use of myrcludex B in CHD and CHB infection.

Financial support

This clinical study was supported by Hepatera Ltd, Moscow, who was also legal sponsor of this study. Other partial funding came from Myr GmbH, Bad Homburg, the Bundesministerium für Bildung und Forschung (BMBF), "Innovative Therapieverfahren" and the "Deutsche Zentrum für Infektionsforschung (DZIF)" (German Center for Infection Research, DZIF TTU 05.901, TTU 05.804, TTU 05.904, TTU 05.704). AB has received personal funding from the Medical Faculty of the Heidelberg University. FAL is member of the Hartmut Hoffmann-Berling International Graduate School of Molecular and Cellular Biology (HBIGS), Heidelberg University. Further financial support was received from the Hans Lutz Merkle Foundation, and the Robert Bosch Foundation (Stuttgart, Germany).

Conflict of interest

SU is co-applicant and co-inventor of patents protecting myrcludex B. AA is employee of the Myr GmbH, Bad Homburg, partly funding this study.

Authors' contributions

AB, SU, AA, and WEH analyzed the results and wrote the paper. PB, NV, MM, KK, and MP carried out the clinical study. TL carried out the virus kinetic modeling. MS and MH carried out the bile acid analytics. HW and FAL carried out HDV RNA and immunogenicity analytics. All authors significantly contributed to the manuscript.

Acknowledgements

The authors acknowledge the contributions of all volunteers, investigators, study personnel, the sponsor, the staff from data management (dataMatrix, St. Petersburg, Russia), and the analytical laboratory (Prolytik GmbH, Frankfurt, Germany). We are grateful to Katrin Schöneweiss and the technical assistance of Sarah Engelhardt, Franziska Schlund, and Markus König for analyzing samples. We thank the central diagnostic laboratory of the University Hospital Heidelberg and the Heidelberg Virology Department, namely Paul Schnitzler and Steffen Geiss for analyzing the samples.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.04.016.

References

[1] Taylor JM. Virology of hepatitis D virus. Semin Liver Dis 2012;32:195–200.
[2] Alvarado-Mora MV, Locarnini S, Rizzetto M, Pinho JR. An update on HDV: virology, pathogenesis and treatment. Antivir Ther 2013;18:541–548.
[3] Noureddin M, Gish R. Hepatitis delta: epidemiology, diagnosis and management 36 years after discovery. Curr Gastroenterol Rep 2014;16:365.
[4] Wedemeyer H. Hepatitis D revival. Liver Int 2011;31:140–144.
[5] Rizzetto M, Hepatitis D. Virus: introduction and epidemiology. Cold Spring Harb Perspect Med 2015;5.
[6] Rizzetto M. Current management of delta hepatitis. Liver Int 2013;33:195–197.
[7] Goyal A, Murray JM. The impact of vaccination and antiviral therapy on hepatitis B and hepatitis D epidemiology. PLoS One 2014;9 e110143.
[8] Heidrich B, Manns MP, Wedemeyer H. Treatment options for hepatitis delta virus infection. Curr Infect Dis Rep 2013;15:31–38.
[9] Triantos C, Kalafateli M, Nikolopoulou V, Burroughs A. Meta-analysis: antiviral treatment for hepatitis D. Aliment Pharmacol Ther 2012;35:663–673.
[10] Wedemeyer H, Yurdaydin C, Dalekos GN, Erhardt A, Cakaloglu Y, Degertekin H, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. N Engl J Med 2011;364:322–331.
[11] Heidrich B, Yurdaydin C, Kabacam G, Ratsch BA, Zachou K, Bremer B, et al. Late HDV RNA relapse after peginterferon alpha-based therapy of chronic hepatitis delta. Hepatology 2014;60:87–97.
[12] Gunsar F, Akarca US, Ersoz G, Kobak AC, Karasu Z, Yuce G, et al. Two-year interferon therapy with or without ribavirin in chronic delta hepatitis. Antivir Ther 2005;10:721–726.
[13] Niro GA, Ciancio A, Gaeta GB, Smedile A, Marrone A, Olivero A, et al. Pegylated interferon alpha-2b as monotherapy or in combination with ribavirin in chronic hepatitis delta. Hepatology 2006;44:713–720.
[14] Rizzetto M, Smedile A. Pegylated interferon therapy of chronic hepatitis D: in need of revision. Hepatology 2015;61:1109–1111.
[15] Lamers MH, Kirgiz OO, Heidrich B, Wedemeyer H, Drenth JP. Interferon-alpha for patients with chronic hepatitis delta: a systematic review of randomized clinical trials. Antivir Ther 2012;17:1029–1037.
[16] Niro GA, Ciancio A, Tillman HL, Lagget M, Olivero A, Perri F, et al. Lamivudine therapy in chronic delta hepatitis: a multicentre randomized-controlled pilot study. Aliment Pharmacol Ther 2005;22:227–232.
[17] Soriano V, Vispo E, Sierra-Enguita R, Mendoza C, Fernandez-Montero JV, Labarga P, et al. Efficacy of prolonged tenofovir therapy on hepatitis delta in HIV-infected patients. Aids 2014;28:2389–2394.
[18] Soriano V, Barreiro P, de Mendoza C. Tenofovir for hepatitis delta. Hepatology 2016;63:1395–1396.
[19] Rizzetto M, Ciancio A. The prenylation inhibitor, lonafarnib: a new therapeutic strategy against hepatitis delta. Lancet Infect Dis 2015;15:1119–1120.
[20] Yust-Katz S, Liu D, Yuan Y, Liu V, Kang S, Groves M, et al. Phase 1/1b study of lonafarnib and temozolomide in patients with recurrent or temozolomide refractory glioblastoma. Cancer 2013;119:2747–2753.
[21] Koh C, Canini L, Dahari H, Zhao X, Uprichard SL, Haynes-Williams V, et al. Oral prenylation inhibition with lonafarnib in chronic hepatitis D infection: a proof-of-concept randomised, double-blind, placebo-controlled phase 2A trial. Lancet Infect Dis 2015;15:1167–1174.
[22] Poutay D, Sabra M, Abou Jaoudé G, Chemin I, Trepo C, Vaillant A, et al. Nucleic acid polymers are efficient in blocking hepatitis delta virus entry in vitro. The Global Viral Hepatitis Summit. Berlin: German Liver Foundation; 2015.
[23] Replicor homepage; 2015 [cited 2015 23.11.2015]; Available from: http://replicor.com/.
[24] Lamas Longarela O, Schmidt TT, Schoneweis K, Romeo R, Wedemeyer H, Urban S, et al. Proteoglycans act as cellular hepatitis delta virus attachment receptors. PLoS One 2013;8 e58340.
[25] Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. elife 2012;1 e00049.
[26] Petersen J, Dandri M, Mier W, Lutgehetmann M, Volz T, von Weizsacker F, et al. Prevention of hepatitis B virus infection in vivo by entry inhibitors derived from the large envelope protein. Nat Biotechnol 2008;26:335–341.
[27] Urban S, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. Gastroenterology 2014;147:48–64.
[28] Vercauteren K, Brown RJ, Mesalam AA, Doerrbecker J, Bhuju S, Geffers R, et al. Targeting a host-cell entry factor barricades antiviral-resistant HCV variants from on-therapy breakthrough in human-liver mice. Gut 2015.
[29] Schulze A. Analyse der frühen Schritte der Hepatitis B Virus Infektion: Zell-Polarisierung, Differenzierung und Zell-assoziierte Heparansulfat-Proteoglykane als essentielle Faktoren für die Etablierung einer Hepatitis B Virus Infektion. Heidelberg University; 2008.
[30] Schulze A, Schieck A, Ni Y, Mier W, Urban S. Fine mapping of pre-S sequence requirements for hepatitis B virus large envelope protein-mediated receptor interaction. J Virol 2009;84:1989–2000.
[31] Volz T, Allweiss L, Barek MB, Warlich M, Lohse AW, Pollok JM, et al. The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. J Hepatol 2013;58:861–867.
[32] Dandri M, Lutgehetmann M. Mouse models of hepatitis B and delta virus infection. J Immunol Methods 2014;410:39–49.
[33] Lutgehetmann M, Mancke LV, Volz T, Helbig M, Allweiss L, Bornscheuer T, et al. Humanized chimeric uPA mouse model for the study of hepatitis B and D virus interactions and preclinical drug evaluation. Hepatology 2012;55:685–694.
[34] Schieck A, Schulze A, Gahler C, Muller T, Haberkorn U, Alexandrov A, et al. Hepatitis B virus hepatotropism is mediated by specific receptor recognition in the liver and not restricted to susceptible hosts. Hepatology 2013;58:43–53.
[35] Blank A, Markert C, Hohmann N, Carls A, Mikus G, Lehr T, et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor Myrcludex B. J Hepatol 2016;65:483–489.
[36] Schulze A. HBV/HDV infection inhibition study using a human derived liver cell culture. OMZ study report 201. Heidelberg: Otto Meyerhof Zentrum; 2010.
[37] Mederacke I, Bremer B, Heidrich B, Kirschner J, Deterding K, Bock T, et al. Establishment of a novel quantitative hepatitis D virus (HDV) RNA assay using the Cobas TaqMan platform to study HDV RNA kinetics. J Clin Microbiol 2010;48:2022–2029.
[38] Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998;282:103–107.
[39] Canini L, Perelson AS. Viral kinetic modeling: state of the art. J Pharmacokinet Pharmacodyn 2014;41:431–443.
[40] Colombatto P, Civitano L, Oliveri F, Coco B, Ciccorossi P, Flichman D, et al. Sustained response to interferon-ribavirin combination therapy predicted by a model of hepatitis C virus dynamics using both HCV RNA and alanine aminotransferase. Antivir Ther 2003;8:519–530.
[41] Snoeck E, Chanu P, Lavielle M, Jacqmin P, Jonsson EN, Jorga K, et al. A comprehensive hepatitis C viral kinetic model explaining cure. Clin Pharmacol Ther 2010;87:706–713.
[42] Haag M, Hofmann U, Murdter TE, Heinkele G, Leuthold P, Blank A, et al. Quantitative bile acid profiling by liquid chromatography quadrupole time-of-flight mass spectrometry: monitoring hepatitis B therapy by a novel Na-taurocholate cotransporting polypeptide inhibitor. Anal Bioanal Chem 2015;407:6815–6825.

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[43] Keskin O, Wedemeyer H, Tüzün A, Zachou K, Deda X, Dalekos GN, et al. Association between level of hepatitis D virus RNA at week 24 of pegylated interferon therapy and outcome. Clin Gastroenterol Hepatol 2015;13:2342–2349, e1-2.

[44] Wedemeyer H. Re-emerging interest in hepatitis delta: new insights into the dynamic interplay between HBV and HDV. J Hepatol 2010;52:627–629.

[45] Guedj J, Rotman Y, Cotler SJ, Koh C, Schmid P, Albrecht J, et al. Understanding early serum hepatitis D virus and hepatitis B surface antigen kinetics during pegylated interferon-alpha therapy via mathematical modeling. Hepatology 2014;60:1902–1910.

[46] Glebe D, Urban S, Knoop EV, Cag N, Krass P, Grun S, et al. Mapping of the hepatitis B virus attachment site by use of infection-inhibiting preS1 lipopeptides and tupaia hepatocytes. Gastroenterology 2005;129:234–245.

[47] Wooddell CI, Chavez D, Goetzmann JE, Guerra B, Peterson RM, Lee H, et al. Reductions in cccDNA under NUC and ARC-520 therapy in chimpanzees with chronic hepatitis B virus infection implicate integrated DNA in maintaining circulating HBsAg (ID32). In: Diseases AAftSoL, editor. The AASLD Liver Meeting; 2015; San Francisco: Hepatology; 2015.

[48] Op den Brouw ML, Binda RS, van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, et al. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. Immunology 2009;126:280–289.

[49] Shi B, Ren G, Hu Y, Wang S, Zhang Z, Yuan Z. HBsAg inhibits IFN-alpha production in plasmacytoid dendritic cells through TNF-alpha and IL-10 induction in monocytes. PLoS One 2012;7 e44900.

[50] Glebe D, Aliakbari M, Krass P, Knoop EV, Valerius KP, Gerlich WH. Pre-s1 antigen-dependent infection of Tupaia hepatocyte cultures with human hepatitis B virus. J Virol 2003;77:9511–9521.

[51] Pizarro JC, Vulliez-le Normand B, Riottot MM, Budkowska A, Bentley GA. Structural and functional characterization of a monoclonal antibody specific for the preS1 region of hepatitis B virus. FEBS Lett 2001;509:463–468.

[52] Vaz FM, Paulusma CC, Huidekoper H, de Ru M, Lim C, Koster J, et al. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. Hepatology 2015;61:260–267.

[53] Khurana S, Raufman JP, Pallone TL. Bile acids regulate cardiovascular function. Clin Transl Sci 2011;4:210–218.

[54] Rainer PP, Primessnig U, Harenkamp S, Doleschal B, Wallner M, Fauler G, et al. Bile acids induce arrhythmias in human atrial myocardium–implications for altered serum bile acid composition in patients with atrial fibrillation. Heart 2013;99:1685–1692.