Case Report

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Excellent safety and effectiveness of high-dose myrcludex-B monotherapy administered for 48 weeks in HDV-related compensated cirrhosis: A case report of 3 patients

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Summary

Short-term administration of the entry inhibitor myrcludex-B (MyrB) has been shown to be safe and effective in phase II studies in patients coinfected with hepatitis B virus (HBV) and hepatitis delta virus (HDV). However, its effectiveness and safety are unknown during long-term and high-dose treatment of patients with compensated cirrhosis in real-life settings. Herein, we describe the first 3 European patients with HDV-related compensated cirrhosis who were treated with MyrB 10 mg/day for 48 weeks as a compassionate therapy. Liver function tests, bile acids, and virological markers were monitored every 4 weeks. HBV/HDV-specific T cell quantity (up to 48 and 36 weeks) and HBV RNA levels were also assessed in 2 cases. During MyrB treatment, HDV RNA levels progressively declined from 4.4 and 5.6 logs IU/ml to undetectability in 2 cases, and from 6.8 log copies/ml to 500 copies/ml for the other patient. Alanine aminotransferase normalised after 20, 12 and 28 weeks, respectively. A significant improvement in features of portal hypertension, liver function tests and alpha-fetoprotein levels were documented in 2 cases. In the male patient with histological and clinical stigmata of autoimmune hepatitis, IgG and immunoglobulins rapidly normalised. No significant changes in HBV surface antigen levels and circulating HBV/HDV-specific T cells were demonstrated; HBV DNA and HBV RNA levels remained undetectable throughout the study period. MyrB was well tolerated; patients remained fully asymptomatic despite a significant increase of bile acids. In conclusion, this report shows excellent safety and effectiveness of a 48-week course of MyrB 10 mg/day, combined with tenofovir disoproxil fumarate, for the treatment of HDV-related compensated cirrhosis.

Introduction

Hepatitis delta virus (HDV) is a defective RNA virus, discovered in 1977, that requires the presence of hepatitis B virus (HBV) surface antigen (HBsAg) to envelop its viral ribonucleoprotein complex to become infectious. Approximately 15–20 million individuals worldwide have evidence of chronic HDV-induced hepatitis that typically shows a more rapid progression to cirrhosis (4% per year) and provokes more complications than HBV-monoinfection. The only off-label therapy available so far, pegylated-interferon (Peg-IFN), is endowed with significant side effects and only suppresses HDV replication in a minority of patients.[1] However, the therapeutic landscape of HDV therapy is changing rapidly as promising new compounds, given as monotherapies or in combination with Peg-IFN, are now under investigation in phase I and II clinical trials.[2] Among these new strategies, the peptidic entry inhibitor MyrB, targeting the commonly used receptor for HBV and HDV (sodium taurocholate cotransporting polypeptide, NTCP) has interesting properties. In phase II studies, patients treated with MyrB monotherapy at 2 or 5 mg/day for up to 48 weeks experienced a significant reduction in serum levels of HDV RNA coupled with alanine aminotransferase (ALT) normalisation.[3,4] When combined with Peg-IFN for 48 weeks, the virological suppression rates further improved (50% *vs.* 13% of Peg-IFN monotherapy arm), and HBsAg loss occurred in 13% of the patients in the combination arms (*vs.* 0/15 patients in the Peg-IFN monotherapy arm).[5] MyrB was safe and well tolerated despite the significant increase of

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total bile acids,[6] which correlates with an inhibition of the major conjugated bile salt transporter NTCP. Therefore, administration of MyrB may represent a new therapeutic opportunity for patients with HDV, especially for those with an urgent need for treatment, such as cirrhotics or patients in whom IFN is contraindicated due to disease severity or previous non response.[7] Of note, no data are available on high-dose MyrB therapy given for more than 24 weeks.

Therefore, this is the first report aimed at describing the safety and effectiveness of MyrB 10 mg/day administered for 48 weeks in the first 3 European patients with compensated cirrhosis treated outside clinical trials. Following the Ethical Committee approval for MyrB compassionate use for the 2 Italian patients and the written informed consent for the Austrian patient, MyrB was added to the ongoing tenofovir disoproxil fumarate (TDF) therapy.

MyrB was self-administered as 2 subcutaneous injections every 24 hours (2 vials of 5 mg each). Liver function tests, total bile acids and virological HDV and HBV markers were monitored every 4 weeks. HDV RNA was quantified by RoboGene® (Aj-Roboscreen, Jena, Germany; lower limit of detection [LLOD] 6 IU/ml) or by in-house reverse-transcription PCR (RT-PCR) (lower limit of quantification [LLOQ] 100 copies/ml). HBV DNA was quantified by Abbott RealTime HBV (Abbott Diagnostics, Rome, Italy; LLOQ 10 IU/ml) or by Roche Cobas® (AmpliPrep/TaqMan System®, LLOQ 20 IU/ml). HBV genotype was determined by INNO-LiPA HBV genotyping (Fujirebio Europe NV, Ghent, Belgium), while HDV genotype was assessed by sanger sequencing and analyses of the hepatitis delta antigen region: HDV RNA was transcribed using primers random hexamers and SuperScript III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, California, USA). First PCR and Nested PCR were performed using primers synthesised by metabion international AG (Metabion GmbH, Germany) and *TaKaRa Ex Taq* Hot Start Version Kit (TAKARA BIO INC, Kusatsu, Japan). Auto-antibodies and immunoglobulins were assessed every 6 months, liver stiffness by Fibroscan® every 3–6 months, abdominal ultrasound every 6 months.

In 2 of the cases, additional analyses were performed. HBV RNA was tested after every 12 weeks of treatment using a specific real-time PCR technique: to achieve high sensitivity, 200 µl of serum were used for nucleic acid extraction (LLOD 160 copies/ml). In peripheral blood mononuclear cells collected and cryopreserved every 4 weeks, HBV/HDV-specific T cell quantity and function were analysed by direct *ex vivo* IFN-γ ELISPOT methods using overlapping peptides (15 mers/overlap of 10AA), covering both HBV and HDV proteomes.[10] The positivity of spot forming units are determined when the values were above the threshold (which is 3-fold higher than the negative control wells).

Patients provided written informed consent for additional sera sample storage and analysis.

Case 1

A 69-year-old, female, Caucasian HBV e antigen (HBeAg)-negative patient with HDV-related compensated cirrhosis (Child-Pugh Score A5) complicated by portal hypertension (splenomegaly and thrombocytopenia), was evaluated for MyrB treatment in January 2018 since interferon therapy was contraindicated because of age, platelet count and previous non response. She had genotype D for HBV and genotype 1 for HDV.

She had been treated with Peg-IFN alpha 135 µg/week for 18 months in 2011, with normalisation of ALT levels and undetectable HDV RNA during therapy, but virological and biochemical relapse after discontinuation of interferon. To control HBV replication, TDF 245 mg/48 hour was started in September 2012. Since January 2014, ALT levels remained consistently elevated (100–150 IU/L), with HDV viraemia constantly detectable. She had diabetes mellitus and received diet therapy (body mass index [BMI] 23.4 kg/m^2), femoral and lumbar osteopenia, and mild arterial hypertension. The table summarises the most important baseline characteristics. Following MyrB 10 mg/day introduction, HDV RNA progressively declined from 4.4 log IU/ml to became undetectable after 36 weeks, while ALT levels normalised after 20 weeks (Fig. 1). The virological and biochemical responses were maintained during therapy. Serum alpha-fetoprotein (AFP) levels decreased from 6 to 3 ng/ml, and liver function tests as well as platelets counts improved (Table 1). HBsAg levels and liver stiffness values slightly increased (from 10 to 29 IU/ml and from 17.3 to 21.3 kPa, respectively). MyrB injections were well tolerated, no skin reactions or drug-related problems were reported. The significant increase of total bile acid remained asymptomatic (Fig. 1).

Despite the drop in HDV RNA values, we did not detect any increased HDV-specific T cell response, with only a single detectable fluctuation of T cell response against HBV protein (polymerase) at week 20 (Fig. 1, Panel B). HBV RNA serum levels were below the LLOD at the start and at all time points during MyrB treatment (Fig. 1).

Case 2

A 51-year-old, male, Caucasian HBeAg-negative patient with HDV-compensated cirrhosis (Child-Pugh Score A5), complicated by splenomegaly, thrombocytopenia and small oesophageal varices, was evaluated for MyrB therapy in May 2018. He had genotype D for HBV and genotype 1 for HDV.

The patient was known to have HBV/HDV coinfection since June 2010, when a liver biopsy revealed moderate to severe HDV-related hepatitis (Grading 9, Staging 6, according to Ishak score) with a significant plasma cellular component. Since 2012, ALT levels remained elevated (150–200 IU/L) with HDV viraemia constantly detectable. He had diabetes and was on diet therapy (BMI 22.7 kg/m^2), and he received TDF 245 mg/24 hour (since 2012) and vitamin D supplementation. Interferon therapy was contraindicated because of disease severity, low platelet count and autoimmune stigmata at histology. The most important baseline demographic, clinical and virological features are summarised in Table 1.

During MyrB 10 mg/day therapy, HDV RNA declined from 5.6 log IU/ml to became undetectable after 28 weeks, and ALT levels normalised in just 8 weeks (Fig. 1). These responses were maintained through week 48, the last visit documented here. Aspartate aminotransferase, alkaline phosphatase and AFP levels normalised in parallel (AFP from 21 to 5 ng/ml), while gamma glutamyltransferase levels significantly improved. Liver function tests (albumin from 3.6 to 4.4 g/dl, pseudo-cholinesterase from 4,470 to 6,648 IU/L), features of portal hypertension (platelet count from 74 to 111 $\times$ 10^9/L), liver stiffness (from 17.6 to 10.1 kPa) and HBsAg levels (from 9,091 to 7,499 IU/ml) improved. Of note, immunological markers such as IgG and gammaglobulin levels also significantly improved during therapy (Table 1). MyrB was well tolerated; no symptoms were

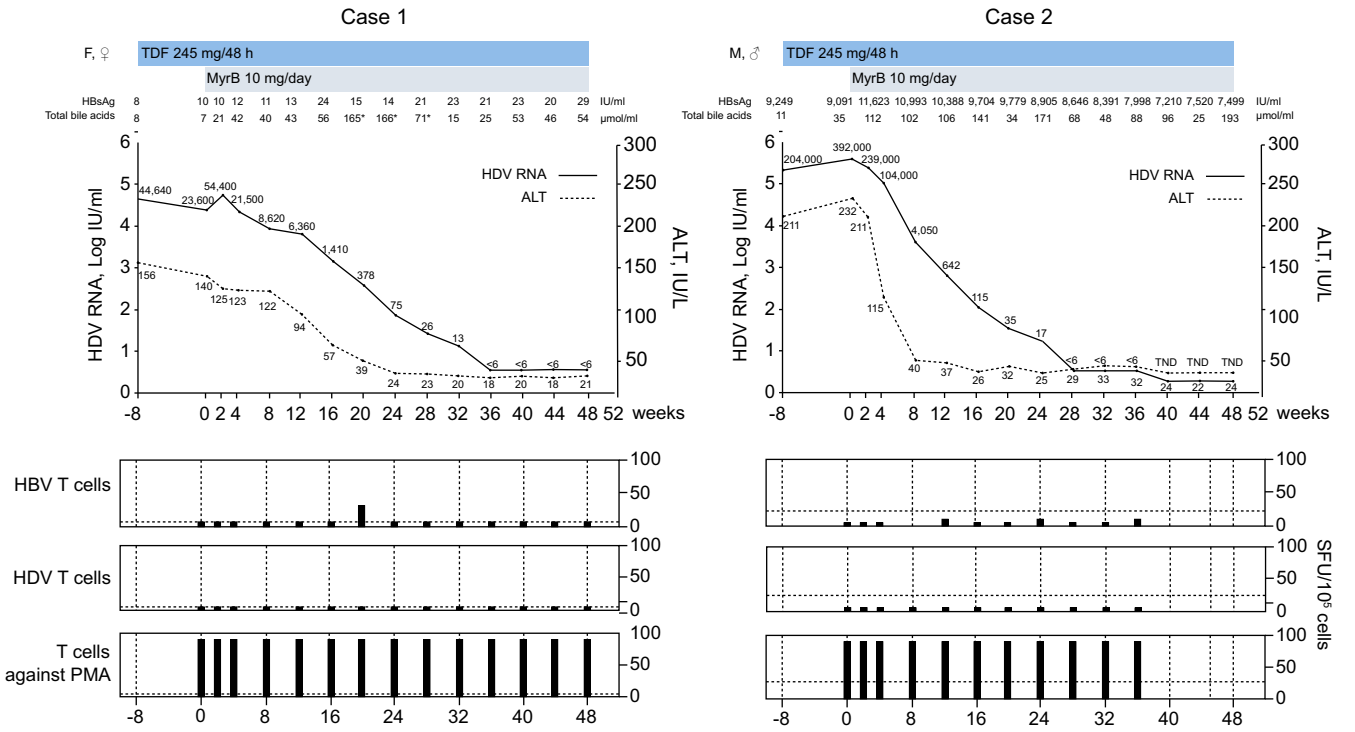

Fig. 1. Changes of HDV RNA, ALT, bile acids and HBsAg levels during MyrB treatment in 2 patients (Case 1 and 2). Panels B, C, D show bars indicating the numbers of spots × 10^5 PBMCs responding to the different peptides mixtures or PMA+Ionomycin (Positive control). ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HDV, hepatitis delta virus; PBMCs, peripheral blood mononuclear cells; MyrB, myrcludex-B.

reported despite an increase of bile acids that was maintained throughout therapy (Fig. 1).

Despite the profound decline in HDV RNA, recovery of HDV or HBV-specific T cell responses was not observed. They remained lower than the positivity level (3-fold higher than background) throughout the study (Fig. 1, Panel B and C). HBV RNA serum levels again were below the LLOD at the start of treatment and at all time points during treatment (Table 1).

Case 3

A 58-year-old female, from Uzbekistan, was referred in 2012 for HBV/HDV coinfection. She was known to be HBsAg positive since 1988. A liver biopsy was never performed but based on the ultrasound findings she was suggested to have compensated cirrhosis. She was HBeAg negative, HBV DNA was 4,850 IU/ml, HDV RNA 6.2 log copies/ml. She started TDF 245 mg/day, and 3 months later she received Peg-IFN alpha 180 μg/week that was however discontinued because of an increase of ALT levels and a significant decrease of platelet count to 5 × 109/L, due to an autoimmune thrombocytopenia with detectable anti-platelet antibodies. She was treated with high doses of prednisolone treatment, tapered over time. The next 3 years she received no additional therapy, her platelet count was stable within the normal range, but her aminotransferases remained between 200 and 450 IU/L due to HDV. As interferon therapy was contraindicated because of her history of autoimmune thrombocytopenia, MyrB 10 mg/day was started in April 2018. The most important baseline demographic, clinical and virological features are summarised in Table 1. HDV RNA progressively declined during MyrB from 6.8 log copies/ml to reach 500 copies/ml by week 48, a 4.1 log copies/ml decline, ALT normalised by week 28, while HBsAg levels remained unchanged. Despite a significantly increase of bile acids, the patient remained asymptomatic (Table 1).

Discussion

This case report demonstrated the effectiveness and safety of 48-week administration of MyrB 10 mg/day in 3 patients with HDV-related compensated cirrhosis. To our knowledge, these are the first cases ever treated with this strategy. Patients showed a fast normalisation of aminotransferases, and a profound reduction of HDV serum RNA levels with clinically relevant improvements of liver function in the context of an excellent safety profile. Responses were maintained during the study period.

These 3 cases help to provide additional knowledge on the safety profile of MyrB under "field conditions" as there are no published studies describing the safety and tolerability of MyrB 10 mg given for 48 weeks. Treatment was well tolerated in all 3 patients, without treatment emergent serious adverse events. As expected, given the mode of action of the drug and in agreement with phase II studies,[6] bile acids significantly increased throughout the study period, but patients remained fully asymptomatic. Interestingly, when blood tests were done after MyrB administration, and not before as recommended, bile acid levels further increased exceeding 100 μmol/L, but again patients remained fully asymptomatic.

Additionally, this new data provides evidence of the effectiveness of this approach, as no study has so far reported on the antiviral and biochemical response to 10 mg/day MyrB administered for 48 weeks. All 3 patients treated with MyrB monotherapy not only achieved the recently recommended

Table 1. Time course of virological and biochemical variables during MyrB 10 mg/day + TDF treatment in the 3 patients (reported on 3-months basis).

Variables	Case 1					Case 2					Case 3				
	Baseline	Week 12	Week 24	Week 36	Week 48	Baseline	Week 12	Week 24	Week 36	Week 48	Baseline	Week 12	Week 24	Week 36	Week 48
AST (IU/L)/ALT (IU/L)	83/140	50/94	31/24	30 /18	22/21	179/232	44/37	38/25	42/32	38/24	111/244	72/97	42/46	35/33	33/35
ALP (IU/L)/GGT (IU/L)	91/27	84/19	83/20	82/ 19	76/23	185/231	100/158	89/138	93/121	86/140	74/44	97/28	95/19	89/16	87/17
Total bilirubin (mg/dl)	0.7	0.5	0.6	0.59	0.5	0.4	0.6	0.6	0.6	0.4	0.36	0.42	0.39	0.42	0.34
Total biliary acids (μmol/L)	7	43	166*	25	54	35	106	171	88	193	<10	184	200	164	179
pCHE (IU/L)	8,880	8,143	8,380	8,453	9,005	4,470	5,503	6,534	7,456	6,648	6,330	6,700	6,340	6,300	6,310
Albumin (g/dl)/gamma globulin (g/dl)	4.3/1.9	4.9/1.9	4.7/1.7	4.2/1.6	4.5/1.6	3.6/2.9	4.0/2.4	4.4/1.9	4.6/1.7	4.4/1.5	4.1/–	4.5/–	4.5/–	4.6/–	4.5/–
IgG (mg/dl)	1,650	–	1,647	t	1,758	3,077	–	2,061	–	1,608	2,350	–	–	–	–
Alpha-fetoprotein (ng/ml)	6	6	3	3	3	21	9	6	5	5	4.7	4.8	4.4	4.2	3.8
PT-INR	1.1	1.06	1.08	1.04	1.02	1.09	1.06	1.17	1.14	1.13	1.0	1.0	1.0	1.0	1.0
WBC (/mm^3)/Hb (g/dl)	5,030/ 14.2	5,300/ 13.7	7,410/ 13.6	7,540/ 13.1	3,850/ 13.2	4,880/ 15.7	6,250/ 16.0	7,000/ 16.3	8,510/ 17.1	6,930/ 16.3	6,540/15.8	5,770/ 15.6	6,290/ 15.2	5,640/ 15.5	6,150/ 14.8
Platelet count (x10^9/L)	95	100	109	117	115	74	77	100	113	111	210	217	226	215	217
Liver stiffness, kPa	17.3	23.6	17.3	–	21.3	17.6	–	14.5	–	10.1	–	–	–	–	–
Spleen length, cm	12.4	–	12.6	–	11.6	14.0	–	14.0	–	13.0	–	–	–	–	–
Creatinine (mg/dl)	1.06	0.91	0.89	0.91	0.94	0.82	0.85	0.83	0.96	0.88	0.8	0.72	0.74	0.81	0.73
Glycaemia (mg/dl)/ triglycerides (mg/dl)	151/87	135/82	115/87	122/96	123/82	118/129	127/94	119/91	123/77	119/ 122	128/142	106/134	89/172	115/ 160	115/ 193
Total cholesterol (mg/dl)/HDL (mg/dl)	157/65	145/59	154/54	136/45	166/63	166/41	171/46	185/54	189/53	169/46	198/–	196/–	198/–	193/–	194/–
HBsAg (IU/ml)	10	13	14	21	29	9,091	10,388	8,905	7,998	7,499	9,780	13,264	9,688	9,462	10,181
HBV DNA (IU/ml)	TND	TND	TND	TND	TND	TND	TND	<10	<10	TND	<20	<20	<20	<20	<20
HBV RNA (cp/ml)	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160	–	–	–	–	–
HDV RNA	23,600[a]	6,330[a]	75[a]	<6[a]	<6[a]	392,000[a]	642[a]	17[a]	<6[a]	TND[a]	5,900,000[b]	400,000[b]	9,500[b]	2,000[b]	500[b]

*MyrB injection was performed before blood sampling. [a]IU/ml; [b]copies/ml.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein cholesterol; MyrB, myrcludex-B; pCHE, pseudo-cholinesterase; PT-INR, prothrombin time-international normalized ratio; TDF, tenofovir disoproxil fumarate; TND, target not detected; WBC, white blood cells.

Reference values: AST, 10–33 IU/L; ALT, 6–41 IU/L; ALP, 35–104 IU/L; GGT, 5–36 IU/L; total bilirubin, 0.12–1.10 mg/dl; total biliary acids <7 μmol/L; pCHE, 4,200–11,250 IU/L; albumin, 3.4–4.8 g/dl; IgG, 700–1,600 mg/dl; INR, 0.84–1.20; WBC, 4,800–10,800/mm^3; Hb, 12–16 g/dl; platelet count, 130–400 x10^9/L; creatinine, 0.5–1.0 mg/dl; glycaemia, 70–110 mg/dl; triglycerides, <150 mg/dl; total cholesterol, <190 mg/dl; HDL, >45 mg/dl; HBsAg, <0.05 IU/ml; HDV RNA, <6 IU/ml or 100 copies/ml.

endpoint of >2 log decline of HDV RNA and ALT normalisation,[8] but also experienced a profound suppression of viral replication with a 4.0 to 5.0 log decline of HDV RNA. Of note, viraemia became undetectable in 2 patients. These results are in line with the published MYR studies results that showed a progressive, dose-dependent decline in HDV RNA. HDV RNA undetectability was achieved in 3.3 to 6.2% of the patients treated with different doses for 24 weeks in the MYR 202 study (personal communication from MYR GmbH), and in 2 out of 15 (13%) patients treated with 2 mg/day for 48 weeks.[5] Of note, 10 mg was shown to induce a steeper HDV RNA decline than 2 mg in the MYR 202 clinical trial during 24 weeks of treatment. The kinetics of biochemical and virological responses in our patients demonstrated that the ALT response was faster than the HDV RNA response, a pattern that is in agreement with the previous phase II studies. Last but not least, virological and biochemical responses were maintained over time with no evidence of virological or biochemical breakthrough, nor HBV DNA increase. Compared to IFN monotherapy which has limited efficacy and significant side effects, this report supports the possibility of an anti-HDV strategy based on long-term administration of MyrB monotherapy, with the aim of suppressing viral load and intrahepatic viral replication to undetectable levels. In patients with contraindications or not responding to IFN, MyrB monotherapy may represent a viable therapeutic option. However, as there are no studies assessing the efficacy and safety of MyrB in decompensated cirrhotics, the use of MyrB in this setting will require dedicated clinical trials.

The study also provides important clinical findings. Biochemical and virological responses preceded the improvements of liver function tests, AFP levels and platelet counts. These findings are of clinical relevance given the well-known aggressiveness of hepatitis delta and the relative short duration of therapy in these patients. Moreover, in Case 2 who had histological and clinical features of autoimmune hepatitis secondary to HDV infection, not a rare condition in HDV-infected patients, IgG and gamma globulins normalised in parallel with HDV RNA decrease. This could be of clinical relevance in patients with HDV infection and autoimmune stigmata that nowadays represents a contraindication to IFN introduction.

Finally, 2 cases were also tested for additional markers, with the aim of investigating the T cell activation pattern. Despite the virological response, we did not detect any sign of recovery of HDV-specific and HBV-specific T cell responses in these 2 MyrB treated patients. However, an analysis of the impact of MyrB therapy on HDV and HBV-specific T cell recovery requires larger studies and patients of younger age, who are often characterised by a more conserved antiviral T cell repertoire. Furthermore, the possibility that HDV inhibition would affect the global immunity profile might not be discounted and a more global analysis of other components of the immune system (B, natural killer and myeloid cells) is needed. HBV RNA levels were under the limit of detection before and during MyrB treatment, which likely reflects low transcription of HBV RNA from covalently closed circular DNA (cccDNA). This could be attributed to the HBeAg-negative status, as well as to the long preceding period of TDF treatment (>6 years) in both patients, or the limited sensitivity of the method. Coinfection with HDV may also have played a significant role. Presuming that cccDNA activity was low, the detected HBsAg was likely predominantly derived from the integrated HBV genome.

Whether a 48-week course of MyrB 10 mg/day could lead to a sustained, *i.e.* off-treatment, virological and biochemical response is a major question. However, this is presently unknown as no studies have so far assessed this regimen and all our patients are planned to continue MyrB beyond week 48. In fact, the MYR 202 study clearly demonstrated that a 24-week course of MyrB does not induce long-term off-treatment responses although approximately 10% of patients maintained normal ALT levels and had HDV RNA <2 log IU/ml 24 weeks after treatment discontinuation. Similar findings were recently reported at the International Liver Congress™ 2019: only 2 of 15 (13%) patients treated with MyrB 2 mg monotherapy for 48 weeks maintained a combined HDV RNA and ALT response 24 weeks post-treatment (H. Wedemeyer, *et al.* EASL 2019 G13; *J Hepatol* 2019;70(S):e81). As suggested by recent *in vitro* models, it is likely that treatment must be extended well beyond 48 weeks. Indeed, MyrB monotherapy will be administered for 3 years in the phase III trial. Whether long-term off-treatment responses can be achieved without the loss of HBsAg will be investigated in upcoming trials.

In conclusion, this is the first report of MyrB administered at the dose of 10 mg/day for 48 weeks in 3 patients with HDV-related compensated cirrhosis in a real-life setting. The excellent virological and biochemical responses induced and maintained by long-term administration of MyrB monotherapy coupled with a favourable safety profile may represent an interesting therapeutic approach for a difficult to treat and aggressive disease such as hepatitis delta. Long-term studies in a larger number of patients are required to confirm these preliminary findings.

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Conflict of interest

Peter Ferenci: advisor and speaker bureau for Gilead Sciences, GSK, MSD, Abbvie; Florian van Bömmel: research grants from Gilead Sciences Inc., MYR Pharma and Roche Diagnostics, speaker and advisor for Gilead Sciences, Roche, Janssen, Abbvie, MSD and BMS; Antonio Bertoletti advisor for Gilead, SpringBank, Vir, Simcere. He is also Scientific Founder of LION TCR pte.; Pietro Lampertico: advisor and speaker bureau for BMS, Roche, Gilead Sciences, GSK, MSD, Abbvie, Janssen, Arrowhead, Alnylam, Eiger, MYR Pharma. The other authors declare that they have no competing interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

AL, PF and PL were involved in patient's care and drafting of the manuscript. AL, MB, RP were involved in data collection. ET, SG, LP, LL, DP were involved in PBMC extraction and cryopreserva-

tion. CT and AB performed T cell analysis. HH, SU, LG, FC, GL and FvB performed molecular and virological analysis. AL, AB, PF and PL were involved in manuscript editing. All authors approved the final version of the manuscript.

Declarations

The uses of myrcludex-B were approved by the local Ethics Committee on a 6-month basis for the Italian patients.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.07.003.

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