Research Article
Viral Hepatitis

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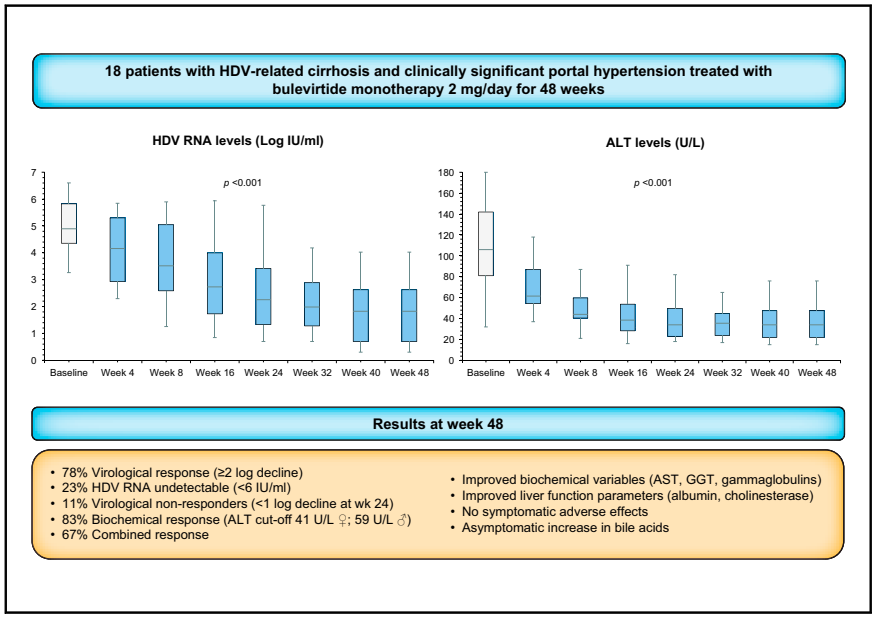
Bulevirtide monotherapy for 48 weeks in patients with HDV-related compensated cirrhosis and clinically significant portal hypertension

Graphical abstract



Highlights

- 48 weeks of bulevirtide 2 mg/day monotherapy was safe in patients with compensated cirrhosis and clinically significant portal hypertension.
- Virological, biochemical, and combined responses were achieved in 78%, 83% and 67% of patients, respectively.
- BLV treatment led to a significant improvement in most biochemical variables and an increase in liver function parameters.
- Treatment was safe and well tolerated, an asymptomatic increase of bile acids was observed.

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Lay summary

Hepatitis delta virus (HDV) is associated with the most severe form of viral hepatitis. A new treatment for HDV called bulevirtide has recently received conditional approval for patients with chronic HDV infection. However, its safety and effectiveness in patients with more advanced liver disease is not known. Herein, we show that it is safe and effective in patients with HDV-related cirrhosis and clinically significant portal hypertension.

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Bulevirtide monotherapy for 48 weeks in patients with HDV-related compensated cirrhosis and clinically significant portal hypertension

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Background & Aims: Bulevirtide (BLV) has recently been conditionally approved for the treatment of chronic hepatitis delta (CHD) in Europe, but its effectiveness and safety in patients with compensated cirrhosis and clinically significant portal hypertension (CSPH) are unknown.

Methods: Consecutive patients with HDV-related compensated cirrhosis and CSPH who started BLV 2 mg/day were enrolled in this single-center study. Clinical/virological characteristics were collected at baseline, weeks 4, 8 and every 8 weeks thereafter. HDV RNA was quantified by Robogene 2.0 (lower limit of detection 6 IU/ml).

Results: Eighteen Caucasian patients with compensated cirrhosis and CSPH under nucleos(t)ide analogue treatment were enrolled: median (IQR) age was 48 (29-77) years, and 67% were male. Median (IQR) platelet count was 70 (37-227) x10^3/µl, liver stiffness measurement (LSM) 16.4 (7.8-57.8) kPa, alanine aminotransferase (ALT) 106 (32-222) U/L, HBsAg 3.7 (2.5-4.3) log IU/ml, HDV RNA 4.9 (3.3-6.6) log IU/ml. During 48 weeks of BLV monotherapy, HDV RNA declined by 3.1 (0.2-4.3) log IU/ml (p <0.001 *vs.* baseline), becoming undetectable in 5 patients (23%). A virological response was observed in 14 (78%) patients while a non-response was observed in 2 (11%). ALT decreased to 35 (15-86) U/L (p <0.001 *vs.* baseline), normalizing in 83% of patients. A combined response was observed in 67% of patients. Aspartate aminotransferase and gamma-glutamyltransferase levels significantly improved. Concerning liver function parameters, albumin values significantly increased and bilirubin remained stable. LSM significantly improved in patients with virological response, while platelet count was unchanged. None of the patients developed decompensating events or hepatocellular carcinoma. BLV was well tolerated, no patient discontinued treatment and the increase in bile acids was fully asymptomatic.

Conclusions: A 48-week course of BLV 2 mg/day monotherapy is safe and effective even for difficult-to treat patients with HDV-related compensated cirrhosis and CSPH.

Lay summary: Hepatitis delta virus (HDV) is associated with the most severe form of viral hepatitis. A new treatment for HDV called bulevirtide has recently received conditional approval for patients with chronic HDV infection. However, its safety and effectiveness in patients with more advanced liver disease is not known. Herein, we show that it is safe and effective in patients with HDV-related cirrhosis and clinically significant portal hypertension.

Introduction

Chronic hepatitis delta (CHD) is a severe form of chronic viral hepatitis that affects approximately 12-72 million patients worldwide.[1–3] Until now, the only therapeutic approach available was the off-label use of a 48-week course of pegylated-interferon-α (PegIFNα), an antiviral strategy characterized by limited off-therapy responses and an unfavorable safety profile.[4–7]

In 2020, the European Medicines Agency provided conditional approval for bulevirtide (BLV) at the dose of 2 mg/day subcutaneously[5] for the treatment of compensated CHD. BLV is a first in class entry inhibitor of HBV; it is a lipopeptide that mimics the Na+-taurocholate co-transporting polypeptide (NTCP) receptor binding domain, blocking HDV/HBV entry exclusively in liver cells and thereby preventing NTCP-mediated intrahepatic HDV spreading.

In phase II trials and in week 48 analysis of the phase III study, BLV treatment significantly reduced HDV RNA levels and improved alanine aminotransferase (ALT) levels, although these benefits were lost in most patients after drug withdrawal.[8–11] When combined with PegIFNα, a synergistic effect on both HDV viremia and HBsAg decline was demonstrated.[9,10] Virological response was mirrored by a significant decline of intrahepatic HDV RNA levels and of the number of HDV antigen (HDAg)-positive cells in a subset of patients treated for 48 weeks.[12] Preliminary real-life studies not only confirmed the

Keywords: Bulevirtide; HDV; Entry inhibitor; HDV RNA; HBcrAg; HBV RNA; HBV; compensated cirrhosis; clinically significant portal hypertension.
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effectiveness and safety of this treatment strategy but also suggested that treatment extension may further improve both virological and biochemical responses.[13–17]

However, to date, the safety and effectiveness of BLV 2 mg/day in patients with compensated cirrhosis and clinically significant portal hypertension (CSPH)[18–22] are poorly known, as these patients have been scarcely represented in clinical trials of BLV and there are only a few case reports in the literature.[15] These difficult-to-manage patients face a substantial risk of developing decompensated liver disease and hepatocellular carcinoma (HCC) within a short period of time. Moreover, treatment with PegIFNα is contraindicated due to the severity of the underlying liver disease.

Our study aimed to explore the safety and effectiveness of BLV 2 mg/day monotherapy in patients with HDV-related compensated cirrhosis and CSPH with or without HCC.

Patients and methods

Patient population

This is a single-center study that was performed at the outpatient Liver Clinic of the Hepatology Division of the Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico. All consecutive patients with HDV-related compensated cirrhosis and CSPH who started BLV 2 mg/day monotherapy from December 2020 were enrolled. CHD was defined as HDV RNA positivity >6 months. Cirrhosis was defined either histologically (METAVIR F4), non-invasively by liver stiffness measurement (LSM)[21] or clinically (blunted nodular liver edges at abdominal ultrasound ± splenomegaly + platelet count under the limit of normal). CSPH was defined as either presence of gastroesophageal varices, LSM >25 kPa or platelet count <100 x 10^3/μl with evidence of porto-systemic collaterals at abdominal imaging.[18–22]

BLV treatment was approved on a case-by-case basis by the Italian Medicines Agency (AIFA) and provided in the context of the 5% AIFA fund program. Informed consent was obtained for all participants, according to Helsinki Declaration and the study was approved by the local IRB (Comitato Etico Area 2 Milano). BLV at 2 mg was self-administered as subcutaneous injections every 24 hours. All patients were already receiving tenofovir disoproxil fumarate or entecavir treatment for HBV.

Follow-up and measurements

All clinical and virological variables were collected at treatment baseline, week 4, 8, 16, 24 and 48. LSM and controlled-attenuation parameter were assessed at weeks 8, 24 and 48, upper endoscopy according to Baveno VI recommendations, and HCC surveillance were performed as per international guidelines.[23]

LSM examination by FibroScan® (Echosens, Paris, France) was performed by a trained operator certified by the manufacturer. LSM was assessed following at least 4 hours of fasting; results were expressed in kPa. Examinations were deemed valid in case of at least 10 valid individual measurements and were classified as very reliable or reliable according to the Boursier criteria (interquartile range/median [IQR/M] ≤10%: very reliable; IQR/M >10% and ≤30%: reliable).[24]

HDV RNA was quantified by RoboGene® HDV RNA quantification 2.0 (Aj-Roboscreen, Jena, Germany; lower limit of detection 6 IU/ml); HBV RNA was quantified by a real-time PCR-based investigation assay (Roche Diagnostics, Pleasanton, Ca, USA, lower limit of quantification [LLOQ] 10 cp/ml).[25] Serum HBsAg was quantified by Elecsys HBsAg II quantitative assay on the Cobas® e801 Analyzer (Roche Diagnostics Gmbh, Mannheim, Germany; LLOQ 0.05 IU/ml). Serum hepatitis B core-related antigen (HBcrAg) levels were measured using LUMIPULSE® G HBcrAg assay (Fujirebio Europe, LOD 2 $\log_{10}$ IU/ml). HBV DNA was quantified by Cobas® HBV Test on the Cobas® 4800 System (Roche Diagnostics, Germany; LLOQ 10 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 HDAg region. HDV RNA was reverse transcribed into cDNA and amplificated with SuperScript™ IV One-Step RT-PCR System (ThermoFisher Scientific, USA) using the outer sense primer (5'-GCCCAGGTCGGACCGCGAGGA-3') and the outer antisense primer (5'-ACAAGGAGAGGCAGGATCACCGAC-3'). Nested PCR amplification was performed with Platinum™ II Taq Hot-Start DNA Polymerase (ThermoFisher Scientific, USA) using the inner sense primer (5'-GAGATGC CATGCCGACCCGAAGAG-3') and the inner antisense primer (5'-GAAGGAAGGCCC TCGAGAACAAGA-3'). PCR amplicons were visualized on 2% TBE agarose gel, purified and sequenced with BigDye™ Terminator v1.1 Cycle Sequencing Kit (ThermoFisher Scientific, USA) on an Applied Biosystem ABI Genetic Analyzer (ThermoFisher Scientific, USA) using the inner primers (supplementary CTAT table).

Study endpoints

The primary endpoint of the study was virological response defined as HDV RNA undetectable or ≥2 log IU/ml decline *vs.*

Table 1. Baseline demographic, clinical and virological features of the 18 enrolled patients with chronic hepatitis delta.

Baseline variables	N = 18
Age, years	48 (29-77)
Males	12 (67%)
Caucasian	18 (100%)
HDV genotype 1	18 (100%)
Child-Pugh score A5°	13 (72%)
Esophageal varices°°	14 (78%)
Spleen size, cm	17 (10-25)
LSM, kPa	16.4 (7.8-57.8)
CAP, dB/m	194 (100-271)
BMI >25 kg/m^2	8 (44%)
Active HCC	2 (11%)
Previous IFN treatment	12 (67%)
TDF or ETV treatment°°°	18 (100%)
Bilirubin, mg/dl	1.3 (0.5-1.8)
AST, U/L	92 (52-214)
ALT, U/L	106 (32-222)
Albumin, g/dl	3.9 (2.9-4.4)
Platelet count, 10^3/μl§	70 (37-227)
MELD score	6 (13-13)
Bile acids, μmol/L[a]	23 (8-306)
HBsAg, Log IU/ml	3.7 (2.5-4.3)
HBeAg negative	17 (94%)
HBV DNA detectable§§	4 (28%)
HBV RNA detectable[c]	1 (6%)
HBcrAg, Log IU/ml[b]	3.8 (2.3-5.0)
HDV RNA, Log IU/ml	4.9 (3.3-6.6)

Values are expressed as n (%) or median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled-attenuation parameter; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; IFN, interferon; MELD, model for end-stage liver disease; TDF, tenofovir disoproxil fumarate.
°Child-Pugh A6 n = 5 (28%), mild ascites in 3 patients.
°°n = 7 under primary prophylaxis.
°°°10 (56%) in TDF.
§33% with platelets <60x10^3/μl.
§§HBV DNA: median 15 (range 14–22) IU/ml.
[a]bile acids normal range between 1 and 6 μmol/L.
[b]HBcrAg levels >3 log U/ml in 17 (90%) patients.
[c]HBV RNA > 10 cp/ml.

baseline at week 48. Secondary endpoints included: 1) safety defined as proportion of patients experiencing treatment-related adverse events; 2) biochemical response, defined as ALT normalization; 3) combined response, defined as HDV RNA undetectable or ≥2 log IU/ml decline *vs.* baseline and ALT normalization; 4) virological non-response, defined as <1 log decline in HDV RNA levels compared to baseline; 5) proportion of patients developing a liver-related complication, *i.e.* decompensation and or HCC. Exploratory endpoints included the proportion of patients with HDV RNA <100 IU/ml and <1,000 IU/ml at week 48, and the proportion of patients with detectable HBV RNA and or HBcrAg.

Statistical analysis

Categorical variables were reported as frequencies (percentages) and continuous variables as median (range). Categorical variables were compared using the Chi-square or the Fischer exact tests, and continuous variables by the Student's *t* test, the Mann-Whitney *U* test or the Kruskal-Wallis test, when appropriate. All tests were 2-sided and used a significance level of 0.05. Repeated analysis of variance was used to compare continuous variables assessed at different timepoints and Bonferroni correction was applied in order to counteract the multiple testing problem.

Results

Baseline features

The baseline demographic, virological and clinical features of the 18 patients enrolled are shown in Table 1. Most patients were middle aged men with elevated HBsAg, ALT, HDV RNA, and LSM levels, with undetectable HBV DNA, negative HBeAg, low platelet counts and previous non-response to IFN. All patients were Caucasian with compensated cirrhosis (72% Child-Pugh A5, 28% A6) and HDV genotype 1 infections. All patients were receiving nucleos(t)ide analogue therapy for HBV (10 on tenofovir disoproxil fumarate and 8 on entecavir). While only 1 patient had detectable HBV RNA levels (27 cp/ml), all but one had high levels of HBcrAg levels, the median being approximately 4 logs IU/ml (Table 1). Two patients had active HCC. Cirrhosis was diagnosed by liver biopsy in 9 (50%) patients, and clinically and/or non-invasively in the remaining 9 (50%). CSPH was diagnosed according to the presence of gastroesophageal varices in 14 (78%), and platelet count <100 x 10^3/μl with evidence of porto-systemic collaterals at abdominal imaging in the remaining 4 (22%) patients (Table S1).

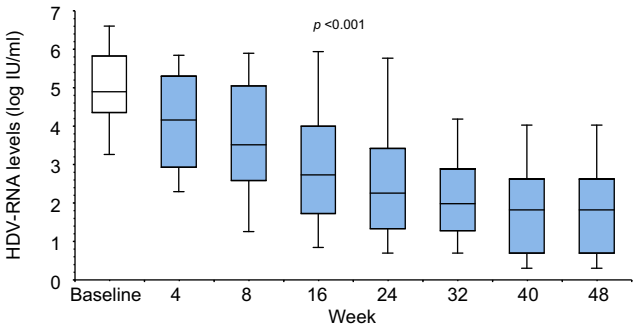

Fig. 1. HDV RNA levels during 48 weeks of BLV 2 mg/day treatment in the overall population. Repeated analysis of variance, level of significance *p* <0.05. BLV, bulevirtide.

Virological and biochemical response

HDV RNA levels progressively declined from 4.9 (3.3-6.6) log IU/ml at baseline to 2.2 (0.3-6.0) log IU/ml at week 48 (*p* <0.001) (Fig. 1, Table 2). The overall median decline *vs.* baseline was 3.1 (0.2-4.3) log IU/ml. By week 48, 5 patients achieved undetectable HDV RNA (<6 UI/ml), while 2 additional patients achieved a viremia of <100 IU/ml: overall, viremia was <100 IU/ml in 7 (39%) patients (Table 2, Fig. S1). When patients were stratified according to baseline viremia, 6 out of 9 patients with pretreatment HDV RNA <5 log IU/ml achieved viremia <100 IU/ml at week 48 compared to one of the remaining patients (67% *vs.* 11%, *p* = 0.01). Virological non-response, defined as <1 log decline of viremia at week 24, was observed in 2 (11%) patients (Fig. S2A-B). Overall, a virological response, defined as undetectable HDV RNA or a ≥2 log decline *vs.* baseline, was observed in 14 (78%) patients (Table 2).

ALT levels rapidly improved in most patients (Table 3, Fig. 2, Fig. S3), decreasing from 106 (32-222) U/L at baseline to 35 (18-

Table 2. Time course of virological variables during 48 weeks of BLV treatment (N = 18).

Variables	Baseline	Week 8	Week 16	Week 24	Week 32	Week 40	Week 48	*p* value
HDV RNA, Log IU/ml	4.9 (3.3-6.6)	3.5 (1.2-5.9)	2.7 (0.9-5.9)	2.3 (0.7-5.8)	2.0 (0.7-5.8)	1.8 (0.3-6.0)	2.2 (0.3-6.0)	**<0.001**
HDV RNA decline, Log IU/ml	-	1.4 (0.4-3.1)	2.2 (0.4-3.6)	2.7 (0.6-3.9)	2.8 (0.4-3.9)	3.1 (0.3-4.6)	3.1 (0.2-4.3)	**<0.001**
HDV RNA decline ≥2 Log IU/ml	-	2 (11%)	7 (39%)	15 (83%)	15 (83%)	14 (78%)	14 (78%)	**<0.001**
HDV RNA decline <1 Log/ml	-	2 (11%)	2 (11%)	2 (11%)	2 (11%)	2 (11%)	2 (11%)	0.97
HDV RNA <1,000 IU/ml	0	8 (44%)	10 (56%)	13 (72%)	14 (78%)	14 (78%)	14 (78%)	**<0.001**
HDV RNA <100 IU/ml	0	2 (11%)	7 (39%)	9 (50%)	10 (56%)	10 (56%)	7 (39%)	**<0.001**
HDV RNA <6 IU/ml	0	0	0	2 (11%)	5 (23%)	6 (33%)	5 (23%)	**0.003**
Virologic response°	**-**	**2 (11%)**	**7 (39%)**	**15 (83%)**	**15 (83%)**	**14 (78%)**	**14 (78%)**	**<0.001**
HBsAg, Log IU/ml	3.7 (2.5-4.3)	3.8 (2.6-4.3)	3.8 (2.6-4.3)	3.8 (2.5-4.3)	3.7 (2.5-4.2)	3.7 (2.5-4.3)	3.7 (2.4-4.2)	0.31
HBV DNA detectable**	4 (28%)	0	0	0	2 (11%)	2 (11%)	1 (5%)	0.08
HBV RNA detectable***	1 (6%)	n.a.	n.a.	0	n.a.	n.a.	0	n.a.
HBcrAg, Log U/ml	3.8 (3.0-5.0)	3.7 (3.0-5.1)	3.8 (3.0-5.0)	3.7 (3.0-5.0)	3.7 (3.0-4.9)	3.7 (3.0-4.9)	3.7 (3.0-4.9)	**0.03**
HBcrAg >3 log U/ml	17 (94%)	16 (89%)	16 (89%)	16 (89%)	16 (89%)	16 (89%)	16 (89%)	0.99

Values are expressed as n (%) or median (range). Bold enhances the concept that virologic response represents the primary study endpoint.
Categorical variables were compared using the $\chi 2$ or the Fisher's exact tests (level of significance *p* <0.05); repeated analysis of variance was used to compare continuous variables assessed at different timepoints (level of significance *p* <0.05). Bonferroni correction was applied in order to counteract the multiple testing problem.
BLV, bulevirtide; HBcrAg, hepatitis B core-related antigen.
°Virological response: HDV RNA undetectable or ≥2 log IU/ml decline *vs.* baseline.
**HBV DNA >10 IU/ml.
***HBV RNA >10 cp/ml.

Table 3. Time course of biochemical variables during 48 weeks of BLV treatment (N = 18).

Variables	Baseline	Week 8	Week 16	Week 24	Week 32	Week 40	Week 48	*p* value
Bilirubin, mg/dl	1.3 (0.5-1.8)	1.0 (0.4-2.9)	0.9 (0.5-2.4)	1.0 (0.3-2.5)	1.0 (0.5-2.5)	0.9 (0.4-4.1)	1.2 (0.5-4.6)	0.51
AST, U/L	92 (52-214)	52 (26-123)	42 (26-141)	38 (24-134)	39 (25-97)	36 (23-86)	39 (21-92)	**<0.001**
ALT, U/L	106 (32-222)	44 (21-114)	39 (16-91)	34 (18-82)	36 (17-80)	34 (15-76)	35 (15-86)	**<0.001**
GGT, U/L	52 (13-262)	43 (11-270)	35 (6-229)	30 (6-237)	29 (7-199)	27 (7-179)	23 (6-158)	**0.01**
Albumin, g/dl	3.9 (2.9-4.4)	3.9 (3.1-4.8)	3.9 (3.0-4.4)	3.9 (3.5-4.6)	4.0 (3.5-4.5)	4.1 (3.5-4.7)	4.0 (3.6-4.7)	**0.03**
Platelet count, 10^3/µl	70 (37-227)	68 (40-210)	67 (35-228)	70 (33-219)	70 (44-192)	77 (37-211)	73 (24-221)	0.93
Bile acids, µmol/L[a]	23 (8-306)	60 (11-490)	48 (11-710)	37 (7-748)	49 (10-748)	61 (7-416)	63 (10-416)	**0.04**
Creatinine, mg/dl	0.8 (0.7-1.0)	0.9 (0.6-1.1)	0.9 (0.7-1.2)	0.9 (0.7-1.1)	0.9 (0.7-1.1)	0.9 (0.6-1.1)	0.9 (0.6-1.1)	0.66
AFP, µg/L°°	9 (3-596)	9 (3-846)	8 (2-495)	6 (3-14)	5 (2-17)	5 (2-15)	5 (2-15)	0.29
IgG, mg/dl	2,168 (1,047-4,059)	2,056 (1,009-3,208)	1,570 (988-2,329)	1,666 (980-2,286)	1,604 (953-2256)	1,611 (996-2312)	1,643 (901-2200)	**<0.001**
Gamma globulins, g/dl	2.0 (1.0-3.4)	2.0 (0.9-2.8)	1.7 (1.0-2.6)	1.6 (1.0-2.1)	1.5 (1.0-2.2)	1.5 (0.9-2.2)	1.5 (0.9-2.1)	**<0.001**
CHE, U/L	4,471 (1,807-8,378)	4,599 (2,337-8,861)	4,949 (2,715-8.759)	4,982 (2,854-6.849)	4,997 (2,837-7,793)	5,550 (2,465-8,826)	5,396 (2,229-8,826)	**0.04**
LSM, kPa	16.4 (8-58)	21.8 (9-49)	-	17.4 (6-48)	-	-	13.7 (5-30)	0.06
Biochemical response*	1 (6%)	9 (50%)	14 (78%)	13 (72%)	14 (78%)	16 (89%)	15 (83%)	**<0.001**
Combined response°	-	0	5 (28%)	12 (67%)	11 (61%)	13 (72%)	12 (67%)	**<0.001**

Values are expressed as n (%) or median (range). Bold enhances statistically significant comparisons.
Categorical variables were compared using the $\chi 2$ or the Fisher's exact tests (level of significance p <0.05); repeated analysis of variance was used to compare continuous variables assessed at different timepoints (level of significance p <0.05). Bonferroni correction was applied in order to counteract the multiple testing problem.
AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BLV, bulevirtide; CHE, cholinesterase; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma.
*Biochemical response: ALT normalization.
°Combined response: HDV RNA undetectable or ≥2 log IU/ml decline *vs.* baseline and ALT normalization.
°°Both patients with HCC, one of them with high AFP levels, underwent successful percutaneous ablation.
[a]bile acids normal range between 1 and 6 µmol/L.

86) U/L at week 48 (p <0.001). The proportion of patients with normal ALT levels increased from 1 (6%) at baseline to 15 (83%) at week 48. At week 48, 12 patients (67%) achieved a combined virological and biochemical response. When different ALT cut-offs were considered (31 U/L for females, and 41 U/L for males), the corresponding rates of biochemical and combined response were 56% and 50%. Significant improvements were also observed for aspartate aminotransferase (p <0.001) and gamma-glutamyltransferase levels (p = 0.01) (Table 3, Figs. S4-5).

Other virological markers and clinical response

HBsAg, HBV DNA levels (Fig. S6) as well as HBeAg status remained unchanged throughout therapy, while a decrease in HBcrAg median values was observed (baseline *vs.* week 48; p = 0.03) (Table 2). The overall HBV RNA/HBcrAg pattern remained unchanged, as all patients remained HBV RNA negative and HBcrAg positive during 48 weeks of BLV treatment.

Concerning markers of liver function, albumin and cholinesterase (CHE) levels significantly improved, so that 4 out of 5 Child-Pugh A6 patients at baseline improved to Child-Pugh A5 at week 48. In addition, immunoglobulin G significantly declined from 2,168 to 1,643 mg/dl (p <0.001) and gamma globulin levels declined from 2.0 to 1.5 g/dl at week 48 (p <0.001) (Table 3; Figs. S7-10). Bilirubin, prothrombin time (assessed by international normalized ratio), platelet count and LSM remained unchanged during the first 48 weeks of therapy, although LSM values showed a declining trend (16.4 kPa *vs.* 13.7 kPa, p = 0.06) (Figs. 3-4). Median alpha-fetoprotein values decreased from 9 (3-

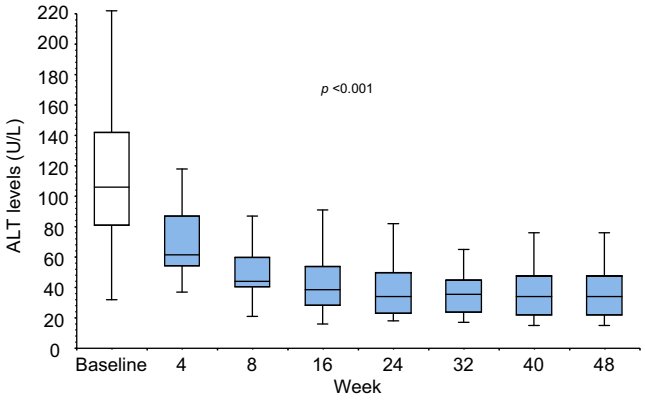

Fig. 2. ALT levels during 48 weeks of BLV 2 mg/day treatment in the overall population. Repeated analysis of variance, level of significance p <0.05. ALT, alanine aminotransferase; BLV, bulevirtide.

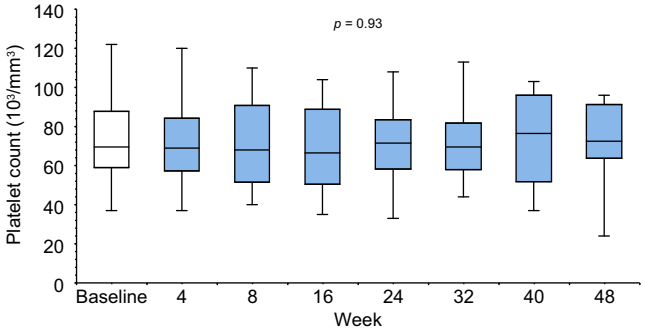

Fig. 3. Platelet count during 48 weeks of BLV 2 mg/day treatment in the overall population. Repeated analysis of variance, level of significance p <0.05. BLV, bulevirtide.

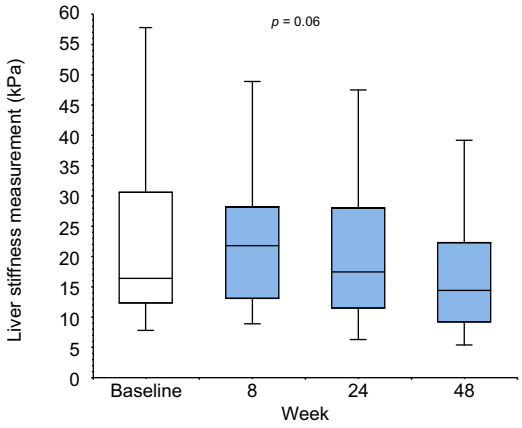

Fig. 4. LSM values during 48 weeks of BLV 2 mg/day treatment in the overall population. Repeated analysis of variance, level of significance p <0.05. BLV, bulevirtide; LSM, liver stiffness measurement.

596) to 5[2–15] μg/L, although this change was not significant (p = 0.28) (Table 3).

When restricting the analysis to the 16 patients achieving a virological response, similarly to the overall analysis, a significant reduction in aspartate aminotransferase, ALT, gamma-glutamyltransferase, immunoglobulins, gamma globulins and HBcrAg levels was observed, as well as significant increases in albumin, bile acid and CHE levels. Conversely, LSM decline became significant (16.4 kPa at baseline *vs.* 14.1 kPa at week 48, p = 0.03).

At week 48, 13 out of 18 patients qualified for a repeat upper endoscopy: among the 9 patients not receiving endoscopic (banding) prophylaxis, varices remained stable in 8 patients (no varices in 3, F1 in 5) and regressed in 1 patient (F1 to no varices).

The 2 patients with active HCC at baseline had early-stage tumors (BCLC stage A) and underwent successful percutaneous radiofrequency ablation without complications during BLV treatment. Overall, none of the patients developed decompensating events (including ascites, encephalopathy, bleeding) or *de novo* or recurrent HCC during the study period.

Adverse events

None of the patients discontinued BLV and no symptomatic adverse effects were reported, including injection site reactions. Median bile acids increased (Fig. 5) from 23 (8-306) μmol/L at baseline to 60 (11-490) μmol/L at week 8 and maintained fluctuating levels during the course of BLV treatment, reaching 63 (10-416) μmol/L at week 48 (baseline *vs.* week 48: p = 0.04). All patients remained fully asymptomatic for pruritus. Notably, all patients had pretreatment bile acid levels exceeding the upper limit of normal (6 μmol/L). Overall, BLV was well tolerated in these difficult-to-manage patients with compensated cirrhosis and CSPH, including in patients with active HCC. BLV administration was also safe in the 2 patients receiving anti-coagulants (rivaroxaban and warfarin).

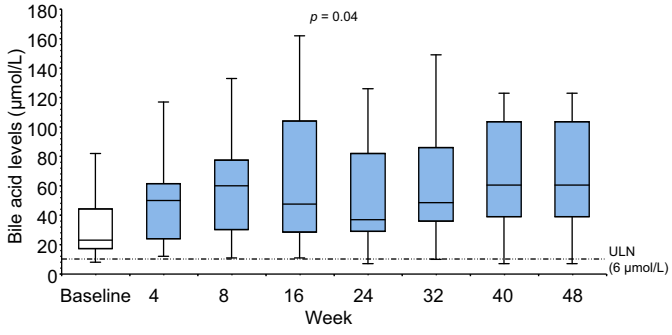

Fig. 5. Bile acids levels during 48 weeks of BLV 2 mg/day treatment in the overall population. Repeated analysis of variance, level of significance p <0.05. BLV, bulevirtide.

Discussion

This study describes the effectiveness and safety of BLV 2 mg/day administered as monotherapy for 48 weeks in patients with HDV-related compensated cirrhosis and CSPH and/or active HCC. To our knowledge, this is the first report showing that BLV is effective and safe in these difficult-to-treat patients with advanced compensated cirrhosis who have been underrepresented in the phase II and III studies of new anti-HDV compounds.

The virological response to BLV 2 mg monotherapy observed in our study in these difficult-to-treat patients was similar to the response previously reported in studies enrolling patients with milder liver disease.[8–11] In fact, most patients achieved a week 24 virological response that was maintained through week 48 despite the presence of advanced cirrhosis, the decline of viremia comparing favorably with that already reported in previous studies.[8–11] Our data are also in line with a recently published Austrian real-life study, reporting an overall 65% virological response after 48 weeks of BLV 2 mg/day: in this paper, 10 out of 16 patients with cirrhosis included had a platelet count below 100,000/μl.[17] In our study, the slight increase in median HDV RNA values (1.8 log *vs.* 2.2 log) between week 40 and week 48 was related to partial non-compliance in 3 patients, that resulted in viremia increasing <1 log in all cases. In contrast to what has been suggested by the Austrian real-life study,[17] the rates of virological non-response to BLV 2 mg at week 24 in our real-life study were only 11%. Overall, these preliminary findings support the effectiveness of BLV 2 mg/day even in these difficult-to-treat patients.

Another important therapeutic endpoint for any liver disease is the achievement of ALT normalization, which has been associated with improved outcomes, independently of the underlying etiology.[26] This endpoint is even more relevant for patients with CHD in whom ALT levels are significantly elevated, heralding progression of the disease and poor survival.[5–7] In our study, most patients achieved a biochemical response during a relatively short period of time, 24 weeks of therapy, the rates of ALT normalization being even higher than those reported in previous studies in patients with milder HDV-related liver disease.[11] Interestingly, the proportion of patients who normalized ALT levels at different time points paralleled the percentage of patients in whom viremia dropped below 1,000 IU/ml. This relationship should be further investigated.

In addition, we assessed the impact of BLV monotherapy on the most important clinical parameters in patients with advanced CHD. Surrogate markers of liver function, such as albumin and CHE significantly improved by week 48, leading to Child-Pugh improvement to A5 in 4 out of 5 patients with Child-Pugh A6 at baseline. Bilirubin and international normalized ratio levels were largely unchanged, and no patient developed any clinical complication. Markers of portal hypertension such as

platelet count and LSM remained largely unchanged. However, LSM improved if the analysis was restricted to the virological responders; we could hypothesize that this could be due to the maintenance of increased ALT in non-responder patients, thus preventing LSM improvement in these patients. Given the fact that CHD is a rapidly progressive liver disease, these initial findings are promising but require confirmation in the long term. While the optimal duration of BLV treatment is currently unknown and it is agreed that treatment could be continued upon clinical benefit, we decided to prolong therapy beyond week 48 in all patients based on our results.

This study provided new insights into the usefulness of new HBV biomarkers in the setting of HDV coinfection. While quantification of HBV RNA and HBcrAg levels have been recently shown to be useful in the management of untreated or treated HBV-monoinfected patients,[27–30] their role in patients with CHD is poorly known. In a previous multicenter study involving a large number of untreated patients with CHD, we have shown that most of these patients tested negative for HBV RNA but positive for HBcrAg.[31] The present study not only confirms this divergent pattern in the pretreatment samples but also provides evidence that this virological condition is maintained during treatment with BLV monotherapy, thus extending the preliminary results generated in 2 patients with CHD treated with BLV for up to 3 years.[15,31] Since both markers are thought to mirror the transcriptional activity of the covalently closed circular DNA of HBV, and indeed in HBV-monoinfected patients their levels tend to display a parallel time course, the exact mechanism responsible for a divergent pattern in patients with HDV remains to be fully investigated. Interestingly, during 48 weeks of BLV treatment, a decline in median HBcrAg levels was observed: whether HBcrAg levels could be linked to HDV RNA kinetics, as well as the meaning of the observed HBcrAg decline, needs to be further evaluated. In line with previous reports, we did not observe any significant decline of HBsAg levels during BLV treatment.

The safety and tolerability of any new compound intended for treatment of patients with advanced liver disease is of paramount relevance. This is even more important if the antiviral therapy must be administered long-term.[5] Previous studies have shown that BLV 2 mg monotherapy is generally well tolerated, even if associated with a dose dependent yet asymptomatic increase in bile acids.[5,10,11,13–17] Despite the severity of liver disease of the patients included in our study, this pattern was indeed confirmed, with an overall modest increase in bile acids. No symptoms were reported in the 1 patient with high pretreatment levels of bile acids that further increased during therapy. Nevertheless, these encouraging preliminary findings must be taken with caution, given the 48-week duration of therapy.

We acknowledge that this study has some limitations. The limited duration of treatment, 48 weeks, prevents the generation of robust long-term clinical response data. Moreover, our results cannot be extended to decompensated (Child-Pugh B or C) patients. Since all patients were Caucasian and infected with HDV genotype 1, our findings cannot be generalized to other clinical settings. Finally, we did not include a control group. However, this is the first study to specifically explore the effectiveness and safety of BLV 2 mg/day in difficult-to-manage patients, such as those with compensated cirrhosis and CSPH. The single-center design of the study allowed for predefined and homogeneous management of patients coupled with the up-to-date virological methodology, including quantification of HBV RNA and HBcrAg levels. A control group would not be ethically acceptable, given the aggressiveness of this disease, if left untreated.

In conclusion, this is the first report describing the favorable safety profile and effectiveness of BLV 2 mg/day monotherapy administered for 48 weeks in patients with HDV-related compensated cirrhosis and CSPH. These encouraging yet preliminary results significantly expand the current knowledge on this new anti-HDV compound, which appears to be the first effective alternative to liver transplantation for these patients.

Abbreviations

ALT, alanine aminotransferase; BLV, bulevirtide; CHD, chronic hepatitis delta; CHE, cholinesterase; CSPH, clinically significant portal hypertension; HBcrAg, hepatitis B core-related antigen; HCC, hepatocellular carcinoma; HDAg, hepatitis D antigen; LSM, liver stiffness measurement; NTCP, Na+-taurocholate co-transporting polypeptide; PegIFNα, pegylated-interferon-alpha.

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Conflict of interest

Elisabetta Degasperi: Advisory Board: AbbVie; Speaking and teaching: Gilead, MSD, AbbVie; Alessandro Loglio: Travel grant for MYR Pharma, Speaker bureau for Gilead Sciences; Fabien Zoulim: Advisor for Aligos, Antios, Arbutus, Assembly, Gilead, GSK, MYR Pharma, Roche Pietro Lampertico: Advisor and speaker bureau for BMS, Roche, Gilead Sciences, GSK, MSD, Abbvie, Janssen, Arrowhead, Alnylam, Eiger, MYR Pharma, Antios, Aligos. Other authors have nothing to disclose.

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

Authors' contributions

Concept and design: Elisabetta Degasperi, Alessandro Loglio, Pietro Lampertico. Data collection: Elisabetta Degasperi, Maria Paola Anolli, Alessandro Loglio, Marta Borghi, Riccardo Perbellini, Dana Sambarino, Floriana Facchetti, Sara Monico, Andrea Costantino, Mirella Fraquelli. Writing of the article: Elisabetta Degasperi, Alessandro Loglio, Pietro Lampertico. Statistical analysis: Elisabetta Degasperi, Pietro Lampertico. Virological analysis: Caroline Scholtes, Fabien Zoulim, Sara Uceda Renteria, Ferruccio Ceriotti. Critical revision of the manuscript: Pietro Lampertico, Fabien Zoulim. All authors approved the final version of the manuscript.

Data availability statement

Data from the present study is kept confidential but can be provided upon direct request to the authors.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.07.016.

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Author names in bold designate shared co-first authorship

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