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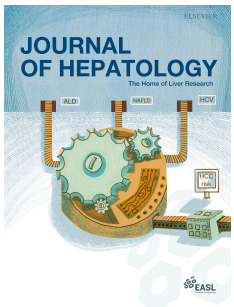

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PII: S0168-8278(21)02119-X

DOI: https://doi.org/10.1016/j.jhep.2021.10.012

Reference: JHEPAT 8478

To appear in: *Journal of Hepatology*

Received Date: 17 June 2021

Revised Date: 27 September 2021

Accepted Date: 12 October 2021

Please cite this article as: Loglio A, Ferenci P, Uceda Renteria SC, Tham CYL, Scholtes C, Holzmann H, van Bömmel F, Borghi M, Perbellini R, Rimondi A, Farina E, Trombetta E, Manunta M, Porretti L, Prati D, Ceriotti F, Zoulim F, Bertoletti A, Lampertico P, Safety and effectiveness of up to 3 years' bulevirtide monotherapy in patients with HDV-related cirrhosis, *Journal of Hepatology* (2021), doi: https://doi.org/10.1016/j.jhep.2021.10.012.

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Safety and effectiveness of up to 3 years' bulevirtide monotherapy in patients with HDV-related cirrhosis

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Keywords:

Bulevirtide; HDV; Entry inhibitor; T-cell; HDV-RNA; HBcrAg; HBV-RNA; HBV

Main text word count: 2581

Abstract word count: 256

No. of tables: 1

No. of figures: 1

No. of supplementary tables: 1

Financial Supports:

This work was supported by a grant from "Ricerca Corrente RC2021/105-01", Italian Ministry of Health, and by a grant from the French National Research Agency Investissements d'Avenir Progam (CirB-RNA project-ANR-17-RHUS-0003)

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Conflict of interest:

Alessandro Loglio: travel grant for MYR Pharma, speaker bureau for Gilead Sciences. 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, Spring-Bank, Vir, Simcere; he is also Scientific Founder of LION TCR pte.; 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. The other authors declare that they have no competing interests.

Authors' contributions:

AL, PF and PL were involved in patients' care and drafting of the manuscript. AL, HH, MB, AR, EF, RP were involved in data collection. ET, MM, LP, DP were involved in PBMC extraction and cryopreservation. CT and AB performed T-cell analysis. CS, FvB and FZ performed HBV-RNA and HBcrAg analysis. HH, SU, FC performed molecular and virological analysis. AL, AB, PF, FZ and PL were involved in manuscript editing. All authors approved the final version of the manuscript.

Data availability statement

The data is available after a justified request.

ABSTRACT

The entry-inhibitor Bulevirtide (BLV) received conditional approval by EMA in July 2020 for the treatment of adult patients with compensated chronic hepatitis Delta. However, the effectiveness and safety of BLV administered as monotherapy beyond 48 weeks in difficult to treat HDV cirrhotic patients is presently unknown. Here we describe the first patients with HDV-related compensated cirrhosis who were treated with BLV (10 mg/day as a starting dose) for up to three years as compassionate use. Patients were also monitored for HBcrAg and HBV-RNA levels and HDV and HBV specific T-cells markers.

In the patient who stopped BLV at week 48 after achieving a virological and biochemical response, the initial virological and biochemical rebound was followed by ALT normalization coupled with low HDV-RNA and HBsAg levels. In the two patients treated continuously for 3 years, virological and biochemical responses were maintained throughout the treatment period even after dose reduction. In a patient with advanced compensated cirrhosis, liver function tests significantly improved, esophageal varices disappeared, and histological/lab features of autoimmune hepatitis resolved. Overall, no safety issues were recorded, as bile salt increase was asymptomatic. While serum HBV-RNA levels remained undetectable in all patients, HBcrAg levels showed a progressive, yet modest decline during long-term BLV-treatment. No HDV-specific Interferon-γ producing T-cells were detected, neither after HDV reactivation (after BLV withdrawn in Patient 1) nor during 3 years of BLV treatment. In conclusion, this report shows that continuous administration of BLV monotherapy for three years provides excellent virological and clinical response in HDV cirrhotic patients who had contraindications to IFN-based therapies.

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LAY SUMMARY

- HDV-RNA levels became undetectable, and ALT normalized in all three patients treated with Bulevirtide (BLV). Virological and biochemical responses were maintained even after dose reduction.
- Improvement of liver function tests, regression of esophageal varices and recovery of HDV-related autoimmune disease were documented in the male cirrhotic patient long-term treated with BLV.
- An asymptomatic increase of bile acids was the only drug-related clinical adverse event.

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INTRODUCTION

Chronic hepatitis Delta (CHD) is a rare but severe form of chronic viral hepatitis that affects approximately 12-72 million patients worldwide[1]. It is sustained by the hepatitis D virus (HDV), a small defective virus that requires the helper function of HBV to replicate and propagate[2]. In the last 30 years, the only therapeutic approach has been the off-label use of a 48-week course of Interferon (IFNα)[3]. However, this antiviral strategy is characterized by limited off-therapy responses and an unfavorable safety profile[3,4].

The unmet medical need of an effective and safe therapeutic option for CHD patients coupled with the recent identification of the entry receptor for HBV and HDV, has fostered the research in this field. Among the several compounds now being tested in clinical studies, Bulevirtide (BLV) is the only drug that has received a conditional approval from the European Medicine Agency in July 2020, at the dose of 2 mg/day s.c.[4]. Previously named Myrcludex–B and now commercialized as Hepcludex® in European Union, BLV is a first in class entry-inhibitor of HBV, a subcutaneously delivered lipopeptide that mimics the Na+-taurocholate co-transporting polypeptide (NTCP) receptor binding domain, blocking the HDV/HBV entry exclusively in liver cells. In Phase II trials, BLV administration induced HDV-RNA reduction and ALT improvement during a 24- and 48-week treatment that was however followed by a relapse after treatment cessation in most patients. When combined with Peg-IFNα, a synergic effect on HDV-RNA and HBsAg decline was demonstrated[5].

In this brief report we describe for the first time the safety, effectiveness and clinical response to BLV administered for up to 3 years as a monotherapy in patients with HDV-related compensated cirrhosis. In addition, we enriched the study by an integrated analysis of both standard and

innovative HBV markers, such as serum HBV core-related Antigen (HBcrAg) and HBV-RNA, coupled with an immunological monitoring of IFN-γ HDV and HBV specific T-cells.

PATIENTS AND METHODS

This brief report study is based on the data generated in three CHD patients who received BLV ina single patient compassionate use program. All instances of compassionate use were approved by a local Ethic Committee [6] and informed consent was obtained for all subjects, according to Helsinki Declaration. BLV at 10 mg as the initial dose was self-administered as subcutaneousinjections every 24 hours. Liver function tests, total bile acids and virological HDV and HBV markers were monitored every 4 weeks for 2 years and then every 8-weeks; liver stiffness by Fibroscan® every 6 months, upper endoscopy according to Baveno VI recommendations, and HCC surveillance as per international guidelines[3].

HDV-RNA was quantified by RoboGene® (HDV-RNA quantification 2.0; Aj-Roboscreen, Jena, Germany; lower limit of detection (LOD) 6 IU/mL); HBV-RNA was quantified by an in-house real-time PCR technique (Leipzig, LOD 160 cp/mL) in the first year, and by a real-time PCR based investigation assay (Roche Diagnostics, Pleasanton, Ca, USA, LLOQ 10 cp/mL) in the following 2 years[7]. Serum HBcrAg levels were measured using LUMIPULSE® G HBcrAg assay (Fujirebio Europe, LOD 2 $\log_{10}$ U/mL). HBV DNA was quantified by Abbott RealTi*m*e HBV (Abbott Diagnostics, Rome, Italy; LOQ 10 IU/mL) or by Roche Cobas® (AmpliPrep/TaqMan System®, LOQ 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

synthesized by metabion international AG (Metabion GmbH, Germany) and *TaKaRa Ex Taq* Hot Start Version Kit (TAKARA BIO INC, Kusatsu, Japan). In peripheral blood mononuclear cells (PBMC) collected and cryopreserved every 4 weeks, HDV/HBV-specific T-cell quantity and function were analyzed by direct ex-vivo IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assays, using a panel of 313 overlapping peptides (15-mers overlapping by 10AA) covering the full proteome sequence of HBV genotype D (Accession number AF121241) pooled in 8 individual mixtures containing the indicated peptides number: HBV-X (29 peptides), Nucleocapsid (41 peptides), envelope 1 and 2 (38-peptides each), polymerase 1, 2, 3, and 4 (42,42,42 and 41 peptides, respectively)[8]. Another panel of 42 overlapping peptides (15-mers overlapping by 10 AA) covers the full proteome sequence of HDV genotype 1. Both HBV- and HDV-specific T-cell responses were analyzed in IFN-γ ELISPOT assays ex vivo. Briefly, 96-well plates (Multiscreen-HTS; Millipore, Billerica, MA) were coated overnight at 4°C with 5 μg/ml capture mouse anti-human IFN-γ monoclonal antibody (Purified NA/LE anti-human IFN-γ). Plates were then blocked with RPMI medium 1640 containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin–L-Glutamine for 2 hours. A total of 3×10^5 PBMCs were seeded per well for each individual peptide mixture. Plates were incubated for 18 hours at 37°C in the absence or presence of peptides (at a final concentration of 2 μg/ml). After the incubation, plates were incubated with biotinylated anti-human IFN-γ and streptavidin-HRP and developed using the chromogen substrate solution according to the recommended protocol from BD (Becton Dickinson). The colorimetric reaction was stopped after 10 to 15 minutes by washing the plates with distilled water. Plates were air dried, and spots were counted using an automated ELISPOT reader (ImmunoSpot reader; CTL Technologies, OH). The number of peptide-specific IFN-γ-secreting cells was calculated by subtracting the non-stimulated control value from that of the stimulated sample. Positive controls consisted of PBMCs stimulated with phorbol myristate

acetate (10 ng/ml) and ionomycin (100 ng/ml). In the direct ex vivo assays, wells were considered positive when the number of spot-forming units (SFU) was above 5 and at least three times the mean value of the unstimulated control wells. Negative control was stimulated with RPMI containing the DMSO concentration present in the diluted peptide mixtures (0.2%). Only experiments with a positive result in the positive control were considered[6,9].

The first 48-week results of these three patients have been previously published[6]. All patients were already under Tenofovir Disoproxil Fumarate (TDF) treatment.

RESULTS

Patient 1

A 69 year-old, Caucasian, HBeAg-negative female with HDV-related compensated cirrhosis complicated by portal hypertension (splenomegaly and thrombocytopenia) that contraindicated IFN therapy. Comorbidities included diabetes mellitus, femoral and lumbar osteopenia, mild arterial hypertension and uterine polyps under assessment. This patient had genotype D of HBV and genotype 1 of HDV; she was treated with TDF.

During 10 mg/day BLV treatment, ALT rapidly normalized, HDV-RNA became undetectable and clinical parameters improved (Figure-Panel A, Supplementary Table). Following the diagnosis of an endometrial carcinoma at week 52, BLV was withdrawn as a precautious measure to avoid any possible drug-to-drug interaction with chemotherapy. After BLV discontinuation, HDV-RNA rapidly rebounded and ALT flared up peaking at week 28 (333 IU/L), but both markers fell within the normal range by week 48 off-therapy (Figure-Panel A, Supplementary Table). HDV reactivation was not associated to any sign of clinical decompensation. HBsAg levels, that had slightly increased during BLV treatment, progressively and significantly declined after BLV was withdrawn, paralleling HDV-RNA rebound and ALT flare (Figure-Panel A). In summary, at

96-week off-therapy, HBsAg and HDV-RNA levels were very low and ALT normal. No significant increases in HDV or HBV specific IFN-γ T-cell response was observed neither during BLV treatment nor during virological and clinical relapse, nor HBcrAg levels that remained always below the LOD except at BLV baseline and at ALT peak secondary to HDV relapse (Figure-Panel A).

Bile acids, that increased during BLV administration, rapidly normalized after drug withdrawal. The asymptomatic increase of serum bile acids levels >160 µmol/l that we observed at week 20 and 24 was likely due to the fact that BLV was self-administered before blood sampling, and not after as usually done.

Patient 2

The second patient was a 51 year-old male, Caucasian, HBeAg-negative with HDV-compensated cirrhosis complicated by thrombocytopenia, small esophageal varices (EV) and autoimmune hepatitis (liver biopsy performed in 2010) that contraindicated IFN-treatment[6]. He had diabetes on diet therapy (Table). This patient, on TDF therapy, had genotype D of HBV and genotype 1 of HDV.

Treatment with BLV 10 mg/day induced a rapid biochemical and virological responses that was maintained up to week 144 without any sign of virological or biochemical breakthrough even after BLV dose reduction to 5 mg/day at week 76 (Figure-Panel B): we decided to reduce BLV daily dose only to simplify therapy, i.e. only one injection every morning, given the excellent HDV suppression lasting for >1 year. During BLV treatment, liver function tests as well as alpha-fetoprotein (AFP) and IgG levels rapidly normalized, platelet count improved to almost normal levels, while HBsAg levels and the other HBV markers remained stable over time. Serum HBV-RNA levels remained undetectable while HBcrAg levels showed a progressive, yet modest

decline (from 4.5 to 3.4 Log U/mL). In a liver biopsy performed after 72 weeks of BLV treatment, we observed a reduction of plasma cells infiltration compared to what had been observed in the previous liver biopsy performed in 2010, with an improvement of histological features of autoimmune hepatitis.

Notably, platelet count significantly increased, liver stiffness improved, and esophageal varices disappeared after 20 months of BLV treatment (Table). The latter finding was confirmed in two consecutive endoscopies 12-months apart. No recovery of HDV nor HBV specific IFN-γ producing T-cells function was observed at any time point during BLV administration (Figure-Panel B). BLV was well-tolerated as no local or systemic adverse events were reported, the increase of bile acids levels was dose-related and fully asymptomatic.

Patient 3

The third patient was a 58 year-old female, native to Uzbekistan, HBeAg-negative with HDV-related compensated cirrhosis complicated by an autoimmune thrombocytopenia with detectable anti-platelet antibodies for which she was treated in the past with high doses of steroids (Table). This patient, on anti-HBV therapy with TDF, had genotype D of HBV and genotype 1 of HDV.

BLV administration led to a rapid biochemical response that was followed by a virological response with HDV-RNA becoming undetectable at week 52, both responses were maintained up to week 144 despite BLV dose reduction to 5 and 2 mg/day (Figure-Panel C). Liver function tests as well as HBV markers remained stable over time while HBcrAg levels showed a progressive but modest decline (Table, Figure-Panel C). No local or systemic side effects were recorded apart from a dose-related, fully asymptomatic increase of bile acid, that had a decrease from a mean level of 203 to 152 umol/L after BLV dose reduction (10→5 mg/day). Autoimmune thrombocytopenia did not recur.

DISCUSSION

To our knowledge this is the first report assessing the safety, effectiveness and clinical response of BLV administered as monotherapy for up to three years in patients with HDV-related compensated cirrhosis in whom Peg-IFN therapy was contraindicated. The long-term administration of BLV monotherapy in these difficult to treat patients was associated with positive virological and clinical results coupled with a favorable safety profile. All patients reported excellent compliance to BLV therapy (2 subcutaneous injections every morning), as confirmed by the increase of bile salts levels, a biomarker of target engagement.

One of the most important findings of the study is the positive virological and biochemical response observed as all three patients achieved and maintained undetectable HDV-RNA, which was tested by a very sensitive assay. These findings are unprecedented for at least three reasons: first, no studies to date have reported the outcome of BLV treatment beyond week 48; second, no data exists to demonstrate that virological response was maintained upon dose reduction; third, the effectiveness of the administration of this drug for such a long-term in difficult to treat patients such as those with advanced compensated cirrhosis, including one case with clinical significant portal hypertension (CSPH), has never been reported so far.

Another important finding was the clinical response that we have observed in these patients. One patient who had compensated cirrhosis with CSPH, showed a clinically relevant improvement of liver function tests, AFP and platelet levels, as well as portal hypertension features, with the regression of EV. To our knowledge, this is the first report of EV disappearance in a HDV patient, an event that has been already demonstrated in patients with HBV-related cirrhosis long-term treated with nucleos(t)ide analogs[3]. In the other two cirrhotic patients, liver function tests and synthetic function of the liver remained stable up to 3 years of therapy, a favorable finding for a

chronic liver disease characterized by high rates of progression to end-stage-liver disease and HCC[2].

There is currently no safety data published or presented beyond week 48 with BLV in HDV patients, regardless of the severity of liver disease. Extension of BLV treatment to 3 years in these two compensated HDV cirrhotic patients was well tolerated, not associated with any drug-related local or systemic adverse event, confirming the preliminary favorable week 48 data from phase II studies[4,5]. No itching was reported even though high levels of bile acids were observed in patients with advanced liver disease treated with BLV 10 mg/day, first results of an observation lasting 3 years in a real-life setting.

It is well known that a significant proportion of the patients with viral hepatitis may have or may develop autoimmune phenomena *via* molecular mimicry, ranging from isolated non-organ specific autoantibodies positivity to florid autoimmune hepatitis. Patient 2 was indeed a representative case, with hypergammaglobulinemia, autoantibodies and interface hepatitis with plasma cells infiltration, associated with CHD. Upon achieving virological response to BLV, the laboratory and histological features of autoimmunity rapidly improved, a strong argument in favor of a causal relationship between HDV replication and autoimmunity. As far as we know, this is the first case of HDV-related autoimmune hepatitis cured by antiviral therapy. A similarly favorable outcome was observed in Patient 3, who did not have a recurrence of autoimmune-mediated thrombocytopenia during BLV treatment.

These cases were also instrumental in assessing two recently developed HBV markers, such as HBV-RNA and HBcrAg, in the setting of HDV patients treated with BLV[10]. In the two patients treated with BLV for 3 years, moderate to high serum levels of HBcrAg were documented while serum HBV-RNA was persistently undetectable. Since both markers are expected to mirror the covalently close circular DNA (cccDNA) transcriptional activity of HBV, these findings may be

related to the fact that HDV could directly interfere with HBV replication processes associated with extremely low levels of cccDNA[4,11]. To confirm these results and investigate the underlying molecular mechanisms, additional studies are needed.

Last but not least, we were able to perform immunological studies in two BLV-treated patients. No recovery of HBV or HDV-specific IFN-γ producing T-cell was identified ex vivo, regardless of the duration of therapy. Of note, recovery of the peripheral HBV and HDV T-cell was not observed even in Patient 1, despite a significant ALT flare following BLV discontinuation. Direct ex-vivo Elispot can only detect robust expansion of HBV and HDV-specific T cell frequency and we cannot exclude that more detailed analysis, such as T-cell analysis after a round of in-vitro expansion[12] might be able to detect subtle modifications of frequencies. However, the lack of recovery of circulating HBV and HDV-specific IFN-γ producing T-cells in these treated patients could also be explained by the mature age of the patients (>50 years), and the preferential homing of HBV and HDV specific T-cells into the liver[12].

CONCLUSIONS

This is the first report that describes the safety, virological and clinical responses of BLV monotherapy administered for up to 3 years in patients with HDV-related compensated cirrhosis treated *via* compassionate use. Forty-years after the discovery of the Delta virus, the results conveyed by this first report on long-term BLV monotherapy will pave the way for the management of this severe form of chronic liver disease.

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ABBREVIATIONS

HBcrAg, HBV core-related Antigen; CHD, chronic hepatitis Delta; cccDNA, covalently close circular DNA; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; EV, esophageal varices; NUC, nucleos(t)ide analogs; TDF, tenofovir disoproxil fumarate; PBMC, peripheral blood mononuclear cells; ALT, alanine aminotransferase; BLV, bulevirtide.

DECLARATIONS

The use of Bulevirtide was approved by the local Ethics Committee on a 6-month basis for the Italian patients, with written informed consent from the Austrian patient. Informed consent for additional analyses and serum storage was obtained by all patients.

ACKNOWLEDGEMENTS

We thank Dr. Alexander Alexandrov from MYR Pharma for the free supply of Bulevirtide, and Fujirebio Italy for the free supply of HBcrAg kits. We thank Charlotte Fenwick, native English speaker, for the language revision of the paper.

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FIGURE LEGEND

Figure: Changes of HDV RNA, ALT, bile acids, HBsAg, HBcrAg, HBV DNA and HBV RNA levels during Bulevirtide (BLV) treatment in the three patients.

(A) Patient 1 (*BLV was administered just before blood sampling at week 20, 24, 28*); (B) Patient 2; (C) Patient 3; (D and E) Immunological parameters variation in Patient 1 and 2 (Bars show the numbers of spots x 10^5 PBMC responding to the different peptides mixtures or PMA+Ionomicyne [Positive control] while in the horizontal axis Time as weeks of treatment or off-treatment).

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Table – Time course of virological, biochemical, and clinical variables during BLV and TDF treatment in Patients 2 and 3 (reported on a 6-month basis).

Variables	Patient 2							Patient 3						
	Baseline	**Week 24**	**Week 48**	**Week 72**	**Week 96**	**Week 120**	**Week 144**	**Baseline**	**Week 24**	**Week 48**	**Week 72**	**Week 96**	**Week 120**	**Week 144**
AST (U/L) / ALT (U/L)	179 / 232	38 / 25	38 / 24	34 / 23	37 / 26	35 / 23	32 / 20	111 / 244	42 / 46	33 / 35	37 / 35	37 / 29	25 / 30	29 / 22
ALP (U/L) / GGT (U/L)	185 / 231	89 / 138	86 / 140	84 / 128	66 / 103	74 / 107	72 / 77	74 / 44	95 / 19	87 / 17	92 / 14	86 / 13	85 / 15	91 / 14
Total bilirubin (mg/dL)	0.4	0.6	0.4	0.4	0.8	0.7	0.6	0.4	0.4	0.3	0.4	0.5	0.5	0.3
pCHE (U/L)	4,470	6,534	6,648	6,692	7,633	7,029	7,421	6,330	6,340	6,310	5,990	6,110	6,180	4,960
Albumin / Gamma globulin (g/dL)	3.6 / 2.9	4.4 / 1.9	4.4 / 1.5	4.2 / 1.3	4.6 / 1.2	4.4 / 1.1	4.4 / 1.0	4.1 / -	4.5 / -	4.5 / -	4.6 / -	4.5 / -	4.6 / -	4.6 / -
IgG (mg/dL)	3,077	2,061	1,608	1,642	1,608	1,422	1,147	2,350	-	-	-	-	-	1,210
Alpha-fetoprotein (ng/mL)	21	6	5	5	4	6	4	5	4	5	3	3	3	3
PT-INR	1.09	1.17	1.13	1.13	1.15	1.23	1.24	1.0	1.0	1.0	1.1	1.0	1.0	1.0
White blood cells (mmc)	4,880	7,000	6,930	10,930	6,350	11,555	9,780	6,540	6,290	6,150	4,580	4,800	4,725	4,580
Hemoglobin (g/dL)	15.7	16.3	16.3	16.5	16.9	17.5	17.0	15.8	15.2	14.8	14.6	14.5	15.0	14.8
Platelet count ($x10^9$/L)	74	100	111	110	105	124	133	210	226	217	212	206	208	220
Liver Stiffness, kPa	17.6	14.5	10.1	11.9	16.5	7.7	10.9	-	-	-	10.1	-	-	-
CAP, dB/m	-	-	-	222	297	-	268	-	-	-	-	-	-	-
Esophageal varices	Small	-	Small	-	No	-	No	-	-	-	-	-	-	-
Spleen length, cm	14.0	13.0	13.0	13.0	13.0	13.0	13.0	-	-	-	12.0	-	-	-
Creatinine (mg/dL)	0.82	0.83	0.88	0.85	1.00	0.90	0.86	0.80	0.74	0.73	0.72	0.74	0.76	0.74
Glycemia (mg/dL) / Triglycerides (mg/dL)	118 / 129	119 / 91	119 / 122	111 / 71	120 / 69	122 / 56	111 / 59	128 / 142	89 / 172	115 / 193	104 / 89	109 / 126	99 / 101	96 / 106
Total Cholesterol / HDL (mg/dL)	166 / 41	185 / 54	169 / 46	170 / 48	193 / 55	180 / 44	164 / 42	198 / -	198 / -	194 / -	172 / -	150 / -	189 / -	180 / -

BLV dose: In *Patient 2*, BLV dose was reduced from 10 to 5 mg/day at week 76; in *Patient 3*, BLV dose was reduced from 10 to 5 mg/day at week 108 and to 2 mg/day at week 128. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; pCHE, pseudo-cholinesterase; CAP, Controlled Attenuation Parameter. Reference values: AST, 10–33 U/L; ALT, 6–41 U/L; ALP, 35–104 U/L; GGT, 5–36 U/L; Total bilirubin, 0.12–1.10 mg/dl; pCHE, 4,200–11,250 U/L; Albumin, 3.4–4.8 g/dl; IgG, 700-1600 mg/dL; INR, 0.84–1.20; White cells, 4,800–10,800/mmc; Hemoglobin, 12–16 g/dl; Platelet, 130–400 $x10^9$/L;

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Creatinine, 0.5–1.0 mg/dl; Glycemia, 70-110 mg/dL; Triglycerides, <150 mg/dL; Total cholesterol, <190 mg/dL; HDL, >45 mg/dL.

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Supplementary Fig. 1: Graphical Abstract

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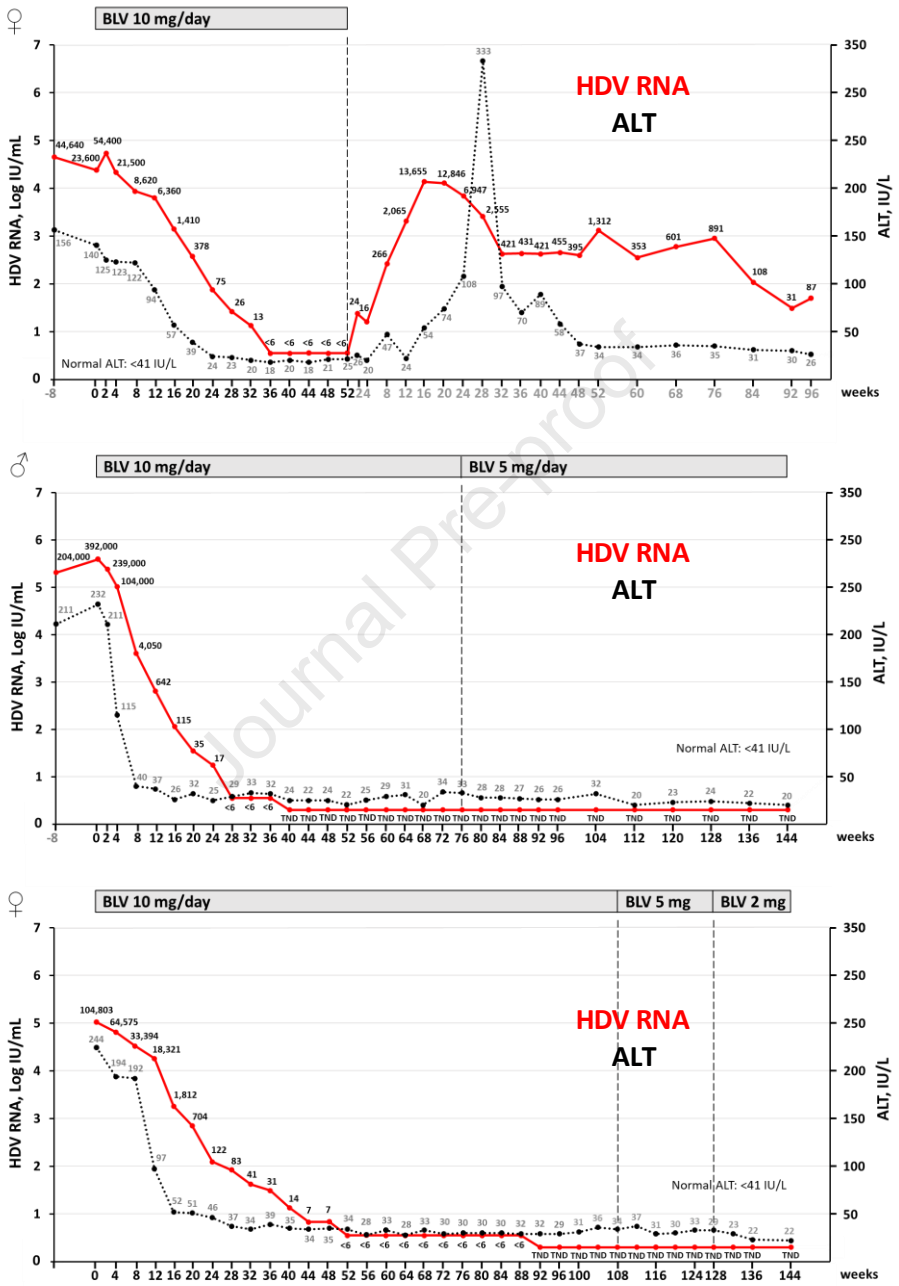
3 patients with HDV compensated cirrhosis + contraindications to Interferon treatment

started entry-inhibitor Bulevirtide (BLV) at 10 mg/day *as per* compassionate use



HDV RNA undetectable in all patients

Virological and biochemical response maintained over 3 years of BLV even after dose reduction

Regression of small esophageal varices

Recovery of HDV-related autoimmune features

No adverse events

Figure

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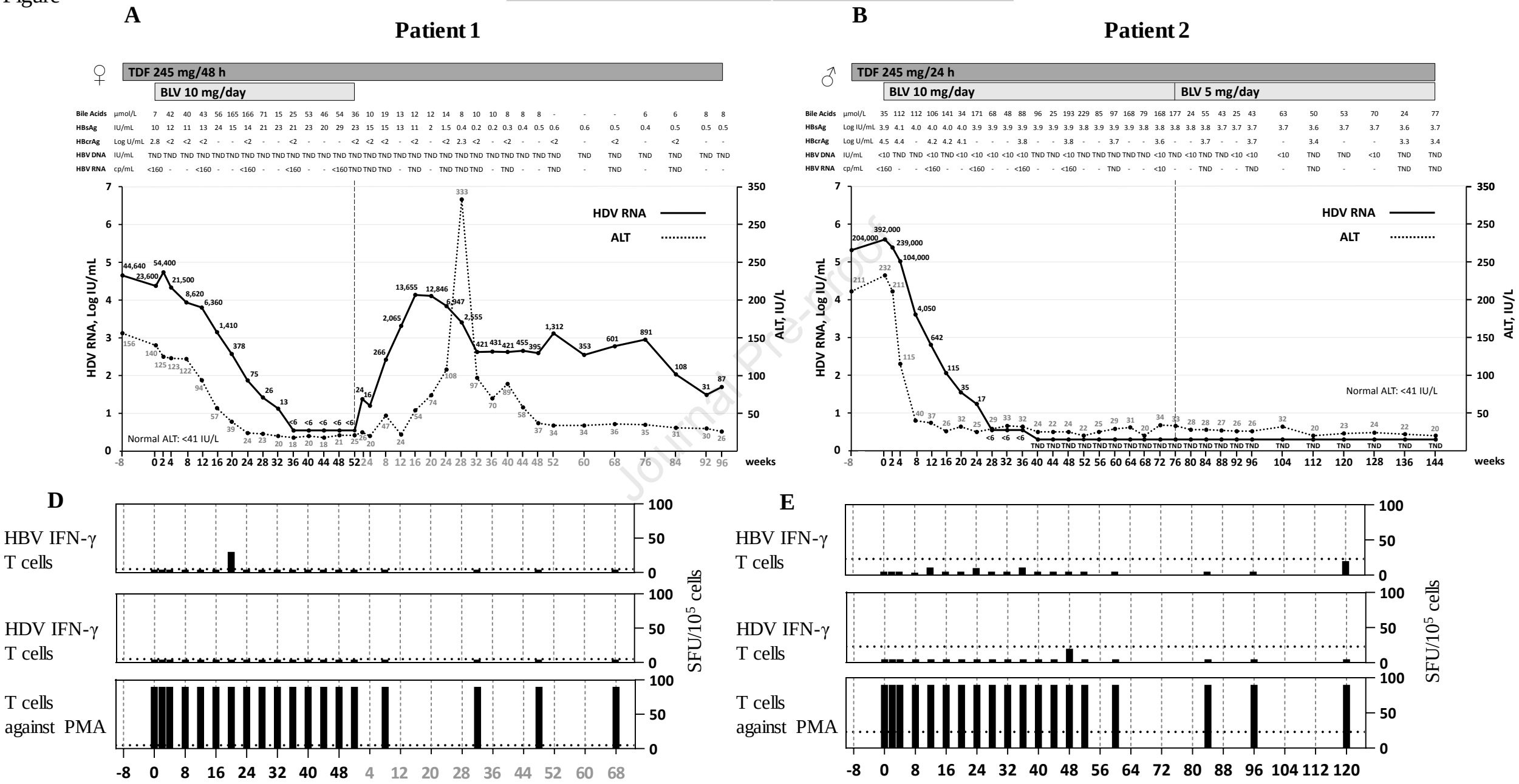

Figure

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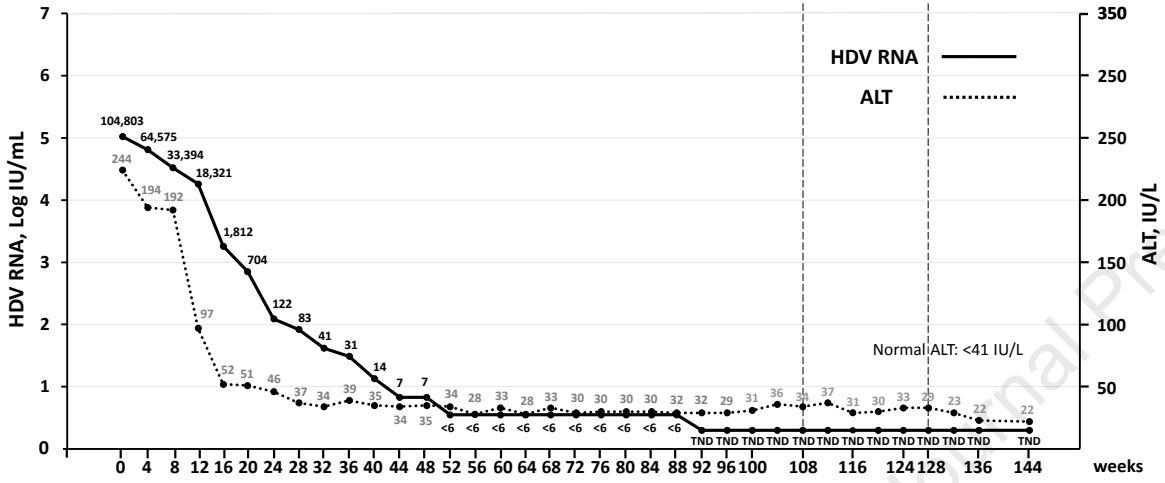
Patient 3

♀

TDF 245 mg/24 h

BLV 10 mg/day | **BLV 5 mg** | **BLV 2 mg**

Bile Acids	µmol/L	<10	-	233	184	218	150	200	211	190	164	190	179	179	-	-	202	-	-	246	-	-	212	-	195	202	212	251	237	132	139	162	174	152	154	168	132
HBsAg	Log IU/mL	4.0	4.0	4.0	4.1	4.1	4.0	4.0	4.0	4.0	4.0	3.9	4.0	4.0	-	-	4.0	-	-	4.2	-	-	4.0	-	3.9	3.9	3.9	4.0	3.9	3.9	3.9	3.9	3.9	4.0	3.8	3.9	3.9
HBcrAg	Log U/mL	4.6	-	4.4	4.6	4.2	4.4	4.4	4.3	-	4.3	4.3	4.1	3.9	4.1	4.2	4.0	4.2	4.0	4.1	4.2	-	4.1	-	-	4.2	4.0	3.9	3.9	-	-	-	-	-	-	-	-
HBV DNA	IU/mL	<20	-	-	<20	-	-	<20	-	-	<20	-	-	<20	-	-	TND	-	-	TND	-	-	<20	-	-	<20	-	-	TND	-	-	<20	-	<20	-	-	TND



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