ORIGINAL ARTICLE

A Phase 3, Randomized Trial of Bulevirtide in Chronic Hepatitis D

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ABSTRACT

BACKGROUND

Coinfection with hepatitis D virus (HDV) accelerates the progression of liver disease associated with chronic hepatitis B. Bulevirtide inhibits the entry of HDV into hepatocytes.

METHODS

In this ongoing phase 3 trial, patients with chronic hepatitis D, with or without compensated cirrhosis, were randomly assigned, in a 1:1:1 ratio, to receive bulevirtide subcutaneously at 2 mg per day (2-mg group) or 10 mg per day (10-mg group) for 144 weeks or to receive no treatment for 48 weeks followed by bulevirtide subcutaneously at 10 mg per day for 96 weeks (control group). Patients will complete 96 weeks of additional follow-up after the end of treatment. The primary end point was a combined response at week 48 of an undetectable HDV RNA level, or a level that decreased by at least 2 $\log_{10}$ IU per milliliter from baseline, and normalization of the alanine aminotransferase (ALT) level. The key secondary end point was an undetectable HDV RNA level at week 48, in a comparison between the 2-mg group and the 10-mg group.

RESULTS

A total of 49 patients were assigned to the 2-mg group, 50 to the 10-mg group, and 51 to the control group. A primary end-point response occurred in 45% of patients in the 2-mg group, 48% in the 10-mg group, and 2% in the control group ($P<0.001$ for the comparison of each dose group with the control group). The HDV RNA level at week 48 was undetectable in 12% of patients in the 2-mg group and in 20% in the 10-mg group ($P=0.41$). The ALT level normalized in 12% of patients in the control group, 51% in the 2-mg group (difference from control, 39 percentage points [95% confidence interval {CI}, 20 to 56]), and 56% in the 10-mg group (difference from control, 44 percentage points [95% CI, 26 to 60]). Loss of hepatitis B virus surface antigen (HBsAg) or an HBsAg level that decreased by at least 1 $\log_{10}$ IU per milliliter did not occur in the bulevirtide groups by week 48. Headache, pruritus, fatigue, eosinophilia, injection-site reactions, upper abdominal pain, arthralgia, and asthenia were more common in the 2-mg and 10-mg groups combined than in the control group. No treatment-related serious adverse events occurred. Dose-dependent increases in bile acid levels were noted in the 2-mg and 10-mg groups.

CONCLUSIONS

After 48 weeks of bulevirtide treatment, HDV RNA and ALT levels were reduced in patients with chronic hepatitis D. (Funded by Gilead Sciences; MYR 301 ClinicalTrials.gov number, NCT03852719.)

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Hepatitis D virus (HDV) is a satellite RNA virus that requires hepatitis B virus (HBV) surface antigen (HBsAg) for entry into hepatocytes and for propagation. HDV is estimated to affect between 10 million and 20 million persons worldwide.[1] In the United States, 5 to 10% of persons who are positive for HBsAg are also seropositive for anti-HDV antibody, which means that HDV infection meets the threshold for designation as an orphan disease (i.e., a disease occurring in <200,000 persons).[1]

Long-term coinfection with HBV and HDV is considered to be the most serious type of chronic viral hepatitis.[2-4] Relative to HBV monoinfection, HBV–HDV coinfection accelerates the course of disease, thus increasing the risk of cirrhosis, hepatocellular carcinoma, the need for liver transplantation, and early death.[5-8] Oral antiviral drugs currently available for the treatment of HBV infection are effective as monotherapy in suppressing HBV replication, but they do not effectively suppress HDV replication.[9,10] Pegylated interferon alfa is used off-label for treating HDV infection, but it is contraindicated in a large proportion of patients, is associated with toxic effects, and has a low likelihood of resulting in a sustained virologic response (which occurs in <30% of patients at 6 months after treatment),[9,11,12] even when the treatment duration is prolonged to 96 weeks.[11] In addition, late relapses occurring 6 months or later after interferon treatment have been described.[13,14]

In the absence of approved HDV therapies, the Food and Drug Administration (FDA) recommends that investigational antiviral treatments for chronic hepatitis D should be capable of decreasing viral replication and reducing liver inflammation as evidenced by a biochemical response. Because a 2-$\log_{10}$ decline in the HDV RNA level has been associated with clinical benefit, the FDA recommends, as a surrogate end point for treatment, an undetectable HDV RNA level or a level that is decreased by at least 2 $\log_{10}$ IU per milliliter, in combination with normalization of the alanine aminotransferase (ALT) level.[15,16]

Sodium taurocholate cotransporting polypeptide (NTCP), a carrier protein expressed on the basolateral membrane of hepatocytes, plays a crucial role in enterohepatic circulation as a bile acid transporter.[17] In 2012, NTCP was identified as the entry receptor for both HBV and HDV.[18] Bulevirtide — a synthetic lipopeptide derived from the pre-S1 domain of the large envelope protein of HBsAg — inhibits the entry of HBV and HDV into hepatocytes by binding to and inactivating NTCP.[19,20] In a phase 2 trial, bulevirtide was shown to induce pronounced declines in HDV RNA and ALT levels.[21,22] The results of that trial, combined with data from the phase 1 study,[23] provided a basis for conditional approval in the European Union and full approval in Russia of bulevirtide, administered at a dose of 2 mg per day, for treating chronic hepatitis D in adults with compensated liver disease.[24] Bulevirtide has not been approved by the FDA.

In this ongoing phase 3 trial, which includes 144 weeks of treatment and 96 weeks of post-treatment follow-up, we are assessing the efficacy and safety of bulevirtide monotherapy in patients with chronic hepatitis D. We now report data on the prespecified primary end point: a combined virologic and biochemical response, as assessed by analysis of the HDV RNA and ALT levels, respectively, at week 48.

METHODS

STUDY DESIGN

In this multicenter, open-label, randomized trial, eligible patients were assigned in a 1:1:1 ratio, with stratification according to the presence or absence of cirrhosis, to receive immediate subcutaneous treatment with bulevirtide at 2 mg per day (one injection; 2-mg group) or 10 mg per day (two injections; 10-mg group) for 144 weeks or to receive no treatment for 48 weeks followed by subcutaneous treatment with bulevirtide at 10 mg per day for 96 weeks (control group). All the patients entered a 96-week follow-up period at the end of the treatment period.

TRIAL OVERSIGHT

Four of the authors contributed to the conception and design of the trial. All the authors contributed to the collection and interpretation of the data and to the drafting of the original manuscript. Gilead Sciences, the trial sponsor, conducted the data analysis and provided writing assistance. All the authors approved the final version of the submitted manuscript and vouch for the fidelity of the trial to the protocol (available with the full text of this article at NEJM.org), the accuracy and completeness of the data, and the integrity of the analysis.

The protocol was approved by the review board or ethics committee at each trial site before the trial was initiated. The trial was conducted in accordance with the International Council for Harmonisation Good Clinical Practice guidelines and the principles of the Declaration of Helsinki.

PATIENTS

Eligible patients were 18 to 65 years old; had chronic hepatitis D, with anti-HDV antibody or HDV RNA detected in serum by polymerase chain reaction (PCR) at least 6 months before screening; had HDV RNA detected by PCR at screening; and had an ALT level of more than 1 times but less than 10 times the upper limit of the normal range at screening. Key exclusion criteria were decompensated cirrhosis, defined as class B or C cirrhosis according to the Child–Turcotte–Pugh classification (in which class A [score of 5 or 6] indicates mildly impaired liver function, B [scores of 7 to 9] moderately impaired liver function, and C [scores of 10 to 15] severely impaired liver function); receipt of interferon therapy within 6 months before screening; and a platelet count of less than 60,000 cells per cubic millimeter. Full inclusion and exclusion criteria are provided in the protocol.

Patients receiving treatment with nucleoside or nucleotide analogues (NAs) for chronic hepatitis B at screening were allowed to continue their treatment as prescribed. NA treatment was initiated in the remaining patients at the baseline visit or during the trial if NA use was indicated according to existing HBV treatment guidelines.[25,26]

ASSESSMENTS

Quantification of HDV RNA levels was performed with the RoboGene HDV RNA quantification kit, version 2.0 (Roboscreen), which has a lower limit of quantification of 50 IU per milliliter and a lower limit of detection of 6 IU per milliliter. An undetectable HDV RNA level was defined as a level below the lower limit of quantification, with the HDV RNA target not detected. Normalization of the ALT level was defined as an ALT level within the normal range as assessed according to central laboratory criteria (≤31 for women and ≤41 U per liter for men in Russia, and ≤34 and ≤49 U per liter, respectively, in all other locations). Additional cutoff values for normalization of ALT levels as defined by the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) were used in sensitivity analyses.[25,26]

At baseline and week 48, liver stiffness was assessed with use of transient elastography (FibroScan, Echosens). A baseline liver biopsy was recommended and performed if the patient was willing to undergo the procedure and no contraindications were present as assessed by the investigator. If a liver biopsy had been performed within 1 year before screening and biopsy specimens were available for assessment, the requirement for a baseline liver biopsy was waived. A follow-up liver biopsy was performed at week 48 only in patients with an available baseline biopsy result. Additional information on assessments is provided in the Supplementary Methods section in the Supplementary Appendix (available at NEJM.org).

END POINTS

The primary end point was a combined response at week 48 of an undetectable HDV RNA level or a level that decreased by at least 2 $\log_{10}$ IU per milliliter from baseline (virologic response), and normalization of the ALT level (biochemical response). The key secondary end point was an undetectable HDV RNA level at week 48, in a comparison between the 2-mg group and the 10-mg group.

STATISTICAL ANALYSIS

A hierarchical testing procedure was used with two sequential comparisons — a comparison of the 10-mg group with the control group and of the 2-mg group with the control group — for the analysis of the primary end point. If the null hypotheses of both comparisons of the primary end point were rejected, the hierarchical testing procedure continued with a prespecified comparison of the key secondary end point between the 2-mg and 10-mg groups. The overall significance level was 0.05, which was split into a level of 0.01 in the prespecified formal interim analysis at week 24 and a level of 0.04 in the primary analysis at week 48. On the basis of results from the phase 2 MYR 202 trial,[22] we expected that a primary end-point response would occur in 45% of patients in the 2-mg group, 45% in the 10-mg group, and 8% in the control group. We calcu-

lated that with a sample size of 47 patients per treatment group and using Fisher's exact test, the trial would have 97.8% power to detect a difference between the 2-mg or 10-mg group and the control group in the percentage of patients with a combined response, at a two-sided significance level of 0.04. The trial lacked the power to detect a difference between the 2-mg and 10-mg groups in the percentage of patients with a combined response. Additional information is provided in the Supplementary Methods section in the Supplementary Appendix.

RESULTS

PATIENTS

A total of 150 patients were enrolled: 49 were assigned to receive 2 mg of bulevirtide, 50 to receive 10 mg of bulevirtide, and 51 to receive delayed treatment (Fig. S1 in the Supplementary Appendix). Demographic and clinical characteristics at baseline were generally balanced across the trial groups (Table 1). Patients in the trial were representative of the population with chronic hepatitis D in the regions where this trial was conducted (Table S1). The majority of the patients were men and were White; the mean age was 42 years. Approximately half the patients had cirrhosis. Nearly all the patients had HDV genotype 1 infection, and 84% (126 patients) had HBV genotype D infection. Interferon therapy had previously been received by 56% of the patients. A total of 55% of the patients were receiving NAs up to the start of the trial and continued NA therapy during the trial, and 8 patients (5%) started NA therapy at trial initiation; thus, 60% in total received NAs during the trial (Table 1 and Table S2). No patients stopped NA therapy during the 48-week trial period.

RESPONSE TO TREATMENT

Combined Response

The primary combined end-point response occurred in 22 of 49 patients (45%) in the 2-mg group and 24 of 50 (48%) in the 10-mg group, as compared with 1 of 51 (2%) in the control group (Table 2). The percentage of patients with a combined response was significantly greater in the 2-mg group than in the control group (difference, 43 percentage points; 96% confidence interval [CI], 27 to 58) and in the 10-mg group than in the control group (difference, 46 percentage points; 96% CI, 30 to 61) ($P<0.001$ for the comparison between each dose group and the control group). Ad hoc univariate analysis of the 2-mg and 10-mg groups showed that the percentage of patients with a combined response was generally consistent across all subgroups (Fig. S2D and S2E). Additional information is provided in the Supplementary Results section in the Supplementary Appendix.

HDV RNA and HBsAg Responses

At week 48, the HDV RNA level was undetectable in none of the patients (0 of 51) in the control group, 12% (6 of 49) in the 2-mg group, and 20% (10 of 50) in the 10-mg group. The difference between the 10-mg group and the 2-mg group was 8 percentage points (96% CI, −8 to 24; $P=0.41$).

A virologic response (an undetectable HDV RNA level or a level that decreased by ≥2 $\log_{10}$ IU per milliliter from baseline) at week 48 occurred in 4% of patients in the control group, 71% in the 2-mg group, and 76% in the 10-mg group. Figure S3 shows the percentage of patients with a virologic response between baseline and week 48. The percentage of patients in whom the HDV RNA level decreased by at least 2 $\log_{10}$ IU per milliliter from baseline at week 48 is shown in Table S3. Figure 1A shows the decline in HDV RNA levels over time; in 9 of 47 patients (19%) in the 2-mg group and 2 of 46 (4%) in the 10-mg group, the HDV RNA level did not decrease by at least 1 $\log_{10}$ IU per milliliter from baseline at week 48. Across treatment groups, no patients had HBsAg loss. Additional information is provided in the Supplementary Results section in the Supplementary Appendix.

ALT Response

Normalization of ALT levels at week 48 occurred in 51% of patients in the 2-mg group, 56% in the 10-mg group, and 12% in the control group (Table 2 and Fig. 1B). These percentages were similar to the percentages obtained when normalization of the ALT level was assessed according to cutoff values defined by the EASL and the AASLD (Fig. S4).

Liver Stiffness and Histologic Activity

At week 48, liver stiffness appeared to have been reduced in the 2-mg and 10-mg groups as compared with the control group; the level of im-

Table 1. Demographic and Clinical Characteristics at Baseline.*

Characteristic	Control (N=51)	Bulevirtide, 2 mg (N=49)	Bulevirtide, 10 mg (N=50)	Overall (N=150)
Age — yr	41±7.5	44±9.0	41±8.5	42±8.4
Male sex — no. (%)	26 (51)	30 (61)	30 (60)	86 (57)
Body-mass index†				
Mean	25±3.9	24±3.1	25±3.6	25±3.6
<30 — no. (%)	46 (90)	48 (98)	45 (90)	139 (93)
Race — no. (%)‡				
White	40 (78)	41 (84)	43 (86)	124 (83)
Asian	11 (22)	8 (16)	6 (12)	25 (17)
Black	0	0	1 (2)	1 (<1)
Cirrhosis — no. (%)§	24 (47)	23 (47)	24 (48)	71 (47)
Liver stiffness — kPa¶	15±9.0	14±8.2	15±9.3	15±8.8
HDV RNA level — $\log_{10}$ IU/ml	5.1±1.36	5.0±1.32‖	5.1±1.40	5.0±1.34‖
HDV genotype — no. (%)				
1	51 (100)	49 (100)	48 (96)‖	148 (99)
5	0	0	1 (2)	1 (<1)
HBV genotype — no. (%)				
A	4 (8)	2 (4)	3 (6)	9 (6)
D	39 (76)	44 (90)	43 (86)	126 (84)
E	0	0	1 (2)	1 (<1)
Could not be determined	8 (16)	3 (6)	3 (6)	14 (9)
HBeAg not detected — no. (%)	47 (92)	45 (92)	43 (86)	135 (90)
HBsAg level — $\log_{10}$ IU/ml	3.7±0.47	3.7±0.52	3.6±0.59	3.7±0.52
HBV DNA level — $\log_{10}$ IU/ml	0.9±0.99	1.3±1.30‖	1.1±1.26‖	1.1±1.19**
HIV infection — no. (%)	0	1 (2)	1 (2)	2 (1)
ALT level — U/liter	102±61.9	108±62.5	123±80.6	111±69.0
Vitamin D level — ng/ml	26±12.2	28±12.3	26±14.8	—
Concomitant NA therapy during trial — no. (%)	32 (63)	31 (63)	27 (54)	90 (60)
Previous interferon therapy — no. (%)	29 (57)	26 (53)	29 (58)	84 (56)

* Plus–minus values are means ±SD. ALT denotes alanine aminotransferase, HBeAg hepatitis B virus e antigen, HBsAg hepatitis B virus surface antigen, HBV hepatitis B virus, HDV hepatitis D virus, NA nucleoside or nucleotide analogue.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ Race was determined by the investigators.
§ All patients with cirrhosis had class A cirrhosis according to the Child–Turcotte–Pugh classification, in which class A (score of 5 or 6) indicates mildly impaired liver function, B (scores of 7 to 9) moderately impaired liver function, and C (scores of 10 to 15) severely impaired liver function.
¶ Liver stiffness was assessed by transient elastography (FibroScan, Echosens). Levels range from 2.5 to 75 kPa (normal range, 2.5 to 7 kPa), with higher levels indicating increasing severity of liver scarring.
‖ Data from 1 patient were missing.
** Data from 2 patients were missing.

provement was similar in the 2-mg and 10-mg groups (Table S4). Results of liver biopsies at baseline and week 48 were available for 55% of the patients (82 of 150). The changes from baseline in fibrosis stage and histologic activity index at week 48 are provided in Table S6. At week 48, the 2-mg and 10-mg groups had the highest percentage of patients with an improved histo-

Table 2. Responses at Week 48.*

Response	Control	Bulevirtide, 2 mg	Bulevirtide, 10 mg
Combined response			
Overall			
No./total no. (%)	1/51 (2)	22/49 (45)†	24/50 (48)†
95% CI — %	0–10	31–60	34–63
According to cirrhosis status — no./total no. (%)			
Present	—	8/23 (35)	12/24 (50)
Absent	—	14/26 (54)	12/26 (46)
Undetectable HDV RNA level			
Overall			
No./total no. (%)	0/51 (0)	6/49 (12)‡	10/50 (20)
95% CI — %	—	5–25	10–34
According to cirrhosis status — no./total no. (%)			
Present	—	5/23 (22)	6/24 (25)
Absent	—	1/26 (4)	4/26 (15)
Undetectable or ≥2 $\log_{10}$ IU/ml decrease in HDV RNA level			
Overall			
No./total no. (%)	2/51 (4)	35/49 (71)	38/50 (76)
Difference from placebo (95% CI) — percentage points	—	68 (52–80)	72 (56–84)
According to cirrhosis status — no./total no. (%)			
Present	2/24 (8)	18/23 (78)	19/24 (79)
Absent	0/27 (0)	17/26 (65)	19/26 (73)
Normalized ALT level			
Overall			
No./total no. (%)	6/51 (12)	25/49 (51)	28/50 (56)
Difference from placebo (95% CI) — percentage points	—	39 (20–56)	44 (26–60)
According to cirrhosis status — no./total no. (%)			
Present	4/24 (17)	9/23 (39)	12/24 (50)
Absent	2/27 (7)	16/26 (62)	16/26 (62)

* CI denotes confidence interval.
† P<0.001 for the difference from the control group (primary end-point comparison).
‡ P=0.41 for the difference from the 10-mg group (prespecified key secondary end-point comparison).

logic activity index, whereas the control group had the highest percentage of patients with a worsened fibrosis stage.

Immunogenicity and Resistance

Over the treatment period, antibulevirtide antibody developed in 22% of patients (11 of 49) in the 2-mg group and in 18% (9 of 50) in the 10-mg group. However, the percentage of patients with a virologic response did not appear to differ according to the presence or absence of antibulevirtide antibody (see the Supplementary Results section in the Supplementary Appendix).

Resistance analysis was performed in 11 patients who qualified for testing because of virologic breakthrough or infection that did not respond to treatment. No evidence that resistance had developed was documented. Additional details are provided in the Supplementary Results section in the Supplementary Appendix.

SAFETY

Headache, pruritus, fatigue, eosinophilia, injection-site reactions, upper abdominal pain, arthralgia, and asthenia were more common in the 2-mg and 10-mg groups combined than in the control group; all these events were mild to moderate in severity and did not lead to drug discontinuation (Table 3). Pruritus occurred in 14% of patients in the 2-mg and 10-mg groups combined as compared with no patients in the control group. Injection-site reactions occurred in 8 patients (16%) in the 2-mg group and 15 patients (30%) in the 10-mg group.

Serious adverse events occurred in all trial groups, but none in the 2-mg and 10-mg groups were considered to be related to bulevirtide. Grade 3 adverse events considered to be related to bulevirtide occurred in one patient in the 2-mg group (decreased neutrophil count) and in three patients in the 10-mg group (one event each of thrombocytopenia, neutropenia, and leukopenia). There were no grade 4 adverse events.

At baseline, the median total bile acid level was slightly greater than the upper limit of the normal range and was similar in the three groups. A dose-dependent increase in the total bile acid level was noted in the 2-mg and 10-mg groups (Fig. S5 and Supplementary Results section in the Supplementary Appendix). Increased total bile acid levels did not lead to drug discontinuation in any patient.

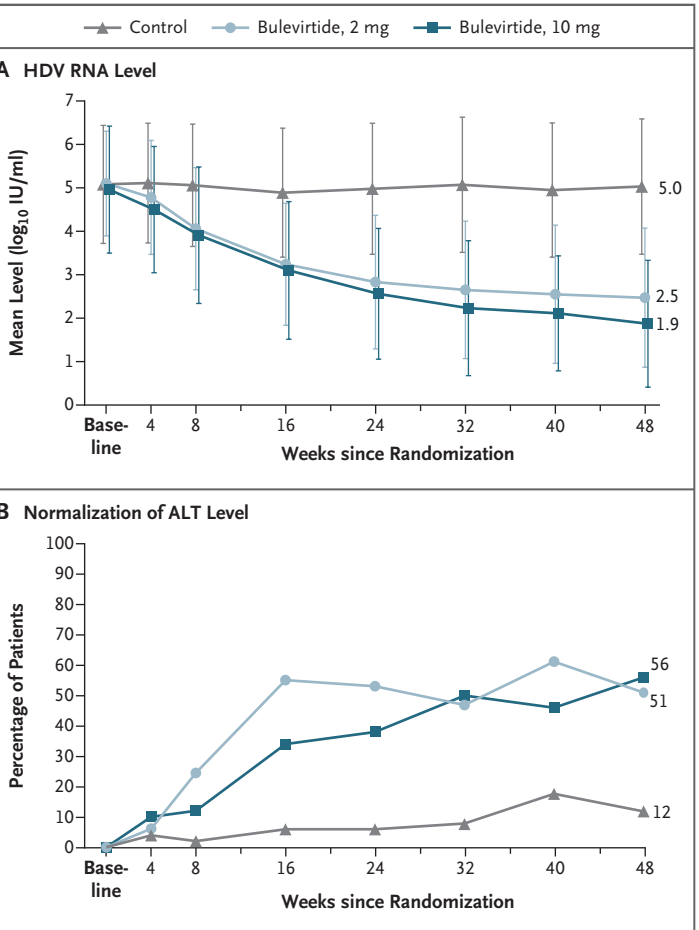

Figure 1. Hepatitis D Virus RNA and Normalization of ALT.

The primary end point of the trial was a combined response at week 48 of an undetectable hepatitis D virus (HDV) RNA level, or a level that decreased by at least 2 $\log_{10}$ IU per milliliter from baseline, and normalization of the alanine aminotransferase (ALT) level. Panel A shows the mean HDV RNA level at baseline and follow-up visits, according to trial group. The baseline level in the 2-mg and 10-mg groups was the last available level measured before administration of the first dose of bulevirtide. The baseline level in the control group was the last available level measured at or before randomization. Data were analyzed according to the group to which the patients were randomly assigned (planned treatment group). Panel B shows the percentage of patients with a normalized ALT level across the 48 weeks of the trial, according to trial group.

DISCUSSION

In this ongoing trial involving patients with chronic hepatitis D who received bulevirtide (2 mg or 10 mg) or no treatment for the first 48 weeks, a significantly greater percentage of patients in bulevirtide groups than in the control group had a virologic and biochemical response (primary combined end-point response), as assessed by analysis of the HDV RNA and ALT levels, respectively, at week 48. This surrogate end point is considered to be a reasonably likely predictor of improved clinical outcomes in patients with hepatitis D[27]; however, longer-term data are needed to confirm the clinical benefit of bulevirtide.

The percentage of patients with a combined response at week 48 was generally similar in the 2-mg and 10-mg groups. Moreover, similar changes in liver stiffness from baseline to week 48 were seen in the 2-mg and 10-mg groups overall, and the percentage of patients with an improvement in the histologic activity index from baseline to week 48 was similar with the two doses in the subgroup of patients with liver biopsy results available at both time points. These results suggest that a bulevirtide dose of 2 mg may be sufficient to block the entry of HBV and HDV into hepatocytes.

Table 3. Adverse Events during the Treatment Period and Laboratory Abnormalities.*

Event	Control (N=51)	Bulevirtide, 2 mg (N=49)	Bulevirtide, 10 mg (N=50)
		no. of patients (%)	
Adverse events that occurred during the treatment period			
Any adverse event			
Overall	39 (77)	40 (82)	44 (88)
Grade 3 or 4	3 (6)	5 (10)	4 (8)
Treatment-related adverse event			
Overall	0	24 (49)	36 (72)
Grade 3 or 4	0	1 (2)	3 (6)
Serious adverse event			
Overall	1 (2)	2 (4)	1 (2)
Treatment-related	0	0	0
Adverse event leading to treatment discontinuation	0	0	0
Non–injection-site event†			
Vitamin D deficiency	8 (16)	6 (12)	8 (16)
Leukopenia	9 (18)	6 (12)	5 (10)
Headache	0	9 (18)	10 (20)
Thrombocytopenia	8 (16)	5 (10)	5 (10)
Pruritus	0	6 (12)	8 (16)
Fatigue	1 (2)	5 (10)	7 (14)
Eosinophilia	0	5 (10)	5 (10)
Neutropenia	3 (6)	2 (4)	5 (10)
Nausea	2 (4)	3 (6)	4 (8)
Abdominal pain upper	1 (2)	0	6 (12)
Arthralgia	0	3 (6)	4 (8)
Asthenia	0	2 (4)	3 (6)
Increased total bile acid level	0	1 (2)	3 (6)
Injection-site event†			
Reaction	0	3 (6)	4 (8)
Erythema	0	2 (4)	4 (8)
Pruritus	0	1 (2)	3 (6)
Swelling	0	1 (2)	3 (6)
Grade 3 or 4 laboratory abnormality occurring in ≥1 patient in any treatment group			
Platelet count			
25,000 to <50,000/mm^3‡	2 (4)	1 (2)	2 (4)
<25,000/mm^3§	0	1 (2)	2 (4)
Neutrophil count of 500 to <1000/mm^3‡	2 (4)	0	2 (4)

* A full list of adverse events that occurred during the treatment period and laboratory abnormalities is shown in Table S7.

† The *Medical Dictionary for Regulatory Activities*, version 24.0, was used to code these adverse events. Adverse events during the treatment period that occurred in a larger percentage of patients in the 2-mg or 10-mg groups or that occurred in 10% or more of patients overall are highlighted here.

‡ The abnormality was assessed as grade 3 in severity.

§ The abnormality was assessed as grade 4 in severity.

Among patients with a combined response, normalization of the ALT level occurred in most patients by week 24, whereas the HDV RNA level continued to decline between week 24 and week 48. The detailed mechanisms associated with this difference in response kinetics remain to be investigated, including the potential effects of bile acids on immune responses and intrahepatic inflammation. Minimal changes in HBsAg levels between baseline and week 48 were observed with bulevirtide treatment, and no patients had HBsAg loss. Because most HBsAg production in HBV antigen–negative patients — the predominant HBV–HDV-coinfected population in this trial — is derived from integrated HBV genomes as opposed to covalently closed circular DNA, we were not surprised to observe a decrease in the HDV RNA level without a corresponding decrease in the HBsAg level or HBsAg loss.[28] The kinetics of the HDV and HBV responses during treatment require further study, particularly with regard to cell-to-cell transmission and hepatocyte turnover.

Headache, fatigue, eosinophilia, injection-site reactions, pruritus, upper abdominal pain, arthralgia, and asthenia occurred in a greater percentage of patients in the 2-mg and 10-mg groups combined than in the control group. All these adverse events were mild to moderate in severity and did not lead to drug discontinuation.

Dose-dependent increases in bile acid levels occurred with bulevirtide treatment.[29] Bile acid levels were above the normal range in all groups at baseline, a finding that may be explained by the inhibition of NTCP by HBsAg.[30,31] Pruritus associated with bulevirtide occurred in approximately 14% of patients in the 2-mg and 10-mg groups; however, all cases were mild or moderate in severity, and none led to drug discontinuation. We did not observe evidence of cholestatic liver injury, as assessed by detection of an elevated alkaline phosphatase level, or of hepatic dysfunction due to elevated bile acid levels with bulevirtide. It should be noted that extremely elevated plasma bile acid levels induced by a genetic deficiency of NTCP have not been associated with clinical signs of hepatic dysfunction.[32]

The current trial is ongoing to address the efficacy, safety, and side-effect profile of long-term (up to 3 years or 144 weeks) bulevirtide therapy. A phase 2 trial is evaluating the efficacy of bulevirtide administered with pegylated interferon alfa, as compared with bulevirtide alone and pegylated interferon alfa alone, as a potential finite curative treatment strategy in patients with chronic hepatitis D (ClinicalTrials.gov number, NCT03852433). That trial is also exploring the potential synergistic effect of interferon, which prevents cell division–mediated spread of HDV, with bulevirtide.[33]

Our trial has limitations. First, because daily injection of placebo is considered to be unethical, trial-group assignment was not blinded; however, because the assessments of efficacy end points were performed on the basis of objective laboratory measurements, the integrity of the trial data could be maintained. Second, no patients had Child–Turcotte–Pugh class B or C cirrhosis, although 47% of the patients had Child–Turcotte–Pugh class A cirrhosis, and 17% had a baseline platelet count of 100,000 or less per mm^3. Third, not all HDV and HBV genotypes were represented. However, in vitro data suggest that bulevirtide has activity against every HDV and HBV genotype.[34] The predominant HDV genotype in this trial — genotype 1 — is the most common HDV genotype globally,[1] and HBV genotype D, which was seen in the majority of patients, is common in Europe,[35] North America, Russia, and Central Asia.[36] Fourth, most patients in this trial were White, and data on patients of other races were limited or absent. Fifth, this analysis evaluated data at week 48, when patients in two of the groups were receiving treatment, and thus we could not assess end points during the follow-up period after the end of therapy. However, the current trial is designed to assess both longer-term efficacy (through week 144) and a secondary end point of a sustained virologic response at week 168 (24 weeks after the end of therapy). Finally, the diagnosis of cirrhosis was made at the investigator's discretion at the time of screening with the aid of transient elastography findings and clinical data in all patients but without the availability of liver biopsy results in some patients.

In this phase 3 trial involving patients with chronic hepatitis D, a combined response at week 48 occurred in a significantly greater percentage of patients in the bulevirtide groups than in the control group.

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APPENDIX

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