ORIGINAL ARTICLE

Efficacy and Safety of Bepirovirsen in Chronic Hepatitis B Infection

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ABSTRACT

BACKGROUND

Bepirovirsen is an antisense oligonucleotide that targets all hepatitis B virus (HBV) messenger RNAs and acts to decrease levels of viral proteins.

METHODS

We conducted a phase 2b, randomized, investigator-unblinded trial involving participants with chronic HBV infection who were receiving or not receiving nucleoside or nucleotide analogue (NA) therapy. Participants were randomly assigned (in a 3:3:3:1 ratio) to receive weekly subcutaneous injections of bepirovirsen at a dose of 300 mg for 24 weeks (group 1), bepirovirsen at a dose of 300 mg for 12 weeks then 150 mg for 12 weeks (group 2), bepirovirsen at a dose of 300 mg for 12 weeks then placebo for 12 weeks (group 3), or placebo for 12 weeks then bepirovirsen at a dose of 300 mg for 12 weeks (group 4). Groups 1, 2, and 3 received loading doses of bepirovirsen. The composite primary outcome was a hepatitis B surface antigen (HBsAg) level below the limit of detection and an HBV DNA level below the limit of quantification maintained for 24 weeks after the planned end of bepirovirsen treatment, without newly initiated antiviral medication.

RESULTS

The intention-to-treat population comprised 457 participants (227 receiving NA therapy and 230 not receiving NA therapy). Among those receiving NA therapy, a primary-outcome event occurred in 6 participants (9%; 95% credible interval, 0 to 31) in group 1, in 6 (9%; 95% credible interval, 0 to 43) in group 2, in 2 (3%; 95% credible interval, 0 to 16) in group 3, and 0 (0%; post hoc credible interval, 0 to 8) in group 4. Among participants not receiving NA therapy, a primary-outcome event occurred in 7 participants (10%; 95% credible interval, 0 to 38), 4 (6%; 95% credible interval, 0 to 25), 1 (1%; post hoc credible interval, 0 to 6), and 0 (0%; post hoc credible interval, 0 to 8), respectively. During weeks 1 through 12, adverse events, including injection-site reactions, pyrexia, fatigue, and increased alanine aminotransferase levels, were more common with bepirovirsen (groups 1, 2, and 3) than with placebo (group 4).

CONCLUSIONS

In this phase 2b trial, bepirovirsen at a dose of 300 mg per week for 24 weeks resulted in sustained HBsAg and HBV DNA loss in 9 to 10% of participants with chronic HBV infection. Larger and longer trials are required to assess the efficacy and safety of bepirovirsen. (Funded by GSK; B-Clear ClinicalTrials.gov number, NCT04449029.)

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Chronic hepatitis B virus (HBV) infection is a major worldwide health problem, with an estimated 1.5 million new infections and 820,000 deaths each year (predominantly from cirrhosis and hepatocellular carcinoma).[1,2] The goal of therapy is to achieve functional cure — that is, long-term hepatitis B surface antigen (HBsAg) loss, with or without HBsAg seroconversion (positive for antibodies against HBsAg [anti-HBs]), and sustained undetectable HBV DNA after cessation of therapy.[3-5] Despite prolonged treatment with nucleoside or nucleotide analogue (NA) therapy (first-line treatment for HBV infection),[6] fewer than 5% of patients have HBsAg loss after 12 months of treatment,[5,7-11] which underscores the need for therapies capable of achieving functional cure.

Bepirovirsen (GSK3228836), a 2′-O-methoxyethyl modified antisense oligonucleotide, targets all HBV RNAs, including HBV messenger RNA and pregenomic RNA.[10] In a phase 2a trial, 4 weeks of bepirovirsen elicited a rapid and dose-dependent reduction in HBsAg levels and, in some participants, transient HBsAg loss.[12] Immunostimulatory activity of bepirovirsen through toll-like receptor 8 (TLR8) may be correlated with HBsAg reduction.[13]

We conducted a phase 2b trial (B-Clear) to investigate the efficacy and safety of 12- and 24-week bepirovirsen treatment in participants with chronic HBV infection either receiving stable NA therapy or not receiving NA therapy. To assess durability of response, the primary efficacy outcome was HBsAg and HBV DNA loss for 24 weeks after the discontinuation of bepirovirsen treatment in the absence of newly initiated antiviral treatment.

Methods

Participants

Participants were 18 years of age or older with documented chronic HBV infection for at least 6 months and an HBsAg level of more than 100 IU per milliliter. Participants who were receiving NA therapy were on a stable NA regimen and had an HBV DNA level of less than 90 IU per milliliter and an alanine aminotransferase (ALT) level less than or equal to 2 times the upper limit of the normal range. Participants who were not receiving NA therapy had never received such therapy or had ended NA therapy at least 6 months before screening and had an HBV DNA level of more than 2000 IU per milliliter and an ALT level of less than 3 times the upper limit of the normal range. Key exclusion criteria were the presence of hepatitis C, human immunodeficiency, or hepatitis D virus infection; cirrhosis; hepatocellular carcinoma; and interferon-containing therapy within 12 months before screening (Table S7 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

Trial Design

B-Clear was a phase 2b, randomized, parallel-cohort trial conducted from July 27, 2020, to March 18, 2022, at 123 sites in 22 countries (see the Supplementary Appendix). The trial sponsor and participants were unaware of the trial-group assignments, which were known to the investigators. Randomization was performed with the use of an interactive Web-response system, with stratification according to hepatitis B e antigen (HBeAg) status (positive or negative) and baseline HBsAg level (≤3 or >3 $\log_{10}$ IU per milliliter).

Participants were randomly assigned (in a 3:3:3:1 ratio) to receive weekly subcutaneous injections of bepirovirsen at a dose of 300 mg for 24 weeks (group 1), bepirovirsen at a dose of 300 mg for 12 weeks then 150 mg for 12 weeks (group 2), bepirovirsen at a dose of 300 mg for 12 weeks then placebo for 12 weeks (group 3), or placebo for 12 weeks then bepirovirsen at a dose of 300 mg for 12 weeks (group 4) (Fig. 1). Loading doses of bepirovirsen (300 mg, in groups 1, 2, and 3) or placebo (in group 4) were administered on days 4 and 11. Participants were followed for up to 55 weeks, with a 24-week treatment period and a 24-week follow-up period. Participants receiving NA therapy continued such therapy during the trial.

Trial Oversight

The sponsor, GSK, designed and oversaw the trial conduct and data collection and analysis. Professional writers paid by the sponsor prepared the first draft of the manuscript under the authors' direction. The manuscript was reviewed and edited by all the authors. All the authors made the decision to submit the manuscript for publication and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol (available at NEJM.org). An independent data monitoring committee reviewed unblinded

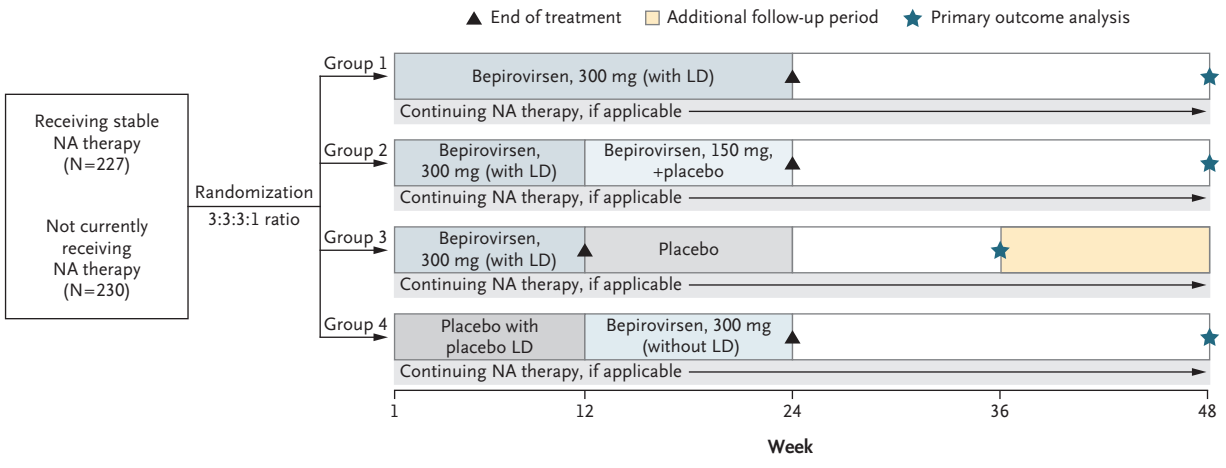

Figure 1. Trial Design.

Participants receiving stable nucleoside or nucleotide analogue (NA) therapy were expected to continue NA therapy during the trial; participants not receiving NA therapy at trial entry were expected to continue without NA therapy during the trial. Participants receiving stable NA therapy and participants not currently receiving NA therapy underwent randomization separately. Doses were administered once weekly as two subcutaneous injections (two syringes total; one syringe contained either bepirovirsen at a dose of 150 mg or placebo). The loading dose (LD) of bepirovirsen (300 mg, in groups 1, 2, and 3) or placebo (in group 4) was administered on days 4 and 11.

data. The trial was conducted in accordance with the Declaration of Helsinki, the Council for International Organizations of Medical Sciences International Ethical Guidelines for Biomedical Research Involving Human Subjects, the Good Clinical Practice guidelines of the International Council for Harmonisation, and all applicable laws and regulations in participating countries. The protocol and amendments were reviewed and approved by local institutional review boards or independent ethics committees. Written informed consent was obtained from all the participants.

OUTCOMES

The primary composite efficacy outcome was an HBsAg level below the lower limit of detection (0.05 IU per milliliter) and an HBV DNA level below the lower limit of quantification (20 IU per milliliter) maintained for 24 weeks after the planned end of bepirovirsen treatment in the absence of any medication initiated for the purpose of suppressing HBV replication (groups 1, 2, and 3) (Table S1). Group 4 (placebo-first group) was included to allow evaluation of the effect of a loading dose and evaluation of safety in the first 12 weeks, but the trial was not powered to assess the primary outcome in this group. An additional prespecified analysis used a modified version of the primary outcome that permitted "blips" (single-time-point increases in the HBsAg level to greater than or equal to the lower limit of detection or in the HBV DNA level to greater than or equal to the lower limit of quantification) in the response (Table S8).

Secondary efficacy outcomes included the difference between groups 1 and 2, groups 1 and 3, and groups 2 and 3 in the proportion of participants having a primary-outcome event; the proportion of participants having an HBsAg level below the lower limit of detection and an HBV DNA level below the lower limit of quantification at the end of treatment; log changes from baseline in HBsAg and HBV DNA levels (according to category of HBsAg or HBV DNA level); actual values and change from baseline in HBsAg, HBV DNA, HBeAg, and anti-HBs levels; and ALT normalization (ALT level less than or equal to the upper limit of the normal range) in the absence of newly initiated antiviral treatment in participants with an ALT level above the upper limit of the normal range at baseline.

Safety outcomes included clinical assessments, laboratory measurements, and adverse events. Increases in ALT levels, as well as class effects of antisense oligonucleotides (renal injury, injection-site reactions, thrombocytopenia, and vas-

cular inflammation and complement activation), were evaluated as adverse events of special interest (Table S9).

STATISTICAL ANALYSIS

We planned to enroll approximately 440 participants: approximately 66 participants receiving NA therapy and 66 not receiving NA therapy in each of groups 1, 2, and 3 and approximately 22 participants receiving NA therapy and 22 not receiving NA therapy in group 4. This sample size was chosen on the basis of a Bayesian model and selected to provide at least a 75% posterior probability of the true response rate exceeding a fixed threshold under a range of assumed values for the threshold of interest and true response rate (Table S3).

An estimation approach with no hypothesis testing was used to analyze the primary outcome. A Bayesian hierarchical model (including baseline stratification factors) was used to calculate the point estimate of the primary outcome and 95% credible interval. If the Bayesian hierarchical model did not converge, a post hoc unstratified Bayesian analysis was performed (see the Methods section in the Supplementary Appendix).

Efficacy objectives were assessed with the use of estimands (i.e., precise descriptions of the treatment effect reflecting the clinical question posed by a given clinical-trial objective). The primary estimand was the proportion of participants in groups 1, 2, and 3 who had a primary-outcome event, regardless of completion of treatment, interruptions in treatment, or adherence to treatment had they not been affected by wide disruptive events (e.g., the coronavirus disease 2019 pandemic). A receiver-operating-characteristic analysis explored a range of baseline HBsAg cutoff points as a predictor of response.

Safety analyses included a descriptive summary of adverse-event incidence (including comparison of the first 12 weeks of treatment in groups 1, 2, and 3 with placebo in group 4), vital signs, and laboratory data. Efficacy analyses were conducted in the intention-to-treat population, which included all randomly assigned participants, on the basis of the trial-group assignment. Safety analyses were conducted in all the participants who had undergone randomization and received at least one dose of bepirovirsen or placebo and were based on the trial agent received. As prespecified, analyses of all outcomes were conducted separately for participants receiving NA therapy and those not receiving NA therapy. Additional details are available in the Supplementary Appendix.

RESULTS

PARTICIPANTS

The intention-to-treat population included 457 participants (227 receiving NA therapy and 230 not receiving NA therapy); 13 participants (6%) receiving NA therapy and 23 (10%) not receiving NA therapy prematurely discontinued bepirovirsen or placebo, of whom 5 participants (2%) and 8 (3%), respectively, discontinued owing to adverse events (Fig. S2). The demographic and clinical characteristics of the participants at baseline were similar across trial groups (Table 1). In general, participants were representative of the population with chronic HBV infection (Table S10).

PRIMARY OUTCOME

In group 1, a primary-outcome event occurred in 6 participants (9%; 95% credible interval, 0 to 31) receiving NA therapy and in 7 (10%; 95% credible interval, 0 to 38) not receiving NA therapy. In group 2, a primary-outcome event occurred in 6 participants (9%; 95% credible interval, 0 to 43) receiving NA therapy and in 4 (6%; 95% credible interval, 0 to 25) not receiving NA therapy. In group 3, a primary-outcome event occurred in 2 participants (3%; 95% credible interval, 0 to 16) receiving NA therapy and in 1 (1%; post hoc credible interval, 0 to 6) not receiving NA therapy (Table 2).

When we used the modified primary-outcome definition that allowed for "blips" (single-time-point increases in the HBsAg level to greater than or equal to the lower limit of detection or in the HBV DNA level to greater than or equal to the lower limit of quantification) in response, in group 1, a total of 7 participants (10%; 95% credible interval, 0 to 36) receiving NA therapy and 10 (14%; 95% credible interval, 0 to 64) not receiving NA therapy had a response. Additional results are presented in Table S11.

In group 1, a total of 16% of the participants receiving NA therapy and 25% of those not receiving NA therapy with a low HBsAg level (≤ 3 $\log_{10}$ IU per milliliter) at baseline had a primary-outcome event, as compared with 6% and 7% of participants, respectively, with a high HBsAg level (>3 $\log_{10}$ IU per milliliter) at baseline (Fig. S3). A

Table 1. Demographic and Clinical Characteristics of the Participants at Baseline (Intention-to-Treat Population).*

Characteristic	Receiving NA Therapy				Not Receiving NA Therapy			
	Group 1 (N=68)	Group 2 (N=68)	Group 3 (N=68)	Group 4 (N=23)	Group 1 (N=70)	Group 2 (N=68)	Group 3 (N=68)	Group 4 (N=24)
Age — yr	49.0±11.5	46.1±12.6	47.4±11.2	49.8±11.2	44.5±11.1	43.8±9.9	40.7±11.1	42.4±12.0
Male sex — no. (%)	48 (71)	49 (72)	51 (75)	17 (74)	33 (47)	41 (60)	39 (57)	11 (46)
Body-mass index†	24.66±4.07	24.30±4.14	24.92±2.90	23.67±2.48	25.25±4.77	25.26±4.30	24.52±3.65	23.70±4.42
Race or ethnic group — no. (%)‡								
Asian	36 (53)	35 (51)	36 (53)	12 (52)	37 (53)	44 (65)	38 (56)	12 (50)
White	30 (44)	32 (47)	26 (38)	11 (48)	24 (34)	20 (29)	24 (35)	11 (46)
Black	2 (3)	1 (1)	4 (6)	0	9 (13)	4 (6)	6 (9)	1 (4)
American Indian or Alaska Native	0	0	1 (1)	0	0	0	0	0
Mixed race	0	0	1 (1)	0	0	0	0	0
ALT ≤ULN — no. (%)§¶	62 (91)	60 (90)	62 (91)	21 (91)	50 (71)	48 (71)	47 (69)	15 (62)
HBsAg ≤3 $\log_{10}$ IU/ml — no. (%)¶	19 (28)	23 (34)	19 (28)	3 (13)	12 (17)	15 (22)	11 (16)	5 (21)
HBsAg — $\log_{10}$ IU/ml	3.29±0.62	3.26±0.61	3.33±0.59	3.43±0.43	3.72±0.77	3.65±0.72	3.66±0.67	3.76±0.79
HBV DNA — $\log_{10}$ IU/ml	0.48±0.64	0.39±0.60	0.55±0.66	0.40±0.62	5.02±1.53	5.14±1.56	5.57±1.65	5.00±1.55
Negative HBeAg status — no. (%)¶	50 (74)	47 (70)	44 (65)	16 (70)	49 (70)	52 (76)	52 (76)	17 (71)
Receiving current NA therapy for ≥3 yr — no. (%)¶	47 (69)	45 (67)	43 (63)	20 (87)	N/A	N/A	N/A	N/A
Current NA drugs — no. (%)¶								
Entecavir	38 (56)	25 (37)	20 (29)	10 (43)	N/A	N/A	N/A	N/A
Tenofovir disoproxil	24 (35)	36 (54)	33 (49)	11 (48)	N/A	N/A	N/A	N/A
Tenofovir alafenamide	9 (13)	6 (9)	17 (25)	2 (9)				
Lamivudine	1 (1)	1 (1)	1 (1)	0	N/A	N/A	N/A	N/A
Adefovir dipivoxil	0	1 (1)	0	0	N/A	N/A	N/A	N/A
Emtricitabine	0	0	1 (1)	0	N/A	N/A	N/A	N/A

* Plus–minus values are means ±SD. Participants were randomly assigned (in a 3:3:3:1 ratio) to receive weekly subcutaneous injections of bepirovirsen at a dose of 300 mg for 24 weeks (group 1), bepirovirsen at a dose of 300 mg for 12 weeks then 150 mg for 12 weeks (group 2), bepirovirsen at a dose of 300 mg for 12 weeks then placebo for 12 weeks (group 3), or placebo for 12 weeks then bepirovirsen at a dose of 300 mg for 12 weeks (group 4). Loading doses of bepirovirsen (300 mg, in groups 1, 2, and 3) or placebo (in group 4) were administered on days 4 and 11. HBeAg denotes hepatitis B e antigen, HBsAg hepatitis B surface antigen, HBV hepatitis B virus, NA nucleoside or nucleotide analogue, and N/A not applicable.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡ Race or ethnic group was reported by the participant.

§ The upper limit of the normal range (ULN) for alanine aminotransferase (ALT) is 40 IU per liter for men and 33 IU per liter for women.

¶ Data were missing for 1 participant in group 2 who was receiving NA therapy, so the denominator is 67 rather than 68.

Table 2. Primary Outcome (Intention-to-Treat Population).*

Variable	Receiving NA Therapy				Not Receiving NA Therapy			
	Group 1 (N=68)	Group 2 (N=68)	Group 3 (N=68)	Group 4 (N=23)	Group 1 (N=70)	Group 2 (N=68)	Group 3 (N=68)	Group 4 (N=24)
Primary-outcome event — no. of participants (%)†	6 (9)	6 (9)	2 (3)	0	7 (10)	4 (6)	1 (1)	0
Point estimate of response — % (95% credible interval)	9 (0–31)	9 (0–43)	3 (0–16)	2 (0–8)‡	10 (0–38)	6 (0–25)	2 (0–6)‡	2 (0–8)‡

* Participants were randomly assigned (in a 3:3:3:1 ratio) to receive weekly subcutaneous injections of bepirovirsen at a dose of 300 mg for 24 weeks (group 1), bepirovirsen at a dose of 300 mg for 12 weeks then 150 mg for 12 weeks (group 2), bepirovirsen at a dose of 300 mg for 12 weeks then placebo for 12 weeks (group 3), or placebo for 12 weeks then bepirovirsen at a dose of 300 mg for 12 weeks (group 4). Loading doses of bepirovirsen (300 mg, in groups 1, 2, and 3) or placebo (in group 4) were administered on days 4 and 11.

† The primary outcome was an HBsAg level below the lower limit of detection (0.05 IU per milliliter) and an HBV DNA level below the lower limit of quantification (20 IU per milliliter) maintained for 24 weeks after the planned end of bepirovirsen treatment, without newly initiated antiviral medication.

‡ Shown are point estimates and credible intervals from post hoc unstratified Bayesian analysis owing to nonconvergence of the prespecified stratified Bayesian hierarchical model. Additional details are provided in the Methods section in the Supplementary Appendix.

receiver-operating-characteristic analysis in group 1 indicated that an HBsAg level of approximately 3000 IU per milliliter (3.5 $\log_{10}$ IU per milliliter) at baseline may be an appropriate cutoff point for predicting response (Fig. S4). At the cutoff point of 3000 IU or less per milliliter in group 1, a total of 5 of 43 participants (12%) receiving NA therapy and 6 of 24 (25%) not receiving NA therapy had a primary-outcome event.

Among HBeAg-negative participants, a primary-outcome event occurred in those receiving NA therapy and in those not receiving NA therapy (in group 1, 10% of participants receiving NA therapy and 14% of those not receiving NA therapy). Among HBeAg-positive participants, a primary-outcome event occurred only in those receiving NA therapy (in group 1, 6% of participants receiving NA therapy and 0% of those not receiving NA therapy) (Fig. S3).

SECONDARY OUTCOMES

Between-Group Differences in Primary Results

The differences between groups 1 and 2, groups 1 and 3, and groups 2 and 3 in the proportion of participants having a primary-outcome event are shown in the Results section in the Supplementary Appendix.

HBsAg and HBV DNA Levels

Decreases in HBsAg and HBV DNA levels were dependent on the duration of bepirovirsen treatment (Figs. S5 and S6). The percentage of participants within each category of HBsAg level over time is shown in Figure 2 and Figure S7. For many participants, HBsAg and HBV DNA levels increased after treatment discontinuation (Figs. S7 and S8). Individual HBsAg levels over time according to baseline HBsAg level in group 1 are shown in Figure S9.

In group 1, 43 participants (63%) receiving NA therapy and 41 (59%) not receiving NA therapy had an HBsAg level of less than 100 IU per milliliter by the end of treatment; the values were 26 (38%) and 20 (29%), respectively, at 24 weeks after the end of treatment (Fig. 2). By the end of treatment in group 1, a total of 18 participants (26%) receiving NA therapy and 20 (29%) not receiving NA therapy had an HBsAg level below the lower limit of detection; at 24 weeks after the end of treatment, the values were 8 (12%) and 10 (14%), respectively (Fig. 2 and Fig. S10). In group 1, a total of 34 participants (50%) receiving NA therapy and 35 (50%) not receiving NA therapy had a decrease of at least 3 $\log_{10}$ IU per milliliter in the HBsAg level at the end of treatment; the incidence of relapse at the end of the trial was lowest in group 1 (Fig. S11).

Among participants not receiving NA therapy, the number in group 1 who had an HBV DNA level below the lower limit of quantification was 26 (37%) at the end of treatment and 19 (27%) at 24 weeks after the end of treatment, as compared

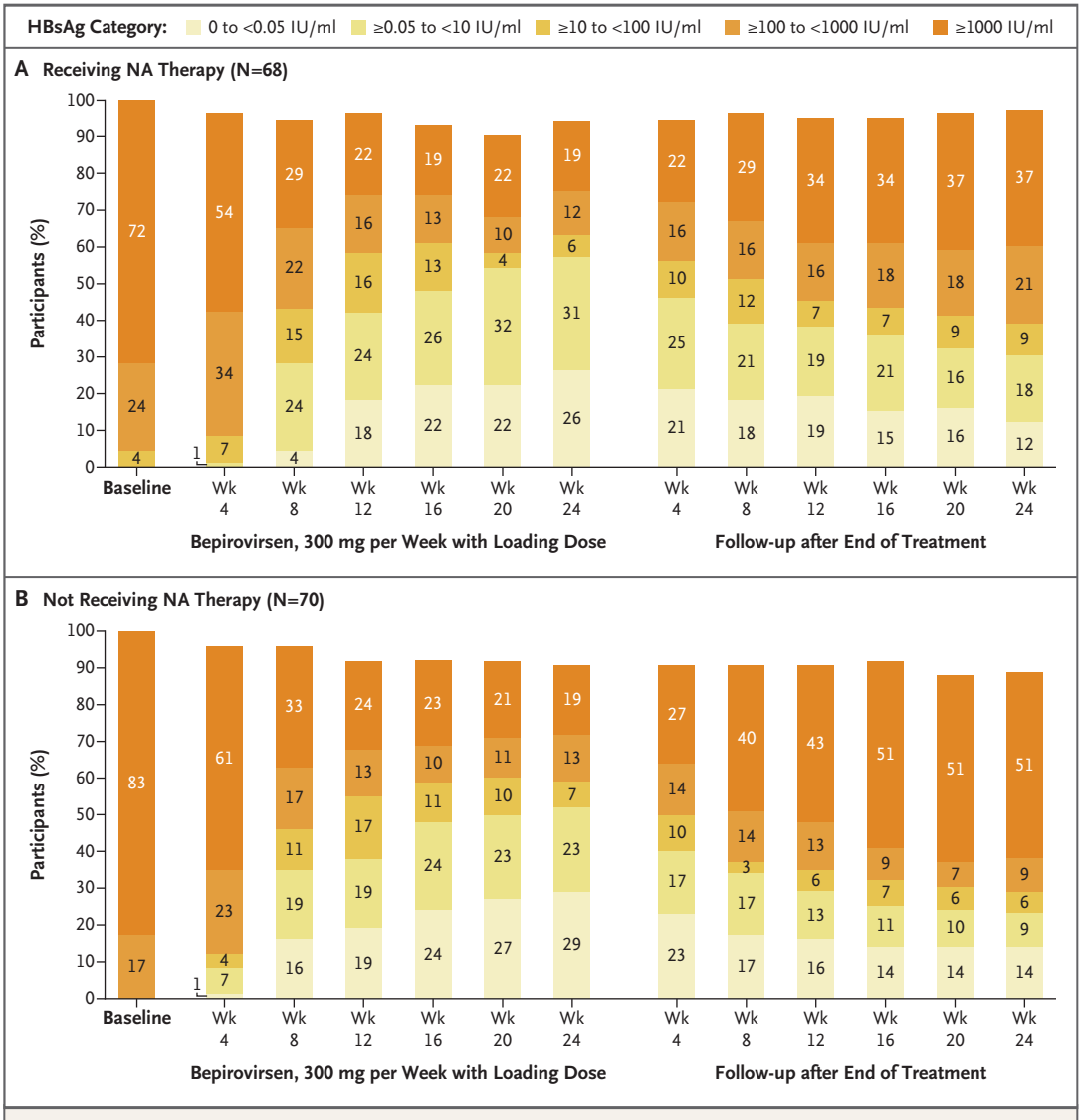

Figure 2. Percentage of Participants within HBsAg Categories over Time (Group 1, Intention-to-Treat Population).

Shown are five categories of hepatitis B surface antigen (HBsAg) level. Participants in group 1 received loading doses of bepirovirsen on days 4 and 11. Percentages were calculated on the basis of the total number of participants in the intention-to-treat population. (At the time of the European Association for the Study of the Liver presentation,[14,15] percentages were calculated on the basis of available data at the trial visit of interest.)

with 20 (29%) and 15 (22%), respectively, in group 2; 18 (26%) and 9 (13%), respectively, in group 3; and 4 (17%) at both time points in group 4. Data excluding participants who received newly initiated antiviral medication are shown in Figure S8. Among participants in group 1 not receiving NA therapy, a decrease of at least 3 $\log_{10}$ IU per milliliter in the HBV DNA level was observed in 27 participants (39%) at the end of treatment and in 18 (26%) at 24 weeks after the end of treatment (Fig. S12).

Levels of HBeAg and Anti-HBs

Of the participants who were positive for HBeAg and negative for antibodies against hepatitis B e (anti-HBe) at baseline, 10 of 63 (16%) receiving NA therapy and 8 of 41 (20%) not receiving NA therapy had HBeAg loss at 24 weeks after the

end of treatment; 7 participants (11%) receiving NA therapy and 8 (20%) not receiving NA therapy had seroconversion (HBeAg loss and positivity for anti-HBe). Of the participants who had a primary-outcome event, 7 (50%) receiving NA therapy and 6 (50%) not receiving NA therapy had anti-HBs at the end of the trial.

ALT Levels

Overall, 39 of 225 participants receiving NA therapy (17%) and 93 of 227 not receiving NA therapy (41%) had a transient increase in the ALT level to at least 3 times the upper limit of the normal range between randomization and the end of follow-up (Table S6). At baseline, most participants (91% of those receiving NA therapy and 70% of those not receiving NA therapy) had an ALT level at or below the upper limit of the normal range (Table 1). The median time to ALT normalization for participants with an ALT level above the upper limit of the normal range at baseline is shown in Figure S13 and Table S12.

ADVERSE EVENTS

During weeks 1 through 12, when comparison between bepirovirsen and placebo was possible, adverse events were more common during bepirovirsen treatment in groups 1, 2, and 3 (in 78%, 85%, and 76%, respectively, of participants receiving NA therapy and in 90%, 82%, and 87%, respectively, of those not receiving NA therapy) than during receipt of placebo in group 4 (in 43% of those receiving NA therapy and 54% of those not receiving NA therapy) (Table 3). For example, injection-site reactions, pyrexia, fatigue, and increased ALT levels were reported more frequently with bepirovirsen than with placebo (Tables S13 and S14). The majority of adverse events reported were those captured under adverse events of special interest.

During weeks 1 through 12, no participants in group 4 (placebo) had serious adverse events; five participants receiving NA therapy (one in group 1, one in group 2, and three in group 3) and three participants not receiving NA therapy (all in group 1) had serious adverse events. Grade 3 or 4 adverse events occurred in 7 to 13% of participants receiving NA therapy and in 10 to 14% of those not receiving NA therapy in groups 1, 2, and 3, as compared with 0 in group 4. After the treatment period (during weeks 25 through 48), the frequency of reported adverse events was generally similar among the trial groups and of low frequency for individual reported events (Tables S15 and S16).

During weeks 1 through 48, the proportion of participants who reported adverse events was higher among those not receiving NA therapy than among those receiving NA therapy (Table 3), with a higher incidence of increased ALT level, increased aspartate aminotransferase level, decreased platelet count, decreased complement factor C3 level, and decreased complement factor C4 level (Tables S17 and S18). In patients receiving NA therapy and in those not receiving NA therapy, the most common adverse events were injection-site reactions (erythema, pain, and pruritus). Overall, 17 participants had adverse events leading to discontinuation of bepirovirsen or placebo, with 0 to 4% frequency among participants receiving NA therapy and 0 to 7% frequency among those not receiving NA therapy (Table 3).

Grade 3 or 4 adverse events occurred in 74 participants overall, with 0 to 16% frequency among those receiving NA therapy and 17 to 23% frequency among those not receiving NA therapy. Serious adverse events were reported in 6 participants (3%) receiving NA therapy and 11 (5%) not receiving NA therapy; 1 serious adverse event in participants receiving NA therapy and 3 in those not receiving NA therapy were considered by the investigator to be related to bepirovirsen treatment (Table 3 and Tables S19 and S20). No deaths were reported. The most common adverse events of special interest were injection-site reactions, reported in 48 to 74% of participants across trial groups and participants receiving or not receiving NA therapy (Table 3).

DISCUSSION

In this phase 2b trial, bepirovirsen at a dose of 300 mg per week for 24 weeks (group 1) resulted in 9 to 10% of participants having HBsAg and HBV DNA loss for 24 weeks after the end of bepirovirsen treatment. Results were similar in participants receiving NA therapy and those not receiving NA therapy.

Results for the modified primary outcome, which permitted "blips" in response, were consistent with the primary analysis, with 10 to 14% of the participants having an outcome event. HBV DNA "blips" after HBV DNA loss are a known phenomenon when stopping NA therapy and are

Table 3. Safety Summary (Safety Population).*

Adverse Event	Receiving NA Therapy				Not Receiving NA Therapy			
	Group 1 (N=68)	Group 2 (N=67)	Group 3 (N=68)	Group 4 (N=23)	Group 1 (N=70)	Group 2 (N=67)	Group 3 (N=68)	Group 4 (N=24)
	number of participants (percent)							
Visits at wk 1–12 (when group 4 received placebo)								
Any adverse event	53 (78)	57 (85)	52 (76)	10 (43)	63 (90)	55 (82)	59 (87)	13 (54)
Any grade 3 or 4 adverse event†	5 (7)	9 (13)	5 (7)	0	10 (14)	9 (13)	7 (10)	0
Any serious adverse event‡	1 (1)	1 (1)	3 (4)	0	3 (4)	0	0	0
Adverse events of special interest§								
Injection-site reaction	38 (56)	47 (70)	43 (63)	3 (13)	50 (71)	41 (61)	46 (68)	3 (12)
Vascular inflammation and complement activation	24 (35)	31 (46)	30 (44)	1 (4)	45 (64)	38 (57)	42 (62)	6 (25)
Thrombocytopenia	12 (18)	9 (13)	11 (16)	3 (13)	21 (30)	17 (25)	19 (28)	4 (17)
Increased ALT level	7 (10)	10 (15)	6 (9)	0	15 (21)	12 (18)	11 (16)	1 (4)
Renal injury	2 (3)	6 (9)	6 (9)	0	5 (7)	3 (4)	9 (13)	1 (4)
All visits								
Any adverse event	56 (82)	59 (88)	53 (78)	16 (70)	65 (93)	60 (90)	62 (91)	19 (79)
Any adverse event leading to discontinuation of trial agent	2 (3)	3 (4)	3 (4)	0	3 (4)	1 (1)	5 (7)	0
Any grade 3 or 4 adverse event†	7 (10)	11 (16)	8 (12)	0	16 (23)	15 (22)	13 (19)	4 (17)
Any serious adverse event‡	1 (1)	1 (1)	4 (6)	0	6 (9)	2 (3)	3 (4)	0
Adverse events of special interest§								
Injection-site reaction	41 (60)	49 (73)	43 (63)	11 (48)	52 (74)	41 (61)	49 (72)	12 (50)
Vascular inflammation and complement activation	30 (44)	34 (51)	31 (46)	10 (43)	49 (70)	40 (60)	46 (68)	12 (50)
Thrombocytopenia	19 (28)	16 (24)	12 (18)	6 (26)	32 (46)	21 (31)	21 (31)	6 (25)
Increased ALT level	7 (10)	10 (15)	6 (9)	6 (26)	20 (29)	19 (28)	16 (24)	5 (21)
Renal injury	4 (6)	9 (13)	6 (9)	2 (9)	7 (10)	6 (9)	9 (13)	3 (12)

* Participants were randomly assigned (in a 3:3:3:1 ratio) to receive weekly subcutaneous injections of bepirovirsen at a dose of 300 mg for 24 weeks (group 1), bepirovirsen at a dose of 300 mg for 12 weeks then 150 mg for 12 weeks (group 2), bepirovirsen at a dose of 300 mg for 12 weeks then placebo for 12 weeks (group 3), or placebo for 12 weeks then bepirovirsen at a dose of 300 mg for 12 weeks (group 4). Loading doses of bepirovirsen (300 mg, in groups 1, 2, and 3) or placebo (in group 4) were administered on days 4 and 11. In group 2, one participant receiving NA therapy and one not receiving NA therapy did not receive any bepirovirsen treatment and were therefore not included in the safety population.

† Adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected version 2.1. Grade 1 indicates a mild event, grade 2 a moderate event, grade 3 a severe event, grade 4 a potentially life-threatening event, and grade 5 death.

‡ A serious adverse event is defined as an adverse event that, at any dose of bepirovirsen or placebo, results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or clinically significant disability or incapacity, or is a congenital anomaly or birth defect.

§ The adverse events of special interest were defined according to standardized *Medical Dictionary for Regulatory Activities* (MedDRA) queries or MedDRA high-level terms or individual preferred terms (see Table S9 in the Supplementary Appendix). The adverse event of special interest "vascular inflammation and complement activation" included preferred terms such as injection-site pruritus and injection-site swelling. Injection-site reactions were the most commonly reported events in the trial.

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suggested to represent spontaneous fluctuations due to a release of virions from hepatic reservoirs.[16,17] The "blips" in HBsAg seen in the B-Clear trial may have a similar cause.

HBsAg loss is rarely achieved with currently available HBV treatments. New therapies are being developed for the treatment of chronic HBV infection, including small interfering RNAs (siRNAs).[18,19] Clinical trials with siRNAs have shown HBsAg reduction to less than 100 IU per milliliter in up to 70% of participants, but no, or very few, participants had HBsAg loss.[20-23] To put the results of the B-Clear trial in context, in group 1, a total of 9% of the participants receiving NA therapy and 10% of those not receiving NA therapy had both HBsAg and HBV DNA loss maintained for 24 weeks after the end of bepirovirsen treatment. The immunostimulatory activity of bepirovirsen may be mediated through TLR8[13]; this mode of action may explain differences seen as compared with other new HBV therapies. These findings may represent progress in the search for achieving a functional cure. Optimization of response will most likely require combination therapy to target multiple steps of the HBV life cycle, stimulate the immune system, or both.[18,19] Studies are ongoing that combine bepirovirsen with other therapies (e.g., an inhibitor of PAPD5 and PAPD7 [ClinicalTrials.gov number, NCT05330455], pegylated interferon therapy [NCT04676724], and ASO-HBV vaccine [NCT03866187]) to enable more patients to have a response.

The B-Clear data suggest that the HBsAg level at baseline may predict response. Participants with a low HBsAg level at baseline were more likely to have a primary-outcome event than those with a high level at baseline, findings that are consistent with previous observations and that highlight the importance of baseline HBsAg levels in predicting response as seen with other HBV therapies.[12,24] We found substantial decreases in HBsAg levels in participants with a high HBsAg level at baseline, but the reductions were often not enough to result in HBsAg loss. A receiver-operating-characteristic analysis suggested that an HBsAg level of 3000 IU per milliliter at baseline may be an appropriate cutoff point as a predictor of response.

Most patients with chronic HBV infection are HBeAg-negative, with the prevalence of this subgroup increasing.[25] In HBeAg-negative patients, HBV sequences are potentially integrated (with some HBV sequences deleted) into the host genome, and the integrated HBV DNA is a primary source of HBsAg.[26] The HBsAg reductions and loss with bepirovirsen that we observed in HBeAg-negative participants suggest that the bepirovirsen target site is preserved in the majority of integrated HBV-derived transcripts. In HBeAg-positive participants, a primary-outcome event occurred only in those receiving NA therapy. Because the primary driver of response seemed to be the HBsAg level at baseline, the apparent lack of response in HBeAg-positive participants who were not receiving NA therapy may be explained by higher HBsAg levels at baseline in this subgroup, with only two HBeAg-positive participants having a low HBsAg level at baseline.

In patients with HBV, ALT flares can be a result of disease activity, immune clearance of HBV-infected hepatocytes, or drug-induced liver injury.[12,27,28] There were two serious adverse events related to ALT changes, which are discussed further in the Results section in the Supplementary Appendix, and one case of Gilbert's syndrome. Otherwise, increases in ALT levels were asymptomatic and resolved without increases in bilirubin or alkaline phosphatase levels and without evidence of liver dysfunction.

During the first 12 weeks of the trial when group 4 received placebo, adverse events (primarily those identified as adverse events of special interest, including an increased ALT level) were more common with bepirovirsen than with placebo. Other commonly reported adverse events were pyrexia and fatigue. Eight serious adverse events occurred in the first 12 weeks of bepirovirsen treatment and none with receipt of placebo. Overall, injection-site reactions were the most common adverse events; two participants (one receiving NA therapy and one not receiving NA therapy) withdrew owing to an injection-site reaction. Among participants receiving NA and those not receiving NA therapy, bepirovirsen at a dose of 300 mg weekly for 24 weeks did not show any marked difference in safety or side-effect profile as compared with other regimens.

In this phase 2b trial, 24-week treatment with bepirovirsen at a dose of 300 mg per week induced HBsAg and HBV DNA loss for 24 weeks after the end of treatment. This efficacy was achieved with a single agent (in 10% of partici-

pants; 95% credible interval, 0 to 38) and in combination with NA therapy (in 9%; 95% credible interval, 0 to 31). Although this is a relatively low percentage of participants overall, it indicates the possibility of enhanced efficacy with the selection of patients according to baseline characteristics (low HBsAg level at baseline), with combination therapies, or both. Durability of response is being investigated in the B-Sure trial (ClinicalTrials.gov number, NCT04954859), which will follow participants for an additional 33 months and includes criteria for stopping NA therapy. Larger trials and longer follow-up are needed to assess the safety and efficacy of bepirovirsen.

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APPENDIX

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