Efficacy and safety of the siRNA JNJ-73763989 and the capsid assembly modulator JNJ-56136379 (bersacapavir) with nucleos(t)ide analogues for the treatment of chronic hepatitis B virus infection (REEF-1): a multicentre, double-blind, active-controlled, randomised, phase 2b trial

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Summary

Background JNJ-73763989 (JNJ-3989), a small interfering RNA, targets all hepatitis B virus (HBV) RNAs, reducing all HBV proteins. JNJ-56136379 (JNJ-6379; also known as bersacapavir), a capsid assembly modulator, inhibits HBV replication. We aimed to evaluate the efficacy (ie, antiviral activity) and safety of these therapeutics in combination with nucleos(t)ide analogues in patients with chronic hepatitis B.

Methods The REEF-1 multicentre, double-blind, active-controlled, randomised, phase 2b study was done at 108 hospitals or outpatient centres across 19 countries in Asia, Europe, and North and South America. We included patients aged 18–65 years with chronic hepatitis B (defined as HBsAg positivity at screening and at least 6 months before screening or alternative markers of chronicity [eg, HBV DNA]), including those not currently treated, virologically suppressed, HBeAg positive, and HBeAg negative. Patients were randomly assigned (1:1:2:2:2:2) via permuted block randomisation according to a computer-generated schedule to receive oral nucleos(t)ide analogues once per day plus placebo (control group); oral JNJ-6379 250 mg daily plus nucleos(t)ide analogues (JNJ-6379 dual group); nucleos(t)ide analogues plus subcutaneously injected JNJ-3989 at doses of 40 mg (JNJ-3989 dual 40 mg group), 100 mg (JNJ-3989 dual 100 mg group), or 200 mg (JNJ-3989 dual 200 mg group) every 4 weeks; or JNJ-6379 250 mg plus JNJ-3989 100 mg every 4 weeks plus nucleos(t)ide analogues (triple group) for 48 weeks followed by a follow-up phase. An interactive web response system provided concealed treatment allocation, and investigators remained masked to the intervention groups until the primary analysis at week 48. The primary endpoint was the proportion of patients meeting predefined nucleos(t)ide analogue-stopping criteria (alanine aminotransferase <3 × upper limit of normal, HBV DNA below the lower limit of quantitation, HBeAg negative, and HBsAg <10 IU/mL) at week 48. All patients who received at least one dose of study drug were included in the analysis population used for primary efficacy assessment, excluding those who withdrew because of COVID-19-related reasons, withdrew before week 44, or had no efficacy data (ie, the modified intention-to-treat population). Safety was assessed in all participants who received at least one dose of study drugs. This trial is registered with ClinicalTrials.gov, NCT03982186. The study has been completed.

Findings Between Aug 1, 2019, and April 26, 2022, 470 patients (310 [66%] male and 244 [52%] White) were randomly assigned: 45 to the control group, 48 to the JNJ-6379 dual group, 93 to the JNJ-3989 dual 40 mg group, 93 to the JNJ-3989 dual 100 mg group, 96 to the JNJ-3989 dual 200 mg group, and 95 to the triple group. At week 48, five (5%; 90% CI 2–11) of 91 patients in the JNJ-3989 dual 40 mg group, 15 (16%; 10–24) of 92 in the JNJ-3989 dual 100 mg group, 18 (19%; 13–27) of 94 in the JNJ-3989 dual 200 mg group, eight (9%; 4–15) of 94 in the triple group, and one (2%; 0–10) of 45 in the control group met nucleos(t)ide analogue stopping criteria. No patients in the JNJ-6379 dual group met stopping criteria. 38 (81%) patients who met nucleos(t)ide analogue-stopping criteria at week 48 were virologically suppressed and HBeAg negative at baseline. Ten (2%) of 470 patients had serious adverse events during the treatment phase, and two patients (one each from the JNJ-3989 dual 200 mg group [exercise-related rhabdomyolysis] and the triple group [increase in ALT or AST]) had serious adverse events related to study treatment. During follow-up, 12 (3%) of 460 patients had a serious adverse event; one (<1%), a gastric ulcer, was considered to be related to nucleos(t)ide analogues and occurred in a patient from the JNJ-3989 dual 200 mg group. 29 (6%) of 460 patients in the treatment phase and in ten (2%) of 460 patients in the follow-up phase had grade 3 or 4 adverse events. Five (1%) of 470 patients discontinued treatment due to adverse events, and there were no deaths.

Interpretation Although treatment with JNJ-3989 led to a dose-dependent response for meeting nucleos(t)ide analogue-stopping criteria, it rarely led to HBsAg seroclearance. However, most patients treated with JNJ-3989 had

clinically meaningful reductions in HBsAg that might contribute to a liver environment conducive to better immune control and, in turn, might improve the response to immune-modulating therapies.

Funding Janssen Research and Development.

Introduction

Chronic hepatitis B infection affects approximately 296 million individuals, around 3·5% of the global population.[1,2] Currently approved therapies include nucleos(t)ide analogues and interferon, which suppress viral replication.[3] With these treatments, the rate of sustained HBsAg seroclearance off-treatment, referred to as functional cure, is low.[4] Pegylated interferon treatment for approximately 1 year results in a slightly higher rate of functional cure versus nucleos(t)ide analogues, but has poorer tolerability.[3,4] Nucleos(t)ide analogues are generally well tolerated, but require long-term, often life-long, treatment.[3] Even with effective suppression of viral replication by nucleos(t)ide analogue treatment and lower disease progression, the risk of hepatocellular carcinoma is not eliminated,[5,6] adding to the need for new treatment strategies.

Novel therapies are being investigated to achieve functional cure, thereby improving long-term clinical outcomes and allowing patients with chronic hepatitis B

Research in context

Evidence before this study

Currently, two classes of drugs are approved for the treatment of hepatitis B virus (HBV), immune modulators (ie, interferons) and antivirals (ie, nucleos[t]ide analogues), neither of which are treatment options that lead to significant rates of functional cure. Thus, new classes of therapies are under investigation, including RNA molecules targeting oligonucleotides (small interfering RNAs [siRNAs] and antisense oligonucleotides), capsid assembly modulators, HBV entry inhibitors, and immune modulators and activators. We searched PubMed for research articles published from database inception to Sept 27, 2022, with no language restrictions. All fields were searched for "siRNA" and "chronic hepatitis B". Although multiple phase 1 trials were found, our search identified only one phase 2 study of an siRNA in people with chronic hepatitis B. Results from a phase 2a trial (AROHBV1001; NCT03365947) showed that short-term treatment with the siRNA JNJ-73763989 at doses from 100 mg to 400 mg, in combination with nucleos(t)ide analogues, led to similar reductions in HBsAg of 1 $\log_{10}$ IU/mL or higher that exhibited long-term persistence, whereas lower doses of JNJ-73763989 (25 mg and 50 mg) led to smaller declines in HBsAg. Results from the phase 2 JADE trial (NCT03361956) showed that the capsid assembly modulator JNJ-56136379 (bersacapavir) at doses of 75 mg and 250 mg in combination with nucleos(t)ide analogues led to greater HBV DNA and RNA suppression than nucleos(t)ide analogue treatment alone, although limited declines were observed for HBsAg. The combination of 200 mg JNJ-73763989, 250 mg JNJ-56136379, and nucleos(t)ide analogues led to similar declines in HBsAg with groups receiving 100 mg to 400 mg of JNJ-73763989 (≥1 $\log_{10}$ IU/mL reduction of HBsAg at the nadir; AROHBV1001).

Added value of this study

This phase 2b study, the first large siRNA study with 48 weeks of treatment and the option to stop nucleos(t)ide analogues, was conducted in a broad population of patients with chronic hepatitis B from several countries who were not currently treated, virologically suppressed with nucleos(t)ide analogues, and HBeAg positive and negative. The results indicate that JNJ-73763989 was generally safe and well tolerated and led to a robust and dose-dependent reduction of HBsAg concentrations. At the end of the 48-week treatment phase, 47 (10%) of 470 patients met the primary endpoint of reaching nucleos(t)ide analogue-stopping criteria (alanine aminotransferase <3 × upper limit of normal, HBV DNA less than the lower limit of quantitation, HBeAg negative, and HBsAg <10 IU/mL) and 19 (4%) additional patients met nucleos(t)ide analogue-stopping criteria during the follow-up phase. The JNJ-73763989 200 mg plus nucleos(t)ide analogues group had the highest proportion of patients who met nucleos(t)ide analogue-stopping criteria during both treatment (18 [19%; 90% CI 13–27] of 94 patients) and follow-up (additional ten [11%] of 464 patients) phases. Although substantial HBsAg reductions were observed with JNJ-73763989 treatment, functional cure (off-treatment HBsAg seroclearance) was not reached with the regimens evaluated: JNJ-73763989 (40 mg, 100 mg, or 200 mg), JNJ-56136379 (250 mg), or triple (JNJ-73763989 100 mg plus JNJ-56136379 250 mg; all patients received nucleos[t]ide analogue treatment). However, the mean HBsAg reduction from baseline was still greater than 1 $\log_{10}$ in all JNJ-73763989 groups at follow-up week 24, indicating meaningful reductions in HBsAg were maintained beyond the end of treatment. In addition, among the patients who stopped nucleos(t)ide analogues, a majority (67% of those who received JNJ-73763989 200 mg with nucleos[t]ide analogues) remained off-treatment at follow-up week 24 with suppressed HBV DNA and low HBsAg.

Implications of all the available evidence

Additional studies are warranted to evaluate JNJ-73763989 in combination with therapies of differing mechanisms of action, such as immune-modulating therapies, to evaluate the potential for achieving functional cure in patients with chronic hepatitis B. Conversely, no additional benefit of JNJ-56136379 was observed.

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See **Online** for appendix

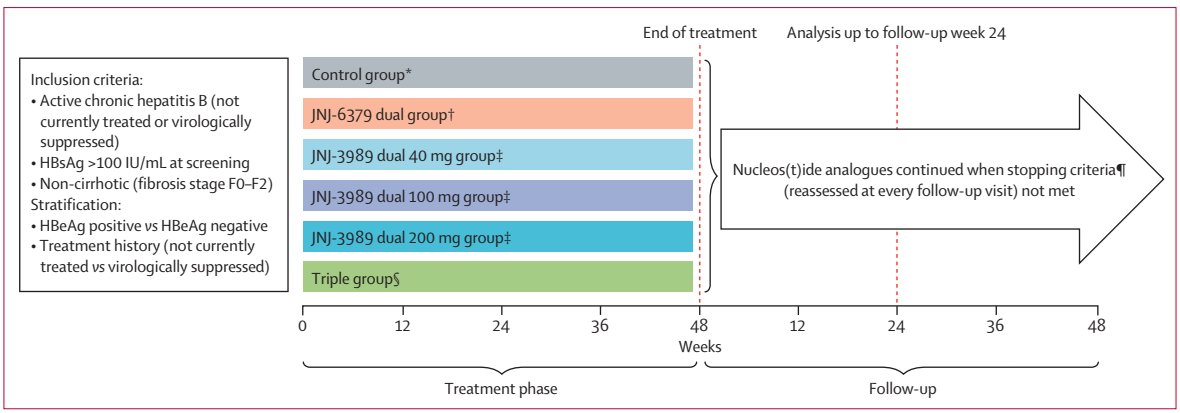

Figure 1: **Study design**
ALT=alanine aminotransferase. HBV=hepatitis B virus. JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379. *Once per day entecavir (0·5 mg), tenofovir disoproxil fumarate (300 mg), or tenofovir alafenamide (25 mg). †Oral JNJ-6379 250 mg once per day plus oral nucleos(t)ide analogues once per day plus JNJ-3989 placebo every 4 weeks. ‡Subcutaneous injection of JNJ-3989 every 4 weeks plus oral nucleos(t)ide analogues once per day plus JNJ-6379 placebo once per day. §Subcutaneous injection of JNJ-3989 100 mg every 4 weeks plus oral JNJ-6379 250 mg once per day plus oral nucleos(t)ide analogues once per day. ¶Nucleos(t)ide analogue stopping criteria were ALT less than 3 times the upper limit of normal, HBV DNA below the lower limit of quanititation, HBeAg negativity, and HBsAg less than 10 IU/mL.

to have finite treatment.[5,7] JNJ-73763989 (JNJ-3989) is a subcutaneously injected, liver-targeted small interfering RNA (siRNA) composed of two triggers (JNJ-73763976 and JNJ-73763924) designed to treat chronic hepatitis B.[8] Engagement of JNJ-3989 by the cellular RNA interference machinery results in specific cleavage of all hepatitis B virus (HBV) RNA transcripts, thereby reducing the levels of all HBV proteins and pregenomic RNA.[8] JNJ-56136379 (JNJ-6379; also known as bersacapavir) is an orally administered capsid assembly modulator (CAM) class E that interferes with the viral capsid assembly process and causes the formation of empty HBV capsids that are structurally normal, but non-functional due to an absence of HBV DNA and RNA, thereby inhibiting HBV replication.[9–14]

Results from the phase 2a clinical trial (AROHBV1001; NCT03365947) showed that short-term (ie, up to 12 weeks of treatment) JNJ-3989 treatments at doses from 100 mg to 400 mg, in combination with nucleos(t)ide analogues, led to similar HBsAg reductions, whereas lower doses (25 mg and 50 mg) led to smaller HBsAg declines.[15] The combination of JNJ-3989, JNJ-6379, and nucleos(t)ide analogue led to similar HBsAg declines versus groups receiving 100 mg or higher doses of JNJ-3989. 15 (38%) of 39 patients with long-term follow-up information maintained a greater than 1 $\log_{10}$ HBsAg reduction from baseline for 1 year or less after the last dose.[14–16] We aimed to determine the dose–response relationship for JNJ-3989 on antiviral activity and to evaluate the efficacy and safety of combination treatment regimens containing JNJ-3989 plus a nucleos(t)ide analogue (with or without JNJ-6379) and JNJ-6379 plus nucleos(t)ide analogues in patients with chronic hepatitis B.

Methods

Study design and participants

REEF-1, a multicentre, double-blind, active-controlled, randomised, phase 2b study, was done at 108 hospitals or outpatient clinics across 19 countries in Asia, Europe, and North and South America. Patients aged 18–65 years with chronic hepatitis B, defined as HBsAg positivity at screening and at least 6 months before screening or alternative markers of chronicity (eg, HBV DNA), and with liver stiffness 9·0 kPa or less on FibroScan within 6 months of screening were included (figure 1). If no FibroScan result was available, a liver biopsy result classified as fibrosis stage F0–F2 within 1 year of screening was required. Patients were classified as not currently treated if they had not received nucleos(t)ide analogue and interferon treatment for at least 6 months before screening or virologically suppressed if they had received nucleos(t)ide analogue treatment of entecavir, tenofovir disoproxil fumarate, or tenofovir alafenamide fumarate for at least 6 months before screening. Not currently treated patients had active hepatitis B with alanine aminotransferase (ALT) above the upper limit of normal (ULN) and less than 10 times the ULN, with HBV DNA of 2000 IU/mL or higher if they were HBeAg negative or with HBV DNA 20 000 IU/mL or higher if they were HBeAg positive. Patients who were virologically suppressed had HBV DNA of less than 60 IU/mL and ALT twice the ULN or less at screening (key inclusion and exclusion criteria are provided in the appendix p 4).

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was consistent with Good Clinical Practice. The protocol (appendix pp 30, 144) was approved by an independent ethics committee and institutional review board. Patients provided written informed consent.

Randomisation and masking

Patients were screened and randomly assigned centrally by a computer-generated schedule that was balanced via permuted block in a 1:1:2:2:2:2 ratio to the following

six treatment groups: placebos plus nucleos(t)ide analogues (control group); JNJ-6379 250 mg plus nucleos(t)ide analogues (JNJ-6379 dual group); nucleos(t)ide analogues plus JNJ-3989 at 40 mg (JNJ-3989 dual 40 mg group), 100 mg (JNJ-3989 dual 100 mg group), or 200 mg (JNJ-3989 dual 200 mg group); and JNJ-3989 100 mg plus JNJ-6379 250 mg plus nucleos(t)ide analogues (triple group; figure 1; appendix p 2). Randomisation was stratified by HBeAg status at screening (positive versus negative) and treatment history (not currently treated versus virologically suppressed). Study investigators remained fully masked to the treatment allocation of patients until week 48 from randomisation, at which time they were informed whether patients were allocated to the control group or one of the investigational groups. Study investigators were fully unmasked at follow-up week 24 from end of treatment. Individual data were provided to the investigators (appendix p 7) and sponsor (interpreted by OL, TV, US, and MBi) from week 48 onwards (including week 44 data).

Procedures

After randomisation, patients received the study drugs for their treatment group assignment, delivered as either once per day oral nucleos(t)ide analogues (entecavir 0·5 mg, tenofovir alafenamide fumarate 25 mg, or tenofovir disoproxil fumarate 300 mg); once per day oral JNJ-6379 250 mg; or subcutaneous injection of JNJ-3989 every 4 weeks at 40 mg, 100 mg, or 200 mg. Patients in the JNJ-3989 groups received oral JNJ-6379 placebo once per day and those in the JNJ-6379 group received subcutaneous injection of JNJ-3989 placebo every 4 weeks. Patients in the control group received both placebos. Treatment with JNJ-3989 and JNJ-6379 occurred for 48 weeks (double-blind phase). Patients who met the nucleos(t)ide analogue-stopping criteria (ALT <3×ULN, HBV DNA below the lower limit of quantitation [LLOQ], HBeAg negative, and HBsAg <10 IU/mL), based on week 44 clinical laboratory tests, terminated nucleos(t)ide analogue treatment at week 48 and began a 48-week treatment-free follow-up phase. Patients who did not meet the nucleos(t)ide analogue-stopping criteria by week 48 continued nucleos(t)ide analogue treatment during the follow-up phase. If nucleos(t)ide analogue-stopping criteria were met during the follow-up phase, which was assessed at every follow-up visit, an additional 48 weeks of follow-up began (extended follow-up). Patients who met nucleos(t)ide analogue-stopping criteria and stopped nucleos(t)ide analogue treatment were closely monitored for nucleos(t)ide analogue-retreatment criteria based on increases in HBV DNA, ALT, or both. Patients could remain in the study without completing 48 weeks of treatment. We report data from up to 24 weeks of follow-up after the end of treatment (appendix p 2).

Outcomes

The primary efficacy endpoint was the proportion of patients meeting nucleos(t)ide analogue-stopping criteria at week 48 based on laboratory tests at week 44, which was centrally assessed. Secondary efficacy endpoints were proportion of patients with HBsAg seroclearance (<0·05 IU/mL) 24 weeks after completion of all study interventions; proportion of patients meeting nucleos(t)ide analogue-stopping criteria during the follow-up phase; and change in HBsAg, HBeAg, HBV DNA, hepatitis B core-related antigen (HBcrAg), and HBV RNA. Additional secondary and exploratory endpoints are provided in the appendix (p 2), as is the methodology used to analyse each virological marker (appendix p 6).

Safety and tolerability were evaluated throughout the study (details provided in the appendix p 2). Safety observations during the treatment phase included all events up to week 48. Safety observations during the follow-up phase included all events observed at the follow-up week 24 analysis, which included data beyond follow-up week 24 for some patients.

Based on the primary efficacy endpoint, the primary hypotheses are as follows: any of the combination regimens is more efficacious than control; there is a positive dose–response signal across the three doses of JNJ-3989 (40 mg, 100 mg, and 200 mg) on the background of nucleos(t)ide analogue versus control; one or both combination regimens of JNJ-6379 dual or triple are more efficacious than control; and the triple combination regimen is more efficacious than the JNJ-3989 dual 100 mg plus nucleos(t)ide analogue regimen, or JNJ-6379 plus nucleos(t)ide analogue regimen.

Statistical analysis

Statistical power to test a dose–response signal (max trend test) was assessed using the generalised version of the MCP-Mod applied to the binary primary efficacy endpoint on the logit scale using data from three JNJ-3989 groups and the control group. The max trend test was used to test the null hypothesis that none of the JNJ-3989 doses were better than control. Assuming a response rate for the highest dose of JNJ-3989 of at least 25%, and a one-sided α level of 5%, the power to conclude a positive dose–response trend over the three JNJ-3989 doses plus nucleos(t)ide analogues is 85% or higher. Assuming a response rate of 5% in the control group, the sample size of 90 in each of the four JNJ-3989 treatment groups and a sample size of 45 participants each in the JNJ-6379 group and control group provides a statistical power of 84% or higher to detect a difference of at least 20% in the primary endpoint between the triple group and control group, and power of 76% or higher for a difference of at least 20% between the JNJ-6379 group and control group, using a fixed-sequence approach for controlling for multiplicity. Testing among the triple, JNJ-3989 100 mg, and JNJ-6379 groups was performed using the min test approach to control for the one-sided type 1 error rate of 0·05.

All efficacy summaries are presented with descriptive statistics by intervention group. If the endpoint is continuous, the descriptive statistics include the number

of participants, mean with SD or SE, and median with IQR. If the endpoint is binary or categorical, the frequency distribution with the number and percentage of participants is presented. Clopper-Pearson exact method was used to calculate 95% CIs.

The primary efficacy analysis was done in the modified intention-to-treat population, which included all patients who were randomly assigned and received at least one dose of study treatment, excluding those who, because of COVID-19 or similar pandemic-related reasons, withdrew prematurely (before week 44) or had no efficacy assessment for the primary endpoint. The COVID-19 Guiding Principles for Study Conduct, Analysis, and Reporting[17] were followed internally. The amendment to the statistical analysis plan was done due to COVID-19 and completed on Dec 2, 2020, which was reviewed and approved by internal assessors (CM, JJ, and MBi). The primary efficacy analysis was performed after all patients completed week 48 or discontinued before the end of treatment. Safety was analysed in all patients who received at least one dose of study drug. Additional details describing statistical analyses are provided in the appendix (p 2–3).

This study is registered at ClinicalTrials.gov, NCT03982186.

Role of the funding source

Employees of Janssen Research and Development were involved in the study design, data collection, data analysis, interpretation of data, and writing of the manuscript.

Results

Between Aug 1, 2019, and April 26, 2022, 470 patients were randomly assigned and received at least one study drug: 45 to the control group, 48 to the JNJ-6379 dual

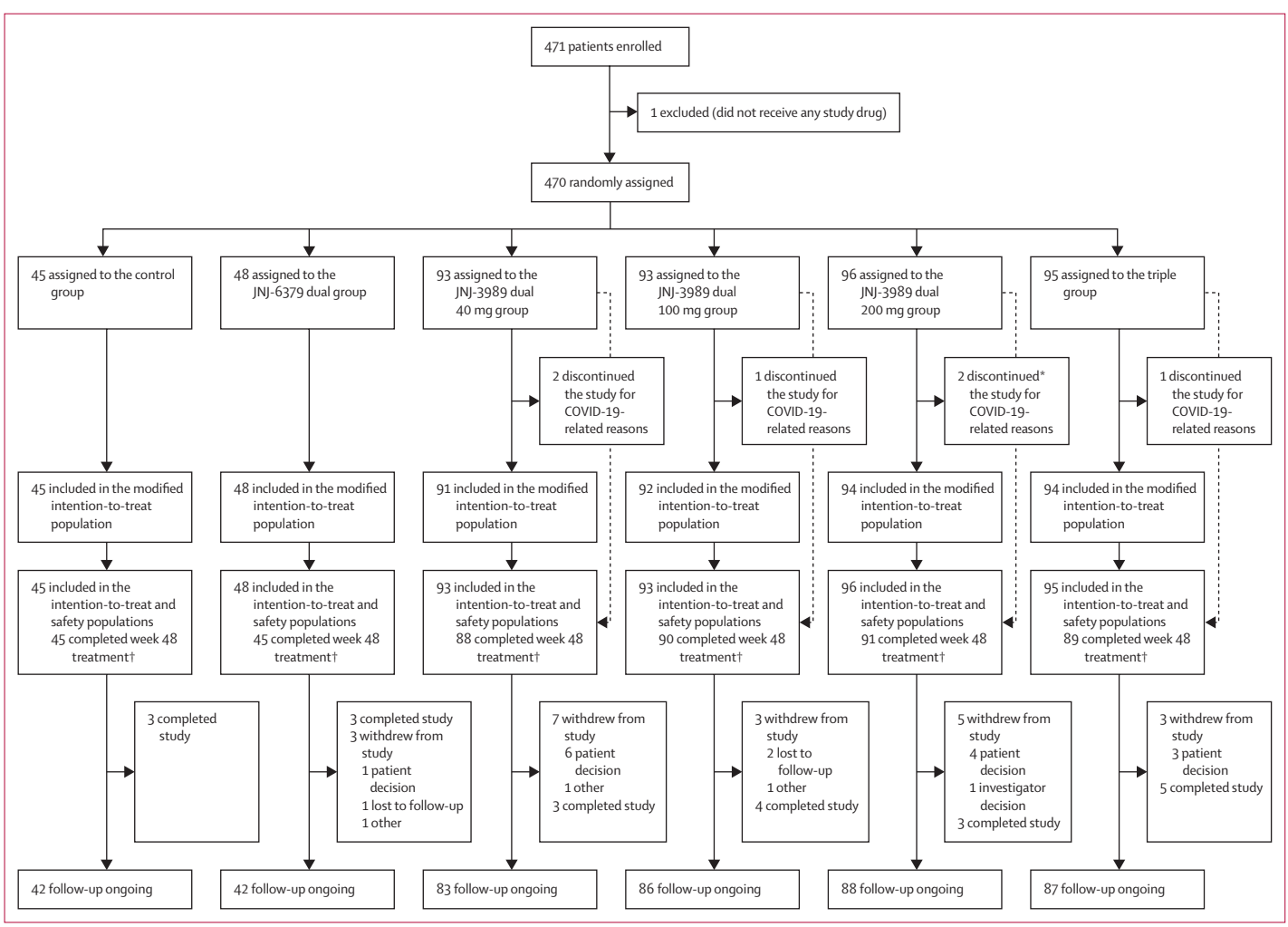

Figure 2: **Trial profile**

JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379. *Includes one patient who discontinued all study treatments due to a COVID-19 1 day before withdrawing from the study, but COVID-19 was not mentioned as a reason for study withdrawal. †The number of patients who completed 48 weeks of treatment is not necessarily equivalent to the number of patients who remained in the study, as some patients did not complete 48 weeks of treatment, but also did not withdraw from the study prematurely and are therefore counted as completing the study or ongoing in the follow-up.

	Control group (n=45)	JNJ-6379 dual group (n=48)	JNJ-3989 dual 40 mg group (n=93)	JNJ-3989 dual 100 mg group (n=93)	JNJ-3989 dual 200 mg group (n=96)	Triple group (n=95)
Sex						
Male	25 (56%)	37 (77%)	61 (66%)	55 (59%)	61 (64%)	71 (75%)
Female	20 (44%)	11 (23%)	32 (34%)	38 (41%)	35 (36%)	24 (25%)
Race						
American Indian or Alaska Native	0	1 (2%)	2 (2%)	0	0	0
Asian	15 (33%)	20 (42%)	42 (45%)	39 (42%)	39 (41%)	35 (37%)
Black or African American	1 (2%)	2 (4%)	9 (10%)	5 (5%)	7 (7%)	6 (6%)
White	28 (62%)	25 (52%)	39 (42%)	48 (52%)	50 (52%)	54 (57%)
Not reported	1 (2%)	0	1 (1%)	1 (1%)	0	0
Age, years	43·0 (36·0 to 50·0)	44·0 (38·0 to 50·0)	43·0 (34·0 to 50·0)	42·0 (35·0 to 50·0)	40·5 (35·0 to 51·5)	42·0 (36·0 to 51·0)
Treatment history						
Not currently treated	16 (36%)	18 (38%)	34 (37%)	34 (37%)	36 (38%)	34 (36%)
Virologically suppressed	29 (64%)	30 (62%)	59 (63%)	59 (63%)	60 (62%)	61 (64%)
HBeAg positive	13 (29%)	15 (31%)	30 (32%)	26 (28%)	30 (31%)	28 (29%)
HBeAg, log_{10} IU/mL for patients who were HBeAg-positive	0·2 (–0·1 to 2·2)	0·7 (–0·3 to 2·2)	1·1 (0·2 to 2·8)	2·2 (–0·1 to 3·1)	1·7 (0·4 to 2·8)	1·2 (0·0 to 2·9)
HBsAg, log_{10} IU/mL	3·9 (3·5 to 4·4)	3·7 (3·2 to 4·2)	3·9 (3·4 to 4·2)	3·8 (3·2 to 4·3)	3·9 (3·3 to 4·3)	3·6 (3·1 to 4·2)
HBsAg <100 IU/mL	0	0	0	1 (1%)	1 (1%)	0
HBsAg <1000 IU/mL	6 (13%)	8 (17%)	13 (14%)	15 (16%)	9 (9%)	17 (18%)
HBsAg <3000 IU/mL	10 (22%)	21 (44%)	30 (32%)	35 (38%)	26 (27%)	43 (45%)
HBV DNA, log_{10} IU/mL	1·2 (0·7 to 5· 5)	1·2 (1·2 to 5·3)	1·2 (0·70 to 4·8)	1·2 (0·7 to 5·4)	1·2 (0·7 to 5·5)	1·2 (0·7 to 4·6)
HBcrAg*, log_{10} IU/mL	3·7 (2·7 to 5·5)	3·9 (3·1 to 5·6)	3·9 (2·7 to 5·8)	4·0 (2·7 to 5·7)	4·0 (2·7 to 6·0)	3·7 (2·7 to 5·2)
HBV RNA*, log_{10} copies per mL	2·2 (2·2 to 4·3)	2·2 (2·2 to 4·4)	2·2 (2·2 to 4·3)	2·2 (2·2 to 4·3)	2·7 (2·2 to 5·1)	2·2 (2·2 to 3·6)
ALT, U/L	28·0 (20·0 to 45·0)	27·0 (18·5 to 55·0)	30·0 (19·0 to 56·0)	30·0 (18·0 to 56·0)	27·0 (19·0 to 55·0)	32·0 (19·0 to 53·0)
Liver stiffness*†, kPa	5·1 (4·4 to 6·4)	5·0 (4·1 to 6·5)	5·2 (4·1 to 6·5)	5·1 (4·3 to 6·1)	5·2 (4·2 to 6·2)	5·1 (4·1 to 6·1)
HBV genotype						
Genotype A	5 (11%)	2 (4%)	8 (9%)	7 (8%)	8 (8%)	11/93 (12%)
Genotype B	2 (4%)	2 (4%)	5 (5%)	5 (5%)	3 (3%)	3/93 (3%)
Genotype C	2 (4%)	7 (15%)	13 (14%)	14 (15%)	16 (17%)	10/93 (11%)
Genotype D	12 (27%)	15 (31%)	17 (18%)	12 (13%)	18 (19%)	17/93 (18%)
Genotype E	0	0	4 (4%)	1 (1%)	1 (1%)	2/93 (2%)
Genotype F	0	0	1 (1%)	2 (2%)	0	2/93 (2%)
Mixed genotype detected	1 (2%)	0	0	0	0	0
Unknown‡	23 (51%)	22 (46%)	45 (48%)	52 (56%)	50 (52%)	48/93 (52%)

Data are n (%) or median (IQR), unless otherwise stated. All groups received nucleos(t)ide analogues. ALT=alanine aminotransferase. HBV=hepatitis B virus. JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379. *Data were missing at baseline for HBcrAg (four patients), liver stiffness (10 patients), and HBV RNA (12 patients). †Measured with FibroScan. ‡HBV genotype could not be determined in any patients who were virologically suppressed and in some patients who were not currently treated due to low HBV DNA levels at baseline.

Table 1: **Baseline demographic and clinical characteristics**

group, 93 to the JNJ-3989 dual 40 mg group, 93 to the JNJ-3989 dual 100 mg group, 96 to the JNJ-3989 dual 200 mg group, and 95 to the triple group (figure 2). 310 (66%) of 470 patients were male, 244 (52%) were White, 190 (40%) were Asian, and mean age was 43·0 years (SD 10·7). Baseline demographics and clinical characteristics were generally similar between groups, except for a larger proportion of male patients in the JNJ-6379 dual group and triple group, and a smaller proportion of male patients and Asian patients in the control group (table 1). Median baseline HBsAg values ranged between 3·6 log_{10} IU/mL and 3·9 log_{10} IU/mL but differed by patient population (table 1; appendix p 9). 298 (63%) patients were virologically suppressed and 328 (70%) were HBeAg negative at screening. Of the 172 patients who were not currently treated, 136 (79%)

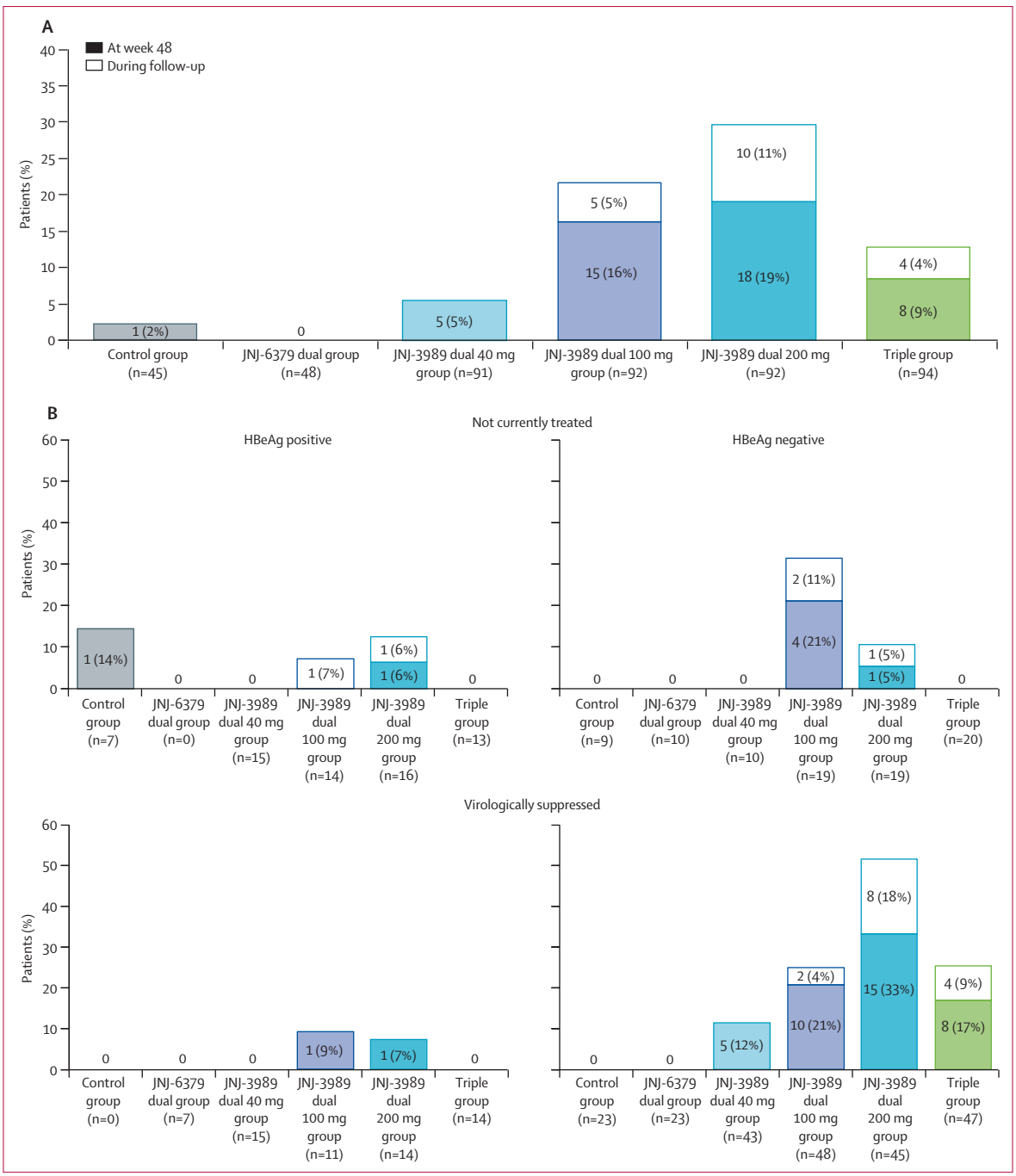

***Figure 3*: Percentage of patients meeting nucleos(t)ide analogue-stopping criteria at week 48 and during the 24-week follow-up overall (A) and by stratification factors (B)**

Nucleos(t)ide analogue-stopping criteria are alanine aminotransferase less than 3 times the upper limit of normal, hepatitis B virus DNA below the lower limit of quantitation, HBeAg negative, and HBsAg less than 10 IU/mL. JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379.

were treatment naive and 75 (44%) were HBeAg positive. Baseline data for subgroups by HBeAg and nucleos(t)ide analogue-treatment history status at screening are shown in the appendix (p 9).

The median duration of follow-up was 37·1 weeks (IQR 32·9–41·0). Of 470 patients, 448 (95%) completed the treatment period (figure 2), with no differences across treatment groups. 23 (5%) patients prematurely discontinued treatment for reasons including adverse events (five patients), loss to follow-up (three patients), patient decision (four patients), treatment failure (two patients), doctor decision (two patients), or other

reasons (seven patients); however, these patients could remain in the study. At follow-up week 24, 428 (91%) patients were still in the study, with 413 (88%) ongoing in the follow-up and 15 (3%) in the extended follow-up; 21 (4%) patients withdrew from the study prematurely. The modified intention-to-treat population included 464 patients and excluded six patients who prematurely discontinued the study for COVID-19-related reasons.

At week 48, 47 patients met the primary endpoint of meeting nucleos(t)ide analogue-stopping criteria: five (5%; 90% CI 2–11) of 91 patients in the JNJ-3989 dual 40 mg group, 15 (16%; 10–24) of 92 patients in the JNJ-3989 dual 100 mg group, 18 (19%; 13–27) of 94 patients in the JNJ-3989 dual 200 mg group, eight (9%; 4–15) of 94 patients in the triple group, and one (2%; 0–10) of 45 patients in the control group; no patients in the JNJ-6379 dual group met stopping criteria (figure 3A). A JNJ-3989 dose–response relationship for the primary endpoint was observed, with the greatest proportion of patients meeting nucleos(t)ide analogue-stopping criteria in the JNJ-3989 dual 200 mg group. 38 (81%) patients who met nucleos(t)ide analogue-stopping criteria at week 48 were virologically suppressed and HBeAg negative at baseline (figure 3B). Few patients from each of the other subgroups met nucleos(t)ide analogue-stopping criteria at week 48: five not currently treated and HBeAg negative, two not currently treated and HBeAg positive, and two virologically suppressed and HBeAg positive (figure 3B). At the follow-up week 24 analysis, 19 (4%) of 464 additional patients had met nucleos(t)ide analogue-stopping criteria after treatment week 48. Of these 19 patients, five were from the JNJ-3989 dual 100 mg group, ten were from the JNJ-3989 dual 200 mg group, and four were from the triple group (figure 3A).

Among patients who did not meet nucleos(t)ide analogue-stopping criteria at week 48, the main reason was not reaching an HBsAg concentration of less than 10 IU/mL for patients who were HBeAg-negative (not currently treated and virologically suppressed) and not reaching HBeAg negativity for patients who were HBeAg-positive (appendix p 21).

Of the 45 patients treated with JNJ-3989 who met nucleos(t)ide analogue-stopping criteria and stopped nucleos(t)ide analogue treatment at week 48, two (one each from the JNJ-3989 dual 200 mg and triple groups) met nucleos(t)ide analogue-retreatment criteria (HBeAg seroreversion, confirmed HBV DNA increase >2000 IU/mL with ALT >5×ULN or HBV DNA increase >20 000 IU/mL) during follow-up (appendix pp 10–11). Of patients who stopped nucleos(t)ide analogue at week 48, off-treatment HBV DNA was below the LLOQ at follow-up week 24 in 11 (73%) of 15 patients in the JNJ-3989 dual 200 mg group, four (31%) of 13 patients in the JNJ-3989 dual 100 mg group, two (25%) of eight patients in the triple group, one (20%) of five patients in the JNJ-3989 dual 40 mg group, and one (100%) of one patient in the control group

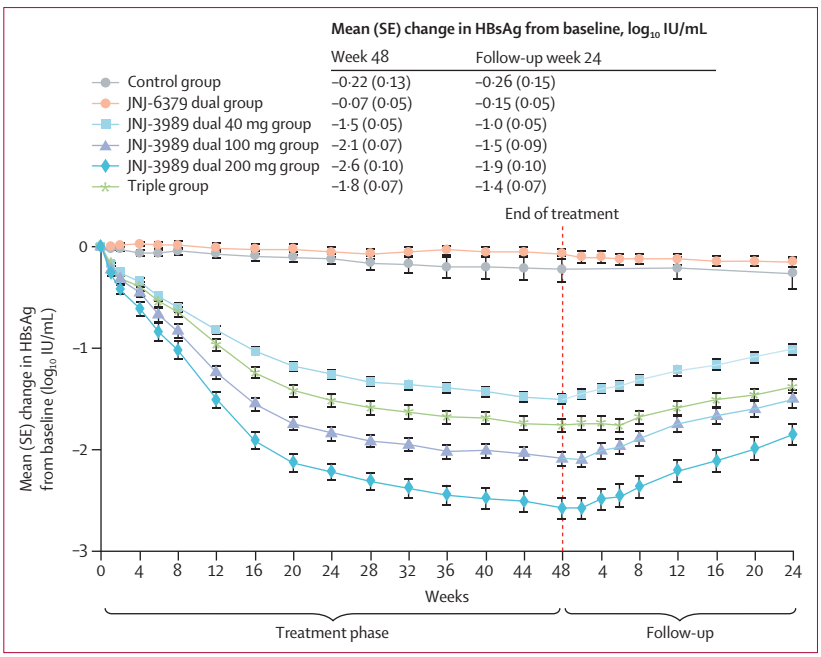

Figure 4: **Mean change in HBsAg from baseline over time**
Error bars show SE. JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379.

(appendix p 10). Of patients in the JNJ-3989 dual 200 mg group who remained off nucleos(t)ide analogue treatment, 15 (100%) of 15 had HBV DNA of less than 2000 IU/mL and ten (67%) had HBV DNA below the LLOQ, with HBsAg of less than 100 IU/mL (appendix p 10).

By week 48, substantial reductions in mean HBsAg were observed in all JNJ-3989 groups versus the control group and JNJ-6379 dual group (figure 4; appendix p 12). Mean HBsAg change from baseline followed a JNJ-3989 dose-dependent pattern. At week 48, 94 (98%) patients in the JNJ-3989 dual 200 mg group had 1 $\log_{10}$ or greater decline from baseline and 67 (74%) of 91 patients with available data at week 48 in the JNJ-3989 dual 200 mg group had a 2 $\log_{10}$ decline or greater from baseline with a mean reduction of 2·6 $\log_{10}$ IU/mL (SE 0·10, range 0·9–6·1; figure 4; appendix p 22). Additionally, a mean of at least 2 $\log_{10}$ IU/mL decline across all subgroups by treatment history (not currently treated or virologically suppressed) and HBeAg status (positive or negative) was observed in this group (appendix p 23). During the follow-up, mean HBsAg concentrations increased in all groups that contained JNJ-3989, but mean reductions from baseline remained greater than 1 $\log_{10}$ IU/mL at follow-up week 24 (figure 4). Among subgroups by HBeAg and treatment history status, the greatest HBsAg reductions were in patients who were not currently treated and HBeAg-positive (appendix p 23).

By week 48, 50 (57%) of 87 patients in the triple group, 61 (69%) of 88 patients in the JNJ-3989 dual 100 mg group, and 68 (75%) of 91 patients in the JNJ-3989 dual 200 mg group had an HBsAg concentration of less than 100 IU/mL (appendix p 13); a smaller, but substantial,

proportion maintained HBsAg at less than 100 IU/mL at follow-up week 24 (37 [44%] of 85 *vs* 31 [38%] of 82 *vs* 39 [47%] of 83; appendix p 13). Although some patients showed a sustained off-treatment response, many had increasing HBsAg concentrations during follow-up (appendix p 24).

Eight patients treated with JNJ-3989 had at least transient HBsAg seroclearance (HBsAg <0·05 IU/mL). At week 48, one (1%) of 88 patients in the JNJ-3989 dual 100 mg group and three (3%) of 91 patients in the JNJ-3989 dual 200 mg group had HBsAg seroclearance (appendix p 25), but all four had detectable HBsAg at follow-up week 24 (appendix p 13). One patient (from the JNJ-3989 dual 100 mg group) had HBsAg seroclearance during the treatment phase but had a transient HBsAg increase at week 48 and HBsAg detectable at follow-up week 24 (appendix p 25). Three patients treated with JNJ-3989 had HBsAg seroclearance 24 weeks after the end of treatment (appendix p 25), one of whom (virologically suppressed, HBeAg negative) stopped nucleos(t)ide analogue at week 52 (follow-up week 4), and thus reached functional cure at 24 weeks off-treatment (20 weeks after stopping nucleos[t]ide analogue). In this patient, HBeAg and HBcrAg remained negative. The other two patients with negative HBsAg at follow-up week 24 had not stopped nucleos(t)ide analogue and had detectable HBeAg, HBcrAg, or both (appendix p 25).

One patient from the control group had HBsAg seroclearance during the 24-week follow-up phase and reached functional cure at follow-up week 24. This patient (who was not currently treated and HBeAg-positive) first had HBeAg seroclearance followed by HBsAg seroclearance, but remained HBcrAg positive.

Declines in HBeAg were observed across all treatment groups (appendix p 26). In patients who were not currently treated and HBeAg positive, who had higher baseline HBeAg concentrations than patients who were HBeAg positive and virologically suppressed at baseline, a JNJ-3989 dose–response trend in HBeAg was seen, with mean reductions from baseline of 1·03 $\log_{10}$ IU/mL (SE 0·17) in the JNJ-3989 dual 40 mg group, 2·09 $\log_{10}$ IU/mL (0·25) in the JNJ-3989 dual 100 mg group, 2·22 $\log_{10}$ IU/mL (0·30) in the JNJ-3989 dual 200 mg group at week 48, and a mean reduction of 1·45 $\log_{10}$ IU/mL (0·63) in the control group (appendix p 14). Numerically, the triple group had the greatest mean reduction in HBeAg (2·48 $\log_{10}$ IU/mL [0·25]; appendix p 14). Limited HBeAg declines were seen in patients who were virologically suppressed and HBeAg-positive, whose baseline concentrations were lower than patients who were not currently treated. Four of five patients who were HBeAg positive remained HBeAg positive despite reaching HBsAg seroclearance (appendix p 24). At week 48, 18 (14%) of 132 patients (four or fewer patients per group) had HBeAg seroclearance (HBeAg <LLOQ; LLOQ=0·11 IU/mL; –0·96 $\log_{10}$ IU/mL) with or without anti-HBe seroconversion, with no clear difference between treatment groups (appendix p 14). HBeAg concentrations remained generally stable or declined from week 48 to follow-up week 24 in the JNJ-3989 groups.

HBV DNA mean changes from baseline values are reported for patients who were not currently treated only (appendix pp 15, 27). At week 48, HBV DNA reductions were observed across all groups, with a trend for greater and dose-dependent mean declines among the JNJ-3989 and triple group versus control for patients who were HBeAg-positive. A high proportion of patients who were HBeAg-negative had HBV DNA below the LLOQ at early timepoints in the study, limiting the interpretation of mean declines within this subpopulation. HBV DNA concentrations remained generally stable or declined from week 48 to follow-up week 24 in the JNJ-3989 groups.

At week 48, reductions in HBcrAg were larger for patients who were not currently treated than for patients who were virologically suppressed (appendix pp 16, 28). The greatest mean reductions from baseline in HBcrAg were observed in patients who were not currently treated and HBeAg-positive from the JNJ-3989 dual 200 mg group (2·56 $\log_{10}$ IU/mL, SE 0·25) and triple group (2·67 $\log_{10}$ IU/mL, 0·33); one of the patients in the triple group had HBcrAg below the LLOQ at week 48. For patients who were not currently treated and HBeAg-negative, a plateau after initial HBcrAg decline was observed that was only partly due to an increasing number of patients with a HBcrAg below the LLOQ (three [16%] of 19 patients had HBcrAg <LLOQ at baseline and nine [50%] of 18 had HBcrAg <LLOQ at week 48 in the JNJ-3989 200 mg group). In patients who were JNJ-3989-treated and virologically suppressed, especially patients who were HBeAg-negative, small declines in HBcrAg were seen. Among these patients, 110 (49%) of 226 had HBcrAg below the LLOQ at baseline and 125 (55%) of 211 had HBcrAg below the LLOQ at follow-up week 24.

Among patients who were not currently treated and HBeAg-positive, a JNJ-3989 dose-dependent mean reduction from baseline in HBV RNA was observed (appendix pp 17, 29). The greatest mean reductions were seen in the two JNJ-6379-containing groups, consistent with the mechanism of action of JNJ-6379 (ie, inhibition of HBV RNA release): 4·88 $\log_{10}$ IU/mL in the JNJ-6379 group and 4·68 $\log_{10}$ IU/mL in the triple group. The proportion of patients with HBV RNA below the lower limit of detection increased from baseline in most of the subpopulations (appendix p 17). 202 (89%) of 227 virologically suppressed and 30 (32%) of 93 patients who were not currently treated and HBeAg-negative had HBV RNA concentrations below the lower limit of detection at baseline or at early timepoints in the study. HBV RNA concentrations remained generally stable or declined from week 48 to follow-up week 24 in the JNJ-3989 groups. In JNJ-6379-containing groups, HBV RNA concentrations frequently plateaued after an initial off-treatment increase.

	Control group (n=45)	JNJ-6379 dual group (n=48)	JNJ-3989 dual 40 mg group (n=93)	JNJ-3989 dual 100 mg group (n=93)	JNJ-3989 dual 200 mg group (n=96)	Triple group (n=95)	Total (n=470)
Adverse events	30 (67%)	41 (85%)	69 (74%)	66 (71%)	62 (65%)	68 (72%)	336 (71%)
Related adverse events	9 (20%)	21 (44%)	28 (30%)	26 (28%)	31 (32%)	33 (35%)	148 (31%)
Adverse events leading to death	0	0	0	0	0	0	0
Serious adverse events	0	2 (4%)	1 (1%)	2 (2%)	3 (3%)	2 (2%)	10 (2%)
Serious adverse events related to study treatment	0	0	0	0	1 (1%)	1 (1%)	2 (<1%)
Adverse events leading to discontinuation of JNJ-6379, JNJ-3989, or placebo	0	1 (2%)	2 (2%)	0	0	2 (2%)	5 (1%)
Grade 3 or 4 adverse events	2 (4%)	7 (15%)	6 (6%)	2 (2%)	5 (5%)	7 (7%)	29 (6%)
Adverse events of special interest	5 (11%)	15 (31%)	15 (16%)	19 (20%)	15 (16%)	26 (27%)	95 (20%)
Renal complications	1 (2%)	4 (8%)	6 (6%)	6 (6%)	2 (2%)	14 (15%)	33 (7%)
Injection-site reactions	0	1 (2%)	3 (3%)	10 (11%)	4 (4%)	5 (5%)	23 (5%)
Haematological abnormalities	3 (7%)	4 (8%)	5 (5%)	4 (4%)	4 (4%)	2 (2%)	22 (5%)
ALT or AST elevations	1 (2%)	4 (8%)	2 (2%)	2 (2%)	3 (3%)	7 (7%)	19 (4%)
Cholesterol increases	0	3 (6%)	1 (1%)	1 (1%)	2 (2%)	1 (1%)	8 (2%)
Most common adverse events (≥5% of patients)							
Headache	7 (16%)	4 (8%)	14 (15%)	17 (18%)	12 (13%)	13 (14%)	67 (14%)
Nausea	1 (2%)	6 (13%)	6 (6%)	6 (6%)	7 (7%)	4 (4%)	30 (6%)
eGFR decrease	1 (2%)	3 (6%)	5 (5%)	5 (5%)	2 (2%)	11 (12%)	27 (6%)
Nasopharyngitis	1 (2%)	3 (6%)	5 (5%)	8 (9%)	4 (4%)	6 (6%)	27 (6%)
Fatigue	3 (7%)	6 (13%)	5 (5%)	3 (3%)	2 (2%)	7 (7%)	26 (6%)
Diarrhoea	2 (4%)	2 (4%)	4 (43%)	10 (11%)	3 (3%)	4 (4%)	25 (5%)
Back pain	1 (2%)	2 (4%)	6 (6%)	6 (6%)	5 (5%)	5 (5%)	25 (5%)

Data are n (%). ALT=alanine aminotransferase. AST=aspartate aminotransferase. eGFR=estimated glomerular filtration rate. JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379.

Table 2: **Adverse events during the double-blind phase (up to week 48) in the safety population**

During the 48-week treatment phase, adverse events were reported by 336 (71%) 470 patients, with 148 (31%) having an adverse event considered to be related to study drugs (table 2). At the time of the follow-up week 24 analysis, 189 (41%) of 460 patients had at least one adverse event during the follow-up phase, with similar frequency across the JNJ-3989 and JNJ-6379 groups (appendix p 18), and five (1%) patients had adverse events that were considered related to study drug. Grade 3 or 4 adverse events were observed in 29 (6%) of 470 patients during the treatment phase and in ten (2%) of 460 patients during the follow-up phase; five (5%) of 95 patients in the JNJ-3989 200 mg group had grade 3 or 4 adverse events. During the treatment phase, five (1%) of 470 patients had an adverse event resulting in discontinuation of treatment with JNJ-3989 or JNJ-6379 (two patients had decreased estimated glomerular filtration rate [eGFR], one had renal impairment, one had insomnia, and one had increased HBV DNA concentrations). The most common adverse events during the treatment phase were headache (67 [14%] of 470 patients), nausea (30 [6%]), decrease in eGFR (27 [6%]), nasopharyngitis (27 [6%]), fatigue (26 [6%]), diarrhoea (25 [5%]), and back pain (25 [5%]). Adverse events of special interest were observed in 95 (20%) of 470 patients during the treatment phase and included renal complications (33 [7%] patients), injection-site reactions (23 [5%]), haematological abnormalities (22 [5%]), ALT or aspartate aminotransferase (AST) elevations (19 [4%]), and cholesterol increases (eight [2%]). Rates of adverse events of special interest during the follow-up phase were low (16 [3%] of 460 patients), and most were haematological abnormalities (seven [2%]). Ten (2%) of 470 patients had serious adverse events during the treatment phase, and two patients (one each from the JNJ-3989 200 mg group [exercise-related rhabdomyolysis] and the triple group [increase in ALT or AST]) had serious adverse events related to study treatment. During follow-up, 12 (3%) of 460 patients had a serious adverse event; one (<1%), a gastric ulcer, was considered to be related to nucleos(t)ide analogues and occurred in a patient from the JNJ-3989 200 mg group. There were no deaths.

During the treatment phase, ALT concentrations in patients who were not currently treated decreased across all treatment groups (figure 5A), and in patients who were virologically suppressed mean ALT values increased slightly for the JNJ-6379 group, JNJ-3989 200 mg group, and triple group, but remained within normal range (figure 5B). ALT reductions during follow-up were also

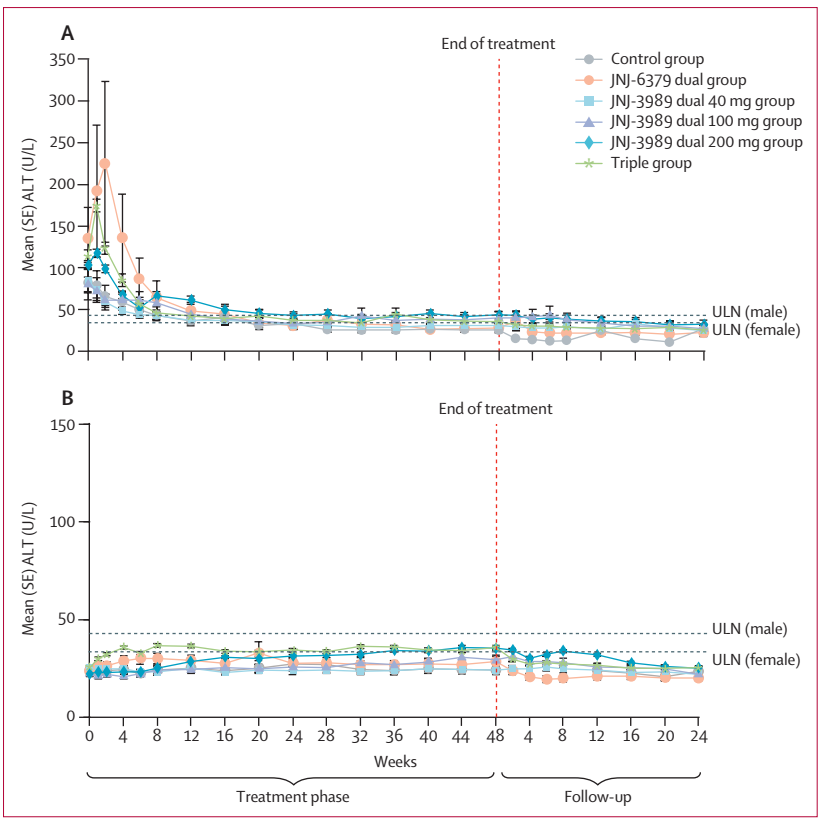

Figure 5: **Mean ALT profiles over time in patients who were not currently treated (A) and patients who were virologically suppressed (B)**

Error bars show SE. ALT=alanine aminotransferase. JNJ-3989=JNJ-73763989. JNJ-6379=JNJ-56136379.

mainly observed in these groups (figure 5). 13 (3%) of 470 patients had a grade 3 or 4 ALT elevation during the treatment phase (appendix p 19). 13 (3%) of 470 patients had an ALT flare (confirmed ALT ≥3×ULN and ≥3×nadir); seven were from the JNJ-3989 200 mg group. One patient who received JNJ-3989 200 mg and stopped nucleos(t)ide analogue treatment at week 48 had an ALT flare during the 24-week follow-up phase and an increase in HBV DNA of more than 20 000 IU/mL. Among the 62 patients who met nucleos(t)ide analogue-stopping criteria and stopped nucleos(t)ide analogue treatment at week 48 or during the follow-up phase, 17 (27%) had virological relapse (confirmed HBV DNA increases >200 IU/mL).

Decreased eGFR (based on serum creatinine; 45 [10%] of 470 patients) and high LDL (18 [4%]) were the most common grade 3 or worse laboratory abnormalities (appendix p 19). Declines in eGFR occurred most frequently in patients treated with JNJ-6379 (with or without JNJ-3989), occurred immediately after baseline, and remained reduced during the 48-week treatment phase. However, these decreases in eGFR showed rapid recovery during follow-up, and there were fewer incidences of grade 3 or worse eGFR decreases and LDL increases during the follow-up phase (appendix p 20).

Discussion

Novel therapeutic approaches for the treatment of chronic hepatitis B aim for sustained virological off-treatment responses. Functional cure is defined as HBsAg seroclearance with or without seroconversion to an anti-HBs antibody-positive state at least 24 weeks after the end of treatment.[18] In our study, a JNJ-3989 dose–response was observed for the primary endpoint (proportion of patients reaching nucleos[t]ide analogue-stopping criteria) and reduction of all viral markers, particularly HBsAg.

Only two of 45 patients in JNJ-3989 groups who met nucleos(t)ide analogue-stopping criteria and stopped nucleos(t)ide analogue at week 48 restarted nucleos(t)ide analogue treatment by follow-up week 24. Of the patients treated with JNJ-3989 at follow-up week 24, 44% overall and 73% in the 200 mg group had off-treatment HBV DNA below the LLOQ. Considering the criteria to stop nucleos(t)ide analogues, it is not unexpected that the virologically suppressed, HBeAg-negative subgroup had the highest proportion of patients who met the primary endpoint. Across all populations, the main reasons that patients did not meet the nucleos(t)ide analogue-stopping criteria were not reaching HBeAg negativity, HBsAg concentrations of less than 10 $\log_{10}$ IU/mL, or both.

A pronounced mean HBsAg decline occurred in all JNJ-3989-containing groups around the first 20 weeks of treatment followed by a more gradual decline up to week 48. The JNJ-3989 200 mg dose resulted in the greatest mean HBsAg reduction (2·6 $\log_{10}$ IU/mL overall and 3·6 $\log_{10}$ IU/mL in patients who were not currently treated and HBeAg-positive) at week 48 and over the 24-week follow-up phase. At week 48, all but two patients in this group had a 1 $\log_{10}$ or greater decline from baseline and 74% had a greater than 2 $\log_{10}$ decline with a range from 0·9 $\log_{10}$ IU/mL to 6·1 $\log_{10}$ IU/mL. Although HBsAg decline was generally more pronounced in patients who were not currently treated and HBeAg-positive, the mechanism explaining this substantial inter-individual variability in HBsAg decline is not understood. Mean HBsAg concentrations in all JNJ-3989 groups increased during the follow-up phase (ie, when patients were off treatment), but the mean reduction from baseline remained 1 $\log_{10}$ IU/mL or greater with a relevant proportion of patients having sustained low off-treatment HBsAg concentrations, for example, ranging from 14·5% of patients with HBsAg less than 100 IU/mL at follow-up week 24 in the JNJ-3989 dual 40 mg group to 47·0% in the JNJ-3989 dual 200 mg group. Data beyond 24 weeks of follow-up are available from earlier studies with JNJ-3989[15,19] and other siRNAs[20–22] and shows that sustained HBsAg and off-treatment HBV DNA suppression are maintained past follow-up week 24. These observations potentially suggest a new steady state between the virus and host immune system in some patients, although this remains to be substantiated.

Unexpectedly, the combination of JNJ-3989 100 mg with JNJ-6379 resulted in less pronounced HBsAg decline than JNJ-3989 100 mg without JNJ-6379 during the 48-week treatment period. This effect was not apparent for HBeAg and HBcrAg. HBV RNA was reduced further when JNJ-6379 was given in the triple group, confirming target engagement and findings from previous studies that JNJ-6379[12,16] potently inhibits HBV DNA and RNA release. There is currently no known explanation for a potential antagonistic effect of JNJ-6379 on JNJ-3989-induced HBsAg decline. Plasma pharmacokinetics of JNJ-6379 and JNJ-3989 in a subset of patients did not indicate an interaction.[23] The absence of a beneficial effect on HBsAg decline of a CAM E class added to an siRNA was confirmed in a study with vebicorvir and AB-729.[24]

One patient in the control group reached functional cure. This patient also reached HBV DNA, HBV RNA, and HBeAg negativity but remained HBcrAg positive. Only eight patients from the JNJ-3989 100 mg or JNJ-3989 200 mg groups had HBsAg seroclearance at least once during the study, of whom five had detectable HBsAg at follow-up week 24. In this study, we observed positivity of HBeAg (and HBcrAg) despite HBsAg levels being negative. Although the rate of HBsAg negativity was greater in a study with the antisense oligonucleotide bepirovirsen,[25] most patients with HBsAg seroclearance in that study had subsequent off-treatment HBsAg increases, showing that direct HBsAg lowering, even to below the LLOQ, for the durations assessed, is insufficient to result in immune control and functional cure in most patients. This suggests that combination treatment with immune-stimulating agents is needed for these drug classes. Importantly, HBsAg of less than 100 IU/mL, a threshold associated with improved immune response and HBsAg seroclearance during the natural course of disease[26,27] was reached by 75% of patients at the end of treatment and by 47% at follow-up week 24 in the JNJ-3989 dual 200 mg group. Thus, those patients who reached low HBsAg concentrations with JNJ-3989 treatment might be good candidates for combination regimens that include immune modulators.

JNJ-3989 dose-dependently reduced HBV RNA. In patients who were not currently treated and HBeAg-positive, greatest mean change from baseline in HBV RNA at week 48 was observed in the triple and JNJ-6379 dual groups followed by the JNJ-3989 200 mg group. Thus, treatment with JNJ-6379 increased the on-treatment reduction in HBV RNA, whereas an increase in HBV RNA was seen followed by levels remaining substantially below baseline levels, especially in the triple group, in the follow-up phase.

For HBeAg and HBcrAg, a JNJ-3989 dose–response was observed and declines seemed dependent on baseline levels, for reasons not completely understood, with the highest declines in patients who were not currently treated and HBeAg-positive who had higher baseline levels than other subgroups. This pattern was not seen for HBsAg, for which reductions appeared unrelated to baseline concentrations. Patients with HBeAg concentrations close to LLOQ, either at baseline or JNJ-3989-induced, infrequently reached HBeAg seroclearance. The clinical relevance of this persisting low-level HBeAg positivity remains unclear. In contrast to HBsAg, mean off-treatment concentrations of HBcrAg and HBeAg generally remained stable or declined.

All regimens were generally well tolerated and safe. During the treatment phase, adverse events were most frequently reported by patients in the JNJ-6379 group, whereas rates were similar across the other groups. Significant ALT elevations were rare (3%) and mainly occurred early in the treatment phase in the not currently treated population. Those flares were generally associated with reduced HBV DNA and ALT and normalised after continued treatment (one patient discontinued treatment at peak of flare). An effect of ALT flares on HBsAg kinetics, as suggested for bepirovirsen,[25] was not seen. The frequency of adverse events during the follow-up phase was similar across all groups. Five (1%) patients discontinued the study early due to adverse events. There were no deaths.

This large study was powered for the overall comparison of study groups and included a wide range of different chronic hepatitis B populations based on HBsAg status, treatment status, race, and age. The sample size of relevant subgroups within study groups was often small, which hindered robust interpretations and might be a study limitation. Furthermore, the relatively short off-treatment period could be a limitation for making conclusions about the sustained effects of the treatment regimens on viral markers.

The combinations of antiviral therapies used in this study were insufficient to achieve functional cure. However, most patients treated with JNJ-3989 had substantial HBsAg reductions that are believed to establish a liver environment that supports better immune control and might allow a better response to immune-modulating therapies. Combination studies aiming for functional cure of chronic hepatitis B involving different mechanisms of action, including immune modulators, are ongoing.

Contributors

M-FY, TA, IMJ, MRB, HLAJ, TT, JLH, TNK, TL, MBe, RK, CG-A, CM, TV, OL, US, and MBi contributed to the design and conduct of the study and interpretation of the data. JJ contributed to the analysis and interpretation of data. All authors were involved in the critical revisions of the manuscript and review for important content, and approved the final manuscript submitted. M-FY and MBi accessed and verified the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

M-FY serves as an adviser or a consultant for AbbVie, AlloVir International, Arbutus Biopharma, Bristol Myers Squibb, ClearB Therapeutics, Dicerna Pharmaceuticals, Gilead Sciences,

GlaxoSmithKline, Janssen, Merck Sharp & Dohme, Roche, and Spring Bank Pharmaceuticals; and receives grant or research support from Assembly Biosciences, Arrowhead Pharmaceuticals, Bristol Myers Squibb, Fujirebio Diagnostics, Gilead Sciences, Merck Sharp & Dohme, Roche, Spring Bank Pharmaceuticals, and Sysmex Corp. TA serves as an adviser or a consultant for AbbVie, Antios Therapeutics, Enyo Pharma, Gilead Sciences, GlaxoSmithKline, Janssen, Roche, and Vir Biotechnology. IMJ serves as a consultant for Aligos, Arbutus, Janssen, and Gilead; has received research funds from Janssen and Assembly Biosciences; and serves on a data monitoring committee for GlaxoSmithKline. MRB serves as a consultant and speaker for AbbVie, Gilead Sciences, Janssen, Eisai, MSD, and Roche. HLAJ received grants from AbbVie, Arbutus, Gilead Sciences, Janssen, and Roche, and is a consultant for Arbutus, Arena, Enyo, Gilead Sciences, GlaxoSmithKline, Janssen, Merck, Roche, Vir Biotechnology, and Viroclinics. TT has received lecturer fees Bristol Myers Squibb, Gilead Sciences, and GlaxoSmithKline; research funding from Janssen and Gilead Sciences; and consulting fees from Janssen. JLH serves as an adviser or a consultant for Aligos, Assembly, Gilead Sciences, Johnson & Johnson, and Roche, and receives grant or research support from Bristol Myers Squibb. TNK, TL, MBe, RK, CG-A, CM, JJ, TV, OL, US, and MBi are Janssen employees and are Johnson & Johnson stockholders.

Data sharing

The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access Project site at http://yoda.yale.edu.

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